INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

INHIBITION OF QUORUM SENSING BY EXTRACTS OF *ALPINIA GALANGA*

Pavithra B.P [1], Soundhari C [2]* and Varsha Gayatri K [1]
[1]Post Graduate Student, Department of Microbiology, Valliammal College for Women, Chennai, India
[2]Associate Professor, Department of Microbiology, Valliammal College for Women, Chennai, India
*Corresponding Author Email: drcsoundhari@gmail.com

Article Received on: 15/09/18 Approved for publication: 25/10/18

DOI: 10.7897/2230-8407.0911266

ABSTRACT

Bacteria communicate with each other by producing and responding to diffusible signal molecules known as auto inducers by a phenomenon called Quorum sensing. Quorum sensing controls virulons in many infectious bacteria. The pathogenicity of an infectious agent can be prevented by blocking this mechanism and thereby attenuate the infectious agents. The lyophilized aqueous and ethanolic extracts of *A. galanga* were subjected to the inhibition of violacein a pigment produced in *Chromobacterium violaceum* under quorum sensing by broth dilution method. There was a concentration dependent inhibition observed from a concentration of 2mg/ml. The aqueous extract *A. galanga* inhibited violacein production completely at a concentration of 2mg/ml & 1mg/ml. The ethanolic extracts of *A. galanga* inhibited violacein production till a concentration of 125µg/ml. Germ tube inhibition was also attempted. The ethanolic extracts of *A. galanga* was found to inhibit germ tube of *Candida albicans* at tested concentration. The lyophilized extracts did not show any significant antibacterial activity. Eighteen bioactive compounds were found in the GC-MS analysis of the ethanolic extracts of *A. galanga*. Cytotoxic results revealed that the extracts were non-toxic from 500µg/ml. The results of the present study identified that the rhizome extracts of *A. galanga* have potential to replace antibiotics because of its quorum sensing inhibitory activity against *C. violaceum* and *C. albicans*.

Key words: quorum sensing, *A. galanga*, violacein, germ tube inhibition

INTRODUCTION

The inappropriate and excessive use of antibiotics has resulted in the development of multi-drug resistant bacterial strains which have become a growing concern worldwide. Million people die because of infectious disease caused by multi-drug resistant pathogens[1].

Bacteria can communicate with each other through producing and responding to small diffusible signal molecules known as autoinducers. The phenomenon is called Quorum sensing[2]. Autoinducers are produced at basal levels and their concentration increases with growth. Because the signals can diffuse through membranes and on reaching a critical concentration, the signal molecules bind to and activate receptors inside bacterial cells which trigger genes that code for several characteristics like bioluminescence, plasmid conjugation, biofilm formation, toxin production, exopolysaccharide production, siderophore synthesis, sporulation, motility, pigment production which determine the pathogenicity of the organism. Because QS controls virulence, blocking the mechanism would prevent pathogenicity, rendering the infectious agents attenuated. QS inhibitor will not interfere with the growth but prevent the pathological consequence and hence act as excellent anti-pathogenic agents. Virulence determinants in pathogens are not strictly essential for viability; hence anti-quorum sensing compounds will not induce mutagenesis which can lead to the development of resistance[3].

Chromobacterium violaceum, a soil-borne Gram negative bacterium. The bacterium produces violet pigment violacein as a result of quorum sensing using its autoinducer N-hexanoyl homoserine lactone (HHL). The autoinducer is released into the environment and diffuses back into the bacterium when a quorum has been reached. The auto-inducer then binds to the transcriptional regulator CviR and participates in the expression of specific genes such as violacein[4].

Candida albicans was the first eukaryotic microorganism shown to exhibit quorum sensing. *C. albicans* causes various forms of candidiasis ranging from mucosal infections to serious systemic infections[5]. Germ tube formation is one of the major virulent traits in *C. albicans* under quorum sensing. There are very few studies which report about the inhibition of germ tube elongation.

The quorum sensing signal molecule identified in *C. albicans* was not an acyl-homoserine lactone or a peptide but the sesquiterpene farnesol (3,7,11-trimethyl-2,6,10-dodecrtriene-1-ol). The accumulation of farnesol blocks the morphological shift from yeasts to hyphae at high cell densities and exogenously added farnesol inhibits germ tube formation as normally triggered by serum, proline, or N-acetylglucosamine[6].

Plants used in traditional system of medicine have been widely exploited around the world in search of phytochemicals. The efficacy of herbals in the treatment of diseases for decades suggests that bacteria, fungi, and viruses may have a reduced ability to adapt to a plant-based antimicrobial regime[7].

