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RESEARCH ARTICLE

SCREENING OF PLANTS FOR ANTI-QUORUM SENSING POTENTIALS AGAINST BIOFILM PRODUCING GRAM NEGATIVE BACTERIA

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ABSTRACT

The gram-negative bacteria produce signaling molecules for combining with the other bacteria producing the same signal such as N-acyl homoserinelactones (AHL) for the formation of biofilm. The anti-quorum sensing potential of *Acalyphaindica, Azimatetracantha, Anethumgraveolens, Calophylluminophyllum, Cassia alata, Erysimumofficinale, Euphatoriumtriplinerve, Leucasaspera, Phyllanthusamarus* and *Vincarosasea* collected from Tamil Nadu were analyzed. These plants were tested for their phytochemical content and major phytocompounds were found to be present in*Acalyphaindica*, *Anethumgraveolens, Phyllanthusamarus*, *Erysimonofficinale*, *Azimatetracantha* and*Euphatoriumtriplinerve*. The chosen plants were tested for its anti-quorum sensing potential using the indicator organism *Chromobacteriumviolaceum.* The pigment violacein was found to be inhibited. Anti-quorum sensing activity was found to be significantly high in *Phyllanthusamarus*, *Acalyphaindica* and *Anethumgraveolens.*

INTRODUCTION

Biofilm are formed by microbial communities of defined structure by getting attached to specific organized communities of cells under controlled signaling molecules using cell-cell signaling known as quorum sensing (Davies *et al.,* 1998). The formation of biofilm is assumed to be in two different stages namely primary attachment onto polymer surfaces and cell-cell proliferation to form multilayered clusters (Hussain *et al.,* 1993). The N-acyl homoserine lactone (AHL) is a small molecule with known function used by the gram negative bacterial species known for its best signaling system. This AHL signaling system has been proved to play an important role in the context of plant, animal and human in developing anti-pathogenic therapies for the diseases acquired. The gram positive bacteria does not have the ability to produce AHL since the nature of the signal molecules used in the gram positive bacteria differs from gram negative bacteria (Matthew R. Parsek *et al.,* 2000). With auto inducers, the bacteria can regulate its behavior according to population density (Teresa R. DeKievit *et al.,* 2000). The local density of the QS bacteria decides its virulence, biofilm formation, adherence, cell to cell signaling and antibiotic resistance (Michael G. Surette *et al.,* 1999). In the medical industry, the biomaterials such as stents, catheters and orthopedic joints has been found that the microbes adhesion to these serve as excellent substrates which lead to biofilm formation (Cardinal *et al.,* 1996, Ell, S. R. *et al.,* 1996, Leonhardt *et al.,* 1999).

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These implanted devices causes infection by formation of biofilm (Schierholz *et al*., 2001). In addition, the infections caused by the biofilm formation affects the central nervous catheters, urinary catheters, orthopedic devices, prosthetic heart valves and even contact lens which results in longer hospitalization, surgery and death (Barie PS *et al.,* 1998, Henke PK *et al.,* 1998, Stewart PS *et al.,* 2001, Linnola R *et al.,* 2001). It was identified that biofilm forming bacteria requires a concentration of 100-1000 times increased dosage than that needed to kill the same species in suspension (Ceri H *et al.,* 1999).

The colonized bacteria within water distribution system pose a threat to human life by the formation of biofilms although many opportunistic pathogens has the ability to survive and proliferate (U.S. Environmental Protection Agency. 1992, Bezanson *et al.,* 1992). It was identified that the disinfectant chlorine used in water systems to prevent contamination does not affect the bacterial formed biofilms (Hoyle *et al.,* 1990). Previous research has proved that the biofilms producing bacteria are even resistant to antibiotics that it cannot be eliminated (Luppens *et al.,* 2002). Because of this many industries are now working with the inhibitory aspects of biofilms negative impact. In addition to the above, many food industries are working towards inhibition of biofilms formation in order to avoid toxin effect in their food product (Wong *et al*., 1988). Herbal based compounds have fewer side effects than synthetic compounds. This valid reason had made people all over the world to research in herbal compounds and to study its disease curing nature (Sasidharan *et al.,* 2011).

Table 1. Qualitative phytochemcial analysis

Azimatetracantha	Solvents	Sugar	Alkaloid	Glycoside	Flavanoid	Tannin	Saponin	Protein
	Hexane	+	-	-	+	-	-	-
	Ethyl acetate	+	-	+	+	-	-	+
	Ethanol	-	-	+	+	-	-	+
assia alata	Hexane	+	-	-	+	-	-	-
	Ethyl acetate	+	-	-	-	+	-	-
	Ethanol	+	+	-	-	+	-	-
Leucusaspera	Hexane	+	-	+	-	-	-	-
	Ethyl acetate	+	-	-	-	+	-	-
	Ethanol	+	-	+	+	-	-	-
Erysimumofficinale	Hexane	+	-	+	-	-	-	-
	Ethyl acetate	+	-	-	+	+	-	-
	Ethanol	+	-	+	+	+	-	-
Vincarocasea	Hexane	+	-	-	-	-	-	-
	Ethyl acetate	+	-	-	-	+	-	-
	Ethanol	+	-	+	-	-	-	-
Phyllanthusamarus	Hexane	+	-	+	-	+	-	-
	Ethyl acetate	+	-	+	+	+	-	-
	Ethanol	+	-	+	+	-	-	-
Euphatoriumtriplinerve	Hexane	+	-	-	-	-	-	-
	Ethyl acetate	+	+	-	-	-	-	-
	Ethanol	+	-	+	-	-	-	-
Acalyphaindica	Hexane	+	-	-	-	-	-	-
	Ethyl acetate	+	+	-	+	+	-	-
	Ethanol	+	-	-	-	-	-	-
Anethumgraveolens	Hexane	+	-	+	-	+	-	-
	Ethyl acetate	+	-	+	+	+	-	-
	Ethanol	+	-	+	+	-	-	-
Callophylum	Hexane	+	-	-	-	-	-	-
	Ethyl acetate	+	-	-	-	-	-	-
	Ethanol	+	+	-	-	-	-	-

