



Effects the plants on quorum sensing and biofilm by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

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Abstract

In this study, used the ainetobacter and pseudomonas is called nosocomial infection and multidrug-resistant and causes many disease such as bacteremia, pneumonia, meningitis, urinary tract infections and wound infections. This study aimed to evaluate the effects antibiotics at minimum inhibitory concentration and sub-minimum inhibitory concentration on biofilm formation. The plant collection from the market except on collection from tree and worked extract plant and applied on bacteria as inhibition factors for bacteria, the first step to determine that's plants have anti-quorum sensing activity by used over lay assay which is component two layer of nutrient agar first layer is solid nutrient agar with volume 10ml and second layer is semi solid with volume 15ml mixing with serratia marscence worked pore with stable diameter put 100ul from crude extract of plants after 24h appear result to determinant of which of plant have anti-quorum sensing or not. Worked biofilm test and mixing extract of plant with bacteria after 24h show results which of plant can inhibit bacteria or not, add one step to sure that is crude extract not kill bacteria take from each concentration 100ul and culture on nutrient agar.

Keywords: Plants, bacteremia, pseudomonas

Introduction

Antibiotics are one of the most important weapons in fighting bacterial infections. It has greatly benefited the human's health since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses. This is not only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of poor communities (Hosseinzadeh S, Jafarikukhdan A, Hosseini A, & Armand R. (2015) ^[1]. The antimicrobial activity of a plant is highly related to secondary substances that are synthesized and produced by these plants. Secondary metabolites are substances of low molecular weight, which were not the products of the primary metabolic pathway of the producing organism. However, it was first thought to be of no advantage to the plant. Nowadays, it has been believed that they have vital functions (Kant RU, Pratibha, D, & Shoeb, A. (2010) ^[2]. Olea europaea preparations have been used widely in folk medicine in Mediterranean area. Olive leaf is one of the potent source of plant polyphenols having antioxidant, antimicrobial, and antiviral properties due to its rich phenolic content (Aytul K.K (2010) ^[3]. The large number of phenolic compounds present in olive leaves aroused the interest of researchers around the world. Also, the studies with animals and humans have reported beneficial health effects such as the capacity of antioxidant, anti-hypertensive, hipo-glicemiant, hypocholesterol, anti-inflammatory, and as co adjuvant in the treatment of obesity (Esmaeili-Mahani S. *et al.* 2010) ^[4, 26]. The discovery of antibiotics has always been a medical advance that has improved the prognosis of infections. However, resistance

to these products has evolved into a major health problem. Some diseases are even resistant to all antibiotics currently available on the market. A reduction in the use of antibiotics must be based on other approaches, namely the use of alternative treatments to antibiotics, such as phytotherapy. In the same objective our study focused on the research of the antibacterial power of Olea Europaea known for its various medicinal properties. "Phytotherapy" is a term that is used in the field of medicine in which, either herbs or their extracts are used as either health promotion agents or to treat diseases (Falzon CC, Balabanova A. Phytotherapy 2017) ^[5]. Although numerous studies have investigated the bactericidal efficacy of herbal extracts of neem and miswak; other natural products that have been reported to exhibit antibacterial properties include Saussurea costus (*S. costus*) and Melaleuca alternifolia (*M. alternifolia*) (Qiu J, Wang J, Luo H, Du X, Li H, Luo M, *et al.* 2011, Graziano TS, Calil CM, *et al.* 2016) ^[6, 7]. The *S. costus* (*India costus*), is a member of the Asteraceae family and is commonly used in countries such as Saudi Arabia, and India for different medical issues such as asthma, breast and hepatic cancer and thyroid diseases (Byambaragchaa M, de la Cruz J, Yang SH, Hwang SG, 2013, Mujammami M. 2020) ^[8, 9]. It is also known as costus, putchuk, or kuth. Likewise, *M. alternifolia*, also known as "tea tree oil" exhibits antimicrobial effects against microbes including *Staphylococcus aureus*, *S. mutans* and *Candida albicans* [Lam NS, Long X, Su XZ, Lu F. 2020, Tullio V, Roana J, Scalas D, Mandras N. 2019) ^[10, 11]. And promotes wound healing (Tullio V, Roana J, Scalas D, Mandras N. 2019) ^[11]. Turmeric extract and the essential oil of *Curcuma longa* inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. A study of chicks infected with the caecal parasite *Eimera maxima* demonstrated that diets supplemented with 1-percent turmeric resulted in a reduction in small intestinal lesion scores and improved

