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EVALUATING THE EFFECT OF *Achillea Fragrantissima* ESSENTIAL OIL ON BACTERIAL BIOFILM AND ITS MODE OF ACTION

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ABSTRACT

The aim of the present study was to evaluate the antibacterial and the antibiofilm activity of essential oil (EO) of *Achillea Fragrantissima* and its mode of action.

Minimum Inhibitory Concentration (MIC) was performed in normal 96-well microtitre plates using a twofold dilution series, while, Minimum Biofilm Inhibitory Concentration (MBIC) assays were performed using a biofilm inoculator with a 96-well plate with peg lids.

The results showed that *Achillea Fragrantissima* EO was able to overcome the resistance of all studied bacterial isolates with MIC values in the range of 0.25-1 mg/ml, while, the MBIC values were in the range of 0.50-2 mg/ml.

The results showed that there were elevated leakage of potassium ions and release of cell materials in all tested bacterial strains treated with *Achillea Fragrantissima* EO indicating damage in the cell membrane. Additionally, it is believed that *Achillea Fragrantissima*

EO was able to inhibit first step in biofilm formation (initial adherence) through manipulating the cell membrane of *Staphylococcus epidermidis* (ATCC 35984).

Keywords: antibacterial, antibiofilm, *Achillea Fragrantissima* , mode of action, essential oil

1. INTRODUCTION

Antibiotic resistance occurs when it loses its ability to inhibit bacterial growth. Thus resistant bacteria grow and multiply in the presence of antibiotics **[1]**, which could be contributed significantly to the irresponsible use of antibiotics, or to the short period of their use or to the in adequate dosages **[2]**.

A biofilm is a community of microbes attached to the surface and surrounded by a matrix of extracellular polymeric substance. The matrix is made up of macromolecules including poly-saccharides, DNA, proteins and lipids **[3, 4]**, and it provides the protection to the microbes against antibiotics, immune system and the surrounding environmental conditions. Bacteria in a biofilm become 100 to 1,000 times more resistant to antibiotics than their planktonic cells counterpart **[5]**.

Various mechanisms play a role in the biofilm resistance including matrix blocking the biofilm penetration by antibiotics, gene transfer among the microbes, slow growth rate of the microbes in the biofilm, and altered metabolism **[6]**.

Biofilm growing bacterial could be the cause of severe chronic infections which are extremely difficult to be treated such as cystic fibrosis, periodontitis, and urinary tract infections **[7]**. Biofilms form on different surfaces including medical implants like catheters, heart valve and artificial and the only way for cure is implant replacement which costs billions of dollars annually **[7]**.

Essential oils (EOs) are secondary metabolites synthesized by aromatic plants, despite of being nonessential for the growth of the plants but they give the plants distinctive properties like the smell and the taste in addition to their defense role against bacteria, viruses, fungi and pests **[8, 9]**.

Because of the antimicrobial, antioxidant, fungicide, and antitumor activities of EO's, they have been used for thousands of years as food additives, fragrance, and in traditional medicine **[9]**.

EO's can be extracted by different methods, however; steam distillation is the most common method **[10]**, their main constituents are including terpenes, terpenoids, and phenylpropanoids **[8]**.

The interest in essential oils and other extracts of plants as sources of natural products has been increased to overcome the increase in bacterial resistance to antibiotics due to their different antimicrobial activities **[11]**.

Achillea fragrantissima is a medicinal plant that has been used in traditional medicine for the treatment of many diseases including gastroenteritis, hypoglycemia, common cold, parasitic and urinary tract infections **[12]**.

The aim of the present study was to evaluate the antibacterial and the antibiofilm activity of EO of *Achillea Fragrantissima* and its mode of action

2. MATERIALS AND METHODS

2.1. Essential oil of *Achillea Fragrantissima*

The chemical composition of *Achillea Fragrantissima* was previously published by our research group **[13]**. Briefly, fresh aerial parts of *Achillea Fragrantissima* was collected, from Mutah, Alkarak, South 0f Jordan, then, chopped and subjected to hydro-distillation using a Clevenger-type apparatus. EO components were analyzed chemically using gas chromatography–mass spectrometry (Agilent (Palo Alto, USA) 6890N gas chromatograph). The identification of EO components was based on a computer search using the library of the mass spectral data and the comparison of the calculated Kovats retention index (KRI) with the available authentic standards and the literature data.

