Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 193(2) (2024) 241-252 EISSN 2392-2192

Antimicrobial effects and phytochemical analysis of *Psidium gujava* leaf extract against *Pseudomonas aeruginosa*

O. C. Anumihe[1,*], U. Ahamefula[1], O. Edward[1], P. Iroka[1], N. Onyewuenyi[1], C. Mbachu[1], O. Ugwuegbu[2]

[1] Department of Biology/Microbiology, Federal Polytechnic Nekede Owerri (FPNO), Imo State, Nigeria

[2] Department of Environmental Biology, Federal Polytechnic Nekede Owerri (FPNO), Imo State, Nigeria

*E-mail address: okechukwuanumihe@gmail.com

ABSTRACT

Antimicrobial properties and phytochemical analysis of *Psidium gujava* leaf extract against *Pseudomonas aeruginosa* was carried out. Fourier transform infrared and Gas chromatographic analysis showed that the extracts contain the following phytochemicals which include proanthocyanin, lunamarin, quinine, epihedrine, anthocyanin, flavan, sapogenin, phenol, flavonones, naringenin, steroids, epicatechin, kaempferol, phytate, flavone, oxalate, catechin, resveratrol and tannin. Among the plant extracts tested, ethanol and hot water extract of *Psidium gujava* showed the highest inhibition on *Pseudomonas aeruginosa* For twitching motility, hot water and ethanolic extracts of exerted the highest inhibition, Hot water extract showed the best inhibition in biofilm formation and cell adhesion, In protelytic activity, cold water extract showed the highest inhibition for *psidium gujava*, on *Pseudomonas aeruginosa* while ethanol had the best inhibition in pyocynin production. The present study revealed the potential for *psidium gujava* leave extracts in treating microbial infections through inhibition of quorum sensing regulated virulence factors for the prevention of bacterial infections.

***Keywords*:** Antimicrobial, Quorum sensing, Biofilm, Inhibition

(Received 15 April 2024; Accepted 14 May 2024; Date of Publication 16 May 2024)

1. INTRODUCTION

1. 1. Background of study

It has long been appreciated that certain groups of bacteria exhibit cooperative behavioral patterns. For example, feeding and sporulation of both myxobacteria and actinomycetes seem optimized for large populations of cells behaving almost as a single multicellular organism. Another example was the investigation of microbial aggregates on tooth surfaces by Antonie van Leeuwenhoek resulted in the identification of microbial biofilms (Donlan, 2002). In 1940, a study by Heukelekian and Heller reported that for marine microbes, growth and activity were enhanced by the presence of a surface onto which they could adhere. During a study of natural marine bacteria populations, it was also discovered that there were many more microbes found attached to solid surfaces than were found in the surrounding medium (Zobell, 1943).

It has become evident that bacterial function and growth within a population is a fundamental aspect of bacterial survival and a typical life style of microorganisms (Davey, 2000).This typical lifestyle of survival has been highlighted in more recent studies (George and Muir, 2007; Clatworthy *et. al.*, 2007; Rasko *et. al.*, 2010; Kobayashi *et. al.*, 2011; Daly *et. al.*, 2015).

Bacterial populations coordinate communal behavior through a process of cell-to-cell signaling mediated by diffusible signal molecules (Schauder and Bassler, 2001).This process, termed quorum sensing (QS), is known to control gene expression responsible for diverse physiological functions including virulence, antibiotic production, and biofilm formation (Rutherford and Bassler, 2012).Gram negative bacteria use a QS system mediated by diffusible signaling molecules of the N-acyl homoserine lactones (AHL) family (Fuqua *et. al.*, 2001).Many pathogenic bacteria use a QS system to regulate genes required for the expression of virulence, thus, inhibition of the QS system is considered as a novel strategy for development of antipathogenic agents, especially for combating bacterial infections caused by antibiotic-resistant strains (Rasko and Sperandio, 2010).

Bacterial QS plays a role in biofilm formation (Kumar *et. al.*, 2016; Kai, 2018). Biofilm is an association of micro-organisms in which microbial cells adhere to each other on a living or non-living surfaces within a self-produced matrix of extracellular polymeric substance. Biofilm allows bacteria to grow in this rhythmic way without any environmental depression even protecting them from antimicrobials and attack from the immune system (Srey *et. al.*, 2013; Solano *et. al.*, 2014). Biofilms are ubiquitous in nature and can be found in industrial places, hotels, waste water channels, bathrooms, labs, hospital settings and commonly occur on hard surfaces submerged in or exposed to an aqueous solution. .