weight gain. Another animal study, in which guinea pigs were infected with either dermatophytes, pathogenic molds, or yeast, found that topically applied turmeric oil inhibited dermatophytes and pathogenic fungi, but neither curcumin nor turmeric oil affected the yeast isolates. Improvements in lesions were observed in the dermatophyte- and fungi-infected guinea pigs, and at seven days post-turmeric application the lesions disappeared. Curcumin has also been found to have moderate activity against *Plasmodium falciparum* and *Leishmania major* organisms (Hour T.C., Chen J., Huang C.Y., 2002) [12]. The Capsicum compounds generate metabolic and structural changes that can culminate in the inhibition or inactivation of microorganisms such as bacteria, fungi, and viruses, the mechanisms of action derived from the interactions of the bioactive compounds of CFs and the microorganisms are described. AMPs from CFs are peptides that act as a form of defense against bacterial attacks (Bacon K, Boyer R. *et al*, 2016) [13, 17]. Alteration of cell membrane potential and permeability, as well as membrane pores induction and cell aggregation, are the antibacterial mechanisms by which AMPs can penetrate the cell membrane and once inside, may interact with proteins and nucleic acids, establish a potential cell-killing mechanism (Afroz M, Akter S, Ahmed A, *et al*, 2020) [14]. In Gram-positive bacteria, AMPs seem to pass the cell wall with relative ease, while in Gram-negative bacteria, the AMPs can cross the cell wall by a charge-exchange mechanism, where cationic peptides compete with Ca^{2+} and Mg^{2+} ions bound to lipopolysaccharide, once they are attached to the outer membrane proteins (Malanovic N, Lohner K (2016) [15]. According to this, it has been proposed membrane models (barrel-stave pore, toroidal pore, and carpet model) associated with cationic AMPs-membrane interactions, membrane disruption, and membrane permeability. By combining both translocation and membrane affinity properties, AMPs can get into bacterial membranes and lead to the disruption of the cell. However, the antibacterial mechanisms of AMPs depend on factors such as peptide structure, the peptide: lipid ratio and the lipid membrane properties (Afroz M, Akter S, Ahmed A, *et al*, 2020) [14]. Otherwise, (Baenas N, Belović M, Ilic N, Moreno DA, García-Viguera C, 2019) [16]. reported that capsianosides can inhibit the growth and survival of Gram-positive bacteria due to their capacity of chelating calcium, which is used to provide an adequate ionic environment in cation-dependent membrane transport system and improves the biofilm architecture development. Conversely, the Gram-negative bacteria such as *S. enterica* and *E. coli* lack of cell wall requirement of calcium, which could mean the ineffectiveness of capsianosides against the formation of biofilms generated by this type of bacteria (Bacon K, Boyer R, Denbow C, O'Keefe S, Neilson A, Williams R, 2016) [13, 17]. Similarly, the antibacterial effect of capsaicin is related to the disruption of peptidoglycan structure due to lipid-lipid interactions providing liquidity in the cell wall (Akyuz L, Kaya M, Mujtaba M, *et al*, 2018) [18]. Also, the antibacterial effect of capsaicin depends on its concentration, and its mechanisms of action involve osmotic stress, destroying cell membrane structures, and inhibiting the expressions of the genes responsible for bacterial cell growth (Adaszek Ł, Gadowska D, Mazurek Ł, Łyp P, Madany J, Winiarczyk S, 2019) [19]. However, it has been reported that bacteria such as *Actinoplanes utahensis* NRLL 12,052, *Bacillus* species isolated from Korean kimchi, and *Streptomyces*

