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Antimicrobial, Anti-Biofilm, Anti-Quorum Sensing, Antifungal and Synergistic Effects of Some Medicinal Plants Extracts

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Abstract

A biofilm is the assembly of microbial cells that are entrapped in an extracellular polymeric substance matrix. Biofilm members are able to talk, communicate, exchange virulence factor with each other according to quorum sensing. Biofilm formation by bacteria is a major factor in pathogenesis and in survival. The objective is to screen plants for their In Vitro antibacterial, anti-biofilm, and anti-quorum sensing. Extracts from the dried aerial parts of plant were prepared using 70% ethanol. All extracts were screened for their antibacterial and antifungal activity, determination the MIC₅₀, anti-biofilm activity, anti-quorum sensing, and synergistic effect. The diameter of inhibition zone resulted from the plant extracts against the bacteria ranged from 0 to 22 mm, while against fungal was 0 to 52 mm. The MIC₅₀ ranged from 0.3-81.3 mg/ml against *Pseudomonas aeruginosa*, 0.02-20.8 mg/ml against fungal. *Pelargonium hortorum* exhibited the highest antimicrobial activity. *Agave sisalana* has the highest anti-biofilm activity, with 87.5% reduction. Screening for anti-quorum sensing assay showed that *P. hortorum* and *Punica granatum* has the best inhibition of motility of the *P. aeruginosa*. *Artemisia absinthium* and *Hibiscus sabdariffa* has the greatest reduction of the pyocyanin pigment in *P. aeruginosa*. *Momordica charantia* showed the greatest reduction in activity of lasA protease (77.1%). *Cinnamomum zeylanicum* had the best synergistic effect. Most of the studied plant extracts showed the ability to inhibit antibacterial antifungal activities anti-biofilm and anti-quorum sensing. Further studies are required to determine the active components and the LD₅₀ of these extracts.

Keywords:

Anti-biofilm,
Antibiotic resistance,
Quorum sensing.

1. Introduction:

The problem of microbial resistance to antibiotics appeared as soon as the mass-production of penicillin was started in 1945 (Shaughnessy, 2007), and has now become a major concern in medicine throughout the world. Bacterial resistance not only can occur to a single class of antibiotic, but

also to multiple classes of antibiotics. The resistance of microorganisms to antibiotics can be categorized in two types: innate resistance, which means that some microorganisms are inherently resistant to many antibiotics; Acquired resistance, which can arise by a number of diverse

mechanisms such as mutation and horizontal gene transfer (Tenover, 2006).

Despite the fact that bacteria are unicellular organisms, they often show group behavior. For example, in living biofilms, individual cells at different locations in the biofilm may have different activities. This led to the proposal that biofilm communities may represent an evolutionary step between unicellular non-specialized organisms and multicellular organisms that possess specialized cells (McLean, Whiteley, Stickler, & Fuqua, 1997).

For optimum performance, bacteria have to monitor their own population density. This can be achieved by quorum sensing. This process relies on the production of a low-molecular-mass signal molecule (often called "autoinducer" or quorumon) (Fuqua, Winans, & Greenberg, 1996).

Biofilm is a community of cells attached to either or to a biotic or abiotic surface enclosed in a complex exopolymeric substance (EPS) (Flemming, Wingender, Griegbe, & Mayer, 2000). According to a 2011 review, biofilms in drinking water systems can serve as a significant environmental reservoir for pathogenic microorganisms (Wiener-Well et al., 2011).

Biofilms are enclosed within an exopolymer matrix that can restrict the diffusion of substances and bind antimicrobials. This will provide effective resistance for biofilm cells against large molecules such as antimicrobial proteins lysozyme and complement (Ishida et al., 1998; Shigeta et al., 1997). In fact, it is estimated that approximately 80% of all bacteria in the environment exist in biofilm communities, and more than 65% of human microbial infections involve biofilms (Donlan, 2002).

