

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2017.601.048>

Anti-Virulence Activity of Selected Natural Products against *Pseudomonas aeruginosa*

Ahmed Ahmed Abd El-Aziz, Tarek El-Said El-Banna, Fatma Ibrahim Sonbol
and Shymaa Mohamed Sallam*

Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Egypt
*Corresponding author

ABSTRACT

Keywords

Pseudomonas aeruginosa, natural products/extracts, virulence factors, outer membrane proteins.

Article Info

Accepted:
23 December 2016
Available Online:
10 January 2017

Pseudomonas aeruginosa is considered as the epitome of an opportunistic pathogens of hospitalized patients. A total of 159 clinical specimens were recovered and molecular identified by PCR assay. The number of isolates which identified as *P. aeruginosa* was 101 clinical isolates. Considering the effect of 14 natural products/extracts on some virulence factors of MDR isolates, sub inhibitory concentration ($\frac{1}{2}$ MIC) of *Syzygium aromaticum* (clove) buds extract inhibit the activity of alkaline protease enzyme on skimmed milk agar. The swarming motility was decreased while phospholipase C enzyme activity was increased by sub inhibitory conc. of clove buds extract. Biofilm formation of *P. aeruginosa* was inhibited by *Psidium guajava* (guava) leaves extract at $\frac{1}{2}$ MIC (showed pink colonies on congo red agar). The effect of different conc. of *Psidium guajava* leaves extract on outer membrane proteins of *Pseudomonas aeruginosa* isolate was determined by SDS-PAGE and analyzed using Image J program. Sub inhibitory conc. ($\frac{3}{4}$ MIC) of guava leaves extract showed lower relative density in protein bands at molecular weights of 70 KDa, 65 KDa, 60 KDa, 59 KDa, 49 KDa, 44 KDa & 36 KDa compared to the control.

Introduction

Pseudomonas aeruginosa is a serious opportunistic pathogen in hospitalized patients. The ability of *P. aeruginosa* to cause a wide range of infections is due to the production of a large array of virulence factors (Balasubramanian *et al.*, 2013). *Pseudomonas aeruginosa* is highly adaptable and have diverse phenotypic characteristics, to inhabit various environmental conditions (Yosuke and Yutaka, 2013). Plant extracts of many plants exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Okigbo and Ogbonnaya, 2006 and Shariff *et al.*, 2006).

The selection of crude plant extracts for screening programs has the potential of being more successful in its initial steps than the screening of pure individual compounds (Uniyal *et al.*, 2006). Inhibition of bacterial QS can prevent the development of bacterial virulence and successful establishment of infections (Schauder and Bassler, 2001 and Robert *et al.*, 2005). Selected natural products which characterized by QSI in their components will be prepared, extracted and investigated their anti pseudomonal activity against MDR isolates. Furthermore, the effect of the tested natural products/extracts on

some virulence factors against MDR *P.aeruginosa* will be investigated. Finally, the analysis of the effect of *Psidium guajava* (guava) leaves extract on the outer membrane proteins of MDR isolate of *P. aeruginosa* will be investigated.

Material and Methods

Isolation of bacterial isolates

The preliminary selection of *Pseudomonas aeruginosa* isolates was done by culturing each specimen (sputum, urine and pus) on MacConkey's agar (Oxoid, UK) plates and the non lactose fermenting colonies were further sub-cultured on Pseudomonas Cetrimide agar (Oxoid, UK) plates as described by Monica Cheesbrough, (2006).

Molecular identification of the recovered isolates

DNA Extraction and PCR amplification

Extraction of bacterial genomic DNA was done by CTAB Method as described by Alexandra, (2009). The purified DNA was amplified using primer highly specific to *P.aeruginosa*. The forward primer PA431CF was (5'- TGGGTCGAAAGGTGGTTGTATC-3') and the reverse primer PA431CR was (5'-GCGGCTGGTGCGGCTGAGTC-3').

PCR amplification was carried out with the following conditions: pre-denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 60 sec., annealing at 63°C for 30 s, and extension at 72°C for 60s and then final extension step at 72°C for 10 min (Choi and Kim, 2013).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis was performed as described by Han *et al.* (2008)

to separate amplified PCR products. The electrophoresis apparatus was attached to an electric power supply at a constant current of 120V for about 80 min.

Polyacrylamide gel staining

Acrylamide gel was stained by silver nitrate as described by Ji *et al.* (2007).

Collection, Extraction and Preparation of natural products

The tested natural products (*Mangifera indica* (Mango) leaves, *Psidium guajava* (Guava) leaves, *Camellia sinensis* (Green tea) leaves, *Citrus limon* (Lemon) leaves, *Cuminum cyminum* (Cumin) seeds, *Syzygium aromaticum* (Clove) flower buds, Bee propolis resin (Bee glue), Sidr Honey, Royal jelly, *Zingiber officinale* (Ginger) rhizome, *Boswellia carterii* (Bitter gum) resin, *Allium cepa* (Onion) bulbs, *Panax ginseng* roots and *Nigella sativa* (Black seed) seeds were obtained from local markets in Egypt and extracted with 80% ethanol as described by Karuppusamy *et al.* (2009).

Antibiotics susceptibility test

Antibiogram was performed using commercially available antibiotics disks (Sigma, USA). The tested *Pseudomonas aeruginosa* isolates were screened for their susceptibility to 16 different antibiotics using disk diffusion method as described by Jan Hudzicki (2009). The inhibition zone diameters were interpreted as susceptible (S), intermediate (I) or resistant (R) according to CLSI 2014 guidelines.

MIC and MBC of natural products/extracts

MIC and MBC of natural products/ extracts were determined against reference *P.aeruginosa* ATCC 27853 and against

multidrug resistant isolates (n=20) with MAR > 0.50 using the broth macrodilution method as described by Carson *et al.* (1995).