Nearly all (99.9%) of micro-organisms have the ability to form biofilm on a wide range of surfaces i.e. biological and inert surfaces (Sekhar, 2009). Biofilm posing a great problem for public health due to its resistant nature to antibiotics and disease associated with indwelling medical devices (Khan, 2014). Bacterial biofilm have been reported to results in nosocomial infections. According to National Institutes of Health (NIH) about 65% of all microbial infections and 80% of all chronic infections are associated with biofilms (Muhsin *et. al.*, 2015). It was found that *H. influenza* has the ability to form biofilm in human body and can escape from human immune system (Sekhar, 2009; Solano *et. al.*, 2014). Biofilm forming capability has been reported in large number of bacterial species such as *P. aeruginosa, S. epidermidis, E. coli spp, S. aureus, E. cloacae, K. pneumoniae* (Fux, 2005; Muhsin *et. al.*, 2015). Over the past 50 years, antibiotics have significantly reduced the rate of mortality caused by bacterial

infections. However, the misuse and abuse of antibiotics in pharmacotherapy have led to the development of widespread resistance in the target organism. The failure of existing antibiotics to control infection makes it crucial to find alternatives to currently available drugs. More recently, several green nonlethal strategies for biofilm control have been developed, because the mode of action of these novel antibiofilm agents is much less susceptible to the emergence of resistance. However although they are promising strategies, they have disadvantages because none have been totally effective (Hibbing and Fuqua, 2012). One promising alternative is the search for naturally occurring compounds of plant origin capable of blocking biofilm formation as well as quorum sensing mediated virulence factors. Hence, the need for this study, which present plant-based alternatives to existing drugs. Since pathogenicity in many bacteria is regulated by QS, inhibition of this system may cause the attenuation of virulence and protect against infection.

2. MATERIALS AND METHODS

2. 1. Collection of plant materials

Leaves of *Psidium gujava* (Guava) were collected from Owerri, Imo state, Nigeria, the individual plant species were authenticated by a taxonomist in the Department of Forestry and Wild Life, Federal University of Technology Owerri, Imo State Nigeria and used in the study.

2. 1. 1. Preparation of Plant Extract

The plant extract were prepared according to method described by Ibe *et. al.*, (2017).The collected plant material will be air-dried under shade at room temperature, finely ground into powder using domestic mixture and will be stored in airtight labeled plastic sampling bags for further studies.

2. 1. 2 Extraction of plant samples

The grounded plant samples were extracted using three solvents i.e cold water, hot water and ethanol.

2. 1. 3. Cold water

10 g of the grounded plant samples were soaked in 100 ml cold distilled water for 72 hours with occasional agitation. The extract of each plant were filtered using (Whatman No.1) filter paper.

2. 1. 4. Hot water

10 g of the grounded plant samples were soaked in 100 ml hot distilled water for 72 hours with occasional agitation. The extract of each plant were filtered using (Whatman No.1) filter paper.

2. 1. 5. Ethanol extracts

Extraction was carried out by the modified method of Hussaini and Mahasneh (2009) .The plant material were extracted at room temperature with ethanol 95% (100 mL/10 g of plant

material). The extract of each plant was filtered using (Whatman No.1) filter paper and evaporated under vacuum at 40 °C using a rotary vacuum evaporator, the concentrated extract thus obtained was collected in screwcap vial and was used for further studies.

2. 2. Quantitative test for phytochemicals

Quantitative phytochemical analysis of the plants extract was performed using a Buck 530 Gas Chromatograph (USA) equipped with an on-column, automatic injector, Electron capture detector, and HP 88 capillary column (100 m × 0.25 µm film thickness). The detector temperature will be set at 280 °C, column temperature was set at 210 °C, and injector temperature will be set at 250 °C while the integrated chart speed will set at 2 cm/min.

2. 3. Test Organism and growth condition

The pure cultures of the test organism i.e *Pseudomonas aeruginosa* and *Staphylococcus aureus* was obtained from the microbiology laboratory, Federal Medical Center, Owerri. The isolates was propagated on nutrient agar plates and maintained on the plates at 4 °C. The isolates were sub-cultured in nutrient agar at 37 °C for 24 hours prior to further studies.

2. 4. Biofilm Inhibition

Biofilm inhibition study was carried out by modified method of micro titre plate crystal violet calorimetric assay. In this method nitrocellulose membrane filter were used. A sterile filter (10 mm) was transferred to a sterile petriplate containing a layer of cotton moisture with water 250 µl of inoculum and 500 µl of respective plant extracts were added to the membrane filter paper (Difco, USA).The inoculated filter paper were incubated at 37 ºC for 72 hours. After incubation, 100 µl of 1% v/v aqueous solution of crystal violet were added to the filter paper and incubated for 30 minutes.The dye were removed and the filter paper was washed using sterile distilled water followed by using 95% ethanol and incubated for 15 minutes. Biofilm inhibition was studied by determination of the absorbance of the ethanol solubilised mixture at 540 nm in an UV spectrophotometer. Control (without bacteria only crystal violet), three replicates was maintained for each treatment. The absorbance of the reaction mixture was read in spectrophotometer at 570 nm.