Forty six components accounting for 100% of EO were identified. The major identified compounds were *trans*-Sabinene hydrate acetate 30.09 %, Iso-Ascaridole 16%, α-Terpinene 14.31%, p-Cymene 7.1%, cis-Carvone oxide 6.08 %, Terpinen-4-ol 2.75%, cis-Pulegol 2.58%, cis-Rose oxide 2.31%,1-Terpineol 1.93%, Z-β-Ocimene 1.9 %, trans-Verbenol 1.88% and trans-Piperitol 1.52%. Oxygenated monoterpene 70.22%, monoterpene hydrocarbon 26.95%, and sesquiterpene hydrocarbon 1.04% were the major components of EO.

2.2. Maintenance and Preparation of Cultures

Six bacterial isolates were used for evaluating the effect of *Achillea Fragrantissima* EO on the bacterial

biofilm, *Staphylococcus epidermidis* (ATCC 35984), Methicillin-susceptible *Staphylococcus aureus* (MSSA) (ATCC 25923), Methicillin-resistant *Staphylococcus* aureus (MRSA) (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), and the non-biofilm forming strain *Staphylococcus epidermidis* (ATCC12228)**.** Cultures were stored on Mueller-Hinton agar (MHB, Merck, Germany) (Oxoid, Hampshire, UK) at 2-4°C and subcultured every 3-4 weeks or whenever required.

2.3. Minimum Inhibitory Concentration (MIC)

MIC was determined using 96 well broth microdilutions according to the method described by Rachid *et al*. **[14]**. *Achillea Fragrantissima* EO stock solutions were prepared by dissolving the EOs in dimethyl sulfoxide (DMSO) (Carlo Erba, France). The stock solutions of *Achillea Fragrantissima* EO in DMSO were diluted in Mueller-Hinton broth (MHB, Merck, Germany) to give EO concentrations of 16000 µg/ml. Then, two fold serial dilutions of *Achillea Fragrantissima* EO in Mueller-Hinton broth (MHB, Merck, Germany), were carried out in microtitre plates to give EO concentrations of 8000, 4000, 2000, 1000, 500, 250, and 125 µg/ml.

Bacterial cells were grown overnight to mid-log phase by inoculating Mueller-Hinton broth (100 ml) and incubating at 37°C until OD_{600} reached approximately 0.6, then, diluted to 1 X 10^6 cfu/ml and seeded (100 µl) to the wells containing *Achillea Fragrantissima* EO, mixed and incubated at 37°C for 24 hrs aerobically. The MIC was taken as minimal concentration of *Achillea Fragrantissima* EO that inhibited visible growth of the strain. Determination of MIC was carried out in triplicate using three independent experiments.

2.4. Minimum bactericidal concentration (MBC)

To determine the MBC values, a volume of 30 µl from each well, which didn't show an apparent growth as confirmed by MIC determination, was taken and plated on Mueller-Hinton agar. The plates were incubated at 37 °C for 48 hrs. The MBC was defined as the lowest EO concentration that can reduce and kill more than 99.9% of the initial inoculum **[15]**.

2.5. Minimum Biofilm Inhibitory Concentration (MBIC) Assay

Biofilm susceptibility assays were performed using MBIC (Minimum Biofilm Inhibitory Concentration) (Innovotech, Inc., Edmonton, AB, Canda), in a biofilm inoculator with a 96-well plate **(Figure 1)** according to the method reported by Ceri *et al* **[16]**.