Screening of different virulence factors of *P. aeruginosa* clinical isolates

Swarming motility assay

Prepared Swarm plates were inoculated by toothpick with individual colonies from a fresh Luria-Bertani (LB) agar plate as described by CheO'May and Nathalie (2011). The inoculum was placed on the agar surface to enable visualization of motility across the agar surface after incubation at 37°C.

Alkaline protease enzyme assay

Bacterial isolates of an 18-24 hours old were screened for alkaline protease enzyme as described by Kumar and Jamwal (2013), by inoculating bacterial isolates as spot on skimmed milk agar and incubated for 24 hours at temperature 37°C. After an incubation period, the diameter of the zone of clearance around the colony, indicating the extent of protease activity.

CTAB-Methylene blue agar assay

The screening of rhamnolipids producing bacterial isolates was described by Siegmund and Wagner (1991). Rhamnolipids producing colonies on CTAB-Methylene blue agar plates were incubated at 37°C for 24 hours and then kept for at least 48 h at room temperature until a blue halo appeared around colonies as described by Zulianello *et al.* (2006).

Gelatinase enzyme assay

A heavy inoculum of an 18-24 hours old at 37°C test bacteria was stab-inoculated into tubes containing Nutrient Gelatin as described by Difco & BBL Manual (2009).

Phospholipase C enzyme assay

Bacterial isolates of an 18-24 hours old at 37°C were screened for Phospholipase C enzyme as described by McMurray and Magee (1972) Using egg yolk agar which showed an opaque zone due to formation of water-insoluble diglyceride suggesting phospholipase C activity. Bacterial isolates were incubated for 24 hours at 37°C. For determination of phospholipase C enzyme activity, Calculation of the precipitation zone ratio was performed according to Price *et al.* (1982).

Biofilm formation

This method is based on the characteristic morphology of biofilm-forming bacteria on Congo red medium as described by Freeman *et al.* (1989). Plates of the Congo red medium were inoculated and incubated aerobically for 24-48 hours at 37°C.

The effect of natural products/extracts on tested virulence factors of MDR isolates

All the tested virulence factors were determined by procedures previously described. The treatment of isolates with natural products/extracts was done by adding the natural products/extracts to the molten Muller-Hinton agar in different concentrations.

Effect of *Psidium guajava* leaves extraction outer membrane proteins profile

The effect of *Psidium guajava* leaves extract ($\frac{1}{4}$ MIC, $\frac{1}{2}$ MIC and $\frac{3}{4}$ MIC) on outer membrane proteins of MDR *Pseudomonas aeruginosa* isolate was determined by SDS-PAGE.

Isolation of outer membrane proteins (OMPs)

Isolation of outer membranes was carried out as described by Burns and Clark (1992) and

Nowsheen and Jain (2008). The crude OMP preparation was stored at -20°C till further use.

Protein Estimation

The protein content in the samples was estimated by Warburg-Christian method as described by Warburg and Christian (1941).

Tris-glycine SDS-polyacrylamide gel electrophoresis of outer membrane proteins

Tris-glycine SDS-polyacrylamide gel electrophoresis method was done as described by Sambrook and Russell (2001).

Staining of SDS-PAGE gel

Reverse staining of SDS-polyacrylamide gel by imidazole-zinc method was used as described by Fernandez-Patron *et al.* (1992).

Analyzing SDS-PAGE gel

ImageJ is a powerful image analysis program that was created at the National Institutes of Health. Image J program was downloaded from the source (<http://rsb.info.nih.gov/ij/>) and Installed on the PC. The analysis steps was described by Tiago and Wayne (2012).

Results and Discussion

Identification of *Pseudomonas aeruginosa* clinical isolates

Only 112 from 159 isolates were grown on Pseudomonas cetrimide agar which considered as preliminary identification of *Pseudomonas* isolates. After molecular identification by PCR assay, only *P. aeruginosa* isolates showed a single amplified product of 232 bp, the number of isolates which identified as *P. aeruginosa* was 101 clinical isolates.

Antibiotics susceptibility test

The incidence of resistance to 16 different antimicrobials ranged from 2.9% to 100%. *P. aeruginosa* isolates showed resistance to Ceftazidime (89.1%), and Azetronam (83.1%) while the sensitivity was showed to Levofloxacin (93.2%), Meropenem (84.2%) and Cefoperazone (79.3%).

MIC and MBC of natural products/extracts

Clove buds extract was the most active against MDR isolates in inhibitory concentration mean of 6.5 % v/v and bactericidal concentration mean of 13.3 % v/v. Other natural products activity against MDR isolates was shown in Table (1).

Screening of different virulence factors of *P. aeruginosa* clinical isolates

The Incidence of the tested virulence factors in clinical *P. aeruginosa* isolates (n=101) was shown in Figure (1).

The effect of natural products/extracts on swarming motility

The swarming diameter average of isolates treated with ½ MIC of clove extract = 10 mm in comparison with swarming diameter average of control isolates = 25 mm. Also, 50% v/v of Sidr Honey and ½ MIC of green tea leaves extract decreased the swarming activity. Although cumin seeds extract showed no change in swarming diameter at ½ MIC & ¼ MIC compared to the control (0%) but affect the way of swarming pattern from dendrites to bull's eye pattern only at ½ MIC as shown in Figure (2). Natural products showed effect on swarming was listed in Table (2) while others unlisted natural products/extracts couldn't affect the swarming motility.

The effect of natural products/extracts on alkaline protease enzyme activity

The sub inhibitory conc. ($\frac{1}{2}$ MIC) of clove buds extract inhibit the activity of alkaline protease enzyme as the clear zone around colonies = 0 mm compared to 5 mm of the control (0%) while ($\frac{1}{2}$ MIC) of cumin seeds extract decrease in the enzyme activity as shown in Figure (3). Natural products showed effect on alkaline protease activity was listed in Table (3) while others unlisted natural products/extracts couldn't affect the alkaline protease activity.