2. 5. Quorum sensing mediated virulence factors

The following quorum sensing mediated virulence factors such as twitching motility, pyocyanin production and proteolytic activity of the test organism were conducted.

2. 6. Twitching Motility

Twitching Motility was determined according to methods described by Karthick and Vivek (2016). 500 µl of respective plant extracts and 250 µl of bacterial inoculums was prepared in LB (Lysogeny broth) broth and were mixed in sterile eppendorf tube, kept in room temperature for 30 minutes. The respective plant extracts treated cultures were stab inoculated through LB agar plates. The plates were incubated at 32 °C for 24 hours. Bacteria grew at the interface between the plastic surface and the agar, which is indicative of twitching motility. To visualize the bacterial growth on the plastic surface, the agar was removed and the plate was

stained with a 1% solution of crystal violet. Twitching motility was determined by measuring the diameter of the stained growth.

2. 7. Pyocyanin Production

Effect of plant extracts on pyocyanine production was done by the modified method described by Karthick and Vivek (2016). Pyocyanine was extracted from the supernatant fraction of test organism grown in trypticase soy broth medium with 500 µl of plant extracts for 24 hours. 5 ml sample of the supernatant was mixed with 5 ml chloroform and the lower organic layer was separated. To this layer 1.5 ml of 0.2 M of HCl will be added and the pyocyanine rich organic layer was separated. The amount of the pyocyanine within the extracted layer was determined by measuring the absorbance at 520 nm.

2. 8. Cell Adhesion

Cell adhesion was studied by using 96 well flat bottom micro well plate was previously coated with bovine serum albumin (BSA). Wells was coated with 150 µl of freshly prepared 1.0% BSA, incubated at 30 °C for 30 minutes. After the incubation period, wells was washed thrice with sterile phosphate buffered saline (PBS).Fifty microlitre of bacterial inocula thus prepared was transferred to the well followed by the addition of 50 µl of the respective plant extracts. Seeded microtitre plate was incubated at 37°C for 24 hours. Cells were allowed to adhere and the non-adhered cells were washed 5 times with PBS at room temperature. Adhered cells were detected by adding 50µl of 0.1% crystal violet per well, incubated at room temperature for 30 minutes. Wells was washed with sterile distilled water to remove excess stain. 10 µl of ethanol was added to fix the adhered cells. 50 µl of 0.2 % Triton X was then be added to the wells for lyse of cells and the absorbance was read at 570nm.

2. 9. Proteolytic activity

Proteolytic activity was carried out by modified method of Karthick and Vivek (2016). Crude enzyme preparation 0.1 ml of tryptic soy broth bacterial culture was inoculated into 100 ml of protease production media (Yeast extract 5 mg/l, Peptone-10 mg/l, Glucose-10 mg/l, Caesin-15 mg/L) supplemented with 200 µl of respective plant extracts. Flasks was incubated at 37 °C for 48 hours. Broth was centrifuged after the incubation period at 10,000 rpm for 10 minutes, the collected supernatant was used as the source of protease enzyme.

3. RESULT

3. 1. Fourier Transform Infrared (FTIR) analysis of extract

The result for the Fourier transform infrared analysis of *Psidium gujava* leave extract is presented in Figures 1.

