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SCREENING OF SOUTH INDIAN HERBS FOR QUORUM QUENCHING PROPERTY.

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ABSTRACT

All bacteria communicates by a process called Quorum sensing. In which the bacteria secrete small molecules are called auto inducer that acts a molecular "Messenger", the auto induces increase with increase in bacterial population and are able to involve in Quorum sensing only after reaching a threshold. Concentration anti quorum sensing agents. Would offer ofcontrolling microbial infection with advantages of reducing risk of resistance development. The continuing search for new and novel anti microbials and anti pathogenic agents has focused on exploiting the fact that plants serving in an environment with high bacterial density has been seen to posses productive means against infection. Naturalproducts especially south Indian folk plants shows the promising results in curing of diseases and bacterial infections in this study 15 plants were investigated and all the plants shows the activity in which *Termanalia chebula* was found to be a leading plants followed by *awsonia inermis, solanum torvum, termanalia arjuna.*

KEY WORDS : ***Chromobacterium, Violaceum, Terminalia, Chebula Quorum Sensing, Pseudomonas aeruginosa***

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INTRODUCTION

Bacteria monitor their environment for the presence of other bacteria by producing and respondingtocertain chemicals signaling molecules. The concentration of these molecules is in direct proportion to the load or density of the bacteria; in other words, there is a direct relationship between the concentrations of chemical signaling molecules and that of the bacteria present in a given environment. This is termed as Quorum Sensing (QS) It allows bacteria to communicate with each other by synchronizing / regulating the expression of several genes that are necessary for bacteria to behave in unison as a group (Anjali *et al.,* 2008) Phytochemicals have been used for thousands of years for the treatment and management of diseases. (Rasmuseen and Givskov 2006). The secretion of enzymes that destroy the auto inducers and the production of auto inducers antagonists are used by competitor bacteria and susceptible eukaryotic host to render QS bacteria mute and deaf respectively. Analogues synthetic strategies are now being explored for the development of novel antimicrobial therapies (Stephan and Bonnie 2011). For many years, control of bacterial infections by inhibiting microbial growth has been a primary approach of antimicrobial chemotherapy. An emergency problem associated with continual indiscriminant are of this therapeutic strategy is the selection of resistant bacteria with higher level of tolerance against broad - spectrum antibiotics. It has been recognized that there is a need for a strategy that can block very basic mechanisms of bacterial communication that appear to control bacterial virulence factors leading to pathogencity (Vattem *et al*., 2008).

The anti-pathogenic activities of the extracts were also explored using to Quorum Sensing inhibition assay. The mutant strain *Chromobacterium violaceum* CV026 was used as a biosensor to detect anti pathogenic activity (MC Lean *et al.,* 2004). It has been reported that many gram-negative bacteria. including pathogenic species (eg *pseudomonas aeruginosa*) use small organic molecules known a N-acylated homoserine lactones AHLs for Cell - Cell communication. (Water and Bassler 2005). *Pseudomonasa* an increasingly prevelant opportunistic human pathogens, is the most common gram-negative bacterium found in nosocomial infections. The capacity of *pseudomonas aeruginosa* to produce such diverse, often over whelming infections is due to an arsenal of virulence factors. Many extra cellular virulence factors secreted by *pseudomonas aeruginosa* have been shown to be controlled by a complex regulatory circuit involving cell-to-cell signaling system that allow the bacteria to produce these factors in a coordinated, cell-density dependent manner (Passador *et al.,* 1995).

Pseudomonas aeruginosa produces several extracellulars products that after colonization can cause extensive tissue damage, blood stream invasion, and dissemination. In vivo studies have shown that mutants defective is the production of exotoxin A, exoenzymes, elastase or alkaline protease are essential for maximum virulence of *pseudomonas aeruginosa* however the relative contribution of a given factors may vary with the type of infection (Nicas TI, *et al*., 1985). Antibiotic resistance provides a great therapeutic and economic burden in the treatment of infectious disease and it may threaten the success of antimicrobial chemotherapy. It is estimated that antibiotic resistance double hospital stay and morbidity single antibiotic resistance itself is a great problem, however the appearance of multiple antibiotic resistant (MDR) strains came a more pronounced obstacle for patients and health care propossionals (Levy S.B. *et al*., 2004, Smith, M.A, 2005). bacteria by reversal of resistance, effect inhibition, inhibition of biofilm formations, interference in bacterial Quorum sensing etc

