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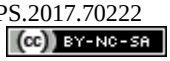
ISSN 2231-3354 

Phytochemical screening, *in vitro* anti quorum sensing activity and antioxidant activity of extracts of *Plumeria alba, Pisonia alba* and *Cynodon dactylon*

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ABSTRACT

Biofilm formation is a major strategy of drug resistant bacteria to thrive and cause infection. This ever increasing drug resistance demands for new drugs and treatment. Our study investigated the potential of phytochemicals in inhibiting biofilm formation through screening of anti quorum sensing activity. Ethyl acetate extracts of leaves of *Plumeria alba and Cynodon dactylon* and methanol extract of leaves *Pisonia alba* were assayed to determine their ability to inhibit the *in vitro* biofilm formation. *C. dactylon* extract showed an inhibition of 94.64±0.44% at 20 µg/mL concentrations against the bio film producing clinical isolate *Pseudomonas aeruginosa*, whereas *Plumeria alba* and *Pisonia alba* extracts showed 90.30±0.27% and 88.25±0.82% inhibition activity respectively. *C. dactylon* extract also exhibited considerable antioxidant activity of 65.42±0.787 through DPPH assay. Thus it indicates that these plants could be prospective agents in treating clinical pathologies through inhibition of cell communication.

INTRODUCTION

One of the biggest challenges in the clinical sector is the increasing drug resistance. A wide approach of survival for infectious bacteria is to form biofilm. They form a protective cover by biofilm and resist killing by biological and chemical bactericidals. This resistance can be attributed to a number of properties including blocking the permeation of antibacterial agents into the matrix of biofilm, multidrug efflux pump expression and enzymatic action of drug modifying enzymes. (Grant and Hung, 2013) (Stewart and Costerton, 2001) (Mah and OToole, 2001). Biofilm also helps to protect the pathogenic microorganisms against host immunity (Bakkiyaraj *et al*., 2010) (Baldassarri *et al*., 2006). The property of biofilm formation can be attributed to quorum sensing. Quorum sensing signals by individual bacterium known as auto inducers aid communication between the various bacteria. This helps them to operate together rather than exerting individual action (Kaufmann *et al*., 2008). This implies that quorum sensing inhibitors (QSIs) can be potent to alleviate the bacterial biofilm formation thus making them susceptible to antibiotics (Brackman *et al*., 2011). Recent reports demonstrate that certain biofilm associated bacteria return to planktonic stage by the secretion of D-amino acids and cis-2-decenoic acid, activating the disassembly of the biofilm. This strengthens the implication that small molecule QSIs can inhibit biofilm formation (Kolodkin-Gal *et al*., 2010) (Davies and Marques, 2009). Using natural products as biofilm inhibitors is safe and less toxic than using synthetic inhibitors. They are more specific than the synthesized chemical inhibitors (Koo and Jeon 2009).

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Plants are rich and inexhaustible source of active ingredients. Since time immemorial, medicinal plants have been known to contain bioactive compounds and were used in some form or other for treatment of various ailments (Rahmatullah *et al*., 2010). These compounds are normally accumulated as secondary metabolites in all plant cells and play a pivotal role in the modern medicine or as the lead compounds for new drug discovery (Vijayalakshmi and Ravindhran, 2012). Plant derived constituents are looked upon as a new therapeutic agents which might also be acts as a nontoxic inhibitors of quorum sensing, thus controlling infections without encouraging the appearance of resistant bacterial strains. The antimicrobial efficiency of plants are as a result of the secondary metabolites produced, say phenols, tannins, flavonoids. (Karuppiah and Mustaffa, 2013) (Koo *et al*., 2003). Hence, there is a strong quest for bio-screening plant extracts for anti-biofilm and quorum sensing inhibitory activity, followed by isolating the compounds responsible for this activity (Zaki *et al*., 2013).