Microbial biofilms cause a variety of problems in different medical, environmental, and industrial settings, ranging from the fouling of ship hulls and the blocking of industrial piping to the colonization of artificial medical implants (Costerton, Stewart, & Greenberg, 1999; Kumar & Anand, 1998), and possibly

many other problems. In this work, 23 plant extracts were investigated for their antimicrobial, anti-biofilm, anti-quorum sensing, and synergistic effects.

2. Materials and Methods:

2.1. Chemicals, Culture Media and antibiotics:

Eight type of media were used for carrying out this study. Brain Heart Infusion Agar (BHIA), Brain Heart Infusion Broth (BHIB) with 1% glucose, Nutrient broth (NB), Mueller Hinton Agar (MH), Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB), and Dulbecco's Modified Eagle Medium DMEM without sodium bicarbonate supplemented with L-glutamine. Swarm plates were prepared using 0.5% Bacto agar, 0.5% peptone, 0.2% yeast extract and 1.0% glucose per 100 ml distilled as media for motility test. Ethanol 70% and Dimethyl sulfoxide (DMSO) were for extraction process and dissolving extracts. 2% crystal violet and 95% methanol for biofilm assay. 0.2 M HCl, chloroform, Na₂PO₄ (pH 4.5) were used.

2.2. Plant Materials Collection:

Four medicinal plants were purchased from Gaza's local markets, and nineteen medicinal plants were collected from different agricultural areas. The tested plants are listed in Table 1.

2.3. Microorganism:

Two isolates of *P. aeruginosa*, one *S. aureus*, and one *Candida albicans* were used. Organism were isolated from clinical specimens submitted to the microbiology laboratories of Al Shifa Hospital, and were maintained on Brain Heart Infusion Agar medium slant at 2-8 C°.

2.4. Preparation of plant extract:

A modification of previously described procedures (Raaman, 2006), was used to prepare the ethanolic extract of the plant as follows. 30 g dried plant materials were mixed with 300 ml ethanol 70% (v/v) for 8 hours in a soxhlet extractor. The extract was then allowed to evaporate in an oven at 37 C° for at least three days. The dried extract was stored in a refrigerator until used.

Table 1 List of medicinal plants tested for their anti-microbial, anti-biofilm and anti-quorum sensing activity

No	Binomial Name	Family*	Vernacular	Part Tested
1	<i>Cinnamomum zeylanicum</i>	Lauraceae	قرفه	Cinnamon sticks
2	<i>Marrubium vulgare</i>	Labiatae	سموه	Leaves
3	<i>Tamarix aphylla</i>	Tamaricaceae	أثله	Leaves
4	<i>Cuminum cyminum</i>	Apiaceae	كمون	Grounded seeds
5	<i>Pelargonium hortorum</i>	Geraniaceae	صقصقي	leaves, flowers and stalks

6	<i>Lawsonia inermis</i>	Lythraceae	حناء	Leaves
7	<i>Salvia officinalis</i>	Lamiaceae	مرميه	Leaves and Flowers
8	<i>Triticum aestivum</i>	Poaceae	نخاله القمح	Straw
9	<i>Artemisia absinthium</i>	Asteraceae	الشيح أو الشيبيا	Leaves
10	<i>Pelargonium graveolens</i>	Geraniaceae	العطره	Leaves
11	<i>Foeniculum vulgare</i>	Apiaceae	شومر	Grounded seeds
12	<i>Hibiscus sabdariffa</i>	Malvaceae	كركديه	Leaves
13	<i>Solenostemon scutellarioides</i>	Lamiaceae	سجاده	Leaves
14	<i>Rosmarinus officinalis</i>	Lamiaceae	اكليل الجبل	Leaves
15	<i>Urtica dioica</i>	Urticaceae	قريص	Leaves
16	<i>Thymus vulgaris</i>	Lamiaceae	زعتري	Leaves
17	<i>Punica granatum</i>	Lythraceae	رمان	peel
18	<i>Agave sisalana</i>	Agavaceae	صبره	Leaves
19	<i>Mentha longifolia</i>	Lamiaceae	نعناع	Leaves
20	<i>Portulaca oleracea</i>	Portulacaceae	بقله	Leaves
21	<i>Matricaria recutita</i>	Asteraceae	بابونج	Leaves, flowers
22	<i>Erythrina crista-galli</i>	Fabaceae	مرجانة	flowers
23	<i>Momordica charantia</i>	Cucurbitaceae	القرع المر	Leaves