In India and Asia, *Alpinia galanga* has been traditionally used for centuries to cure whooping colds in children, throat infection and to control fever. The rhizome part of *A. galanga* is to used treat various kinds of diseases includes diabetes mellitus, spleen trouble, and herpes infection. In the region of Himalaya and Southern region of Western Ghats, *A. galanga* is widely distributed.

The present study was aimed to investigate the anti-quorum sensing activity of extracts of *A. galanga* on violacein production

and germ tube elongation. The present article reports on the efficacy of extracts of *A.galanga* in inhibiting QS mediated virulence factors.

MATERIALS AND METHODS

Collection of Plants

The healthy rhizome of *A.galanga* was collected and shade dried. The accuracy of the plant species was ascertained. The aqueous and ethanolic extracts were prepared and lyophilized[8].The lyophilized extract was stored at -20°C till use.

Collection of Bacterial Culture

Bacterial strain *Chromobacterium violaceum* (MCC2290) was obtained from MCC, NCCS, Pune. The strain was subcultured in Nutrient agar medium at 32°C for 18 hours. For all the experiments, the inoculum was prepared by growing the bacteria in 10ml nutrient broth at 32°C for 24 hours in a shaking incubator (130 r/min).

Candida albicans was obtained from (MCC 1151), NCCS, Pune. The strain was subcultured in Yeast extract-malt extract agar medium at 25°C for 18 hours.

Antimicrobial Susceptible Testing

Preparation of plant extracts stock solution

2mg of the lyophilized aqueous and ethanolic extracts of *A. galanga* were separately weighed and transferred into a sterile tube. It was dissolved in 100µl of DMSO then 900µl of the nutrient broth was added to make 1ml and mixed completely in a cyclomixer. The stock solution was sterilized through a 0.45µm syringe filter.
The working bacterial strains were maintained in nutrient agar slants at 4ºC. Active cultures for the experiment were prepared by inoculating a loop full of the organism from the stock cultures into test tubes containing nutrient broth and incubated for 24 hours. The turbidity was adjusted to 0.5 Mac Farland standards. The antibacterial screening was carried out using the Kirby Bauer standard disc diffusion test**.**

Anti-Quorum Sensing Activity

Violacein inhibition assay

The MIC concentration of aqueous and ethanolic extracts of *A. galanga* was determined using tube dilution technique. Serial two-fold dilution of the extracts were carried out using the Nutrient broth to obtain concentrations 2000, 1000, 500, 250,125, 62.5, 31.25 and 15.6µg/ml. 0.1ml of standard suspension of test organism (0.5 Mc Farland 1-1.5×10^8 CFU/ml) was added to each of the test tubes and incubated at 32°C for 18 hours. The tube containing only broth was maintained as a positive control, the tube containing broth and inoculum was maintained as a negative control. Ampicillin was used as a standard drug control. The presence or absence of pigment at the end of incubation was recorded.

Confirmation of anti-quorum sensing activity

The anti-pathogenic effect observed in the tubes were confirmed further by subculturing loop full of inoculum on a fresh extract free Nutrient agar medium and incubated at 32°C for 18 hours.

Germ Tube Inhibition

Germ tube inhibition was attempted by incubating candida cells in 10% serum at 37°C for 3 hours with varying concentration of extracts of *A. galanga.* 10% serum inoculated with *C. albicans* without extract acted as the control. After the incubation period, the effect of the extract on inhibition of germ tube was determined in comparison with control by microscopic observation.

Antibacterial Activity

Antibacterial activity of extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium according to the recommendation of CLSI guidelines. Ampicillin (20µl/disc) was used as a standard. The plates were incubated at 37ºC for 24 hours. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition.

GC-MS

In order to identify the chemical constituents present in the extracts of *A. galanga*, the extracts were subjected to GC-MS analysis.

Cytotoxic Analysis

The extracts were tested for cytotoxicity (CC_{50}) in Vero cells from an initial concentration of 2000µg/ml to a final concentration of 15.25 µg/ml for determining the maximal toxic free concentration[8].

RESULTS

Lyophilisation

Lyophilized products are extremely hygroscopic hence were sealed in airtight containers following freeze-drying to prevent rehydration from atmospheric exposure. The aqueous and ethanolic extract was obtained after lyophilisation (Fig. 1).