To pursuit for natural biofilm inhibition activity we have studied *Plumeria alba, Pisonia alba and Cynodon dactylon plant* extracts. These medicinal plants have been known to produce bioactive compounds which are used to treat various ailments. *Pisonia alba* belonging to the family Nyctaginaceae is an evergreen tree used as a diuretic (Sunil *et al*., 2009). *Plumeria alba* of the family Apocynaceae, a laticiferous small tree or shrub and the latex is used to alleviate ulcers and herpes. The seeds of this plant have haemostatic potential and bark been used as a plaster over tumours (Chaudhuri *et al*., 2015). *Cynodon dactylon* of the family Poaceae, commonly known as Bermuda grass has been traditionally used against diabetes and jaundice, kidney and urinary diseases and so on (Jarald *et al*, 2008).

It is a universally accepted fact that lately microorganisms have become resistant to several antimicrobials. With the advancement in technology, the bioactive potential of plant derived compounds can be utilized against antibiotic resistant microorganisms (Al-Hussaini and Mahasneh, 2009). Plant derived constituents are looked upon for new therapeutic and anti-pathogenic agents which might be nontoxic inhibitors of quorum sensing, thus controlling infections without encouraging the appearance of resistant bacterial strains (Hentzer and Givskov, 2003).

The phytochemicals' properties can be analyzed and used against microbial growth patterns as plants having phytochemical properties possess strong antioxidant activity. So we performed the anti quorum sensing capability of various extracts of the three plants. Subsequently their antibiofilm potential was also assessed. Potent extracts which showed antioxidant property were analysed for their phytochemicals.

MATERIALS AND METHODS

The plants were collected freshly from the campus of VIT University, Vellore, Tamil Nadu, India in the month of July 2015 and identified by Dr. Benjamin Prasad Kumar, Department of Plant Biology and Biotechnology, Voorhees College, Vellore. A voucher specimen was maintained in our laboratory by an accession number BV/VIT/PPCD/12.07.2015-45 to 47.

Bacterial strains used

*Chromobacterium violaceum (*MTCC 2656), an indicator strain for screening quorum sensing (QS) inhibitors, was purchased from MTCC, IMTECH (Chandigarh, India). A total of 3 clinical isolates, *Staphylococcus aureus, Salmonella typhi* and *Pseudomans aeruginosa* were employed in the study.

Chemicals and media

Methanol, Chloroform, Ethyl acetate, Petroleum ether, DPPH, other chemicals and dehydrated culture media utilized for the study were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). All chemicals involved were of analytical grade.

Extract Preparation and solvent extraction

Freshly collected leaves of *Plumeria alba, Pisonia alba and Cynodon dactylon* were surface sterilized with 0.5% of sodium hypochlorite for 15 minutes and cleaned thoroughly with repeated washing of distilled water. After shade drying the leaves were grounded to get powder and proceeded to extraction technique, which included 5 different solvents with increasing polarity viz., Petroleum ether, Chloroform, Ethyl acetate, Methanol and Distilled water. The dried extract was collected and stored in vials at 4 ºC for further analysis.

Anti-quorum sensing (AQS) activity of the plant extracts

The AQS activity of the plant extracts were checked against an indicator organism, *Chromobacterium violaceum* (MTCC 2656). *C.violaceum* served as a QS system producing a visible violet coloration (Nithya *et al*., 2010). For the assay the prospective QS inhibitors were added in different concentration in wells containing 10 µL (1:100 dilution) of the bacterial culture. The plate was read at OD_{600} upon incubation at 37 ºC for 24 hours. The bacterial cells with DMSO served as control.

Microtitre plate based biofilm formation assay

For detection and confirmation of biofilm formation capability of the clinical isolates *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*, we employed microtitre based technique. Freshly grown culture was diluted to 1:100 into fresh trypticase soy broth for biofilm assays. 100 µL of the dilution per well was added in a 96 well polystyrene microtitre dish.

For quantification, 2-3 replicates were maintained. The microtitre plate was incubated for 24 hrs at 37°C. Upon incubation the cells were dumped by flipping the plate. The plate was cleared of any planktonic cells and media. To the wells 125µL of 0.1% crystal violet dye was added. For staining the plates were incubated at room temperature for 15 to 20 minutes. For quantification of biofilm, the stain was solubilised by addition of

125 µL of 30% acetic acid to the wells. This was maintained static for 10 minutes. Thereafter the contents were added to a new microtitre plate and optical density was analysed at 570 nm with 30% acetic acid as blank (O'Toole, 2011).