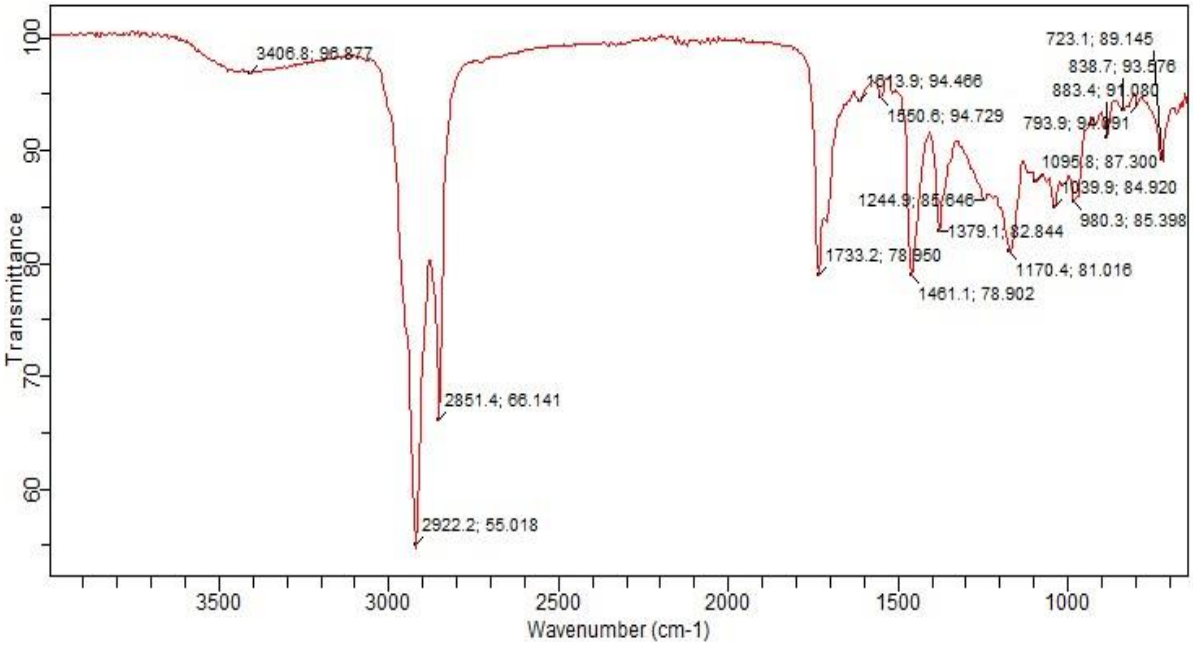

Figure 1. FTIR spectra for *Psidium gujava* leave extract

3. 2. Phytochemical analysis of extract

The GC analysis for phytochemicals in *Psidium gujava* extract is presented in Figures 2.

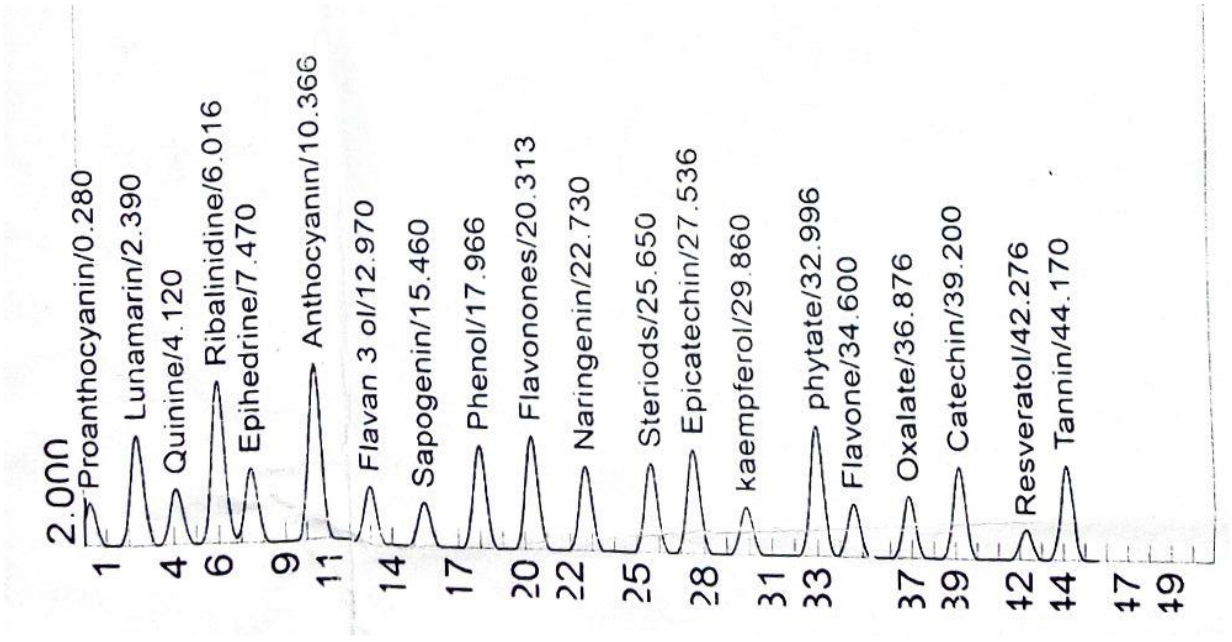

Figure 2. GC spectra for *Psidium gujava* leave extract

Table 1. Phytochemicals, molar mass (g/mol) and concentration (mg/g) of plant extracts

Phytochemicals	Molar mass (g/mol)	Concentration (mg/g)		
		Psidium gujava	***Citrus cinensis***	***Carica papaya***
Proanthocyanin	576.5	0.208	0.323	0.280
Lunamarin	469.311	2.390	2.390	2.390
Quinine	324.42	4.120	6.016	6.016
Rinalindine	275.3	6.016	6.016	6.016
Epihedrine	183.204	7.470	7.470	7.470
Anthocyanin	207.25	10.366	10.366	10.366
Flavan	210.27	12.970	12.970	12.970
Sapogenin	1223.3	15.460	15.460	15.460
Phenol	94.11	17.966	17.966	17.966
Flavanone	222.24	20.313	20.313	20.313
Naringenin	272.257	22.730	4.120	4.120
Steriods	358.4	25.650	25.650	25.650
Epicatechin	442.37	27.536	27.536	27.536
Kaempferol	286.23	29.860	29.860	29.860
Phytate	660.04	32.996	22.730	22.730
Flavone	222.239	34.600	34.600	34.600
Oxalate	88.02	36.676	36.876	36.876
Catechin	290.26	39.200	39.200	39.200
Resveratol	228.25	42.276	42.276	42.276
Tannin	1701.2	44.170	44.170	44.170