mobarraensis possess capsaicin-degradative enzymes (Adams CA, Zimmerman K, *et al*, 2020) [20]. The presence of these types of enzymes could be one of the reasons why some bacteria are not inhibited by capsaicin. Regarding phenolic compounds, it has been reported that ferulic and gallic acids lead to a change in hydrophobicity and a decrease of negative surface charge in cytoplasmic membrane of pathogenic bacteria; causing local rupture and pore formation with leakage of essential intracellular constituents (Bouarab-Chibane L, Forquet V, *et al*, 2019) [21]. This supposes the formation of pores in the cell membrane, generating structural damage and subsequent inhibition of microorganisms. Meanwhile, LAB use hydroxycinnamic acids as external electron acceptors, allowing cofactor recovery and gaining additional metabolic energy to tolerate the stressful conditions generated by phenolic compounds. This adaptation is one of the mechanisms by which LAB are not inhibited by these acids, which represents an advantage over other undesirable microorganisms (Filannino P, Di Cagno R, Gobetti M, 2018) [22]. Based on the above, the antibacterial effects of the compounds present in CFs are not always inhibitory and appear to be strain dependent. Suggesting that plants have evolved mechanisms to recognize bacterial AHLs as signature molecules and mount defensive responses. It seems likely that it is common for plants to interfere with the coordinated expression of virulence or other deleterious traits by either blocking or inappropriately stimulating QS (Cui, X., and R. Harling. 2005, Delalande, L., D. Faure, A. *et al*, 2005) [23, 24]. Indeed, transgenic plants that express bacterial AHLs have altered responses to pathogenic bacteria. In most but not all cases such plants apparently prematurely induce the expression of bacterial virulence traits that otherwise would be expressed only when cell densities are high at later stages of the infection process in plants (Fray, R. G. 2002, Ma'e, A., M. Montesano, V. Koiv, and E. T. Palva. 2001, Toth, I. K. *et al*, 2004) [25, 27]. Apparently, most plants mount a defense against bacterial pathogens only after they recognize AHLs directly, or they mount a defense indirectly after they recognize virulence factors that are expressed only upon AHL production in bacteria (Bauer, W. D., and J. B. Robinson. 2002, Savka, M. A., Y. Dessaux, P. Oger, and S. Rossbach. 2002) [28, 29]. It therefore could be expected that plants defend themselves against deleterious bacteria by producing compounds that interfere with the QS system of bacteria. There has been much interest in developing new strategies for plant disease control based on altering the quorum-sensing process in plants (Cui, X., and R. Harling. 2005, Fray, R. G. 2002, Savka, M. A., Y. Dessaux, P. Oger, and S. Rossbach. 2002) [23, 25, 29]. While *R. trichocalyx*, *A. maritimum*, *Ruta graveolens*, *B. napus*, and *P. sativum* leaf surface extracts caused slight inhibition of QS. *Moringa oleifera* leaves, fruits, roots, and seeds are sources of biologically important phytochemicals and may be used as a consummate nutraceutical molecule, because of its innumerable desirable biological properties. There is an extended interest in using natural antioxidant compounds, as the consumer's pressure on food industry augments, to avoid chemical preservatives, due to the increasing evidence implies that synthetic antioxidant produce toxicity. Many gram negative bacteria, including *Erwinia carotovora*, *Enterobacter agglomerans*, *Chromobacterium violaceum* and *Pseudomonas aeruginosa* use N-acyl homoserine lactones (AHLs) signal molecules to

monitor their own population density. At a threshold population density, AHLs interact with cellular receptors and trigger the expression of a set of target genes, including virulence, antibiotic production, biofilm formation, bioluminescence, mobility and swarming, in a process called “quorum sensing” (QS) (Manefield, M., Rasmussen, T.B., *et al*, 2002) [30]. The discovery of the QS system and its critical role in bacteria virulence and survival has revealed a new target—a novel way to attack and attenuate bacterial pathogenicity. The major advantage of this novel strategy for anti-infective therapy is that it circumvents the problem of antibiotic resistance, which is intimately connected to the use of conventional antibacterial agents, as is specifically interferes with the expression of pathogenic traits rather than to impede growth of the bacteria. To date, the only known QS blockers of non-bacterial origin are halogenated furanones from *Delisea pulchra* (Manefield, M., Rasmussen, T.B., *et al*, 2002) [30]. Anti-QS activity has also been shown in a number of Florida medicinal plants (Adonizio, A.L., Downum, K., Bennett, B.C., Mathee, K., 2006) [31]. And a few other higher plants (Bosgelmez-Tinaz, G., Ulusoy, S., Ugur, A., Ceylan, O., 2007, Rasmussen, T.B., Bjarnsholt, T. *et al*, 2005) [32, 33]. The efficacy and toxicity of previous reported QS blockers (i.e. halogenated furanones) have been important concerns; therefore attention has been focused on identification of such QS blockers from natural and non-toxic sources for the development of novel nonantibiotic drugs for treating bacterial diseases in humans as well as in