Effect of the plant extracts on growth of biofilm isolates

The action of plant extracts on the growth of biofilm formers were analysed by a concentration based study. 20-100µg/mL of methanol extract of *Pisonia alba* and ethyl acetate extract of *Cynodon dactylon* and *Plumeria alba* were prepared as working solutions. 100 µL of overnight cultures *S. aureus, S. typhi* and *P. aeruginosa* adjusted to McFarland standard 0.5 was added in to wells followed by 100 µL of the extracts. After incubation at 37ºC for 24 hours the plate was read spectrophotometrically at OD_{600} for any change in the growth of the biofilm isolates (Edeoga *et al*., 2005).

DMSO and bacterial suspension served as blank and negative control respectively. All treatments and control were maintained in triplicates (Antunes *et al*., 2010)

Inhibition efficacy of plant extracts on biofilm formation

100µLof each working solution varying from the concentrations of 20-100µg/mL was added to the wells containing 100 µL of isolates in trypticase soy broth. The plate was incubated at 37º C for 24 hours. The plate was washed with sterile phosphate buffered saline to remove all unattached cells and 0.4% crystal violet was added to stain the plate.

The plate was de-stained for 30 minutes with 95% ethyl alcohol. At OD_{570}, the contents were analysed spectrophotometrically. Percentage inhibition is determined by the same formula

$$\%\ inhibition = \frac{OD_{control} - OD_{test}}{OD_{control}} x\ 100$$

Minimum biofilm inhibitory concentration (MBIC) of the extracts was decided based on the visible destruction of the biofilm and noticeable decline in the absorbance in comparison with the control (Dhamodaran *et al*., 2010) (Sanchez *et al*., 2016).

Antioxidant assay

DPPH Radical scavenging assay

Antioxidant activity of the solvent extracts was assessed by % DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging method. From the primary stock prepared by dissolving 0.01 g of each plant extract in 1mL of methanol. 200µL was added to make up to a final volume of 2 mL which is the secondary stock solution (Chaudhuri *et al*. 2015).

A series of working solutions viz., 20, 40, 60, 80 and 100µg/mL was made from the secondary stock to a final volume of 2mL (Baldassarri *et al*., 2006). Ascorbic acid served as a standard. 1mL freshly prepared DPPH (was added to all test samples, incubated at 20º C for 40 minutes in dark and their absorbance was measured at 517nm. DPPH radical scavenging assay was calculated according to the formula below:

$$\%\ inhibition = \frac{OD_{control} - OD_{test}}{OD_{control}} x\ 100$$

where, $OD_{control}$ is the optical density of the DPPH radical + methanol, OD_{test} is the optical density of DPPH radical + sample extract/standard (Nithya *et al*., 2010).

Screening for Phytochemicals

In order to analyze the various constituents of the extracts, they were screened for various phytochemicals namely saponins, carbohydrates, proteins, phenolics, sterols, flavonoids, alkaloids, terpenoids and glycosides according to standard protocols (Ho *et al*., 2015).

Analysis of variance

The data were examined statistically by Analysis of Variance (ANOVA) using GraphPad Prism 6.0 (GraphPad software Inc. San Diego, USA)

RESULTS AND DISCUSSION

Successive solvent-solvent extraction

Macerated extraction of phytochemicals by various extracts yielded 0.2-1% of total dry weight of the leaves of all the plants.

Anti-quorum sensing activity of the plant extracts

The indicator organism *Chromobacterium violaceum* was found to be inhibited by *Cynodon dactylon* (Ethyl acetate extract) by 80.34% followed by *Pisonia alba* (Methanol extract) by 49.56% and *Plumeria alba* (Ethyl acetate extract) by 47.90%. The rest of the extracts showed negligible amount of inhibition of the pigment, thus these three extracts were chosen for further studies.