Anti-Quorum Sensing Activity

Violacein inhibition in A.galanga

The inhibitory activity of aqueous and ethanolic extracts of *A. galanga* against the quorum sensing dependent violacein production in *C. violaceum* (MCC2290) was determined by tube dilution assay. The extracts exhibited concentration-dependent inhibitory activity and a significant reduction in violacein content. The aqueous extract of *A. galanga* inhibited violacein production completely at a concentration of 2mg/ml & 1mg/ml (Fig.2). The ethanolic extract of *A. galanga* showed complete inhibition of violacein production from a concentration of 1mg/ml to 125µg/ml. The extract also reduced 50% of violacein up to a concentration of 15.5µg/ml (Fig.3).

Recovery plate

Nutrient agar medium without extracts was inoculated with loopful of inoculum from violacein inhibited tubes. The plates after 18 hours of incubation developed violacein pigment, confirming the inhibition in the tubes was due to the antipathogenic effect of the extracts. However, all the extracts were found to be antibacterial at 1000µg/ml (Fig.4).

Germ Tube Inhibition

The control demonstrated the elongation of germ tube after 3 hours of incubation at 37°C in 10% serum. The ethanolic extracts of *A. galanga* were found to inhibit germ tube in *C. albicans* significantly compared to that of relevant control(Fig.5A, B) .

Lyophilised extracts of plants used in the study

Fig. 1: a. Aqueous and b. Ethanolic extract

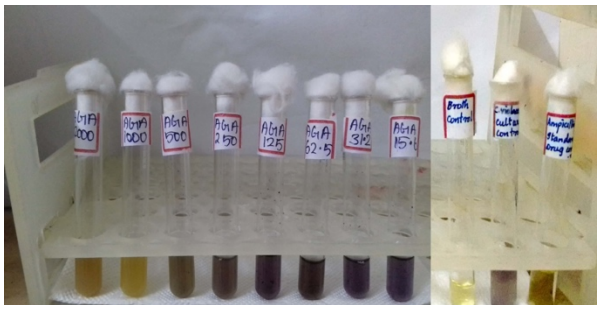

Fig. 2: Inhibition of violacein by aqueous extract of *A. galanga*

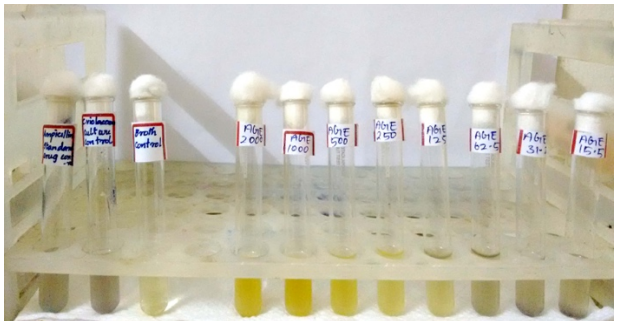

Fig. 3: Inhibition of violacein by ethanolic extract of *A. galanga*

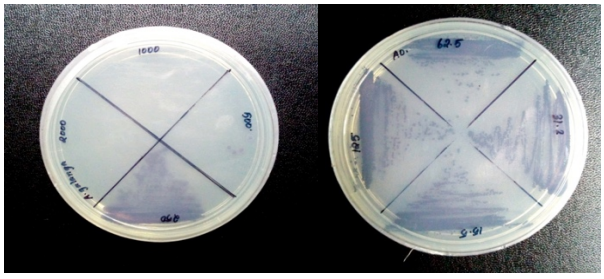

Fig.4: Recovery plates

A **B**

Fig.5: A) Germ tube elongation in control B) Inhibition of germ tube by ethanolic extract of *A. galanga*