*References: (USDA, 2016)

2.5. Extracts stock solutions:

Stock solutions of crude ethanol extracts in dimethyl sulfoxide (DMSO) were prepared, filter-sterilized (0.45 µm) and stored at 4 °C (Rios and Recio, 2005). Concentrations depended in the solubility of the extract in DMSO or DW. The concentrations ranged from 125-333.3 mg/ml as stock solutions.

2.6. Determination of anti-microbial and anti-fungi activity of plant extracts:

2.6.1. Agar well diffusion method:

MHA plates were inoculated with a cotton swab moistened with a McFarland standardized test organism. Holes of 6 mm diameter were punched and were filled with 20 µL of plant extract. The petri dishes were incubated at 37 °C for 24h. Diameters of inhibition growth zone were measured. Culture of fungi were grown on SD agar. The inoculum was prepared in saline solution. Its turbidity was adjusted in accordance with McFarland scale (0.5) (Lahlah, Meziani, & Maza, 2012; Magaldi et al., 2004).

2.6.2. Minimum inhibitory concentration assay:

This assay was adapted from (Mazzola, Jozala, Novaes, Moriel, & Penna, 2009), with a modification in the type of media and the additional use of 2,3,5-triphenyltetrazolium chloride (TTC). In a 96 wells micro-titer plate, 100 µL of nutrient broth medium for bacteria and DMAM medium for fungi was distributed to each well. 100 µL of each extract was added to the

first well. All wells were shaken, 100 µL was withdrawn from the first well and transferred to the next well and serial dilution was performed. Then 50 µL of bacterial suspension were added to each well. Plates were covered and incubated at 37 °C for 24h. 20 µL of 0.1% tetrazolium chloride (TTC) was added to each well, incubated for 15 minutes. Plates were read and MIC was determined as recording the concentration of the well that preceded the red color appearance.

2.6.3. Assessment of biofilm formation:

2.6.3.1. Tube method:

A 0.1 mL of bacterial culture (obtained by adjusting turbidity to 0.5 McFarland standards) was transferred to glass test tubes containing 10 mL TSB tubes which were incubated at 37 °C for 72 hours. The medium was then removed and the tubes were washed with distilled water, air dried and biofilm formation were assayed by crystal violet (Pour et al., 2011). All tests were carried out in triplicates.

2.6.3.2. Microtiter plate method:

Three wells of sterile 96-microtiter U-bottomed plate were filled with 200 µL of bacterial suspension (dilution 1:100 with fresh medium). Negative control contained broth only. After 24 hrs incubation at 37°C, wells were washed three times with 250 µL of DW. After 15 min, plates were stained for 5 min with 0.2 ml of 2% crystal violet per well. Excess stain was removed and rinsed off by placing the plates under running tap water. The plates were air-dried. The adherent cells

were re-solubilized with 160 µl of 3% (V/V) glacial acetic acid per well (Christensen et al., 1985). The optical density (OD) of each was measured at 570 nm. Results interpreted according to the followings criteria; OD <0.005 (-), OD 0.500<1.500 (+), OD >1.500 (++) (Alcaráz, Satorres, Lucero, & Centorbi, 2003).

2.6.4. Biofilm inhibition assay:

This method described by (Musleh & Jebur, 2014; Tang, Pui, Wong, Ahmad, & Radu, 2012), with a modification in type of media used. *P. aeruginosa* from fresh agar were inoculated in BHI broth with 1% glucose and incubated for overnight at 37 °C in stationary condition, dilution 1 in 100 with fresh medium. 96 well U bottom tissue culture plates were filled with 0.2 ml suspension bacteria and 0.2ml plant extract for serial dilution, plates were incubated for overnight at 37°C. After incubation period well was removed by tapping the plate, washed with DW to remove planktonic bacteria. Adherent organisms in plates were stained with crystal violet (0.1% W/V) for 20 min, excess stain was rinsed off by DW and plates were kept for drying, then resuspended each well for 200 95%(v/v) methanol, transferred to 96 well-flat bottom plates. Optical density of stained adherent bacteria were determined with a micro-ELISA reader at wavelength of 620nm.