Microtitre plate based biofilm formation assay

Clinical isolates were screened for biofilm formation by static biofilm formation test. Isolates *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* showed biofilm formation, however *P.aeruginosa* was found as the best biofilm forming isolate. According to Nyenje, 2013, strength of biofilm formation was determined according to the formula ($2 \times Abs_c$) < Abs ≤ ($4 \times Abs_c$). With an absorbance value of 0.473 in comparison with the control which exhibited an absorbance value of 0.131 *P.aeruginosa* was found to be moderately high in biofilm formation.

Effect of the plant extracts on growth of biofilm isolates

The three plant extracts were screened for their effect on antimicrobial activity against the active biofilm producer. The result revealed less than 55% inhibition. The highest activity was exhibited by *Pisonia alba* at 100µg/mL (Table-1).

Table 1. Growth inhibition exhibited by plant extracts against *P.aeruginosa*

Plant Extracts	Growth Inhibition at concentrations as below				
	Concentrations (µg/mL)				
	20	40	60	80	100
Plumeria alba	15.29	22.38	35.82	39.85	46.29
Pisonia alba	45.10	48.85	50.94	51.89	**53.65**
Cynodon dactylon	42.08	42.09	49.42	41.94	39.81

Inhibition efficacy of plant extracts on biofilm formation

Bacteriocidal activity indicates negative results which imply that the bacteria are found resistant to the extracts. However the extracts exerted significant activity against biofilm formation. The bacterial biofilm formation was considerably disturbed as is indicated in figure 1, by the percentage inhibition. The variation was visibly clear in the microtitre as reduction in crystal violet stained cells adhering to the surface of the titre base. *P. aeruginosa* was inhibited at the highest by *C. dactylon* with a percentage of 94.64±0.44. The inhibition percentage exhibited by *Plumeria alba* and *Pisonia alba* was 90.30±0.272 and 88.25±0.827 respectively. The minimum biofilm inhibitory concentration was determined using the microtitre plate technique which was found to be 20 µg/mL for *C. dactylon*, 40 µg/mL for *Pisonia alba* and 60 µg/mL for *Plumeria alba.*

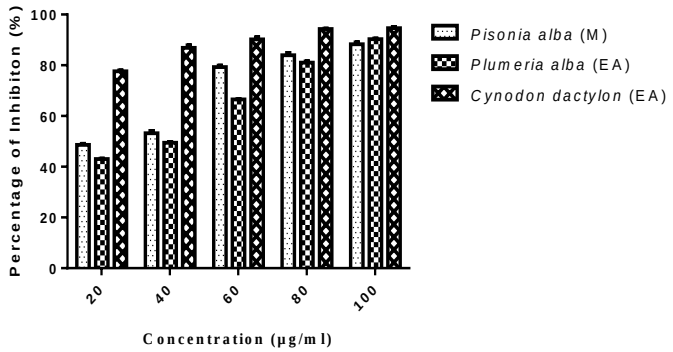

Fig. 1: *C. dactylon* showing highest percentage of inhibition

DPPH Radical scavenging assay

Results of antioxidant assay of the three extracts are as depicted in Fig 2. *C. dactylon* showed the highest antioxidant activity in comparison with all other extracts reaching a value of 65.42±0.787 against ascorbic acid.

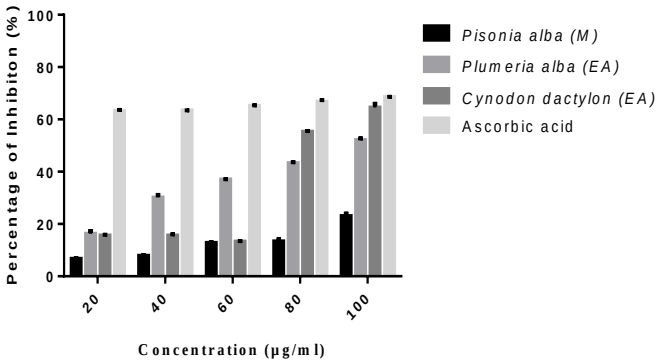

Fig. 2: Antioxidant activity of plant extracts in comparison with ascorbic acid.