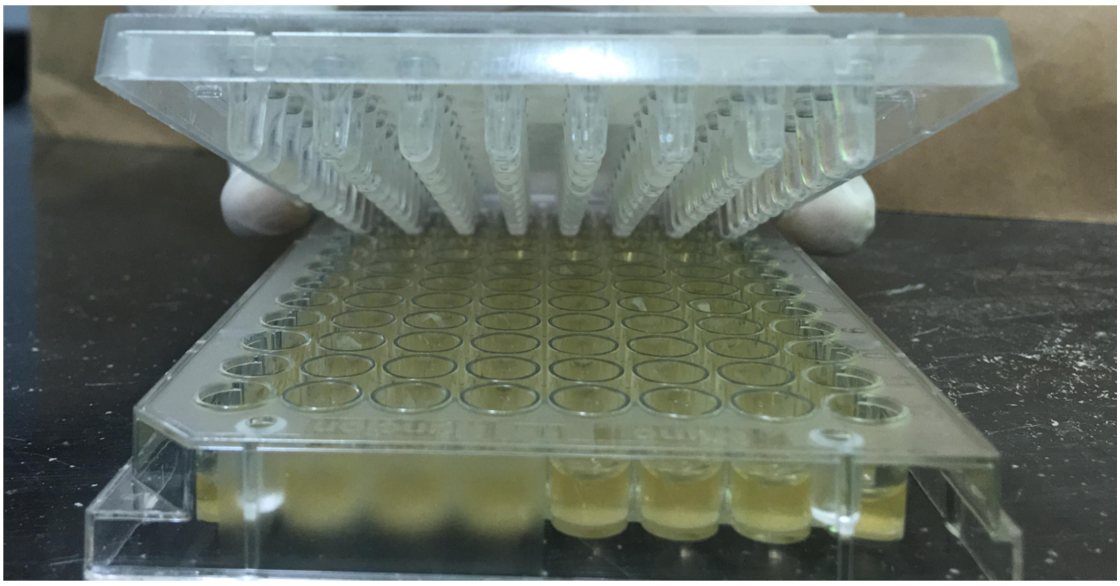

Figure 1: A peg lids biofilm inoculator with a 96-well plate (Innovotech, Inc., Edmonton, AB, Canada)

Bacterial isolates were cultured overnight in Mueller-Hinton agar (MHB, Merck, Germany), diluted to give a final concentration of 1 X 10^6 cfu/ml, then, 150 µl of inoculums was added to each one of the 96-wells of MBIC biofilm inoculators and the peg lid was then fitted on plates. After 24 hrs incubation at 37^0C, biofilms were formed on pegs. Peg lids were rinsed three times in phosphate-buffered saline (PBS) (Sigma Aldrich) to remove the non-adherent cells, then, transferred to a new 96-well plate containing serially diluted EO. The microtiter plate was incubated at 37^0C for 24 hrs. After the incubation, the peg lids were rinsed three times with PBS and placed in a fresh 96-well plate containing 100 ml of Mueller-Hinton broth (recovery plate). The bacteria were removed from the peg lids by sonicating the plates for 5 min at maximum speed with a Decon F51 006 sonicator. The peg lids were discarded and replaced with standard lids. The OD_{650} was determined before and after incubation at 37^0C for 6

hrs. Biofilm susceptibility assays were carried out in three independent experiments in triplicate for each strain. OD_{650} of 0.05 was considered as if the biofilm is absent. MBIC value was read as EO concentration that inhibited visible bacterial growth and confirmed by the non-increase in the OD_{650} reading compared to the initial one. A shift in susceptibility of more than two doubling dilutions in either direction was considered a significant change.

2.6. Minimum Biofilm Eradication Concentration (MBEC)

The values of MBEC were determine by plating 30 µl from each well of the 96-well plate which did not show an apparent growth as confirmed by MBIC value on Mueller-Hinton agar. The plates were incubated at 37 °C for 48 hrs. The MBEC was defined as the lowest EO concentration at which no bacterial growth can be detected on the Mueller-Hinton agar plates.

2.7. Leakage of Potassium Ions

The leakage of potassium ions (K^+) into the bacterial suspension was estimated using a Kalium/Potassium kit (Quantofix, Macherey-Nagel GmbH & Co. KG, Duren, Germany). *S. epidermidis* (ATCC 35984), MRSA (ATCC 43300), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922) were exposed to the EO at MIC value in sterile peptone water (0.1 g/100 ml). The extracellular K^+ concentration was estimated at 0, 30, 60, 90, 120, and 240 minutes. A culture flask without *Achillea Fragrantissima* EO was used as a control.. Results were reported as the amount of free K^+ ions (mg/l) in the bacterial suspension at each time interval.

2.8. Cell Membrane Integrity (Release of cellular material)

EOs at the MIC concentration were added to 2 ml of each one of the following bacterial strains, *Staphylococcus epidermidis* (ATCC12228), MSSA (ATCC 25923), *E. coli* (ATCC 25922) (10^7 cfu/ml) in sterilized peptone water (0.1 g/100 ml), and then, incubated at 37 ″C. After 0, 30, 60, 90, 120, 180 and 240 minutes of treatment, the cells were collected and centrifuged at 3000 rpm. OD_{260} reading of the supernatant was taken using a spectrophotometer. A tube without bacteria in sterilized peptone water was used as a control **[17]**.