2.6.5. The *in vitro* determination of the anti-quorum sensing activities of plant extracts against *P. aeruginosa*:

2.6.5.1. Swarming assay:

This assay was adapted from (Willis & Kinscherf, 1999). Swarm plates were prepared using 0.5% Bacto agar, 0.5% peptone, 0.2% yeast extract and 1.0% glucose per 100 ml DW. 250 µL of plant extract were added to 5 ml of semisolid agar, gently mixed and poured immediately onto the surface of a 10 ml of pre-warmed agar plate as an overlay. Five µl of a standardized inoculum is placed on the center of the plate. Similar volume was placed on the center of a control plate (overlay agar without extract) and the plates were incubated for overnight at 37°C.

2.6.5.2. Pyocyanin assay:

A volume of 7.5 milliliters of the supernatant, after centrifuge at 10000 rpm for 10 min from stationary phase culture (~16 hours old) of *P. aeruginosa* in nutrient broth were mixed with 4.5 ml of chloroform. The mixture is vortexed for 10 seconds and then centrifuged at 10,000 rpm for 10 min. Three milliliters of the lower blue layer, containing pyocyanin and chloroform were collected and extracted with 1.5 ml of

0.2 M HCl, vortexed and centrifuged for 2 min at 10,000 rpm. The absorbance of 2 ml from the pink top layer was measured at 520 nm (Essar, Eberly, Hadero, & Crawford, 1990).

2.6.5.3. Las A Staphylolytic assay:

A 30-ml volume of an overnight *S. aureus* culture was boiled for 10 min and then centrifuged for 10 min at 10,000 g. The resulting pellet was re-suspended in 10 mM Na₂PO₄ (pH 4.5) to an OD₆₀₀ of approximately 0.8. Various plant extracts were tested for their ability to inhibit Las A staphylolytic ability of *P. aeruginosa*. A 50-µl of the sub-lethal concentration of each extract was added to each tube culture containing 1 ml media inoculated with *P. aeruginosa*. A tube with no extract served as a control for the assay.

A 100-µl aliquot of *P. aeruginosa* overnight growth supernatant was added to 900 µl of *S. aureus* suspension. The OD₆₀₀ was determined after 0, 5, 10, 15, 20, 30, 45, and 60 min. for both the tests and control tubes (A. Adonizio, Kong, & Mathee, 2008; A. L. Adonizio, 2008; Kessler, Safrin, Olson, & Ohman, 1993).

2.6.6. Synergistic activity of plant extract with different antibiotic against gram-positive bacteria:

Standardized inoculum of the test microorganism was inoculated on the surface of Muller-Hinton agar plate. Commercial antibiotic discs were placed onto the surface of each inoculated plate. 10 µL of plant extract were dispensed over each disc. Control discs of antibiotic alone and extract alone were also added for comparison. The plates were incubated at 37 °C for 24h. The diameters of clear zones were measured and compared with that of the antibiotic alone and the extract alone (Betoni, Mantovani, Barbosa, Di Stasi, & Fernandes Junior, 2006).

3. Results:

3.1. Antibacterial activity and MIC of crude extracts against two different species of *P. aeruginosa*:

The ethanolic crude extract of all plant showed varying degrees of antibacterial activity against *P. aeruginosa* ranged between 8-22 mm inhibition zone and 0.3-83.3 mg/ml planktonic growth inhibition activity (PMIC₅₀). The largest zone of inhibition was observed for *H. sabdariffa* with inhibition zone diameter of 22 mm for *P. aeruginosa*.

The highest PMIC₅₀ in mg/ml against *P. aeruginosa* was obtained for *P. hortorum*, *H. sabdariffa* and *P. granatum* 0.3 mg/ml, 0.6 mg/ml, 1.3 mg/ml

respectively. The lowest PMIC₅₀ against *P. aeruginosa* was recorded for *F. vulgare*.