The effect of natural products/extracts on phospholipase C enzyme activity

Regarding to phospholipase C enzyme activity, the sub inhibitory conc. of clove buds extract increased the activity of phospholipase C enzyme in conc. dependent way as the average of the precipitation zone ratio of ($\frac{1}{2}$ MIC) = 0.41 compared to the control average = 0.66. Also, 50% v/v Sidr honey increase the activity of phospholipase C as the average of the precipitation zone ratio = 0.41 compared to the control average = 0.63. But, Cumin seeds extract decreased the activity of phospholipase C enzyme from large activity ($P_z < 0.70$) to poor activity ($P_z = 0.80-0.89$) as the average of the precipitation zone ratio at $\frac{1}{2}$ MIC was 0.81 compared to the control average = 0.45 as shown in Figure (4). Natural products showed effect on phospholipase C enzyme activity was listed in Table (4) while others unlisted natural products/extracts couldn't affect the phospholipase enzyme C activity.

The effect of natural products/extracts on biofilm formation

Guava leaves extract at $\frac{1}{2}$ MIC showed pink colonies (inhibition of biofilm formation) while both $\frac{1}{4}$ MIC and the control showed black colonies with dry crystalline

consistency as shown in Figure(5). Other natural products/extracts showed no effect on biofilm formation at different concentrations.

The effect of natural products/extracts on rhamnolipids production and gelatinase enzyme production

All the tested natural products/extracts couldn't affect the production of both rhamnolipids and gelatinase enzyme.

Effect of *Psidium guajava* leaves extract on OMPs profile

The effect of *Psidium guajava* leaves extract at ($\frac{1}{4}$ MIC, $\frac{1}{2}$ MIC and $\frac{3}{4}$ MIC) on outer membrane proteins of MDR *Pseudomonas aeruginosa* isolate was shown in Figure (6). Analysis of the resulted OMP bands to determine the difference in band size and darkness between the control and different treatments of guava leaves extract was done using Image J program. Table (5) showed the summary of relative density of selected protein bands of different concentrations of guava leaves extract compared to the control.

The results showed molecular identification of 101 clinical isolates of *Pseudomonas aeruginosa* out of 112 isolates which grown on Pseudomonas Cetrimide agar. The timely and accurate information provided by PCR assay would help to identify *P. aeruginosa* bacteremia, and initiate adequate therapy from 18 to 24 h earlier than with conventional methods (Reischl *et al.*, 2000 and Shrestha *et al.*, 2002). Because standard phenotypic methods are time consuming and have limitations, highly specific molecular techniques have used to identify bacterial pathogens (Van Delden, 2007).

This research primarily focuses on natural products with QS inhibitors components, which display potential for treating bacterial infections. Because of the wide variety of

molecules present in the natural products/extracts, the antimicrobial activity cannot be attributed to a single mechanism. Instead, different biochemical and structural mechanisms are involved (Carson *et al.*, 2002) at multiple sites within the cell and on the cell surface. (Burt and Reinders, 2003 and Burt, 2004). However, each of these actions cannot be considered separate events but may be a consequence of the other activities. Inhibition of bacterial QS can prevent the development of bacterial virulence and successful establishment of infections (Schauder and Bassler, 2001 and Robert *et al.*, 2005).

Swarming motility plays a critical role in the environmental adaptation of *P. aeruginosa*. The decrease in swarming activity was expected in natural products have QS inhibitor components which interfere with quorum sensing regulation. The way of swarming was changed from dendrites to bull's eye pattern at ½ MIC of cumin extract i.e the swarming pattern changed from dendrites which are long regions of colonization emanating from a central origin (Deziel *et al.*, 2003; Caiazza *et al.*, 2005 and Tremblay *et al.*, 2007) to Bull's eye pattern which results from cyclic and synchronous waves of motility followed by regular periods of swarming cessation (Hoeniger, 1964; Rauprich *et al.*, 1996 and Matsuyama *et al.*, 2000). It may be concluded that the changing of this pattern may be due to a mutation related to secreted molecules : 3-(3-hydroxyalkanoyloxy) alkanolic acids (HAA) precursor which acts as a repellent and di-rhamnolipid that acts as an attractant as (Caiazza *et al.*, 2005 and Tremblay *et al.*, 2007) reported that the dendrites of *P. aeruginosa* result from expansion and repulsion of each other as a result of the complicated interplay between the two secreted molecules.

Pseudomonas aeruginosa secretes alkaline protease (AprA) to enhance its survival. (Bardoel *et al.*, 2011). Alkaline protease of *P.*

aeruginosa inactivate human γ -interferon and human tumor necrosis factor- α (Horvat *et al.*, 1988 and Parmely *et al.*, 1990). γ -interferon is the key factor for innate and adaptive immunity against different infections and for control of tumor formation. A lack of γ -interferon results in autoinflammatory and autoimmune diseases (Schroder *et al.*, 2004 and Schoenborn and Wilson, 2007). The obtained results showed inhibition /or decrease of alkaline protease activity which may be due to the quorum quenching properties in the components of tested natural products/extracts.

Phospholipase C activity in bacteria treated with sub-inhibitory conc. of clove buds extract, green tea leaves extract and lemon leaves extract was increased. Also, 25% & 50% of ginseng and sidr honey increased the activity of phospholipase C. This results may be due to the expression or upregulation of phospholipase enzyme as a result of QS inhibitors in phytochemicals of the tested natural products/extracts. Sophie *et al.*, (2005) reported that the type III secretion regulon is a negative target for QS in *P. aeruginosa*. This is the first *P. aeruginosa* virulence factor for which a negative regulation by RhlR/I-C4-HSL has been demonstrated. Type III secretion system (TTSS) of *P.aeruginosa* is, apparently, imperative to virulence as it delivers, at least, four exoenzymes into host cells: ExoS, ExoT, ExoU and ExoY (Engel and Balachandran, 2009). ExoU is a phospholipase that can cause membrane damage and cell lysis and modulate the inflammatory response (Deng and Barbieri, 2008; Anderson and Frank, 2012).