2.9. Adherence of Bacterial Cells to Polystyrene

Initial adherence of *Staphylococcus epidermidis* (ATCC 35984) to

polystyrene was determined by allowing the bacteria to grown overnight in 10 ml TSB (17 gm Tryptone, 3 gm Soy, 5 gm NaCl, 2.5 gm dipotassium phosphate, 2.5 gm glucose dissolved in deionized distilled H_2O, pH was adjusted to 7.3 up to 1 liter volume) at 37°C and then diluted 1: 100 in fresh Mueller-Hinton broth containing EO of *Achillea Fragrantissima* at the required concentration. A quantity of 5 ml of the bacterial suspensions were poured onto Petri dishes and incubated for 30 min at 37°C. The plates were washed five times using 5 ml PBS, air dried and stained for 1 min with 0.4% crystal violet. The number of the adhered cells per cm^2 was counted in 20 view fields microscopically (CETI 60243T UK). The tested concentrations of EO were 1/10 of MIC, 1/2 MIC, and the full concentration. The assay of *Achillea Fragrantissima* EO concentration was done in triplicate and its adherence in EO treated cells was compared with untreated controls. Assays were performed three times on different days and the same result was obtained for each occasion.

Statistical analysis

All experiments were done in triplicate. The obtained results are expressed as mean values with the standard error. The statistical analyses were performed using Student's t-test to compare the controls and treated samples at a significance level of 5%.

3. RESULTS

3.1 MIC and MBC results

MIC and MBC results of *Achillea Fragrantissima* EO are shown in **Table 1**. Regarding MIC results, the values were in the range of 0.25-1% mg/ml. The most susceptible isolates were *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) with MIC value of 0.25% mg/ml. While, the most resistant isolates were MSSA (ATCC 25923) and MRSA (ATCC 43300) with MIC value of 1% mg/ml.

The MBC values were in the range of 1-2% v/v. The most resistant isolates were MRSA (ATCC 43300) and MSSA (ATCC 25923) with MBC value of 2% mg/ml. The values were higher for MBC than MIC for all the studied isolates.

3.2 MBIC and MBEC for the bacterial isolates

Table 2 shows the results of MBIC and MBEC of *Achillea Fragrantissima* EO. The MBIC values were in the range of 0.50-2% mg/ml. The most susceptible isolate was *Staphylococcus epidermidis*

(ATCC12228) with MBIC value of 0.50% mg/ml. While, the most resistant isolates were MRSA (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus epidermidis* (ATCC 35984) with MBIC value of 2% mg/ml.

The MBEC values were in the range of 1-8% mg/ml. The most susceptible isolate was *Staphylococcus epidermidis* (ATCC12228) with MBEC of 1 mg/ml, while, the most resistant are Methicillin-resistant *Staphylococcus* aureus (MRSA) (ATCC 43300) and *Pseudomonas aeruginosa* (ATCC 27853) with MBEC of 8% mg/ml. The values are higher for MBEC compared to MBIC for all studied isolates.

3.3 Leakage of K^+ Ion

The results of cell membrane permeability based on leakage of K^+ ions are shown in **Figure 2**.

Four bacterial isolates were studied: *Staphylococcus epidermidis* (ATCC 35984), MRSA (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). *Achillea Fragrantissima* EO was added to the studied bacterial isolates at the MIC concentration, a sharp increase in K^+ ions leakage was observed as shown in **Figure 2**. All studied bacterial isolates treated with *Achillea Fragrantissima* EO showed a progressive increase and direct correlation between K^+ ions leakage and incubation time as shown in **Figure 2**. The extracellular K^+ ions concentration is increased progressively from 50 µM to about 300 µM in all studied bacterial isolates.

3.4 Cell Membrane integrity

The integrity of cell membrane was also evaluated by estimating the release of the intracellular compounds especially DNA and RNA extracellulary as shown in **Figure 3**. When *Staphylococcus epidermidis* (ATCC 35984), MRSA (ATCC 43300) and *Pseudomonas aeruginosa* (ATCC 27853) were treated at MIC concentration with *Achillea Fragrantissima* EO, there was a continual increase in OD_{260} reading over the incubation time (Figure 3). There was a progressive increase in release of the intracellular compounds according to time of exposure in all studied bacterial isolates.