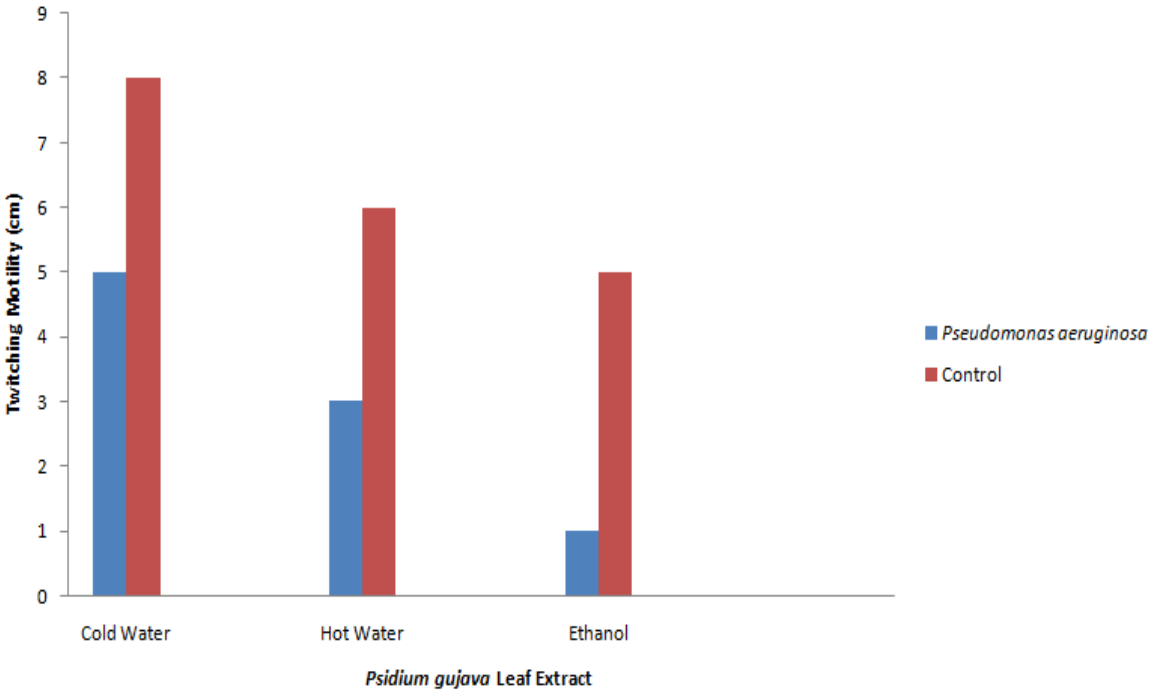

Figure 3. Effect (cm) of *Psidium gujava* extract against twitching motility of *Pseudomonas aeruginosa*

