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Lemon Myrtle (*Backhousia citriodora*) Leaf Extract on Biofilm Formation of *Pseudomonas Aeruginosa*

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Abstract---This study includes 10 samples isolated from a different source. This research paper was to study the antibacterial activity and antibiofilm activity of an alcoholic extract of Lemon Myrtle (*Backhousia citriodora*) leaves, against 10 clinical isolates of *Pseudomonas aerogenoza*. Isolates were diagnosed by the VITEK-2 compact system and (PCR). The capacity of isolates to produce biofilm was tested by using the Microtiter plate technique (96 wells). The results showed that isolates were able to form a biofilm, *also the ability of Backhousia citriodora* was been effective on bacteria and biofilm formation.

Keywords---*Pseudomonas aerogenoza*, Biofilm, *Backhousia citriodora*.

Introduction

Despite the rapid development of the pharmaceutical industry and medications made from pure chemicals, as well as the growing number of microbes resistant to traditional antibiotics, medicinal plants have garnered a lot of attention in

recent decades (Al-Snafi, 2016). As a result, researchers' focus shifted to larger horizons by incorporating compounds derived from natural (plant) sources into the pharmaceutical industry and development, particularly to control numerous microorganisms resistant to most types of traditional antibiotics. Plant extracts are a rich basis of secondary metabolites with antimicrobial action, and among the most potent antibacterial substances are (alkaloids, flavonoids, terpenoids, tannins, saponins, and phenols) (Omojate Godstime et al., 2014). Lemon Myrtle is a shrub in a family of the *Myrtaceae* that is natural to tropical rainforests in Australia's Queensland coastal region, where it can be found 50–800 meters above sea level. (Buchailot et al., 2009). It has a strong lemon scent, contains critical nutrients, and is traditionally used as a spice in Australia and used as antifungal and antimicrobial activity (Wilkinson et al., 2003).

Pseudomonas aerogenoza is a Gram-negative bacterium that is rod-shaped, sporogenous, and monoflagellated, *P. aeruginosa* forms a variety of pigments, such as pyoverdine (fluorescent and yellow-green) and pyorubin (fluorescent and yellow-green) (red-brown), and pyocyanin (blue-green). Many research has suggested that pyocyanin not only supports the persistence of *P. aeruginosa* in the lungs of (CF) patients, but also disrupts a diversity of mammalian cell functions, including ciliary beating, epidermal cell growth, cell respiration, calcium homeostasis and prostacyclin produced from endothelial cells for lung (Winstanley & Fothergill, 2009; Olegovich Bokov et al., 2022). the fact that the precise molecular mechanism mediated by pyocyanin pathology remains unknown, infections with *Pseudomonas aeruginosa* have developed as a serious problem in hospital-acquired infections, particularly in critically ill and immunocompromised patients (Bassetti et al., 2018).

Antibiotic resistance is developing in simplicity *aeruginosa* because of the overuse and misuse of antibiotics. *Pseudomonas aeruginosa* is linked to a wide range of hospital-acquired diseases, including ventilator-associated pneumonia and bloodstream infection. It's the fourth most isolated nosocomial pathogen, responsible for 10% of all hospital-acquired infections. It's also the second most common cause of pneumonia and the third most prevalent gram-negative source of bloodstream infection (BSI) (Afshari et al., 2012). When compared to infection with another gram-positive bacterium, *Pseudomonas aeruginosa* has been linked to a higher rate of mortality in the bloodstream (Jarvis et al., 2007). *P. aeruginosa* is an opportunistic bacterium that causes both chronic and acute illnesses. In addition to its innate resistance to many medications, its capacity to produce biofilm renders immune defense systems a complex biological system and ant biotherapy useless. Free-swimming planktonic differ from their Surface-attached microbial communities counterparts in terms of architectural, phenotypic, and metabolic features.

Emergence of different biofilm phenotypes is highly dependent on *P. aeruginosa* strains and/or trophic conditions, and bacteria propagate from the sessile structure and re-enter the water column in a planktonic state to propagate and colonize additional surfaces (Rasamiravaka et al., 2015). This work aims to find an alternative to antibiotics taken from nature, specifically from plant sources.

Plant products and their effect on preventing biofilm formation

The varieties of microbes that have developed resistance to most antibiotics have increased dramatically in recent years. This leads us to conclude that antibiotics are unable to eradicate pathogenic organisms, which could be due to various causes, including the organisms' ability to build biofilms (Davies & Davies, 2010). Especially after learning about the dangers of biofilm formation and their link to some chronic diseases that are difficult to treat with traditional antibiotics; as a result, scientists began looking for natural alternatives to antibiotics to eliminate the bacteria that form biofilms, and one of those alternatives was extracted from plants. Plants produce secondary metabolic chemicals that aren't involved in the plant's physiological functions. The most well-known plant products with antibacterial properties are phenols, which are one of the plant's main secondary metabolic chemicals. They've gained medical and pharmaceutical significance because of their therapeutic effects and the presence of many substances, including antimicrobial-active chemicals like chalcones (yellow pigments) and flavonoids. Flavonoids and Coumarin are two types of flavonoids. Flavonoids, for example, can prevent biofilm formation in some microbes by inhibiting the Quorum sensing process (Vikram et al., 2010). Alkaloids are one of the first substances to be isolated from plants, and they're well-known for their medicinal qualities. Another study indicated the effect of *Viti's vinifera extract* on the biofilm development of *S. haemolyticus* and *S. aureus* (Al-Mousawi et al., 2020).