3.5 Adherence of Bacterial Cells to Polystyrene

The effects of *Achillea Fragrantissima* EO on the initial adhesion of *Staphylococcus epidermidis* (ATCC 35984) are shown in **Figure 4**. OD_{600}

reading was used to measure the planktonic growth, while, OD_{490} reading was used to measure biofilm growth. The studied EO concentrations were 1/10 of MIC, 1/2 MIC, and full MIC concentration. The addition of Adding *Achillea Fragrantissima* EO to polystyrene Petri dishes containing a suspension culture of the *Staphylococcus epidermidis* (ATCC 35984) isolate resulted in a reduction of the number of individual cells adhering to the polystyrene surface when there was increase in the concentration of EO after 30 minutes incubation period **(Figure 4)**.

Table 1: MIC and MBC of *Achillea Fragrantissima* (mg/ml) for the studied bacterial isolates

Isolate Number	Isolate name	MIC mg/ml	MBC mg/ml
1	***Staphylococcus epidermidis* (ATCC 35984)**	**0.50**	**1**
2	**Methicillin-susceptible *Staphylococcus aureus* (MSSA) (ATCC 25**	**1.00**	**2**
3	**Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 433**	**1.00**	**2**
4	***Pseudomonas aeruginosa* (ATCC 27853)**	**0.25**	**1**
5	***Escherichia coli* (ATCC 25922)**	**0.25**	**1**
6	***Staphylococcus epidermidis* (ATCC12228)**	**0.50**	**1**

Table 2: MBIC and MBEC of *Achillea Fragrantissima* EO (mg/ml) for the studied bacterial isolates

Isolate Number	Isolate name	MBIC mg/ml	MBEC mg/ml
1	***Staphylococcus epidermidis* (ATCC 35984)**	**2**	**4**
2	**Methicillin-susceptible *Staphylococcus aureus* (MSSA) (ATCC 25923)**	**1**	**2**
3	**Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300)**	**2**	**8**
4	***Pseudomonas aeruginosa* (ATCC 27853)**	**2**	**8**
5	***Escherichia coli* (ATCC 25922)**	**1**	**2**
6	***Staphylococcus epidermidis* (ATCC12228)**	**0.50**	**1**

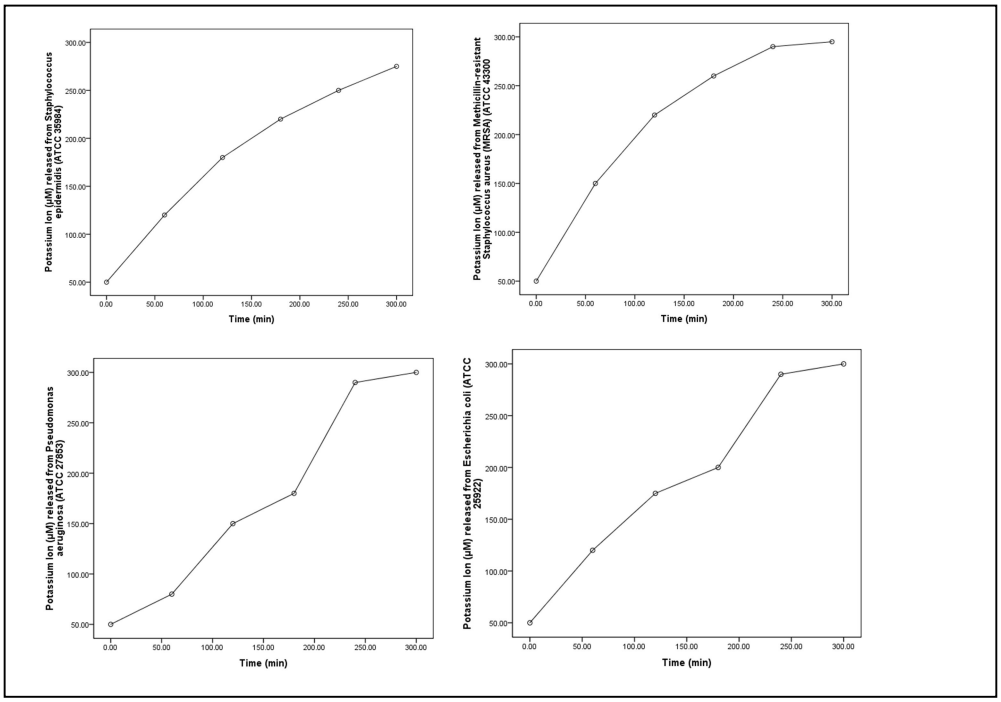

Figure 2: The effect of *Achillea Fragrantissima* EO on K^+ ions release of *Staphylococcus epidermidis* (ATCC 35984), MRSA (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922