3.2. Screening and determination of MIC plant crude extract for *Candida albicans*:

Anticandida activity was evident for all extracts except three (*T. aphylla*, *M. vulgare*, and *C. cyminum*) which failed to inhibit *C. albicans* growth. The extract of *C. zeylanicum* presented the highest antifungal activity with inhibition zone diameter of 52 mm.

3.3. Effect of crude extracts on Biofilm formation of *P. aeruginosa* and *in vitro*:

Eleven of the 23 extracts reduced *P. aeruginosa* biofilm formation (Figure 1) by ≥ 50%. Ethanol extract of *A. sisalana* reduced *P. aeruginosa* biofilm formation by 87.5%. As much as 85.7% and 69.2% by *P. hortorum* and *L. inermis* respectively. A reduction of 60% was obtained by the extract of *C. zeylanicum* and *T. vulgaris*.



Figure 1 Anti-biofilm activity of plant extracts using the crystal violet assay

3.4. Screening for anti-quorum sensing assay:

3.4.1. Swarming method:

Extracts of *P. Hortorum* (0.02 mg/ml), *P. granatum* (0.04 mg/ml) (Figure 3.2), *T. Aphylla* (0.06 mg/ml), *H. sabdariffa* (0.08 mg/ml), *P. graveolens* (0.2 mg/ml), and *R. officinalis* (0.5 mg/ml) exhibited the highest reduction in the swimming motility respectively of *P. aeruginosa* isolate 1.

The highest effect in motility inhibition was for *P. granatum* (0.005 mg/ml), followed by *P. hortorum* (0.02 mg/ml), followed by *P. graveolens* (0.4 mg/ml) against *P. aeruginosa* isolate 2.

Table 2 Effects of crude extracts on biofilm formation and biofilm inhibition of *P. aeruginosa* isolate 2

Plant	Sub-PMIC in mg/mL	(MBIC ₅₀) in mg/mL	%Reduction
<i>C. zeylanicum</i>	3.9	0.1	60
<i>M. vulgare</i>	20.8	0.6	53.3
<i>T. aphylla</i>	4.4	0.5	53.3
<i>C. cyminum</i>	20.8	0.1	46.1
<i>P. hortorum</i>	0.1	0.005	85.7
<i>L. inermis</i>	3.9	0.1	69.2
<i>S. officinalis</i>	9.9	0.05	40

<i>T. aestivum</i>	3.1	1.5	20
<i>A. absinthium</i>	8.9	2.2	25
<i>P. graveolens</i>	1.9	*	
<i>F. vulgare</i>	41.6	*	
<i>H. sabdariffa</i>	0.3	0.01	84.6
<i>S. scutellarioides</i>	5.2	*	
<i>R. officinalis</i>	8.4	*	
<i>U. dioica</i>	10.4	*	
<i>T. vulgaris</i>	10.4	0.6	60
<i>P. granatum</i>	0.6	0.005	61.5
<i>A. sisalana</i>	10.4	0.3	87.5
<i>M. longifolia</i>	5.2	0.3	81.2
<i>P. oleracea</i>	10.4	1.3	66.6
<i>M. recutita L.</i>	10.4	*	
<i>E. crista-galli</i>	8.9	*	
<i>M. charantia</i>	10.4	*	

* No effect; no activity is detected within the concentration range tested.

3.4.2. Pyocyanin production quantitative assay:

Out of 23 extracts screened for anti-quorum sensing activity, only two (*P. graveolens* and *S. scutellarioides*) failed to exhibit anti-QS activity. Unexpectedly, *P. graveolens* extract enhanced green pyocyanin pigment production compared to the control. *H. sabdariffa* and *A. absinthium* were the most effective showing the highest anti-QS activity against *P. aeruginosa* (47.8% inhibition) followed by *C. zeylanicum*, *S. officinalis*, *U. dioica* and *T. aestivum* (44.3% inhibition).



