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# EFFECTIVENESS OF SOME PLANT EXTRACTS AGAINST THE BIOFILM OF *PSEUDOMONAS AERUGINOSA* BACTERIA ISOLATED FROM BURNS

**Wassan Rodha Hassan Al-Shakarje and Hyiam Abdalrodha Al-Awad**
Department of Life Sciences, College of Education for Pure Sciences, University of Kerbala, Iraq
aala77299@gmail.com

## Abstract

This study was conducted to identify the effects of plant extracts *Camellia sinensis*, *Cinnamon cinnamoum*, *Lawsonia inermis* on *Pseudomonas aeruginosa* bacteria producing biofilm and isolated from burn patients in the Imam. Al-Hussein in the Burns department in Kerbala province 50 isolation of the bacteria forming the biofilm can be diagnosed and developed by relying on a number of biochemical tests as well as the technique of Vitek-2 developed the results show that the diagnosed bacteria are bacteria 99% *P. aeruginosa* and has high resistance against most commonly used antibiotics 100%, and showed that the aqueous extract of the three plants did not show any activity against the bacteria *P. aeruginosa* producing the biofilm even at high concentration while showing The alcoholic extract of all the plants used in the study is the opposite as it showed an anti-bacterial activity as the extract of ethanol alcohol for the *Cinnamon cinnamoum* with a concentration of 100% higher inhibition diameter of 16.21 (mm) and record green tea 6.16 (mm) while *Lawsonia inermis* recorded less diameter inhibition Reached 7.98 (mm), as well as the results showed that the value of the Minim inhibition concentration and the Minim inhibition killed concentration rate for green tea extract and cinnamon against the bacteria producing strong biofilm are the 65 (mg/ml), 75 (mg/ml) for concentration respectively while the *Lawsonia inermis* has a concentration value 75 (mg/ml), 85 (mg/ml) for concentration respectively and in the case of bacteria produced for the average bio-membrane the concentration of green tea and cinnamon 35 (mg/ml), 45 (mg/ml) and reached the concentration of *Lawsonia inermis* 40 (mg/ml), 50 (mg/ml) respectively and in the case of bacteria produced the weak biofilm *Camellia sinensis* and *Cinnamon cinnamoun* concentrate 25 (mg/ml), 35 (mg/ml) and the *Lawsonia inermis* concentration is 35 (mg/ml), 45 (mg/ml) for two concentrations respectively.

***Key word*** **:** *Pseudomonas aeruginosa*, Green tea, Cinnamon, Henna.

## Introduction

The ratio of resistance of *P. aeruginosa* bacteria to antibiotic treatment (Appelbaum, 2007) has increased to the search for effective antibacterial substances from medical plant sources that possess many nature-effective molecules (Smadi & Hamed, 2011) which have been used in traditional medicine to address Many diseases have good sources of antibiotics and show a vital effectiveness against many microorganisms, due to the fact that these plants are rich in several types of compounds such as aninat, turbines, flavones, alkaloids and others, which have antibacterial properties. *P. aeruginosa* bacteria is the most common type of species of Pseudomonas and is the highest rate of infection among patients hospitalized, especially burns patients (Elmanama *et al*., 2013). The bacteria also have the ability to invade and colonize the body tissue and enter a circulatory system causes the bacteremia (Davey *et al*., 2000) because of its excellent ability to develop the of virulence factor and the of antibiotic resistant strains (Mansour & Enayat, 2004) Because of the wrong use of these antibiotics (Belb *et al*., 2013) or cut the antidote before completing the duration of treatment (Aledort *et al*., 2000). It has recently been observed that these bacteria are the main cause of increased morbidity and increased mortality among burn patients due to the ability of bacteria to produce the biofilm, which is a microcolonies surrounded by polyocytes produced by bacteria as a protective shield against immune response For the host and antibiotics and invading soft tissues and skin, so the tissues with burns are environment for example for settlement and growth of bacteria causing the serious burns to the (Rook *et al.*, 2010) The role of the biofilm may reach 65% of human infections and it is very difficult to eliminate bacteria with common antibiotics and sterilizers, and the sensitivity test with the Vitek-2 device showed resistance to bacteria producing bio-membranes for most types of antibiotics, there are several Methods for detecting the presence and type of biofilm of which the central method of Congo

red agar(CRA) (Hassan *et al*., 2011) and the microtiter plate method (MTP) (Rukayadi & Hwang, 2006).