Material and Methods

The extracts studied in this work have previously been described (Cock, 2008), Backhousia citriodora leaves were collected from the market. Samples of leaves were dry in a Sunbeam and then pulverized into a powder. At Soxhlet, 1g of powdered leaves was extracted extensively for 24 hours in 50ml ethanol (Ajax, AR grade). The extract was filtered using (Whatman No.5) filter paper, and then passed through 0.22µm filter (Sarstedt) and kept at 4°C until use.

Isolation of bacteria

The samples were isolated from various burn sites and grown on MacConkey agar (Himedia India) for 24 hours.

Detection of biofilm formation:

Biofilm development was distinguished using a modified version of (Mathur et al., 2006). Microtiter test.. *P. aeruginosa* isolates were inoculated overnight in nutritional broth (Himedia), after comparison turbidity with the McFarland tube, which approximations the number of bacterial cells to be 1.5×10^8 cell/ml. Transfer 100µl of Bacterial culture to Microtiter plates (96well flatbottom) and incubate at 37°C for 48 hours, then remove the supernatant and wash the wells with phosphate buffer saline The biofilm was fixed with methanol, and the supernatant was removed once again. After that, the crystal violet (CV) solution was applied to the wells and the excess dye was removed by washing the plates under running tap water after 20 minutes. Finally, by addition 33% Glacial acetic acid, bound crystal violet was freed. At 630nm, the absorbance was measured.

Statistical analysis

The results of this study were analyzed using the statistical program (SPSS) by using a test ANOVA one way, Least Significance Difference (Morgan et al., 2004).

Results

Ability of *P. aeruginosa* to biofilm formation

The findings reveal that bacteria's capacity to build biofilm varies. The ability of bacteria to build biofilm with a strong shape, as shown in Table (1) One of the most essential aspects that donate greatly to the capacity of the microorganism to form disease and its ability to withstand various types of antibiotics. Variations in process of forming the biofilm of studied isolates referred to the effect of conditions, the type of medium used, growth conditions, and (PIA) efficacy in adhesion contribute to the capacity of bacteria to form the biofilm, displayed that the degree of bacteria adhesion to surfaces is mainly dependent on growth conditions and the kind of medium used (Wang, 2008).

Effect of alcoholic extract of Lemon myrtle leaves on biofilm formation

The results shown in table (1) showed the clear variance of the effect of the extract on biofilm formation for all isolates under study, plant The capacity of an alcoholic extract is attributed to the existence of high flavonoids in the leaves, as well as tannins and alkaloids, which are known to include secondary chemicals compounds that hinder the quorum sensing mechanism, which is vital in biofilm development (Ta & Arnason, 2016), we believe that plant extracts, which include a wide range of phytochemicals, will give a decomposable impact to kill germs and Which opens wider horizons for scientists and researchers to eliminate various types of pathogens.

Table 1
Impact of the extract on the biofilm formation towards *P. aeruginosa* (MTP)

Sample	Control (Standard deviation ±average)	Effect of Extract (Standard deviation ±average)
P. aeruginosa 1	0.001 ±0.149	0.017 ± 0.082
P. aeruginosa 2	0.002 ±0.140	0.089 ±0.008
P. aeruginosa 3	0.202 ±8 0.25	0.002 ± 0.130
P. aeruginosa 4	0.0005 ±0.170	0.003 ± 0.056
P. aeruginosa 5	0.0005±0.141	0.0005± 0.100
P. aeruginosa 6	0.001±0 .201	0.077 ±0.025
P. aeruginosa 7	0.001 ±0 .145	0.100±0.001
P. aeruginosa 8	0.0015± 0.129	0.028± 0.117
P. aeruginosa 9	0.001± 0.121	0.002± 0.067
P. aeruginosa 10	0.0026± 0.127	0.099 ±0.0005

Discussion

The use of medicinal plants as a substitute for antibiotics and testing their effect is not new. Since ancient times, medicinal plants and their extracts have been used as drugs and treatments for various types of diseases caused by pathogens, especially *Backhousia citriodora* (Gao et al., 2020; Huldani et al., 2022). Several studies indicated the effect of *Backhousia citriodora* extract in preventing biofilm formation due to its active ingredients in it (Yabuta et al., 2021; Ansari et al., 2022).

Authors' Contribution

Study concept and design: H.A.A

Acquisition of data: H.A.A

Analysis and interpretation of data: H.A.A

Drafting of the manuscript: K.I.Z

Critical revision of the manuscript for important intellectual content: K.I.Z

Statistical analysis: A.A.H

Administrative, technical, and material support: A.A.H

Administrative, technical, and material support:

Ethics

All the procedures are approved by the Department of Medical Laboratory Techniques, Faculty of Medical and Health Techniques, University of Alkafeel, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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