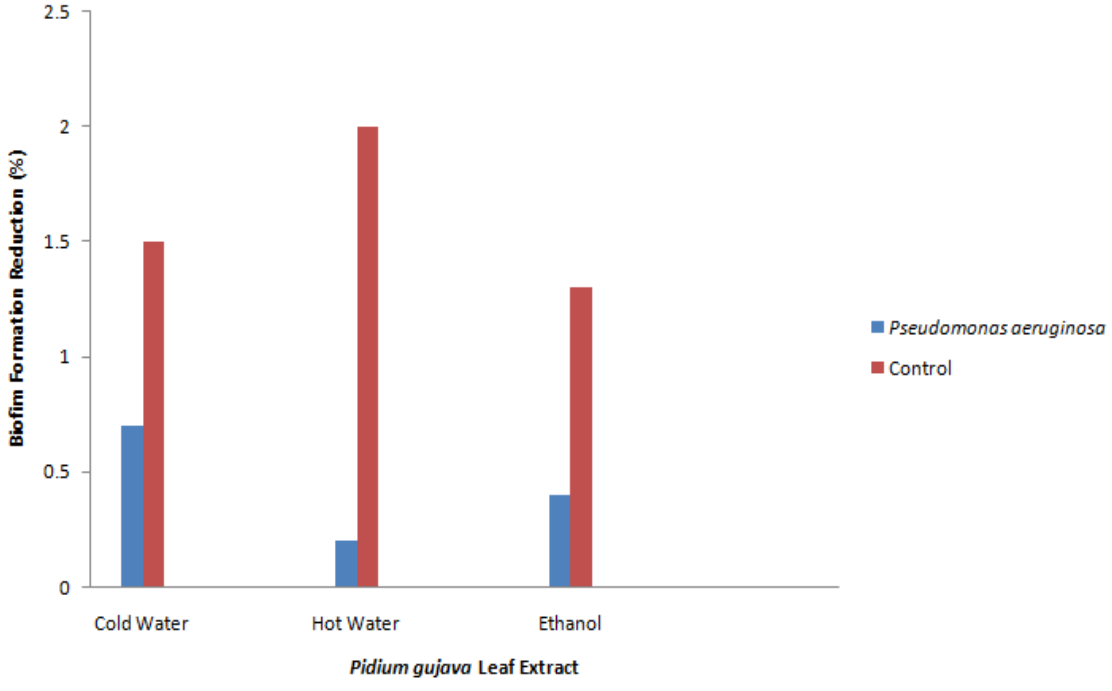

Figure 4. Inhibitory effect (%) of *Psidium gujava* extract on Biofilm Formation of *pseudomonas aeruginosa*

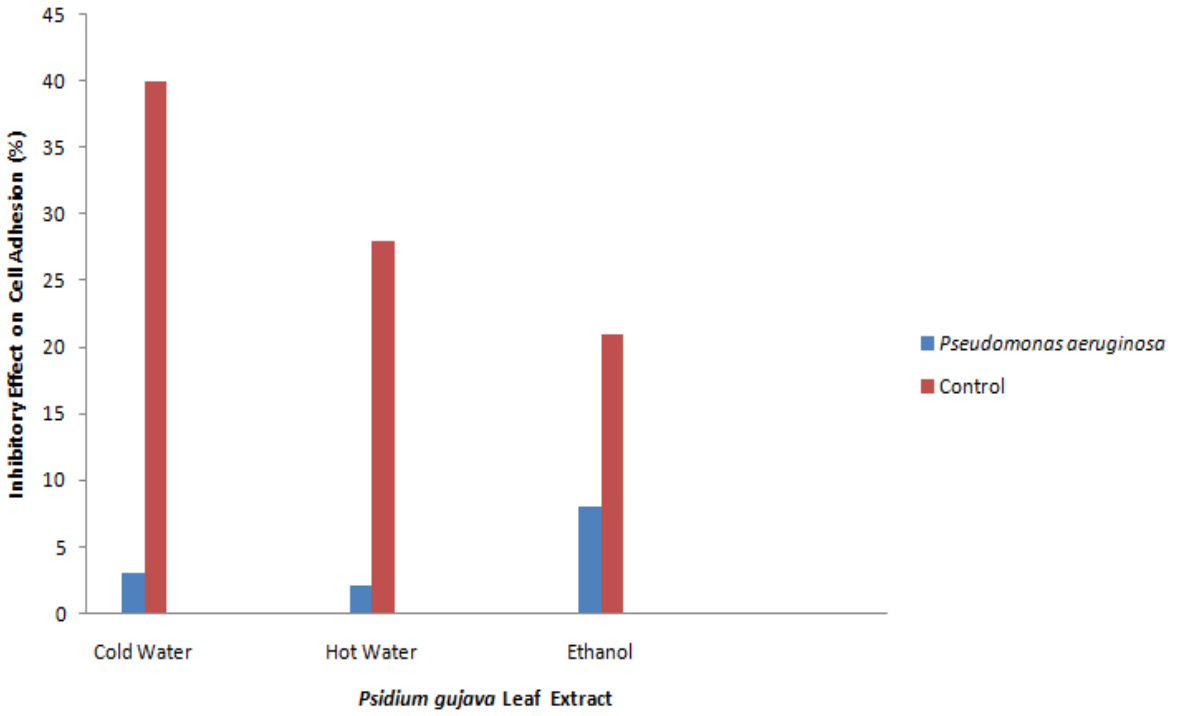

Figure 5. Inhibitory effect (%) of *Psidium gujava* extract on cell adhesion of *pseudomonas aeruginosa*