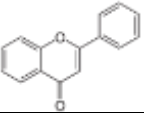
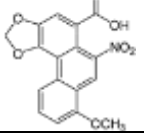
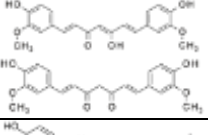
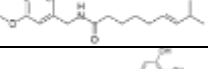
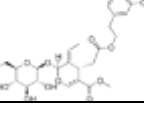
other animals. Therefore, the present work was done to evaluate oxidative DNA damage protecting activity, antioxidant potential and anti-QS activity of *M. oleifera* leaf, fruit and seed.

Material and method

Alcohol extraction preparation:

Ruta or rue, *Moringa oleifera*, *Saussurea costus*, *Curcuma longa*, *Capsicum* and *Olea* leaves were collected from the market of Mosul city, where fresh *Olea* leaves were obtained from the tree, washed with tap water and dried for 24-28 days at room temperature. Follow researcher (grand *et al*, 1988) [38] A modified basic method for research (verpoorte *et al*, 1982) extraction steps were used for the extraction of the bioactive metabolite compounds. To obtain the plants extract the method of. Was followed briefly, fifty grams from each plant were weighted and with 250 ml ethyl acetate as solvent (1:5) mixing by electric blender and filtration by medical sterile gauze. Cell-free supernatant was harvested and centrifuged at 4000 rpm for 15 min. The solvent layer was harvested and then evaporated in a rotary evaporator in order to obtain the crude extracts, To this, 100% of dimethyl sulfoxide (DMSO) was added to constitute a 50 mg/mL stock (w/v) from the crude extract and this stock confirmed for their anti-quorum sensing against *Serratia marcescens* by using agar overlay assay and anti-biofilm against *A. baumannii* isolate 23, *P. aeruginosa* isolate 12 by using 96 well microtiter plate

Table: 1

NO.	Scientific name of plant	Structural formula	Active component
1	Ruta ,rue		Flavonoid
2	<i>Moringa oleifera</i>	R-N=C=S	isothiocyanates
3	<i>Saussurea costus</i>		Aristolochic Acid
4	<i>Curcuma longa</i>		Curcumin
5	<i>Capsicum</i>		capsicium
6	<i>Olea leaf</i>		oleuropein

Screening and Evaluation of Quorum Sensing Inhibition (QSI)

Qualitative Agar Diffusion Assay

Screening for Quorum Sensing Inhibition (QSI) assay was carried out on all prepared extracted plants. Synthesis of red colour pigment (prodigiosin“2-methyl-3-pentyl-6-methoxyprodigiosin”) is a regulated behavior in quorum sensing in *Serratia marcescens*. Quorum sensing inhibition using *Serratia marcescens* as a reporter strain was performed on the extracted plants (screening for QSI). Five hundred microliters of overnight grown culture of *Serratia marcescens* which was obtained from department of

biology/ University of Mosul was inoculated in to 20 mL of nutrient broth medium and incubated for 18 h at 30 °C., Agar overlay assay was done using nutrient broth soft agar (0.8% agar, 150 mL) and maintained at 45 °C, 10 mL of culture (OD600nm = 0.7) were then added before plating the supernatants on the media. The wells were prepared in the plate by using sterile cork borer (6 mm in diameter) and 30µL of plants extracted were placed in each well. Antibacterial activity was revealed through a zone of clearance at the center, while QSI was observed at the periphery as opaque zone of inhibition with intact bacteria. Fresh nutrient broth medium served as the negative control.