Phytochemical screening

Phytochemical screening of *Plumeria alba* exhibited the presence of saponins, glycosides, cardiac glycosides, flavonoids and terpenoids while *Pisonia alba* showed the presence of saponins, glycosides, alkaloids and flavonoids. *Cynodon dactylon* expressed glycosides, flavonoids and terpenoids. Based on the phytochemical content ethyl acetate extract of *Plumeria alba* and *C. dactylon* and methanol extract of *Pisonia alba* were retained for further studies. Figure 3 depicts the phytochemical composition of the plant extracts. Our findings show that *Cynodon dactylon* exhibit significant anti-biofilm at a concentration of 20 µg/mL. This can be attributed to the phytochemical constituent of *C. dactylon.* From the composition of phytochemicals it can be proposed that the flavonoids content contributes to biofilm inhibition. Flavonoids from various sources are potent anti-biofilm agents which inhibit biofilm by bacteria such as *Streptococcus mutans, Aeromonas hydrophila* and *Escherichia coli* (Koo *et al*., 2000) (Vikram *et al*., 2010) (Sanchez *et al*., 2016). Lee *et al.* (2010) reported the action of phloretin, a flavonoid at concentrations of 25 and 50 g/mL against biofilm of *E. coli* O157:H7 by 89 and 93%. However this is the prime report on anti-biofilm activity of *C. dactylon* against *P. aeruginosa*. In addition the extract shows considerable amount of antioxidant activity.

Fig. 3: Phytochemical profile of the plant extracts.

CONCLUSION

Briefly, *C. dactylon can* be a prospective antibiofilm agent which can act against *P. aeruginosa* induced nosocomial infections and the antioxidant activity adds to the beneficial effects. The phytochemicals are a means of biofilm inhibition while antibiotics can wipe out the bacterial population.

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REFERENCES

Al-Hussaini R, Mahasneh AM. Microbial Growth and Quorum sensing antagonist activities of Herbal plant extracts. Molecules, 2009; 14; 3425-3435.

Antunes ALS, Trentin DS, Bonfanti JW *et al*. Application of a feasible method for determination of biofilm antimicrobial susceptibility in staphylococci. Acta Patologica, Microbiologica et Immunologica Scandinavica, 2010; 118,11:873–877.

Bakkiyaraj D, Thevar S , Pandian K. In vitro and in vivo antibiofilm activity of a coral associated actinomycete against drug resistant Staphylococcus aureus biofilms. Biofouling, 2010; 26: 711 - 717.

Baldassarri L, Creti R, Recchia S, Imperi M, Facinelli B,Giovanetti E, Pataracchia M, Alfarone G, Orefici G.Therapeutic failures of antibiotics used to treatmacrolide-susceptible Streptococcus pyogenes infections maybe due to biofilm formation. J Clin. Microbiol, 2006; 44:2721–2727.

Brackman G, Cos P, Maes L, Nelis HJ, Coenye T. Quorum sensinginhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. Antimicrob Agents Chemother, 2011;55: 2655–2661.

Chaudhuri S, Bakshi S,Pande JN, Biswas M. Screening of in-vitro Antioxidant Profile of Different Extracts of the Leaves of Plumeria alba Linn. J. Adv. Pharm. Edu. & Res. 2015 5; 98-102.

Davies DG, Marques CN 2009. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol, 2009; 191: 1393–1403.

Edeoga HO, Okwuand DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol, 2005; 4: 685-688.

Grant SS, Hung DT. Persistent bacterial infections, antibiotic tolerance, and the oxidative stress response. Virulence, 2013; 4: 273–283.

Hentzer, M. Givskov, M. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J. Clini. Invest*. 2003; *112*: 1300-1307.

Ho LK, Chung WE, Choong KE, Cheah YL, Phua EY, Srinivasan R. Anti-Proliferative Activity and Preliminary Phytochemical Screening of Ipomoea quamoclit Leaf Extracts. J. Med. Plants Res, 2015;9:127-134.