It may be concluded that QS represses type III secretion regulon, suggests that the associated virulence functions are likely to be repressed i.e: ExoU (phospholipase) is negatively controlled by QS. So, quorum sensing inhibitors may allow the expression or upregulation of ExoU (phospholipase). It may

be supposed that the increased phospholipase C activity may alter host defence against *P.aeruginosa* pathogen as a resulted effect of the tested natural products/extracts.

Moreover, the sub-inhibitory concentration of cumin extract (which also characterized by QSI phytochemicals components as methyl euogenol) showed decreasing in the activity of phospholipase C. The present results are in agreement with these reported by Kusum *et al.*

(2014), who explored the action of cranberry towards virulence of *P. aeruginosa* through quorum sensing (QS) inhibition and found significant reduction in phospholipase C in presence of cranberry as compared to control. Meyers and Berk (1990) found that the significant reduction in the level of phospholipase C helps pathogen to overcome the action of host defence and contributes towards tissue inflammation.

Table.1 Mean of MIC and MBC ± S.E of natural product extracts against MDR isolates and reference *P.aeruginosa* ATCC 27853 isolate

Natural product extracts	<i>Pseudomonas aeruginosa</i> ATCC 27853		MDR isolates (n=20)	
	MIC (% v/v)	MBC (% v/v)	Mean of MIC ± S.E (% v/v)	Mean of MBC ± S.E(%v/v)
<i>Syzygium aromaticum</i>	5	10	6.5 ± 0.5	13.3 ± 1.7
<i>Psidium guajava</i>	5	10	8.5 ± 1.6	16.5 ± 8.1
<i>Camellia sinensis</i>	10	15	10 ± 0.0	15 ± 0.0
<i>Mangifera indica</i>	10	20	13.5 ± 0.2	38.3 ± 0.0
<i>Cuminum cyminum</i>	20	40	26.6 ± 3.5	>50
<i>Citrus limon</i>	20	>50	27.1 ± 2.0	>50

Table.2 Average of swarming colony diameter (mm) of *P.aeruginosa* isolates treated with different conc. of natural products/extracts

Natural products/ extracts	0% of natural products/ extracts (control)	¼ MIC of natural products/extracts	½ MIC of natural products/extracts
Clove buds extract	25	18	10
Green tea leaves extract	20	15	13
Lemon leaves extract	23	25	18
Cumin seeds extract	25	25	25
Natural products/ extracts	0% of natural products/ extracts(control)	25% of natural products/extracts	50% of natural products/ extracts
Bee glue	18	13	8
Black seed oil	20	20	17
<i>Frankincense</i>	20	20	18
Ginger juice	30	25	20
Ginseng	30	20	15
Sidr honey	22	19	10

Table.3 Alkaline protease activity in term of clear zone diameter average (mm) of *P.aeruginosa* isolates treated with different conc. of natural products/extracts

Natural products/ extracts	0% of natural products/extracts (control)	¼ MIC of natural products/extracts	½ MIC of natural products/extracts
Clove buds extract	5	2	0
Cumin seeds extract	5	4	2
Green tea leaves extract	5	5	3
Guava leaves extract	5	4	2
Natural products	0% of natural products(control)	25% of natural products	50% of natural products
Red Onion juice	4	4	3
Sidr honey	4	2	2

Table.4 Phospholipase C activity in term of precipitation zone ratio of *P.aeruginosa* isolates treated with different conc. of natural products/extracts

Natural products/ extracts	0% of natural products/ extracts (control)	¼ MIC of natural products /extracts	½ MIC of natural products/ extracts
Clove buds extract	0.66 (++++)	0.46 (++++)	0.41 (++++)
Cumin seeds extract	0.45 (++++)	0.60 (++++)	0.81 (++)
Green tea leaves extract	0.68 (++++)	0.64 (++++)	0.52 (++++)
Lemon leaves extract	0.60 (++++)	0.35 (++++)	0.35 (++++)
Natural products	0% of natural products	25% of natural products	50% of natural products
Ginger juice	0.53 (++++)	0.42 (++++)	0.47 (++++)
Ginseng	0.60 (++++)	0.42 (++++)	0.38 (++++)
Sidrhoney	0.63 (++++)	0.57 (++++)	0.41 (++++)

Fig.1 Incidence of virulence factors in *P.aeruginosa* isolates

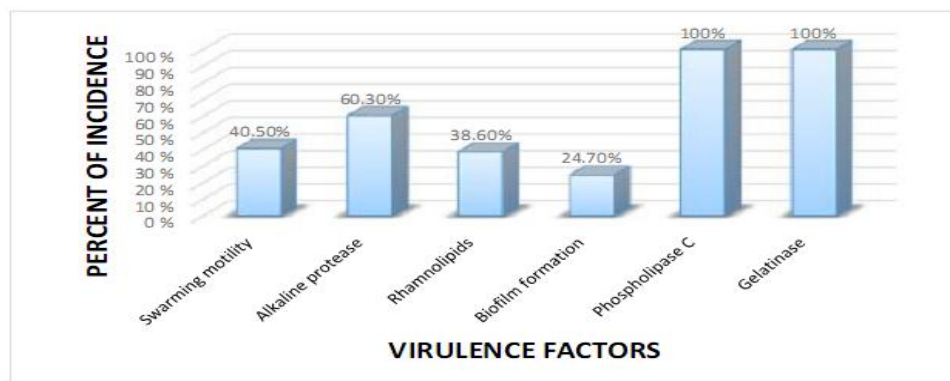


Fig.2 Effect of a-Clove buds b-Sidr Honey c- Green tea leaves d- Cumin seeds on swarming motility