Assay of biofilm production with crude plants extract

Overnight culture of *A. baumannii* isolate 23 and *P. aeruginosa* isolate 12, which were grown in BHI broth at 37°C for 18 h, were then diluted using sterile BHI broth to the 0.5 McFarland standard, and 150µL of the diluted isolates was transferred to wells of a sterile 96-well flat-bottomed polystyrene plate. The crude extract of the dried plants was re-dissolved into 100% of dimethyl sulfoxide (DMSO) 50 mg/mL stock (w/v) using half fold serial dilution. The concentrations for Ruta, Saussurea costus, Curcuma longa, and Olea leaf used were 0.2, 0.2, 0.2 and 0.15 mg/ml, 50µL of each plant crude extract concentration were added to wells that contained diluted *A. baumannii* isolate 23 and *P. aeruginosa* isolate 12. The seventh well was added with only 50µL of sterile BHI broth with 5% DMSO. The plates were incubated for 24h at 37°C. The intact biofilm remaining in each well was stained with a 1% crystal violet (CV) (Sigma) solution at 37°C for 30 min, and proceed by following the same steps in the section; the test

was done in triplicate. Each data point was composed of three independent samples that were averaged.

Colony Count using Spread Plate Method

The cultures of *A. baumannii* isolate 23 and *P. aeruginosa* isolate 12 that had been treated with plants crude extract concentrations in crystal violet assay were diluted using serial dilution to 5 folds dilution. 100µL of the fifth fold dilution was spread on Nutrient agar (NA, Oxoid, UK). The spread plates were incubated for 24h at 37°C. The colonies formed were counted and colony forming numbers were determined. The test was done in triplicate. Each data point was composed of three independent samples that were averaged.

The result

Appear results that capsicum and moringa oleifera do not have quorum sensing inhibition forward serretia marscence and other plants gives quorum sensing inhibition characteristics as such in figure (1).

