Effect of some plant extracts on the Pyocyanin Production from *Pseudomonas Aeruginosa* which Isolated from clinical samples

Farkad Hawas Musa¹, Mohammed abdul aziz ismail¹, Raghad Waleed Khaleel¹, Najeeb Mohammed Hussein*²

¹Department of Biology, College of Education for Pure Sciences, University of Anbar, Iraq
²Department of Biology, College of Sciences, University of Anbar, Iraq

*drnajeebaldulimirartnas@gmail.com

Abstract. In this study, the effect of some plant extracts on the production of the Pyocyanin dye from the bacteria of *Pseudomonas aeruginosa* was known. This study included the use of two types of plants, ginger and ginkgo. These two plants were extracted in a waterway by the Sxolite apparatus. The raw extract of these plants was used, and several concentrations were made of it 20%, 40% and 80%, after which the Pyocyanin concentration was measured. After adding these extracts, the results showed that there were significant differences in the decrease in the production of the Piocyanin dye compared to the control by the bacteria. The decrease in the production varied according to the concentration, and the 80% concentration gave better results. The decrease in dye production compared to the rest of the treatments and compared to control.

Keywords: pyocyanin, production, Ginko, Ginger.

1. Introduction
Pseudomonas aeruginosa (PA) is an opportunistic pathogen responsible for numerous ailments in people, and it is one of the significant reasons for hospital-acquired contamination exhibiting a high drug-resistance profile[3-1]. Pyocyanin is synthesized with the resource of a sequence of complicated steps mediated thru viable of gene products encoded via the functionality of two phzABCDEFG operons[4-6]. The phzH, phzM, phzS genes the pyocyanin synthetic pathway, chorismic ought to be converted into phenazine-1-carboxylic acid via the PhzA-G proteins firstly[7-9]. Subsequently, phenazine-1-carboxylic acid could be converted to pyocyanin with the aid of PhzM andPhzS[10-12]. PYO is accountable for the blue-green coloration typically decided in PA cultures[13, 14]. Besides the virulence difficulty and quorum sensing functions, PYO is moreover a signalling molecule and a redox-active metabolite concerned in a range of giant organic things to do alongside with gene expression, benefiting bacterial cells and biofilm formation[15-17].
2. Material and methods

2.1. Collection of bacterial samples:
Fifty scientific samples had been gathered from Ramadi Teaching Hospital, which had been from several sources along with burns, wounds, UTI infections, and these samples had been amassed through sterile cotton swabs. While urinary tract infections, two (UTI) samples were gathered through the way of a sterile container.

2.2. Bacterial isolation and identification:
Bacterial isolates have been subjected to a variety of cultural and biochemical tests for identification of these isolates[18].

2.3. Ginger extract
Ginger cut into tiny pieces using a mixer device, weighing 40 grams and extracted with cellulite using 200 ml of distilled water. Filter the extract and store in the refrigerator until use[19].

2.4. Ginkgo biloba extract
Weigh 50 g of Ginkgo biloba powder, then add it to 250 ml of distilled water, then heated to a boiling point, then cooled, filtered, and stored in the refrigerator until use.

2.5. Pyocyanin Production:
3 ml from the bacterial suspensions have been filtrated by 0.20 μm pore size. Then absorbance was measured at four hundred nm in a Spectrophotometer. The results have agreed with the following equation. [19].