Acalyphaindica, Azimatetracantha, Anethumgraveolens, Calophylluminophyllum, Cassia alata, Erysimumofficinale, Euphatoriumtriplinerve , Leucasaspera, Phyllanthusamarus and *Vincarosasea* are the plants which are used for screening anti-quorum sensing against the indicator organism *Chromobacteriumviolaceum* in the current study.

MATERIALS AND METHODS

Plant collection

Acalyphaindica, Azimatetracantha, Anethumgraveolens, Calophylluminophyllum, Cassia alata, Erysimumofficinale, Euphatoriumtriplinerve, Leucasaspera, Phyllanthusamarus and *Vincarosasea* were collected from different parts of Tamil Nadu.

Extraction and Drying

All the plant materials (leaves) were shade-dried at room temperature for two weeks, after which it was ground to a uniform powder. The extracts of the leaf samples were prepared in a sequential procedure by soaking 100 g of dried powder in 300 ml of different solvents (Hexane, Ethyl acetate and Ethanol) for 48 h. At the end of each respective extraction, the extracts were filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure in vacuum at 40°C for 25 min using a rotary evaporator (Super fit-ROTAVAP, India).

Qualitative phytochemical test

The qualitative phytochemical tests were done for carbohydrates, alkaloids, glycosides, flavanoids, tannins, saponins and protein (Harborne JB *et al.,* 1973).

Anti-quorum sensing activity

The *Chromobacteriumviolaceum* is used for analyzing the anti-quorum sensing activity of the samples using the Mac-conkey medium. The prepared samples were inoculated into the wells created in plates. All the plants with its respective extracts were screened for its activity against quorum sensing by inhibiting the violace in production of *C. violaceum* (StephaneUroz *et al.,* 2003).

RESULTS AND DISCUSSION

Thephytoconstituents in the plants *Acalyphaindica, Azimatetracantha, Anethumgraveolens,*

Calophylluminophyllum, Cassia alata, Erysimumofficinale, Euphatoriumtriplinerve, Leucasaspera, Phyllanthusamarus and *Vincarosasea* were serially extracted using three solvents hexane, ethyl acetate and ethanol.

Alkaloids were present in *Acalyphaindica, Anethumgraveolens, Phyllanthusamarus* and *Euphatoriumtriplinerve.* In addition, the presence of Flavanoid was indicated in *Acalyphaindica, Azimatetracantha, Erysimonofficinale, nethumgraveolens* and *Phyllanthusamarus*. Phenol, tannin, phytosteroids, alkaloids, flavonoids, coumarins, cardiac glycosides and terpenoids were found to be present in almost every plant except few (Table. 1). *Chromobacteriumviolaceum* is a gram-negative bacteria used as a model for screening the plants activity against quorum sensing and this gram-negative bacteria can be grown using the nutrient agar as supportive medium. *C. violaceum* produces a natural antibiotic called violacein which produces smooth low convex colonies with a dark violet metallic sheen at a optimal temperature of 30-35 ºC. The production of purple pigment in response to appropriate AHL indicates the absence of anti-quorum sensing activity of the plant. Decolorization of the purple color indicates the anti-quorum sensing activity of the plant by preventing the cell signaling between the organisms for the formation of the biofilm. But inhibiting the quorum sensing of a bacteria does not mean that it kills the bacteria but inhibits its signaling molecules from quorum sensing receptor (Jacob M. Hornby *et al.,* 2001).

Plants like *Anethumgraveolens, Acalyphaindica, Erysimumofficinale* and *Phyllanthusamarus* showed significant phytochemcial activity (Table. 1) which were further screened for anti-quorum sensing potentials. *Euphatoriumtriplinerve* and *Azimatetracantha* showed only moderate activities (Table 1). Comparing the solvents used for the extracts for significant zone, Ethyl acetate was used for further proceedings. The most effective substances from plants acting negatively on QS were compounds from *Acalyphaindica, Anethumgraveolens, Phyllanthusamarus* (Table.2). The future in depth study is to be done by isolating the compounds from *Acalyphaindica, Anethumgraveolens, Phyllanthusamarus* to which its anti-quorum sensing potential can be attributed.

Table 2. Screening of anti-quorum sensing property

Plants	Ethanol	Ethyl acetate	Hexane
Azimatetracantha	Absent	Present	Present
Anethumgraveolens	Present	Present	Present
Acalyphaindica	Present	Present	Absent
Callophylum	Absent	Absent	Present
Erysimumofficinale	Present	Present	Present
Phyllanthusamarus	Present	Present	Present
Euphatoriumtriplinerve	Present	Present	Absent

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