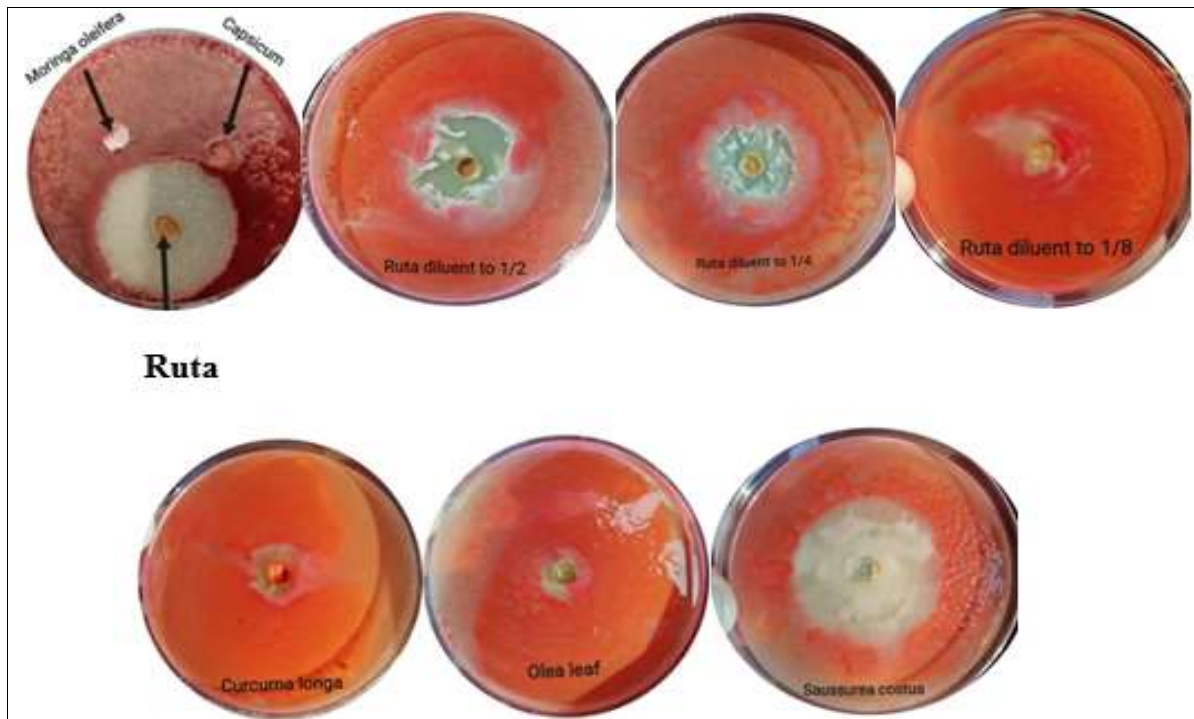
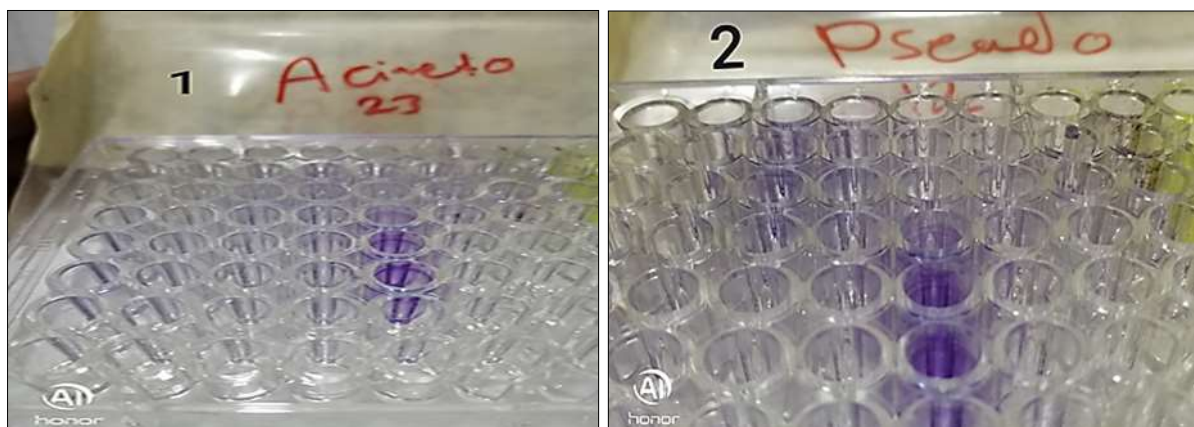


Fig 1



1. This picture explain that effect plants on acinetobacter

2. This picture explain that effect plant on pseudomonas

Fig 2

The figures explain effects the plant on bacteria used in study.