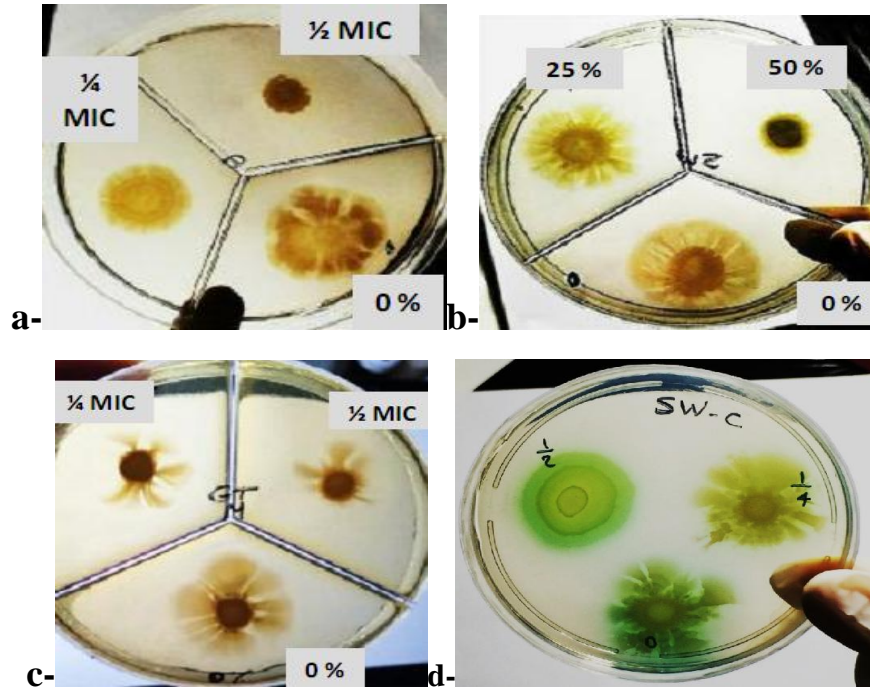


Fig.3 Effect of a-Clove buds b- Cumin seeds on alkaline protease enzyme activity

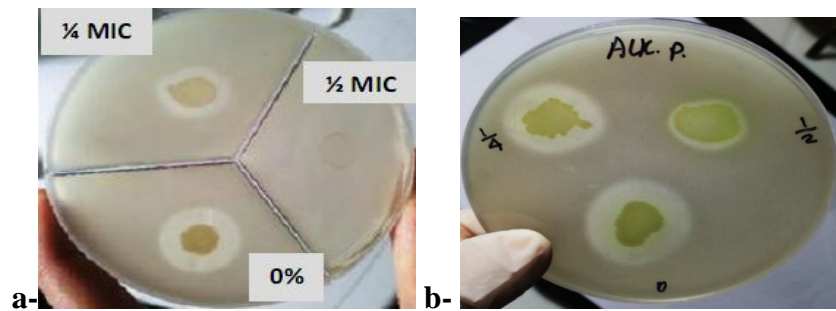


Fig.4 Effect of a-Clove buds b- Sidr honey c- Cumin seeds on phospholipase C enzyme

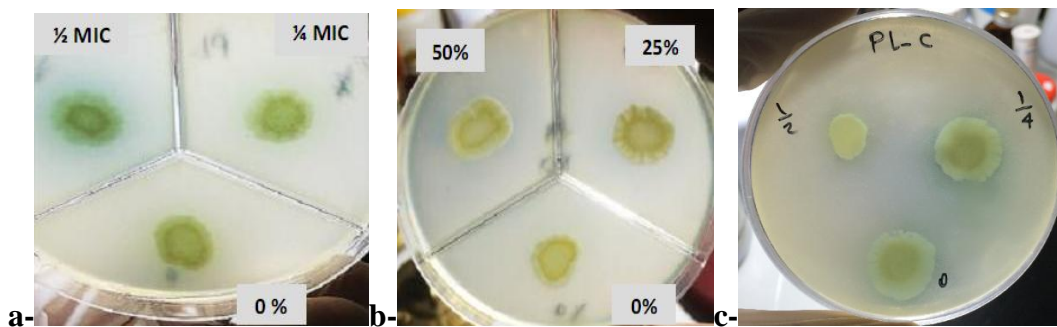


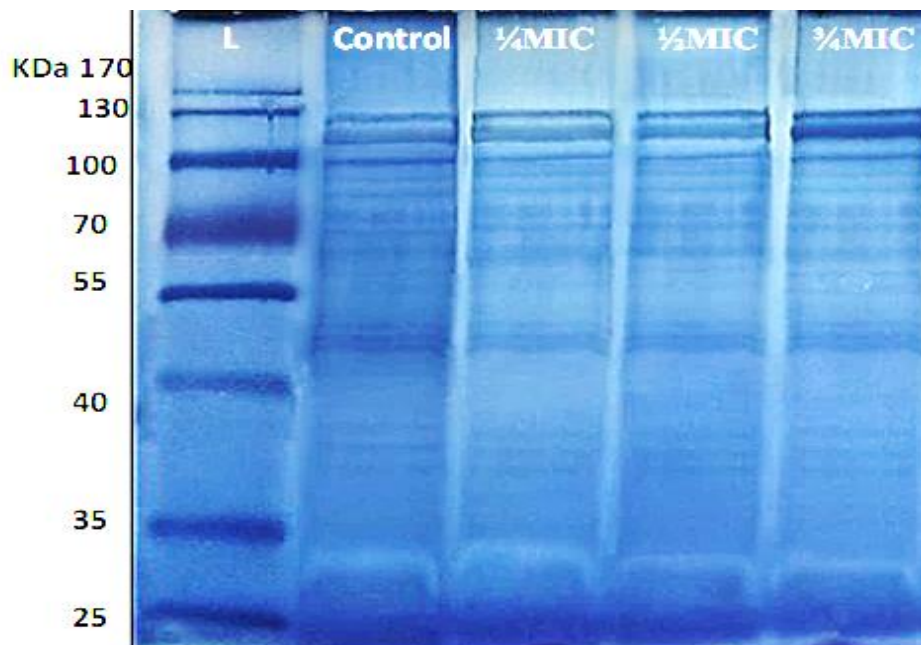
Table.5 The summary of relative density of OMP bands