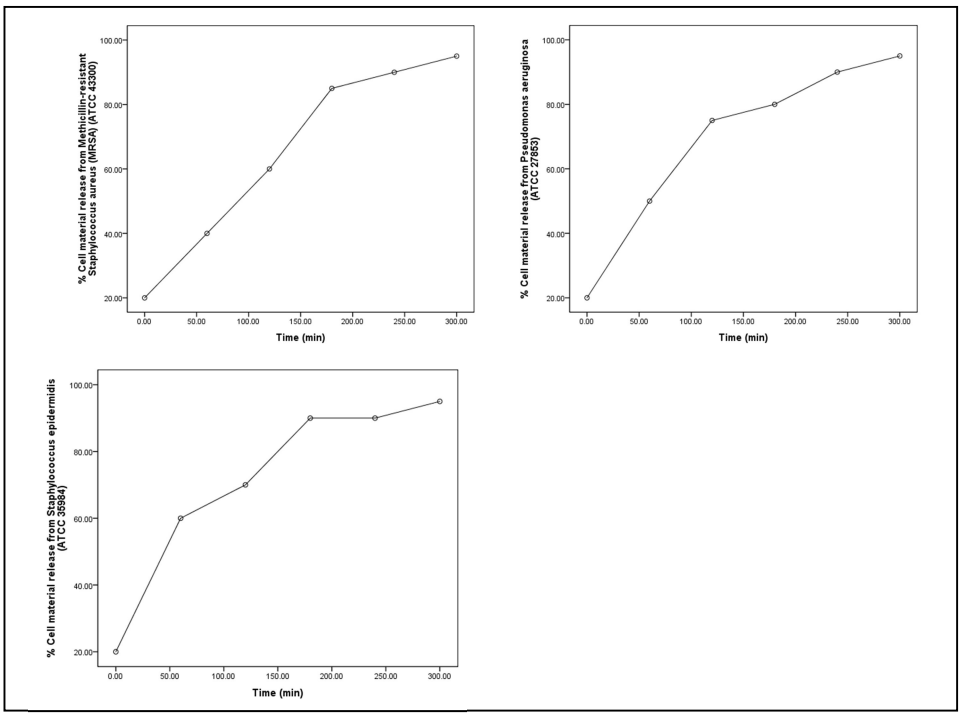

Figure 3: The effect of *Achillea Fragrantissima* EO on intracellular compounds release of MSSA (ATCC 25923), *Staphylococcus epidermidis* (ATCC12228), *Escherichia coli* (ATCC 25922)

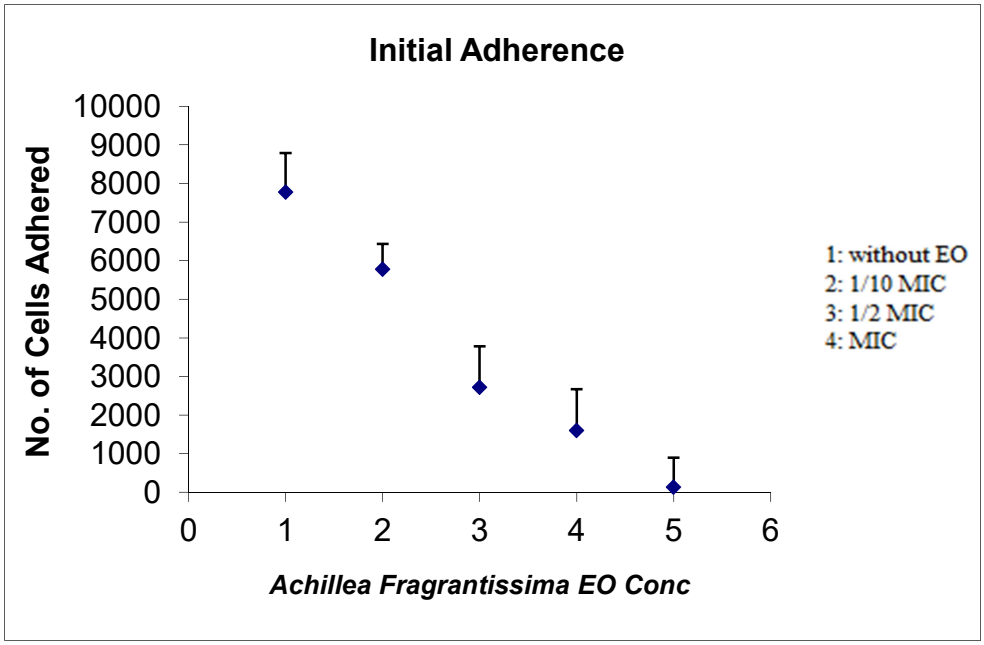

Figure 4: The effect of *Achillea Fragrantissima* EO on initial adhesion of *Staphylococcus epidermidis* (ATCC 35984); 1: without EO, 2: 1/10×MIC 3: 1/2×MIC, 4: MIC