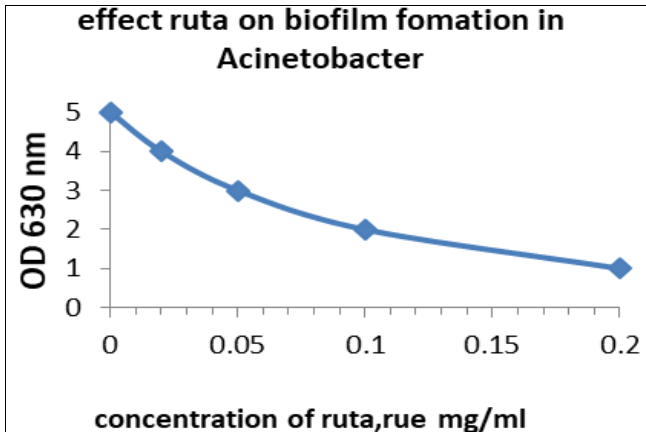


Fig 3

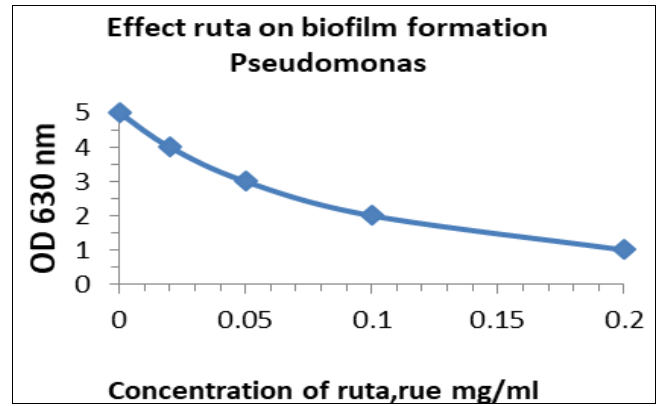


Fig 7

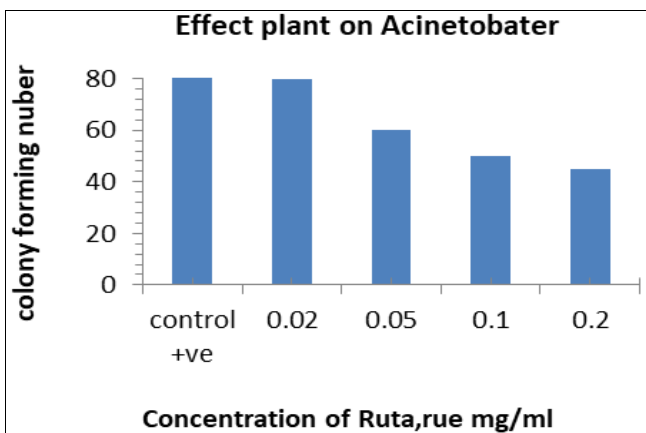


Fig 4

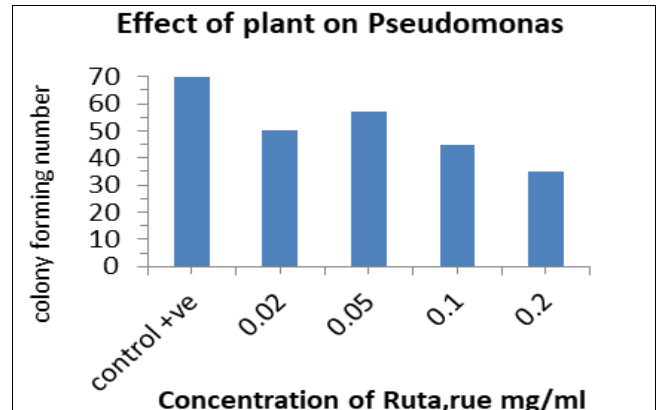


Fig 8

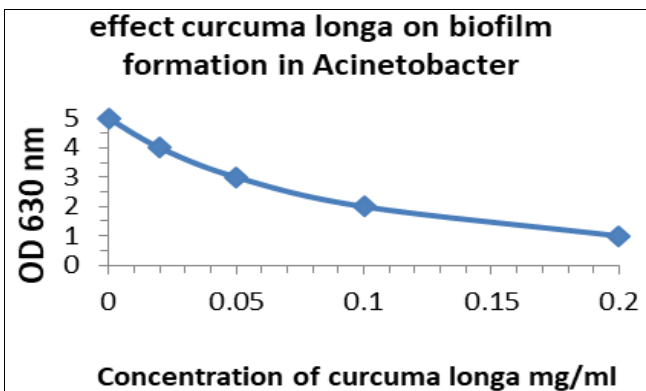


Fig 5

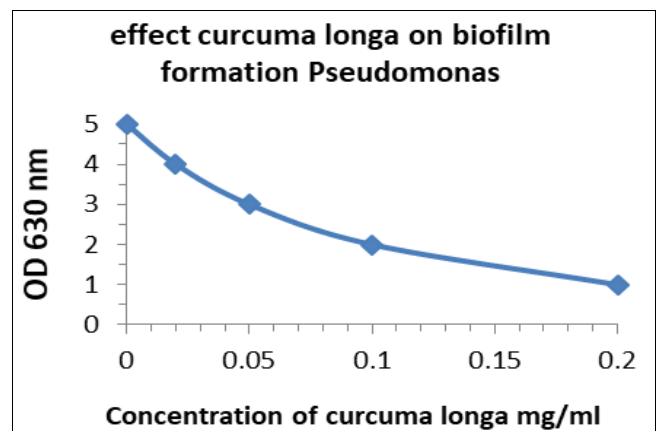


Fig 9

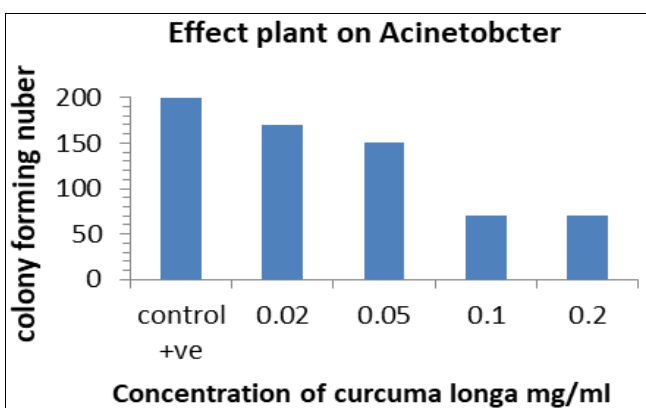


Fig 6

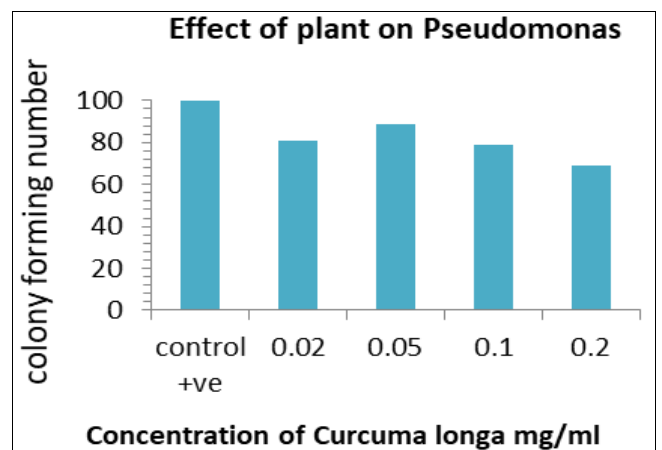


Fig 10

Discussion

Appear result that olea leaf, ruta, curcuma longa and saussurea cost that have anti quorum sensing activity forward serretia, the capsicum and moringa oleifera not have anti quorum sensing forward serretia hence application crud extract on bacteria pathogen (acinetobacter and pseudomonas). And appear effect on biofilm. Because of emergence multi druge resistant bacteria to antibiotics hence, this study was aimed to investigate the effect of crude of plant on biofilm formation capacity. More previous studies that indicate most natural source as plant that have anti quorum sensing because of this plant contain compound same as AHL-dependent quorum sensing in bacteria opposite chemical compound such as antibiotics that is bacteria have many different ways of antibiotics resistant and because of few used for plants as drug not bacteria plant resistant. The chemical structure and bioactivity mechanisms of plant-derived molecules that act as inhibitors of AHL-dependent QS in bacteria. Characterized of compound as: (i) highly stable compounds that are resistant to degradation by host metabolism, a factor that allows for their transport to