<i>Mr</i> (KDa)	Relative density			
	Untreated	2.5% Guava leaves extract	5% Guava leaves extract	7.5% Guava leaves extract
115	1	1.096	1.093	1.154
107	1	0.733	0.612	0.621
89	1	0.640	0.519	0.530
70	1	0.566	0.518	0.505
65	1	0.524	0.490	0.459
60	1	0.489	0.458	0.423
59	1	0.476	0.454	0.414
52	1	0.467	0.409	0.371
49	1	0.511	0.438	0.409
47	1	0.545	0.486	0.459
44	1	0.539	0.432	0.358
39	1	0.634	0.542	0.417
38	1	0.638	0.516	0.410
36	1	0.609	0.475	0.376

Fig.5 Inhibition of biofilm formation by $\frac{1}{2}$ MIC *Psidium guajava* leaves extract.



Fig.6 Effect of *Psidium guajava* leaves extract on OMPs.



It is seemed that there are two opposite results but it may be deduced that the different composition of tested natural products/extracts may affect the phospholipase regulation in more than one regulatory mechanism through QS systems. Surely, this point need further experiments and research.

The present results also revealed that guava leaves extract at sub inhibitory concentration ($\frac{1}{2}$ MIC) showed inhibition of biofilm formation in congo red agar. The possibility that the observed biofilm inhibitory effect was related to quorum sensing inhibition (QSI) effect of guava leaves components such as polyphenols, tannin, triterpernoids and guaijaverin.

Both quorum sensing and biofilm formation are important in the pathogenesis of *P. aeruginosa* infections. Quorum sensing regulates many different functions and its control of these functions can change depending on environmental conditions. Supporting this notion, several different quorum sensing-regulated functions

have been shown to impact biofilm formation at different stages. (Mary *et al.*, 2006).

Another goal of this study is to investigate the mode of action of *Psidium guajava* (guava) leaves extract on the outer membrane proteins of MDR isolate of *P. aeruginosa*. Sub inhibitory conc. ($\frac{1}{2}$ MIC) of guava leaves extract showed lower relative density in protein bands at molecular weight of 107 KDa & 89 KDa in comparison to the control while $\frac{3}{4}$ MIC of guava leaves extract showed lower relative density in protein bands at molecular weights of 70 KDa, 65 KDa, 60 KDa, 59 KDa, 49 KDa, 44 KDa & 36 KDa compared to the control. The protein bands of estimated molecular weights of 70, 44 and 38 KDa are most likely representing the porin proteins Opr C, E and F respectively (Eisaku and Taiji, 1989). Decreasing the relative density in relation to the control indicated downregulation in expressed protein and may point to a mechanism leading to bacterial death. Guava leaves composed of different components such as polyphenols which may be

responsible of resulted changes in outer membrane proteins of MDR *P. aeruginosa* isolate.

Researchers are increasingly investigating herbal products for new anti-pathogenic agents that might act as nontoxic QS inhibitors, thus controlling infections without encouraging the appearance of resistant bacterial strains (Hentzer and Givskov, 2003). QS inhibition, results in reduction of bacterial virulence and control of infection, So that the host immune system can clear out the bacteria. since bacterial growth is not affected by QS inhibitors, the development of resistance risk will be lower (González and Keshavan, 2006).

References

- Alexandra, W. 2009. Worden Lab. DNA Extraction CTAB Method.
- Anderson, D.M. and Frank, D.W. 2012. Five mechanisms of manipulation by bacterial effectors: a ubiquitous theme. *PLoS Pathog.*, 8:e1002823. doi:10.1371/journal.ppat.1002823.
- Balasubramanian, D., Schneper, L., Kumari, H. and Mathee, K. 2013. A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. *Nucleic Acid Res.*, 41:1-20.
- Bardoel, B.W., van der Ent, S., Pel, M.J., Tommassen, J., Pieterse, C.M., van Kessel, K.P. and van Strijp, J.A. 2011. *Pseudomonas* evades immune recognition of flagellin in both mammals and plants. *PLoS Pathog.*, 7(8):1-11.
- Bhaskarwar, B., Itankar, P. and Fuluke, A. 2008. Evaluation of antimicrobial activity of medicinal plant *Jatropha podagrica* (Hook). *Rom Biotechnol Lett.*, 13(5): 3873-3877.
- Burns, and Clark. 1992. Salicylate-Inducible Antibiotic Resistance in *Pseudomonas cepacia* Associated with Absence of a Pore-Forming Outer Membrane Protein, *Antimicrobial Agents And Chemother.*, 36(10): 2280-2285.
- Burt, S. and Reinders, R.D. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157: H7. *Lett. Appl. Microbiol.*, 36: 162–167.
- Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods— A review. *Int. J. Food Microbiol.* 94: 223–253.
- Caiazza, N.C., Shanks, R.M., O'Toole, G.A. 2005. Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. *J. Bacteriol.* 187 (21): 7351–7361.
- Carson, C.F., Cookson, B.D. and Riley, T.V., 1995. Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oils of *Melaleuca alternifolia*. *J. Antimicrobial Chemother.*, 35: 421-424.
- Carson, C.F., Mee, B.J. and Riley, T.V. 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *Antimicrob. Agents Chemother.*, 46: 1914–1920.
- CheO'May and Nathalie Tufenkji. 2011. The Swarming Motility of *Pseudomonas aeruginosa* Is Blocked by Cranberry Proanthocyanidins and Other Tannin-Containing Materials, *Appl. Environ. Microbiol.*, 77(9): 3061–3067.
- Choi, H.J. and Kim, M.H. 2013. Improved PCR for identification of *Pseudomonas aeruginosa*, *Appl. Microbiol. Biotechnol.*, 97: 3643–3651.
- CLSI-Clinical and Laboratory Standards Institute. 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24.
- Deng, Q. and Barbieri, J.T. 2008. Molecular mechanisms of the cytotoxicity of ADP-ribosylating toxins. *Annu. Rev. Microbiol.* 62:271–288.
- Deziel, E., Lepine, F., Milot, S. and Villemur, R. 2003. *rhlA* is required for the production of a novel biosurfactant

- promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkyl)-kanoyloxyalkanoic acids (HAAs), the precursors of rhamnolipids. *Microbiol.*, 149: 2005–2013.
- Difco, BBL Manual, 2009. Manual of Microbiological Culture Media, 2nd edition, Becton Dickinson and Company. Maryland, USA: 402-403.
- Eisaku Yoshihara and Taiji Nak. 1989. Identification of porins in the outer membrane of *pseudomonas aeruginosa* that form small diffusion pores, the *J. Biol. Chem.*, 264(11): 6297-6301.
- Engel, J. and Balachandran, P. 2009. Role of *Pseudomonas aeruginosa* type III effectors in disease. *Curr. Opinion in Microbiol.*, 12: 61-66.
- Fernandez-Patron, C., Castellanos-Serra, L. and Rodriguez, P. 1992. Reverse staining of sodium dodecyl sulfate polyacrylamide gels by imidazole-zinc salts: Sensitive detection of unmodified proteins. *Biotechniques*, 12: 564-573.
- Freeman, J., Falkiner, F.R. and Keane, C.T. 1989. New method for detecting slime production by coagulase negative staphylococci. *J. Clin. Pathol.*, 42: 872-4.
- González, J.E. and Keshavan, N.D. 2006. Messing with bacterial quorum sensing. *J. Microbiol. Mol. Biol. Rev.*, 70(4):859-75.
- Han, Y.C., Teng, C.Z., Hu, Z.L. and Song, Y.C. 2008. An optimal method of DNA silver staining in polyacrylamide gels *Electrophoresis*, 29:1355 – 1358.
- Hentzer, M. and Givskov, M. 2003. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J. Clin. Invest.*, 112: 1300–1307.
- Hoeniger, J.F.M. 1964. Cellular changes accompanying the swarming of *Proteus mirabilis*. I. Observation of living cultures. *Can. J. Microbiol.*, 10: 1–9.
- Horvat, R.T. and Parmely, M.J. 1988. *Pseudomonas aeruginosa* alkaline protease degrades human gamma interferon and inhibits its bioactivity. *Infect Immun.*, 56: 2925-2932.
- Jaeger, K.E., Schneidinger, B., Liebeton, K., Haas, D., Reetz, M.T., Philippou, S., Gerritse, G., Ransac, S., and Dijkstra, B.W. 1996. Lipase of *Pseudomonas aeruginosa*: molecular biology and biotechnological application. In *Molecular Biology of Pseudomonas*. Nakazawa, T., Furukawa, K., Haas, D., and Silver, S. (eds). American Society for Microbiology Press : 319–330.
- Jan Hudzicki, 2009. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, American Society for Microbiology, <http://www.microbelibrary.org/library/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol>.
- Ji, Y.T., Qu, C.Q. and Cao, B.Y. 2007. An optimal method of DNA silver staining in polyacrylamide gels *Electrophoresis*. 28:1173-1175.
- Karuppusamy, S. and Rajasekaran, K.M. 2009. High Throughput Antibacterial Screening of Plant Extracts by Resazurin Redox with Special Reference to Medicinal Plants of Western Ghats. *Global J. Pharmacol.*, 3(2): 63-68.
- Kumar Bajaj, B. and Jamwal, G. 2013. Thermostable alkaline protease production from *Bacillus pumilus* D-6 by using agro-residues as substrates. *Advances in Enzyme Research*. 1 (2):30-36.
- Kusum, H., Ravi, K. and Himanshi, S. 2014. Attenuation of quorum sensing controlled virulence of *Pseudomonas aeruginosa* by cranberry. *Indian J. Med. Res.* 139: 446-453.
- Mary, J.O. and Matthew, R. 2006. Does *Pseudomonas aeruginosa* use intercellular signalling to build biofilm communities?, *Cellular Microbiology*. 8(12): 1841–1849.
- Matsuyama, T., Takagi, Y., Nakagawa, Y., Itoh, H., Wakita, J. and Matsushita, M. 2000. Dynamic aspects of the structured cell population in swarming colony of *Proteus mirabilis*. *J. Bacteriol.* 182:385–393.
- McMurray, W.C. and Magee, W.L. 1972.