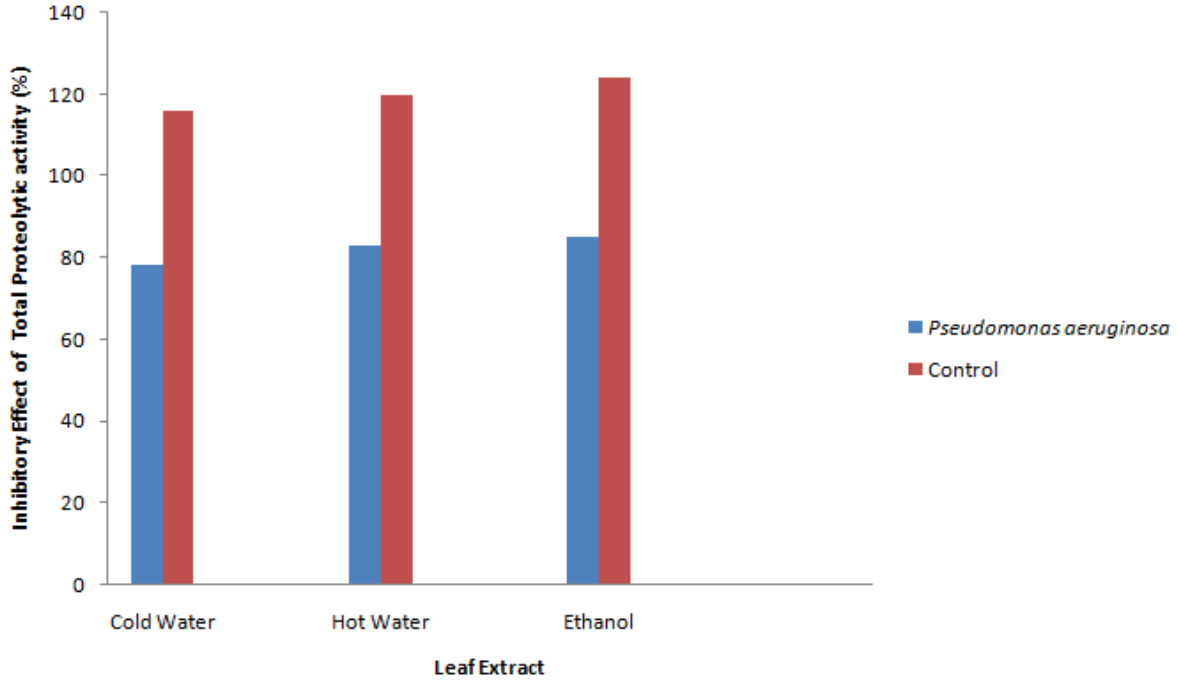

Figure 6. Inhibitory effect (%) of *Psidium gujava* extract on Proteolytic activity of *pseudomonas aeruginosa*

Table 2. Inhibitory effect (%) of plant extract against pyocyanin production of *Pseudomonas aeruginosa*

Plants	Extract	Inhibitory effect (%)
Psidiumgujava	Cold water	15.17±2.08
	Hot water	14.07±0.51
	Ethanol	8.50±0.10

4. DISCUSSION

The FT-IR spectrum was used to identify the functional groups of the active components present in the leave extract based on the peaks values in the region of Infra-Red radiation either directly or by inference. When the extracts were passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio. The associated changes in the spatial arrangement of the groups involved are reflected in the infrared spectrum as additional bands and added complexity. The results summarized in Table 1. From the spectral analysis for the extracts, the characteristic peak area ranges from 723.1 cm^{-1} to 3406.8 cm^{-1} for *Psidium gujava*, Results showed that the plant extract contain 16 (sixteen) distinctive functional groups including alcohol (OH), alkane (CH), amine (NH), aromatic (CH), α,β-unsaturated ketone (C=C), nitro compound (N-O), sulfonamide (S=O), secondary alcohol (C-O), sulfoxide (S=O), alkene (C=C), carboxylic (OH), Isothiocyanate (N=C=S), flouro compound (C-F), aliphatic ester (C-O), alkyne (CΞC) and thiocyanate (S-CΞN).These characteristic functional groupsreflected the biochemical compositions, especially the phenolic compounds, carboxylic acids, alcohols, carbohydrates, and proteins in the plant, responsible for several medicinal properties and biological activities.

From the results obtained in the present study, it could be concluded that the extracts may act as source of therapeutic agent. The richness of the samples–OH group enhances its ability for forming hydrogen bonding capacity and confirmed therefore, the higher potential of its antioxidant and antimicrobial activities (Diaz *et al.*, 2012).

The GC analysis for phytochemicals in the plant extract is presented in Figures 4. to 6. The phytochemicals present in leave extracts generally include proanthocyanin, lunamarin, quinine, epihedrine, anthocyanin, flavan, sapogenin, phenol, flavonones, naringenin, steroids, epicatechin, kaempferol, phytate, flavone, oxalate, catechin, resveratrol and tannin. The results for the various extracts on the quorum sensing mediated virulence factors are presented in figure 7 to 10.

The summary of findings of the study includes;

1) The plant extract contain 16 (sixteen) distinctive functional groups including alcohol (OH), alkane (CH), amine (NH), aromatic (CH), α,β-unsaturated ketone (C=C), nitro compound (N-O), sulfonamide (S=O), secondary alcohol (C-O), sulfoxide (S=O), alkene (C=C), carboxylic (OH), Isothiocyanate (N=C=S), flouro compound (C-F), aliphatic ester (C-O), alkyne (CΞC) and thiocyanate (S-CΞN).