the site of action; (ii) low molecular weight molecules that can penetrate bacterial cells and interact with target proteins; and (iii) high-specificity phytochemicals that directly interact with QS activators. Because the whole plant extracts typically contain more than one anti-QS compound and individual constituent phytochemicals are found to be less efficacious than the whole extract (Joshi, C.; Patel, P.; Kothari, V, 2019) ^[34]. a closer look at natural QS inhibitors bioactivity mechanisms may be helpful not only to reconstruct plant-derived molecular mixtures (Deryabin, D.G.; Tolmacheva, A.A, 2015) ^[35]. But also for the development of new artificial compositions which exhibit a super-additive effect on quorum sensing. In our opinion, compositions of plant-derived molecules exhibiting a different mode of action are promising, which potentially allow a multiple block on QS-related processes in bacteria. This novel combinatorial approach to QS-disruption will be experimentally verified in our subsequent studies. Fundamentally, the presented data show the phylogenetically developed variety of anti-infectious strategies relevant in plant health and performed by QS inhibition in phytopathogens (Ansari, F.; Ahmad, I, 2018) ^[36]. That provides new insight into the interactions between plant and bacterial species. In this context, increased understanding of plant-derived molecule modes of action on QS in bacteria should lead to the development of effective QS-disrupting strategies.

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