- Phospholipid metabolism. *Annu. Rev. Biochem.* 41: 129-160.
- Meyers, D.J. and Berk, R.S. 1990. Characterization of phospholipase C from *Pseudomonasaeruginosa* as a potent inflammatory agent. *Infect Immun.* 68 : 659-66.
- Monica Cheesbrough, 2006 district laboratory practice in tropical countries, Cambridge University Press.2:1-434.
- Nowsheen, H. and Jain, S.K. 2008. Characterization of an Outer Membrane Protein of Salmonella enterica Serovar Typhimurium That Confers Protection against Typhoid. *Clin. vaccine immunol.*, 15(9):1461–1471.
- Ochsner, U.A. and Reiser, J. 1995. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonasaeruginosa*. *Proc. Natl. Acad. Sci., USA.* 92:6424–6428.
- Ochsner, U.A., Koch, A.K., Fiechter, A. and Reiser, J. 1994. Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *J Bacteriol.*, 176:2044–2054.
- Okigbo, R.N. and Ogbonnaya, U.O. 2006. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post harvest yam (*Dioscorea* spp.) rot. *Afr. J. Biotechnol.* 5: 727-731.
- Parmely, M., Gale, A., Clabaugh, M., Horvat, R. and Zhou, W.W. 1990. Proteolytic inactivation of cytokines by *Pseudomonas aeruginosa*. *Infect Immun.*, 58: 3009-3014.
- Pearson, J.P., Passador, L., Iglewski, B.H. and Greenberg, E.P. 1995. A second N-acylhomoserine lactone signal produced by *Pseudomonasaeruginosa*. *Proc. Natl. Acad. Sci. USA*, 92:1490–1494.
- Price, M.F., Wilkinson, I.D. and Gentry, L.O. 1982. Plate method for detection of phospholipase activity in *Candida albicans*. *Sabouraudia.* 20: 7-14.
- Rauprich, O., Matsushita, M., Weijer, C.J., Siegert, F., Esipov, S.E. and Shapiro, J.A. 1996. Periodic phenomena in *Proteus mirabilis* swarm colony development. *J. Bacteriol.*, 178:6525–6538.
- Reischl, U., Linde, H.J., Metz, M., Leppmeier, B. and Lehn, N. 2000. Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR. *J. Clin. Microbiol.*, 38: 2429-2433.
- Robert, R., Joseph, R., Diana, R., Kamal, K., Rachel, C., Nicholas, T., Oksana, D., and Jeffrey, M. 2005. Bacterial Communication (“Quorum Sensing”) via Ligands and Receptors: A Novel Pharmacologic Target for the Design of Antibiotic Drugs. *J. Pharmacol. Exp. Ther.*, 312(2): 417-423.
- Sambrook, J., Russell, D.W. 2001. Molecular Cloning: A laboratory manual., 3rd ed. Cold Spring Harbor Laboratory Press. New York.
- Schauder, S. and Bassler, B.L. 2001. the language of bacteria. *Genes Develop.*, 15:1468-1480.
- Schoenborn, J.R. and Wilson, C.B. 2007. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv. Immunol.*, 96: 41-101.
- Schroder, K., Hertzog, P.J., Ravasi, T. and Hume, D.A. 2004. Interferon-gamma: an overview of signals, mechanisms and functions. *J. Leukoc. Biol.*, 75:163-189.
- Shariff, N., Sudarshana, M.S., Umesha, S. and Hariprasad, P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *Afr. J. Biotechnol.*, 5: 946-950.
- Shrestha, N., K. Tuohy, M.J., Hall, G.S., Isada, C.M. and Procop, G.W. 2002. Rapid identification of *Staphylococcus aureus* and other Agene from BacT/ALERT blood culture bottles by using the Light Cycler system. *J. Clin. Microbiol.*, 40: 2659-2661.
- Siegmund, I. and Wagner, F. 1991. New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar. *Biotechnology techniques*, 5(4): 265-268.

- Sophie, B., Chantal, S., Patricia, N., André Lazdunski, and Alain, F. 2005. Quorum sensing negatively controls type iii secretion regulon expression in *Pseudomonas aeruginosa* PAO1. *J. bacterial.*, 187 (11): 3898–3902.
- Tiago, F. and Wayne, R. 2012. ImageJ User Guide, IJ 1.46r.
- Tremblay, J., Richardson, A., Lepine, F. and Déziel, E. 2007. Self-produced extracellular stimuli modulate the *Pseudomonas aeruginosa* swarming motility behavior. *Environ. Microbiol.*, 9:2622–2630.
- Tyagi, A.K. and Malik, A. 2010. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*. *BMC Complementary and Alternative Med.*, 10: 65.
- Uniyal, S.K., Singh, K.N., Jamwal, P., Lal, B. 2006. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. *J. Ethnobiol. Ethnomed.*, 2: 1-14.
- Van Delden, C. 2007. *Pseudomonas aeruginosa* bloodstream infections: how should we treat them? *Int. J. Antimicrob Agents*, 30(1): 71-75.
- Vijay, K., Davender, K., Lalit, K., Sushil, N., Chand, R. and Rajinder, P. 2012. Screening, isolation and production of lipase/esterase producing *Bacillus sp.* strain DVL2 and its potential evaluation in esterification and resolution reactions, *Arch. Appl. Sci. Res.*, 4(4):1763-1770.
- Warburg, O. and Christian, W. 1941. Isolierung und Kristallisation des Gärungsferments Enolase. *Biochem. Z.*, 310: 384-421.
- Winson, M.K., Camara, M., Latifi, A., Foglino, M., Chhabra, S.R., Daykin, M., Bally, M., Chapon, V., Salmond, G.P.C., Bycroft, B.W., Lazdunski, A., Stewart, G. and Williams, P. 1995. Multiple N-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA*, 92: 9427–9431.
- Winson, M.K., Camara, M., Latifi, A., Foglino, M., Chhabra, S.R., Daykin, M., Bally, M., Chapon, V., Salmond, G.P.C., Bycroft, B.W., Lazdunski, A., Stewart, G.S. and Williams, P. 1995. Multiple N-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA*, 92: 9427–9431.
- Yosuke, T. and Yutaka, Y. 2013. Interspecies Interaction between *Pseudomonas aeruginosa* and Other Microorganisms. *Microbes Environ.*, 28(1): 13–24.
- Zulianello, L., Canard, C., Köhler, T., Caille, D., Lacroix, J.S. and Meda, P. 2006. Rhamnolipids are virulence factors that promote early infiltration of primary human airway epithelia by *Pseudomonas aeruginosa*. *Infection and Immunity*, 74: 3134-3147.

How to cite this article:

Ahmed Ahmed Abd El-Aziz, Tarek El-Said El-Banna, Fatma Ibrahim Sonbol and Shymaa Mohamed Sallam. 2017. Anti-Virulence Activity of Selected Natural Products against *Pseudomonas aeruginosa*. *Int.J.Curr.Microbiol.App.Sci.* 6(1): 396-410.
doi: <http://dx.doi.org/10.20546/ijcmas.2017.601.048>