4. DISCUSSION

The emergence of a multidrug-resistant pathogen and biofilm-related disease

pushed and encouraged the scientists to start looking for alternatives for antibiotics, EOs are having unique potentials such as the chemical variety, the accepted safety and the efficacy to be used as novel antimicrobial agents.

The results showed that *Achillea Fragrantissima* EO had a good antibacterial activity against all studied bacterial isolates. The MIC and MBC values of *Achillea Fragrantissima* EO were ranging from 0.25-1 mg/ml and 0.5-2 mg/ml, respectively, which proves that *Achillea Fragrantissima* EO is able to overcome the resistance shown by all studied bacterial isolates and their biofilm, specifically to the most notorious organisms MRSA (ATCC 43300) and *P. aeruginosa* (ATCC 27853).

Achillea Fragrantissima EO contains sixty four components as identified by our research group [13], thus its antimicrobial activity is not attributable to a single molecule or a single mechanism like antibiotics but to multiple molecules and mechanisms.

In the current study, Gram-positive bacteria were more resistant to *Achillea Fragrantissima* EO than the Gram-negative ones. This could be attributed to the difference in the bacterial cell wall structure and composition, which is more complex in the Gram-negative bacteria compared to the Gram-positive ones [19]. It is made up of a thin peptidoglycan layer next to the cytoplasmic membrane and an outer membrane having hydrophilic channels, known as porins, which block the entry of hydrophobic molecules like EOs [20]. In contrast, Gram-positive bacteria lack the outer membrane which renders them to be more susceptible for the hydrophobic molecules like EOs because of their ability to penetrate the cell wall and to act on various cell components **[19, 20]**. In the present study, the obtained results of MIC and MBIC assays showed that *Achillea Fragrantissima* EO had a strong and consistent inhibitory effect on the different studied bacterial isolates.

The function of cell membrane is to hold different components of the cell together and protect it from extracellular environment. Thus, the release of various intracellular compounds is considered as an indicator for the cell membrane damage. Two tests have been conducted to demonstrate effect of *Achillea Fragrantissima* EO on cell membrane integrity; the leakage of K^+ ions and the release of the intracellular compounds

especially DNA and RNA. The results clearly showed that there was elevated leakage K^+ ions associated with the release of intracellular compounds in accordance with time of exposure to the EO in all studied bacterial isolates treated with *Achillea Fragrantissima* EO. This indicates that the cell membrane was damaged by EO versus the control group. EOs are thought to attach to the cell membrane of the organisms, then, penetrate the phospholipid bilayer causing membrane proteins damage and destabilization of bacterial membranes associated with the loss of essential intracellular compounds like proteins and nucleic acids, furthermore, EOs can inhibit ATP generation and related enzymes leading to cell lysis and leakage of the intracellular compounds **[20, 21]**.

Biofilms are microbial communities attached to surfaces and covered by extracellular polymeric substance in extracellular matrix. *Achillea Fragrantissima* EO was able to inhibit the biofilm formation in all studied bacterial isolates at concentrations 0.5-2 mg/ml. The first step in biofilm formation is a reversible process which involves the attachment of bacteria to a substrate, then, followed by irreversible step when microorganisms adhere to each other and to the substrate **[22]**, so, if the first step of biofilm can be prevented, the biofilm formation cannot be completed. The results show that *Achillea Fragrantissima* EO reduced the number of *Staphylococcus epidermidis* (ATCC 35984) cells adhered to polystyrene which means that the first step of the biofilm formation is prevented. It is believed that *Achillea Fragrantissima* EO caused damage and changes in the cell membrane of *Staphylococcus epidermidis* (ATCC 35984) and prevented its adherence to the polystyrene surface **[23]**.