Figure 2 Anti-quorum sensing activities against *Pseudomonas aeruginosa* swarming motility. (A) *Punica granatum* (0.04 mg/ml) (B) Negative control (Table 2)

3.4.3. LasA protease activity:

Plant extracts showed variable abilities in reducing the lasA protease activity of *P. aeruginosa*. *M. charantia* showed the greatest reduction in activity (77.1%), followed by *M. longifolia* (66.8%), *A. sisalana* (65.2%), *T. vulgaris* (63.3%), *T. aphylla* (63%), The least reduction in activity was observed in plant *H. sabdariffa*, *T. aestivum* *L. inermis*. In fact, some plant

extract has increased the activity such as *C. cyminum* and *S. officinalis*.

3.5. Antibacterial and synergistic activity of different plant extract with different antimicrobials against *S. aureus*:

The overall effect of *L. inermis* as measured by the average zone of inhibition on all tested isolates was the highest. *C. zeylanicum* had synergistic effect with most of the tested antimicrobials, followed by *L. inermis*, and by *P. hortorum* (Table 3).

Table 3 Synergistic activity of different plant extract with different antimicrobials against *S. aureus*

S. aureus isolate	Antimicrobials tested with		
	<i>Lawsonia inermis</i>	<i>Cinnamomum zeylanicum</i>	<i>Pelargonium hortorum</i>
1	-	-	-
2	CN	CN, P	-
3	-	P, CN	P
4	E, CLR, P,	-	-
5	CLR, FOX	CLR, CN, FOX	E, TM, NOR, OX, FOX, P
6	TM, CN	CN, P	P
7	FOX, CLR, CN	P, FOX, CLR, CN	FOX, CLR, CN,
8	C, CN	C, CN	-
9	E, CLR	E, CLR, P	-
10	-	E, CLR	-
11	CLR, E, TM	-	-
12	FOX, P	FOX, P	-
13	CPT	CLR, P, CPT	CPT
14	CN	P, CN	-
15	CLR	CLR	-

CN: Getamycin, P: Penicillin G, E: Erythromycin, CLR: Clarithromycin, FOX: Cefoxitin, TM: Timethoprim, NOR: Norfloxacin, OX: Oxacillin, CPT: Ceftaroline.

4. Discussion:

Our study provided evidence that extract of *C. zeylanicum* possesses significant antibacterial and antifungal activity against *P. aeruginosa*, *C. albicans*, and multi drug resistant *S. aureus*. This activity may be due to the major components for *C. zeylanicum* which include cinnamaldehyde and eugenol (Budri et al., 2015). Keskin & Toroglu (2011), obtained similar results. This study showed that *H. sabdariffa* had a high antibacterial activity against *P. aeruginosa*. Similar result obtained by Elmanama, Alyazji, & Abu-Gheneima (2011). This high activity of *H. sabdariffa* against bacteria may be attributed to the fact that it contains active components (alkaloids, flavonoids, phenolics, and bitterpenoids) (Islam et al., 2008; Lee et al., 2007).

The activity of the extracts on biofilm formation inhibition was tested using the crystal violet method, which is widely used by microbiologists. A low concentration of the extract may be required to prevent biofilm first attachment, while higher concentration to disrupt preformed biofilm (Stewart, 2002). In this study *L. inermis* showed remarkable potential as a possible anti-biofilm agent, as it was active upon *P. aeruginosa* in the planktonic state, no articles on anti-biofilm activity of crude extract *L. inermis* was published, however it failed to reduce QS in most assays. Leaves of the henna are strikingly most effective against the spectrum of bacterial isolates tested. This may be attributed to the added presence of chlorophyll, henna leaves contain up to 5% by weight of the compound (2-hydroxy-1,4-naphthoquinone), chemically active constituents such as quinines (O. A. Habbal, Al-Jabri, El-Hag, Al-Mahrooqi, & Al-Hashmi, 2005; Maekawa et al., 2007).

M. longifolia extract showed strong anti-biofilm activity against *P. aeruginosa* compared to the untreated control. These medicinal plants could be used to manage *Pseudomonas* pathogenesis and hinder its dissemination.