**The importance of plant *Camellis sinensis*, *Cinnamon cinnamon* and *Lawsonia inermis* as antibacterial**

Green tea contains multi-phenol compounds, which include flavonoids, aninat, carotenoids, phenolic salts and phenolic acids, which are found in almost all plant parts such as leaves, flowers, fruits, stems, roots, bark, and seeds, which are secondary metabolic products. In addition, multiple phenol compounds affect the prevention of several diseases in (Hong *et al*., 2009) green tea was used by some peoples as a treatment for many diseases because of its impact on them as demonstrated by the inhibitory effect on many microorganisms (Hosseinin *et al.,* 2007).

The *cinnamon* contains volatile tannins, Komar and other ingredients including gum, resins, sugars and phenols by 10.4%, where every 100g of *cinnamon*, according to the American Ministry of Agriculture contains calories: 247, saturated fat: 0.34, carbohydrates : 80.59, Fiber: 53.1, proteins: 3.99 and Cholesterol: 0 (McCann, 2003) cinnamon is an anti-viral medicinal plant and is anti-bacteria and is a research reference to the need to use cinnamon as an anti-fungal, bacteria and virus to contain effective substances that improve the performance of the immune system to be considered a safe source for the production of drugs and medicines (Tipu *et al*., 2006). The *Lawsonia inermis* plant contains high concentrations of phenolic compounds, flavonoids, alkarbohidat, proteins, Tannins, alkaloids and fatty acids (Chaudhary *et al*., 2010) *Lawsonia inermis* was used in the treatment of skin scabies in humans and Animals (Mostafa, 2007) and studies have shown that extract Henna has the efficacy of inhibiting against some species bacteria (Arum *et al*., 2010).

The importance of research is to study the activity of the anti-leaf extracts of green tea leaf, cinnamon leaf and henna leaf to the *P. aeruginosa* bacteria, which are composed of bio-biofilm and isolated from the burn and antibiotic-resistant patients, in order to take advantage of the natural materials of these Plants in the future as bio-active and alternative substances for antibiotics in the treatment of burn patients.

## Materials and Method

The leaves of the tea green, cinnamon bark and henna powder were obtained from local markets in Kerbala province, the plants were cleaned and grinded by an electric and the powder was preserved in clean plastic bottles until use.

**Preparation of plant extracts for green tea, cinnamon and henna**

Aquatic extracts: Taken plant powders prepared for each plant by dissolving 50 g of powdered powder in 500 ml of distilled water (V: W 10:1) and left for 24 hours at room temperature after which the mixture was filtered with layers of medical gauze to get rid of plankton and then put the solutions in clean and sterilized tubes In the centrifuge at a speed of 3000 rpm then the extracts were filtered using filter paper (Whatman No.1,15 cm) to obtain a clear solution and then dried the extracts using an air incubator not exceeding the temperature of 40 م° and then keeping the dry extracts in the freezer bottles in the refrigerator until use and preparation Hot water extract in the same way with the replacement of cold water with boiling water (Amra *et al*., 2006).

**Alcoholic ethanol extracts**

Prepared to thawed 50 g of plant powder per plant in 500 ml of ethanol alcohol in clean and sterile glass bottles with autoclave then the bottles were coated with opaque paper away from the light for 72 hours and then filtrate with a medical gauze and then the centrifugal at 3000 cycles/minute and then filtrate (Whatman N 0.1, CM15) then dry the extract from the organic solvent and save the dry extracts at temperature – 20° C until use ( Huda *et al*., 2009).

**Isolation of *P. aeruginosa* bacteria**

In addition, 50 isolates were obtained from the burns department at Al-Imam Hussein Medical Hospital city in Kerbala province and developing colonies were diagnosed in agricultural settings used for purification and isolation depending on several biochemical tests and using the identification systems Microbiology (Bio Mérieux API 20 E system, France) and used the (VIETK-2 COMPACT system) to obtain a more accurate diagnosis as well as an antibiotic isolation sensitivity test .

**Testing the effectiveness of extracts towards isolated *P. aeruginosa* bacteria**

The activity of the extracts was tested by well diffusion assay method (Sengul *et al*., 2009) to detect of inhibition activities of preparations against bacteria *p. aeruginosa,* which produced the biofilm for the preparation of aquatic extracts, taking (2) grams of dry extracts per plant and thawed in (10) ml distilled water Sterile, we have a stock solution with a concentration of 200 g/ml sterility of the solution by filtration papers and (Filter Millipore 0.22) to obtain a stock solution clear and sterilized 100% and preparation the concentration (100, 75, 50, 25) mg/ml.

**Select the lowest MIC damper focus and focus the lowest killer MBC:**

MIC and MBC are determined depending on the method of the tubes and the appearance of the bacterial growth in the tube for each concentration (Mehta *et al*., 2016). A series of concentrations were prepared based on the concentration in which the least concentrated diameter was inhibited and the concentration that showed no efficacy against bacterial and as follows (25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85) mg/ml per extract.