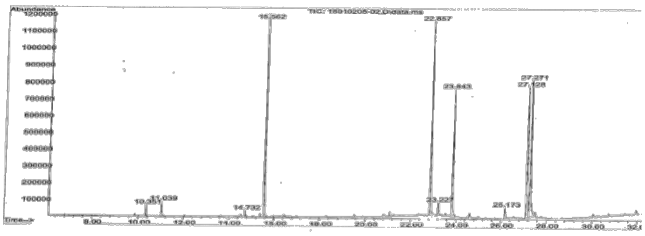

Fig. 6: GC-MS graphical representation of ethanolic extract of *A. galanga*

Table 1: GC-MS table for ethanolic extract of *A. galangal*

S.No.	RETENTION TIME (Rt)	AREA (%)	CHEMICAL COMPOUND
1.	16.173	65.18%	2,4-dimethyl- Tranylcypromine
2.	27.120	3.54%	4-methoxy-.alpha.-(2-nitrocyclopentyl)-
3.	21.292	3.54%	(E)- Oleic Acid
4.	23.849	2.80%	4-fluro-N, N-dibenzyl-
5.	15.096	2.69%	2-methyl-4-trifluromethyl-
6.	22.858	2.54%	2(5H)-Furanone, 5-(bromomethyl)-5- phenyl- .alpha.-d-Mannoforanoside
7.	16.420	2.52%	Hydrazinecarboxmide
8.	17.701	2.51%	methy 1-ester
9.	19.578	2.30%	n-Hexadecanoic acid
10.	13.858	2.20%	(E)-.beta.-Famesene
11.	18.401	2.06%	2,6-Dimethyl-4-hydroxybenzaldehyde 2-Chloro-7-methoxynaphthalene 5-Formylsalicylaldehyde
12.	27.263	1.69%	1,3,8-trihydroxy-6-methyl-
13.	16.268	1.48%	thylester
14.	18.277	1.47%	Farnesol
15.	21.225	0.97%	9,12-Octadecadienioc acid (Z,Z)-
16.	31.959	0.83%	.gamma.-Sitosterol
17.	15.563	0.81%	Diethyl phthalate
18.	17.006	0.78%	(R)- o-Toluidine

Antibacterial Activity

The antibacterial activity of ethanolic extract of *A. galanga* was carried out against Gram positive *Staphylococcus sp* and Gram negative bacteria *E. coli, Salmonella sp, Shigella sp, Klebsiella sp,* using agar disc diffusion method. The extracts at a concentration of 2000µg, 1000µg, 500µg showed inhibition. The zone (mm) around the disc was measured

GC-MS

Eighteen compounds were found in the GC-MS analysis of the ethanolic extracts of *A. galanga*. The active principles with their retention time (Rt) and concentration percentage (area%) are represented in Fig.6 and Table No.1.

Cytotoxic Analysis

The estimation of maximal toxic free concentration (CC_{50}) of the lyophilized extracts done on Vero cell line in varying concentrations from 1000µg/ml to 7.8µg/ml was estimated. The cell viability was found to be 61.85% at 500µg/ml proving *A. galanga* extracts to be nontoxic at the concentrations tested.

DISCUSSION

Antibiotic-resistant bacterial strains with different mechanisms are found continually. In order to overcome or minimize resistances, the research has been focused on inhibiting QS mediated traits like pigment production and germ tube formation using plant extracts. Since QS does not kill the bacteria the chances of development of resistance is low. Hence there is an increasing need for the discovery of new anti QS compounds.

According to the World Health Organization (WHO), 80% of the world population still rely on plant-based traditional medicines for primary health care globally. Biological products remain a reliable source of lead drugs. In addition to inhibition of microbial growth, natural products also play a significant role in interfering microbial cell-cell communication processes.

Lyophilization is a technique by which water is separated from a frozen phase by the principle of sublimation. This method is highly efficient. Most of the phytochemicals are preserved using this method as this method eliminates the risks of heat degradation. Hence the study adopted the usage of lyophilized extracts.

A. galanga is an ancient medicinal plant used in traditional system of medicine to cure various ailments. This study is perhaps the first attempt demonstrating QS inhibitory potential of *A. galanga* against the QS-dependent phenotypic expression in *C. violaceum* and *C. albicans.* The anti-QS potential of the rhizome extract was observed against the QS marker strain *C. violaceum* which showed complete inhibition of violacein pigment till a concentration as low as 125 µg/ml by *A. galanga*. Syed musthafa et al. [9] reported the bark extract of *Rhizophora annamalayana* at a concentration of 1mg/ml could inhibit violacein production in *C. violaceum.* Sybiya Vasantha Packiavathy[10] reported the QS inhibitory potential of the methanolic extract of *Cuminum cyminum* at a concentration of 2 mg/ml to inhibit violacein production. Hence by comparing the earlier reports from the results of the present work, the active principles present in *A. galanga* showed high efficacy and pronounced QS mediated inhibition of violacein (Fig.2 & 3). The extracts demonstrated that the inhibition of violacein production without any interference in the growth of *C. violaceum* indicating the anti-QS of the tested extracts. The extracts at lower concentrations tested also showed a significant reduction in violacein production which was observed as a reduction in the intensity of the purple pigment.

The ability to switch between yeast and hyphal-form is an important aspect of Candidal pathogenesis . There are very few studies which report about the inhibition of germ tube elongation by certain compounds. Penetration of host tissue by fungi is through hyphal extension. Extension of hyphae is also essential for, inter epithelial dissemination, penetration into adjacent epithelial cells following the initial epithelial invasion and these subsequent events may be responsible for the gross damage of epithelial tissues[11]. With this idea, the present study tested the extracts for their efficacy in inhibition of germ tube formation. The ethanolic extract was found to inhibit the formation of germ tube in *C. albicans* (Fig.5 B) at 1mg/ml.