To the best of our knowledge, no reports are available regarding the anti-biofilm activity of *M. longifolia* extract against *P. aeruginosa*. Menthol is the most important component responsible for most of the pharmacological effects of the *M. longifolia*. It is a waxy, crystalline substance, clear or white in color, which is solid at room temperature and melts at slightly high temperatures (Gulluce et al., 2007; Mimica-Dukić, Božin, Soković, Mihajlović, & Matavulj, 2003). There are different mechanisms of quorum sensing inhibition by active component from plant extract, either by inhibition the production of the virulence factor pyocyanin, inhibition two QS system effective compound, and inhibition QS system receptors (O'Loughlin et al., 2013). The result of this study showed that the crude extract of *Tamarix aphylla* had a high QS inhibitory effect against *P. aeruginosa* motility. Tamaricaceae have many chemical compounds; for example, 62 different chemical compounds were identified in *T. boveana* (Saidana et al., 2008). Composition of polyphenols (phenolic acids and flavonoids) of *T. aphylla* which is a source of bioactive compounds has been studied, and it was found that leaves contain higher amount of polyphenols than the stems (Mahfoudhi, Prencipe, Mighri, & Pellati, 2014). QS can be measured both qualitatively and

quantitatively. Pyocyanin assay is a quantitative method which measure the pigment reduction. When treated with 0.1, 0.2 and 0.3 mg/ml of trans-cinnamaldehyde, the production of pyocyanin was significantly reduced as the production are 65.54%, 63.03% and 57.94%, respectively (Chang et al., 2014). In our study, the inhibition effect of Cinnamomum crud extract significantly reduced the production of pyocyanin to 44.3%, at concentration 3.9 mg/ml. The plant extracts were examined for their ability to interfere with the QS-dependent production of the *P. aeruginosa* virulence factors LasA. The assay of staphylolytic activity that we used for this purpose is sensitive and specific (Gustin, Kessler, & Ohman, 1996).

L. inermis, *P. hortorum* and, *C. zeylanicum* extracts have antibacterial activities and exhibited synergistic effects when used with commercial antimicrobials. Therefore, our data clearly demonstrate the importance of plant extracts in the control of resistant bacteria, which are becoming a threat to human health. Aside for being active against resistant isolates, they exhibited synergistic effects with some of the available antibiotics. This also should be investigated further for possible utilization in the drug industry.

5. Conclusions and Recommendations:

Locally available plants demonstrated considerable antibacterial, anticandidal, anti-biofilm, anti-quorum sensing activities. Further studies on purified components instead of the crude extract to determine the biologically active component for each plant. Stability studies and LD₅₀ are also recommended. Palestine has a unique flora and was not fully explored for their biologically active components, which may represent a cheap resource for future medicine. There are very few reports are available on the anti-biofilm and anti QS activities. Hence, we recommend further studies aiming at finding the anti-biofilm and anti QS activities of different plant extracts. The crude extract of 23 plant showed variable antibacterial and antifungal activity against *P. aeruginosa* and *C. albicans*. The highest antimicrobial against *P. aeruginosa* was observed for *P. hortorum*, *H. sabdariffa*, and *P. granatum*. The extract of *C. zeylanicum* presented the highest antifungal activity with inhibition zone diameter of 52 mm, but the highest growth inhibition activity PMIC50 against *C. albicans* was for the plant extract of *P. graveolens*, *P. hortorum*, *C. cyminum*, and *C.*

zeylanicum respectively. The extract of *A. sisalana*, *P. hortorum*, and *H. sabdariffa* constitute an interesting source for anti-biofilm agents in the development of new strategies to treat infections caused by *P. aeruginosa* biofilm. Plant extracts showed variable abilities in inhibited quorum sensing through inhibiting the different virulence factors activity of *P. aeruginosa*. *P. granatum*, and *P. hortorum* exhibited the highest reduction in the swimming motility of *P. aeruginosa* two isolates. *H. sabdariffa*, and *A. absinthium* were the most effective showing the highest inhibition of quorum sensing activity in the form of reduced pyocyanin production by *P. aeruginosa*. Plant extracts showed variable abilities in reducing the lasA protease activity of *P. aeruginosa*. However, *M. charantia* showed the greatest reduction in lasA activity. The crude extract of three plant (*C. zeylanicum*, *L. inermis*, and *P. hortorum*) showed various degrees of antibacterial activity against *S. aureus*. *P. hortorum* showed the most inhibition activity against *S. aureus* isolates. They also exhibited variable synergistic activities when tested with commercially available antibiotics to inhibit the growth of *S. aureus*; *C. zeylanicum* had synergistic effect with most of the tested antimicrobials, followed by *L. inermis*, and by *P. hortorum*.