## Results and Discussion

The results of the present study show that the aquatic extracts in both hot and cold types of green tea, cinnamon and henna showed no effect in inhibiting the *P. aeruginosa* bacteria produced for the biofilm and isolated from burns and even when the concentration of 200 mg/ml and agreed on these results with the study The antibacterial of other plants has several extracts on gram negative bacteria where it was found that the aquatic extract is ineffective unlike other extracts (Malu *et al*., 2009).

**Table 1 :** Variations in the average of the inhibition diameters depending on the type and concentration of the extract.

| Conclusion | Concentration | Number | Average | Standard deviation | LSD |
|---|---|---|---|---|---|
| Green tea | 25 mg/Ml lsd= 0.53 | 50 | 0.50 | 1.06 | 2.07 |
| | 50 mg/mL lsd=1.8 | 50 | 3.45 | 5.42 | |
| | 75 mg/mL lsd= 2.28 | 50 | 8.84 | 6.59 | |
| | 100 mg/mL lsd= 2.06 | 50 | 11.85 | 6.10 | |
| | **Total** | 200 | 6.16 | 6.87 | 1.29 |
| Cinnamon | 25 mg/mL | 50 | 1.02 | 2.03 | 2.10 |
| | 50 mg/mL | 50 | 3.60 | 5.50 | |
| | 75 mg/mL | 50 | 12.12 | 6.45 | |
| | 100 mg/mL | 50 | 16.21 | 6.20 | |
| | **Total** | 200 | 8.24 | 8.15 | 1.29 |
| Henna | 25 mg/mL | 50 | 0.08 | 0.20 | 1.03 |
| | 50 mg/mL | 50 | 1.25 | 1.93 | |
| | 75 mg/mL | 50 | 4.38 | 4.08 | |
| | 100 mg/mL | 50 | 7.98 | 2.73 | |
| | **Total** | 200 | 3.42 | 4.04 | 1.29 |
| Total | 25 mg/mL | 150 | 0.53 | 1.37 | 1.17 |
| | 50 mg/mL | 150 | 2.77 | 4.69 | |
| | 75 mg/mL | 150 | 8.45 | 6.60 | |
| | 100 mg/mL | 150 | 12.01 | 6.22 | |

As for ethanol alcohol extracts for green tea, cinnamon and henna, show results and effects Differentiated among them in inhibiting the *P. aeruginosa* bacteria and showing the presence of statistically significant differences at the probability level of P ≥0.05 in the countries of inhibition zones between green tea and cinnamon for cinnamon, as well as between cinnamon and henna for cinnamon and between green tea and henna for green tea and as illustrated in table (1) as The average number of inhibition diameters for the four concentrations (100, 75, 50, 25) for each extract of extracts was green tea, cinnamon and henna (6.16, 8.24, 3, 42) mm respectively, as for MIC and MBC value of ethanol alcohol extracts vary depending on the type of plant extract, the results showed that The value of MIC and MBC for green tea and cinnamon in the case of isolates produced for strong biofilm is 65 mg/ml and 75 mg/ml respectively as for the construction was 75 mg/ml and 85 mg/ml, where the alcoholic henna extract recorded the highest concentration of inhibition and killing foe bacteria compared to the extract of green tea and cinnamon which Show better effectiveness with less concentration of inhibition and killing of bacteria *P. aeruginosa* as shown in Fig. 1.

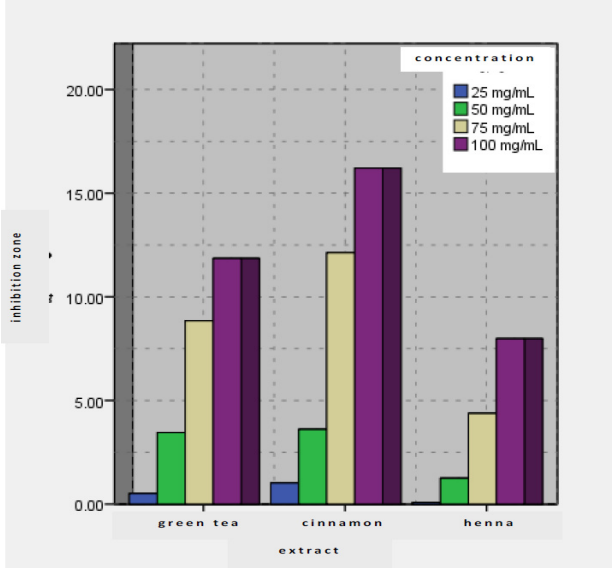


**Figure 1 :** Effect of concentrations of ethanol alcohol extacts on bacterial *P. aeruginosa* growth

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