The GC MS analysis revealed the presence of major compounds in the ethanolic extracts of *A. galanga* was found to be (2,6-Dimethylphenyl) borate Benzoic acid at Rt 16.173 (65.18%), Ethyl homovanil late Propan-2-one at Rt 27.130 (23.14%), (Fig.6, Table 1).

Most of the characterized anti QS compounds have not yet been tested for their toxicity[12], hence the extracts were tested for their toxic free concentrations on Vero cells. The extracts were found to be non-toxic from 500 µg/ml concentration.

Our findings clearly show the significant anti-QS activity of *A. galanga* extracts against the QS marker strain *C. violaceum* and Germ tube formation of *C. albicans*. This is perhaps the pioneering work demonstrating the Anti-QS activity of *A. galanga* extracts.

CONCLUSION

From the current study, it can be concluded that medicinal plants play a significant role in exhibiting anti-microbial and anti-quorum sensing activity. Our results showed the ethanolic extract of *A. galanga* was highly significant in exhibiting anti-quorum sensing activity by inhibiting violacein. Thus, quorum sensing could be an alternate strategy to overcome the development of drug resistance. Being the first assessment, germ tube inhibition was also observed in the current study.

ACKNOWLEDGMENT

The authors thank Dr.I. Seethalashmi for her support.

REFERENCES

1. Wally Mutungwa, Narendranath Alluri, Mala Majumdar. Anti-quorum sensing activity of some commonly used traditional Indian species. Int J Pharm Pharm Sci 2015; 7(11).
2. Caetano Antunes L, Rosana Ferreira BR, Michelle Buckner MC, Brett Finlay B. Quorum sensing in bacterial virulence. Micro Soc 2010; 156: 2271-82.
3. Varsha Shukla, Zarine Bhathena. Broad spectrum anti-quorum sensing activity of tannin-rich crude extracts of Indian medicinal plants. Scientifica 2016.
4. Choo JH, Rukayadi Y, Hwang JK. Inhibition of bacterial quorum sensing by vanilla extract. Lett Appl Microbiol 2006; 42: 637-41.
5. Calvin Boon, Yinyue Deng, Lian-Hui Wang, Yawen He, Jin-Ling Xu, Yang Fan et al. A novel DSF-like signal from *Burkholderia cenocepacia* interferes with *Candida albicans* morphological transition. The ISME journal 2008; 2: 27-36.
6. Mohammed Alem AS, Mohammed Oteef DY, Hugh Flowers T, Julia Douglas L. Production of tyrosol by *Candida albicans* biofilms and its role in quorum sensing and biofilm development. M Bio 2006; 5(10): 1770 – 9.
7. Pooja Gupta D, Tannaz Birdi J. Development of botanicals to combat antibiotic resistance. J Ayurveda Integr Med 2017; 8: 266- 75.
8. Soundhari C and Rajarajan S. In vitro screening of lyophilized extracts of *A.galanga* L. and *Oldenlandia umbellate* L. for antimycobacterial activity. Int J Biol Pharm Res 2013; 4(6): 427-32.
9. Khadar Syed Musthafa, Sunil Kumar Sahu, Arumugam Veera Ravi, Kandasamy Kathiresan. Anti-quorum sensing potential of mangrove *Rhizophora annamalayana.* World J Microbiol Biotechnol 2013; 29 (10): 1851-8.
10. Issac Abraham Sybiya Vasantha Packiavathy, Palani Agilandeswari, Khadar Syed Musthafa, Shunmugiah Karutha Pandian, Arumugan Veera Ravi. Antibiofilm and quorum sensing inhibitory potential of C*uminum cyminum* and secondary metabolite methyl eugenol against Gram negative bacterial pathogens. Food Res Intl 2012; 45: 85-92.
11. Madhushree Routh, Nitin Chauhan, Sandeep Rajput B and Mohan Karuppayil S. Cancer Drugs Inhibit Germ Tube Elongation in *Candida albicans*. J Sci Res Rep 2014; 3(1): 97-109.
12. Khan MSA, Zahin M, Hasan S, Husain FM, Ahmad I. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. Lett Appl Microbiol 2009; 49: 354-60.

Cite this article as:

Pavithra B.P *et al*. Inhibition of quorum sensing by extracts of *Alpinia galangal*. Int. Res. J. Pharm. 2018;9(11):101-105 http://dx.doi.org/10.7897/2230-8407.0911266

Source of support: Nil, Conflict of interest: None Declared