MATERIALS AND METHODS

Plant Material and Extract Preparation

The leaf of *Terminalia Arjuna, Tragia involucrata, Clerodendrum Phlomids, Solanum trilobatum, Solanum surattense, Solanum nigrum, Solanum torvum and Clerodendrum inerme*, The seed of *Lawsonia inermis, Myristica fragrans, Terminalia chebula, Piper longum and Solanum melongena.* The root of *Glycyrrhiza Glabra, Withania somnifera,* used in this study were obtained from the local market and were identified based on its physical characteristics. Air dried and powered plant materials approximately from 100 to 300g were submitted to sequential maceration with 500ml of water at room temperature. After each step the extracts were filtered and the solvents were removed under vacuum at 30^0C until dry aqueous extracts were obtained

Strains and Media

Pseudomonas aeruginosa MTCC 2453 and *Chromobacterium violaceum* 2656 were collected from Microbial type culture collection center. IMTECH, Chandiragh. The purchased culture was propagated in the nutrient broth and incubated at 37^0 C For 24 hours of incubation The grown culture was subcultured at regular intervals of 30 days and stored at 4^0 c for future use. The Luria Bertani (LB) medium was used throughout the study.

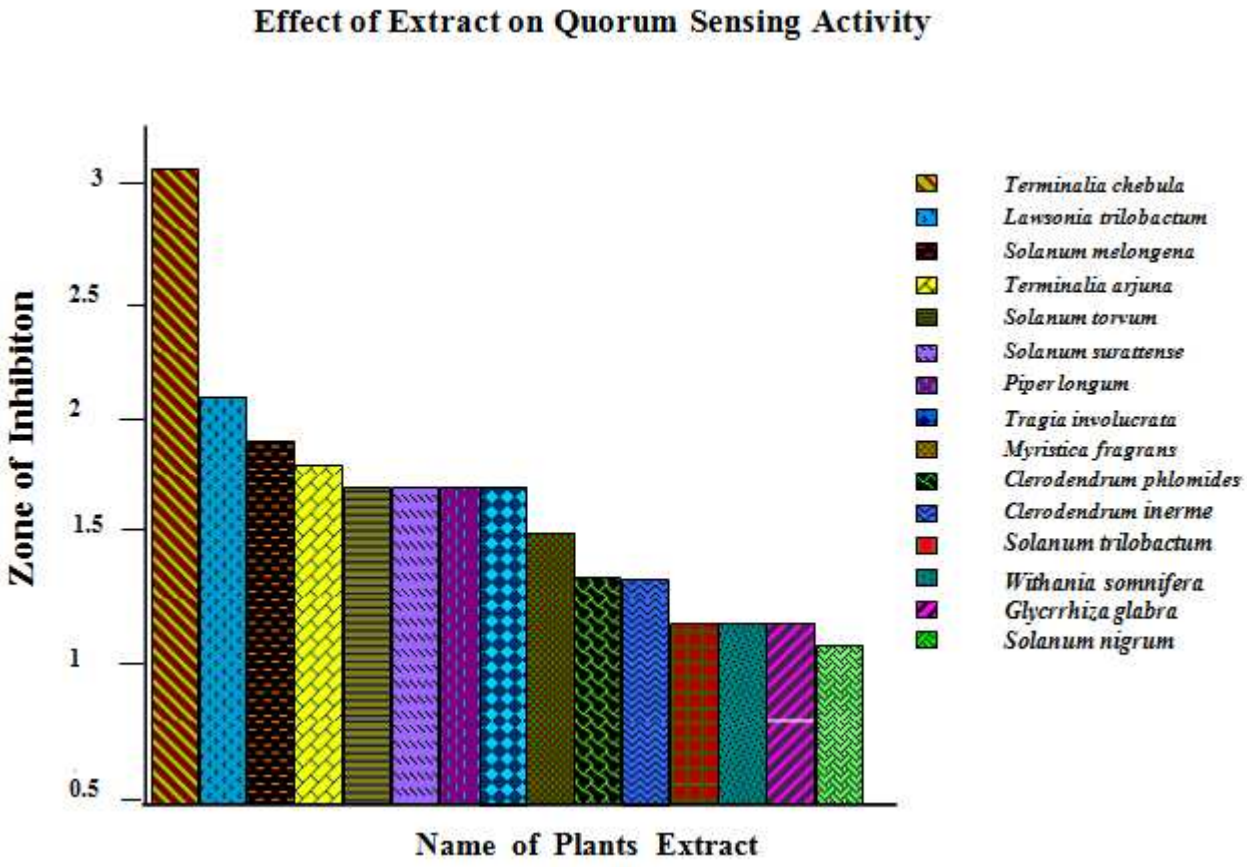
Culture Condition

The entire assay except those for biofilm formation the culture condition were as follows. Overnight cultures of Pseudomonas aeruginosa were grown in LB medium at 37^0 C with shaking. The cultures were then diluted 100- fold in fresh LB medium and allowed to grow to an optical density at 600nm (OD_{600}) 1.7 (early stationary phase). At this point, the culture was divided into 10ml aliquots and additional 1ml of fresh medium containing plant extract or media control was added to a final concentration of 1mg/1ml extract. Cultures were recovered at late stationary phase (approximately 72h after addition). The cells were separated from the growth medium by centrifugation at 10,000xg for 10min (Turbo et al., 2003).

AHL Bioassay

The extracted plant material was placed directly on to LB plates spread with *Chromobacterium violaceum* supplemented with AHL were incubated at 30^0C and QS inhibition was detected by a ring of colorless. Purified halogenated furanone (100µg) was used as a positive control for QS inhibition and ethanol (20µg) as a negative control. The ethanol was allowed to evaporate from the control and sample discs before testing to eliminate toxicity.