5. CONCLUSION

Achillea Fragrantissima EO possesses antibacterial and antibiofilm efficacy against all studied bacterial isolates. Its antibacterial mode of action is believed to be through permeabilization of the cell membrane associated with membrane-disrupting.

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REFERENCES

[1] Bin Zaman S, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus*. 2017; 9: 1-9.

[2] Ventola CL. The Antibiotic Resistance Crisis Part 1: Causes and Threats. *P&T*. 2015; 40: 277-283.

[3] Al-Sarayreh S, Alsharafa K, Al-Tarawneh I, Al-Qudah M, AlShuneigat J. Anti-biofilm properties of *Melissa officinalis* essential oil. *Indian Journal of Natural Sciences*. 2016; 6: 10488-10493.

[4] Donlan RM. Biofilms: Microbial Life on Surfaces. Emerging Infectious *Diseases*. 2002; 8: 881-890.

[5] Olsen I. Biofilm-specific antibiotic tolerance and resistance. *Eur J Clin Microbiol Infect Dis*. 2015; 34: 877-86.

[6] Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB. Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms. *Heliyon*. 2019; 5: e02192.

[7] Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon*. 2018; 4: e01067

[8] Al-Shuneigat J, Al-Sarayreh S, Al–Saraira Y, AlQudah M , Al-TarawnehI , Al-Dalaen S. Chemical composition and antimicrobial activity of the essential oil of wild Thymus vulgaris grown in South Jordan. *Journal of Pharmacy and Biological Sciences*. 2014; 9: 78-82

[9] Upadhyay RK. Essential oils: anti-microbial, antihelminthic, antiviral, anticancer and anti-insect properties. *J. Appl. Biosci*. 2010; 36: 1-22

[10] Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W.. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical

Review. *Medicines*. 2016; 3: 1-16

[11] Mohiuddin AK. Chemistry of Secondary Metabolites. *Annals of Clinical Toxicology*. 2019; 2: 1-22.

[12] Saeidnia S, Gohari AR, Mokhber-Dezfuli N, Kiuchi F. A review on phytochemistry and medicinal properties of the genus Achillea. *DARU*. 2011; 19: 173-186

[13] Al- Sarayreh S, Al-Shuneigat J, Al-Qudah M and Al-Tarawneh I. Chemical Composition and Antioxidant Activity of Essential Oil of *Achillea fragrantissima. Indian Journal of Natural Sciences*. 2020; 10: 18001-18007.

[14] Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W. Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesion expression in biofilm-forming *Staphylococcus epidermidis*. *Antimicrob Agents Chemother*. 2000; 44: 3357-3363.

[15] Jardak M, Elloumi-Mseddi J, Aifa S, Mnif S. Chemical composition, anti-biofilm activity and potential cytotoxic effect on cancer cells of Rosmarinus officinalis L. essential oil from Tunisia. *Lipids Health Dis*. 2017: 16: 1-10

[16] Ceri H, Olson M, Morck D, Storey D, Read R, Buret A, Olson B. The MBEC Assay System: multiple equivalent biofilms for antibiotic and biocide susceptibility testing. *Methods Enzymol*. 2001; 337: 377-385.

[17] Yang XN, Khan I A, Kang SC. Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf essential oil of *Forsythia koreana* deciduous shrub. *Asian Pac J Trop Med*. 2015; 8: 694-700.

[18] Heilmann C, Gerke C, Premington FP, Gotz F. Characterization of Tn917 insertion mutant of *Staphylococcus epidermidis* affected in biofilm formation.

Infect Immun. 1996; 64: 277-282.

[19] Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. 2013; 6: 1451-1474.

[20] Bajpai VK, Sharma A, Baek KH. Antibacterial Mode of Action of the Essential Oil Obtained from Chamaecyparis obtusa Sawdust on the Membrane Integrity of Selected Foodborne Pathogens. *Food Technol. Biotechnol*. 2014; 52: 109-118

[21] Khameneh B, Iranshahy M, Soheili V, Bazzaz BSF. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance and Infection Control*. 2019; 8: 1-28.

[22] Berne C, Ducret A, Hardy GG, Brun YV. Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiol Spectr*. 2015; 3: 1-45.

[23] Al-Shuneigat J, Al- Sarayreh S, Al-Qudah M, Al–Saraireh Y. Antibacterial and antibiofilm activity of essential oil of *Achillea biebersteinii*and its mode of action. *Journal of Pharmacy & Pharmacognosy Research*. 2020; 8: 155-166.