Jarald EE, Joshi SB, Jain DC, Antidiabetic activity of aqueous extract and non polysaccharide fraction of Cynodondactylon Pers., Ind J of Exp Biol, 2008;46:660-667.

Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed, 2013; 9: 737–742.

Kaufmann GF, Park J, Janda KD Bacterial quorum sensing: a new target for anti-infective immunotherapy. Expert Opin Biol Ther, 2008; 8: 719–724.

Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R. D-Amino acids trigger biofilm disassembly. Science, 2010; 328: 627–629.

Koo H, Jeon JG. Naturally occurring molecules as alternative therapeutic agents against cariogenic biofilms. Adv Dent Res, 2009; 21: 63-68.

Koo H, Hayacibara MF, Schobel BD, Cury JA, Rosalen PL, Park YK ,Vacca-Smith AM, Bowen WH. Inhibition of *Streptococcus mutans* biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. J. Antimicrob. Chemother, 2003; 52:782–789.

Lee JH, Regmi SC, Kim JA,Cho MH, Yun H, Lee CS, Lee J. Apple Flavonoid Phloretin Inhibits Escherichia coli O157:H7 Biofilm Formation and Ameliorates Colon Inflammation in Rats, INFECT IMMUN, 2011; 79: 4819–4827.

Mah TF, O'Toole G A. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001;9: 34- 39.

Nithya C, Aravindraja C, Pandian SK. *Bacillus pumilus* of Palk Bay origin inhibits quorum-sensing-mediated virulence factors in Gram-negative bacteria. Res Microbiol, 2010; 16: 293–304.

Nyenje ME , Green E, Dip RNN, Evaluation of the Effect of Different Growth Media and Temperature on the Suitability of Biofilm Formation by *Enterobacter cloacae* Strains Isolated from Food Samples in South Africa. Molecules, 2013; 18: 9582-9593.

O'Toole GA. Microtiter Dish Biofilm Formation Assay. J. Vis. Exp, 2011;47:2437.

Rahmatullah M, Samarrai W, Jahan R, Rahman S, Sharmin N, Miajee EU, Chowdhury MH, Bari S, Jamal F, Bashar A, Azad AK, Ahsan S. An Ethnomedicinal, Pharmacological and Phytochemical Review of Some Bignoniaceae Family Plants and a Description of Bignoniaceae Plants in Folk Medicinal Uses in Bangladesh. Adv Natur App Sci, 2010;3: 236-253.

Sánchez E, Catalina Rivas Morales,1 Sandra Castillo,2 Catalina Leos-Rivas,1 Ledy García-Becerra,1 and David Mizael Ortiz Martínez1. Antibacterial and Antibiofilm Activity of Methanolic Plant Extracts against Nosocomial Microorganisms. J Evid Based Complementary Altern Med, 2016. dx.doi.org/10.1155/2016/1572697

Stewart P S, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001; 358: 135 - 138.

Sunil C , Latha PG, Suja SR, Shine VJ, Shyamal S, Anuja GI , Sini S , Rajasekharan S, Agastian P , Ignacimuthu S , Kalichelvan V. Effect of Ethanolic Extract of Pisonia alba Span. Leaves on Blood Glucose levels and Histological Changes in Tissues of Alloxan-Induced Diabetic rats, Int J Appl Res Natur Pro, 2009;2: 4-11.

Vijayalakshmi R, Ravindhran R. Preliminary comparative phytochemical screening of root extracts of Diospyrus ferrea (Wild.) Bakh and Aerva lanata (L.) Juss. Ex Schultes. Asian J Pla Sci Res,2012;2: 581 - 583.

Vikram, A, Jayaprakasha GK, Jesudhasan PR, Pillai SD, Patil BS. Suppression of bacterial cell-cell signalling, biofilm formation and type III secretion system by citrus flavonoids. J. Appl. Microbiol, 2010; 109: 515–527.

Zaki A, Shaaban M , Hashish ne , Amer MA, Lahloub M. Assessment of Anti-Quorum Sensing Activity for Some Ornamental and Medicinal Plants Native to Egypt. Sci Pharm. 2013;81:251-258.

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