2) The phytochemicals present in leave extracts generally include proanthocyanin, lunamarin, quinine, epihedrine, anthocyanin, flavan, sapogenin, phenol, flavonones, naringenin, steroids, epicatechin, kaempferol, phytate, flavone, oxalate, catechin, resveratrol and tannin
3) For twitching motility, ethanol and hot water extract of *Psidium gujava* showed the highest inhibition for *Pseudomonas aeruginosa*
4) For biofilm formation, both hot water and ethanolic extracts of *Psidium gujava* exerted the highest inhibition
5) In cell adhesion, hot water extract for *psidium gujava* showed the highest inhibition for *pseudomonas aeruginosa*
6) In protelytic activity, cold water extract showed the highest inhibition for *psidium gujava*, on *Pseudomonas aeruginosa*
7) In pyocyanin production, ethanol extract showed the highest inhibition for *Psidium gujava* on *Pseudomonas aeruginosa*

5. CONCLUSION

The continuous emergence of multidrug-resistant bacteria caused increased need of anti-pathogenic and anti-infective strategy to combat bacterial infections. Natural products provide alternative medicine for treating emerging bacterial infections without leading to antibiotic resistance. The presence of active compounds exhibiting anti-QS activity in the plant extracts maybe useful for the development of anti-infective drugs.

5. 1. Recommendation

Future study is necessary to unveil the detailed mechanism of the studied extract (isolating single compound from crude extract) and mode of actions with proteolytic activity, biofilm inhibition etc to confirm the realistic action on vivo research considering symbiotic,synergistic and antagonistic growth inhibition.

References

[1] Davey M.E. and O'Toole G.A. (2000). Microbial biofilms: from ecology to molecular genetics. *Microbiol .Mol. Biol. Rev.* 64, 847-867.

[2] Donlan R.M. (2002). Biofilms: Microbial life on surfaces. *Emerging Infect. Dis.* 8, 881-890.

[3] Fuqua C., Parsek M.R. and Greenberg E.P. (2001). Regulation of geneexpression by cell-to-cell communication: acyl-homoserinelactone quorum sensing. *Annu Rev Genet*, 35: 439-468.

[4] Fux C (2005). Survival strategies of infectious biofilms. *Trends Microbiol*. 13: 34-40

[5] George E.A. and Muir T.W. (2007). Molecular Mechanisms of agr Quorum Sensing in Virulent Staphylococcus aureusylococci. *Chem-BioChem.* 8(8): 847-55. doi: 10.1002/cbic.200700023

[6] Heukelekian H.and Heller A. (1940). Relation between food concentration and surface for bacterial growth. *J. Bacteriol.* 40, 547-558.

[7] Hibbing M.E. and Fuqua C. (2012). Inhibition and dispersal of Agrobacterium tumefaciens biofilms by a small diffusible Pseudomonas aeruginosaaeruginosaexoproduct(s). *Arch Microbiol*; 194(6): 391-403.

[8] Karthick R.N.S. and Vivek J.M. (2016). Anti-quorum sensing activities of medicinal plant extracts against quorumsensing mediated virulence factors of Pseudomonas aeruginosaaeruginosa. *Der Pharmacia Lettre*, 8 (8): 412-423

[9] Khan S. (2014). Isolation of Shigella species and their resistance patterns to a panel of fifteen antibiotics in mid and far western region of Nepal. *Asian Pacific J Tropical Dis.* 4: 30-34.

[10] Kumar J.S., Umesha S., Prasad K.S., Niranjana P. (2016). Detection of quorumsensing molecules and biofilm formation in Ralstoniasolanacearum. *Current Microbiol*, 72(3): 297-305.

[11] Muhsin J., Ufaq T., Tahir H. and Saadia A. (2015). Bacterial Biofilm: Its Composition, Formation and Role in Human Infections. *Research & Reviews: Journal of Microbiology and Biotechnology*, 18, 87–92.

[12] Rasko D.A., and Sperandio V. (2010). Anti-virulence strategies to combat bacteria-mediated disease. *Nature reviews Drug discovery*, 9(2): 117-28. doi: 10.1038/nrd3013

[13] Rutherford S. T.and Bassler B.L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring HarbPerspect Med.* 2. a012427.

[14] Schauder S. and Bassler B.L. (2003). The languages of bacteria. *Genes Dev* 15: 1468-80.

[15] Srey S., Jahid I.K. and Ha S.D. (2013). Biofilm formation in foodindustries: a food safety concern. *Food Control* 31, 572–585

[16] Zobell, C.E. (1943). The effect of solid surfaces upon bacterial activity. *J. Bacteriol.* 46, 39-56