In light of the above conclusions and based on the results of this study, the following conclusions are suggested. Further studies to confirm the findings of this study using other methods and experimenting on purified components instead of the crude extract to determine the biologically active component for each plant. Stability studies for plants extracts will be of value before any further testing. Further investigations in animal models to determine plant extract toxicity (LD50) and to human cells. It is recommended that plant extracts with bioactivities against biofilm and QS be further studied to check their efficacies *In Vivo*.

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التأثيرات المضادة للميكروبات، للأغشية الحيوية، لإستشعار النصاب، للفطريات والتأثير التآزري لبعض مستخلصات النباتات الطبية

كلمات مفتاحية:

التأثير المضاد للأغشية الحيوية،
مقاومة المضادات الحيوية،
النصاب الاستشعاري.

الببوفيلم هو تجمع الخلايا الميكروبية المحصورة في مادة البوليمر النسيجية الغشائية. ينتج الببوفيلم بواسطة آليات معقدة من خلال جينات خاصة، جميع البكتيريا المشاركة في تكوين الببوفيلم لها القدرة على التحدث، التواصل، وتبادل عوامل الضراوة فيما بينها بواسطة النصاب الاستشعاري. يعد الببوفيلم من أهم عوامل بقاء البكتيريا وقدرتها على التسبب بالأمراض. الهدف الرئيسي لهذه الدراسة هو فحص قدرة النباتات للنشاط المضاد للميكروبات، للببوفيلم، لاستشعار النصاب داخل المختبر. جُهزت المستخلصات من خلال تجفيف النبتة في الهواء وتم استخلاصها بواسطة جهاز السكوكسلت واستخدام الايثانول بتركيز 70% كمذيب. وجميع المستخلصات تم فحصها لدراسة النشاط الميكروبي والفطري وتحديد MIC50، المضادة للببوفيلم النصاب الاستشعاري والتأثير التآزري. إن قطر منطقة التثبيط من المستخلصات ضد البكتيريا تتراوح ما بين 0-22 ملجم/مل و ضد الخميرة تتراوح بين 0-52 ملجم/مل حيث إن MIC50 ضد الزانفة الزنجارية يتراوح بين 0.3-81.3 ملجم/مل. يعد مستخلص نبتة الصقلى أكثر المستخلصات فعالية ضد البكتيريا بينما مستخلص الصبرة يمتلك أعلى تأثير ضد تكوين الببوفيلم حيث يثبط بنسبة 87.5%. تم تحديد التأثير ضد النصاب الاستشعاري بواسطة الفحص ضد الحركة حيث أن مستخلص الصقلى وقشر الرمان كان أفضل مثبط للحركة الزانفة الزنجارية. وباستخدام القياس الكمي للبيوسيانسن فان مستخلص الشبج والكرديه تمتلكان أفضل مثبط ضد الزانفة الزنجارية. مستخلص نبتة الأمبلايا كانت لها أعلى نسبة تثبيط للانزيم (Laz A Protease) بنسبة 77%. مستخلص القرقة يمتلك أفضل تأثير تآزري المكورة العنقودية الذهبية. وجد أن أغلب المستخلصات تمتلك نتائج واعدة لما لها من عدة خصائص ضد التأثير التآزري، الفطري، الأغشية الحيوية، والنصاب الاستشعاري. لذلك نوصي بإجراء العديد من الدراسات لتحديد المركبات الفعالة LD50 لهذه المستخلصات.