S.No	Botanical Name	Family	Parts used	Folk Name	Medicinal Uses
					Flatulence, Constipation, Piles, Cough and
2	*Solanum torvum*	Solanaceae	Leaves	Sundakai	Tolerate diseases
3	*Solanum surattense*	Solanaceae	Leaves	Karimulli	Toothache, cough, Asthma, Catarrh.
4	*Myristica fragrans*	Myristicaceae	Seed	Jathikai	Skin diseases, leprosy, colds, cough, pimples.
5	*Piper longum*	Piperaceae	Seed	Arisi Thippili	Throat irritation
6	*Terminalia arjuna*	Combretaceae	Leaves	Maruthu	Heart disease, hemorrhages and ulcer.
7	*Solanum melongena*	Solanaceae	Seed	Kandakathri	Venereal disease, fever, dysentery.
8	*Tragia involucrata*	Euphorbiaceae	Leaves	Kanjsori	Curing eczema, fevers, wheezing and diabetes.
9	*Clerodendrum phlomides*	Lamiaceae	Leaves	Thaluthalai	Swelling.
10	*Solanum trilobactum*	Solanales	Leaves	Thuthuvalai	Cooking, Cough and mildly toxic.
11	*Lawsonia inermis*	Lythraceae	Leaves	Maruthani	Skin dyeing, reducing Heat
12	*Clerodendrum inerme*	Verbenaceae	Leaves	Bechangu	Hypertension dysentery, cough, Skin rashes and heart related disorders.
13	*withania somnifera*	Solanales	Leaves	Ashwagantha	Tumors, tubercular glands, Carbuncles, and ulcers.
14	*Solanum nigrum*	Solanaceae	Leaves	Manathathakkali	Mouth ulcers, pepticulcers
15	*Glycrrhiza Glabra*	Fabaceae	Root	Athimathuram	Mouth ulcers, pepticulcers



RESULT AND DISCUSSION

Screening results of 15 different medicinal plant extract for their ability to inhibit QS regulated violacein production against *C. violaceum.* The plant extracts namely Terminalia Chebula showed the greater inhibition, where as *Lawsonia trilobactum, solanum melongena, Terminalia arjuna* are the good sources of producing AHL antagonists (Table:1). The present study proves the potentiality of the Indian medicinal plants as a source of anti QS compounds. Ethnomedicinal uses of these plants are focused on the development of molecules that are structurally analogues to the AHL or Autoinducers. Such molecules are potential use as an antimicrobial drug aimed at bacteria as anti QS molecules. Further researches are undergoing to view as plants are potential targets for novel antimicrobial drug.

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