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\text{Pyocyanin activity (U/ml) = Absorption of the sample test - Absorption of control (broth only).}
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3. Result and Discussion

Table 1 shows the number of isolated isolates from pathological samples, where the number of isolated isolates from burn cases was 20 isolates with an isolation rate of 40%. As for isolates that were isolated from urinary tract infections ten isolates with an isolation rate of 20% and 15 isolates from wounds by 30% As for the isolates obtained from middle ear infections, five isolates increased by 10%.

| Source of sample | Number | percentage |
|------------------|--------|------------|
| Burn             | 20     | 40%        |
| Urine            | 10     | 20%        |
| Wound            | 15     | 30%        |
| Ear              | 5      | 10%        |
| Total            | 50     | 100%       |

Table 2 shows the effect of ginger extract on the production of pyocyanin, where several concentrations of ginger extract were prepared and studied its effect on the production of this dye. The best results are for a reduction in pyocyanin production compared with control and other treatments.

| Concentration   | Frequency | Frequency | Frequency | Frequency | Frequency |
|-----------------|-----------|-----------|-----------|-----------|-----------|
| 20 % of ginger extract | 0.55      | 0.57      | 0.58      | 0.54      | 0.52      |
| 40 % of ginger extract | 0.44      | 0.45      | 0.46      | 0.43      | 0.42      |
| 80 % of ginger extract | 0.22      | 0.20      | 0.19      | 0.18      | 0.19      |
Table 3 shows the statistical analysis of the effect of ginger plant extract on the production of pyocyanin dye from Pseudomonas aeruginosa bacteria, where the results of the table showed significant differences for all treatments with the treatment of the control in the low dye of the pyocyanin.

Table 3. ANOVA table of the impact of ginger extract on pyocyanin production of P. aeruginosa.

| ANOVA production of pyocyanin (U/ml) | Sum of Squares | df | Mean Square | F     | Sig. |
|-------------------------------------|----------------|----|-------------|-------|------|
| (Combined)                          | 5.866          | 3  | 1.955       | 451.306 | .000 |
| Between Groups                      |                |    |             |       |      |
| Linear Term                         | 4.610          | 1  | 4.610       | 1063.961 | .000 |
| Deviation                           | 1.256          | 2  | .628        | 144.979 | .000 |
| Within Groups                       | .069           | 16 | .004        |       |      |
| Total                               | 5.935          | 19 |             |       |      |

Table 4 shows the effect of an extract on the production of the pyocyanin tincture produced by the bacterium Pseudomonas aeruginosa where the results demonstrated significant differences for the effect of this extract on the production of this dye and the percentage of decrease in the production of this dye increased with an increase in the concentration of the extract compared to the control, the concentration of 80% of the extract He gave the best results in the decrease in the production of this dye, compared with the rest of the treatments and with control.

Table 4. The impact of Ginkgo biloba extract on pyocyanin manufacturing of P. aeruginosa.

| Concentration          | pyocyanin production U/ml |
|------------------------|---------------------------|
|                        | Frequency | Frequency | Frequency | Frequency | Frequency | Frequency |
| 20 % of Ginkgo biloba extract | 0.77       | 0.73       | 0.72       | 0.76       | 0.73       |
| 40 % of Ginkgo biloba extract | 0.55       | 0.51       | 0.53       | 0.57       | 0.54       |
| 80 % of Ginkgo biloba extract | 0.33       | 0.32       | 0.31       | 0.32       | 0.30       |
| Control                | 1.9        | 1.8        | 1.9        | 1.7        | 1.8        |

Table 5 shows the effect of Ginkgo biloba extract on the production of pyocyanin, where several concentrations of ginger extract were prepared and studied its effect on the production of this dye. The best results are for a reduction in pyocyanin production compared with control and other treatments.

Table 5. ANOVA table of the effect of Ginkgo biloba extract on pyocyanin manufacturing of P. aeruginosa.

| ANOVA production of pyocyanin (U/ml) | Sum of Squares | df | Mean Square | F     | Sig. |
|-------------------------------------|----------------|----|-------------|-------|------|
| (Combined)                          | 4.788          | 3  | 1.596       | 362.302 | .000 |
| Between Groups                      |                |    |             |       |      |
| Linear Term                         | 4.166          | 1  | 4.166       | 945.671 | .000 |
| Deviation                           | .622           | 2  | .311        | 70.617  | .000 |
| Within Groups                       | .070           | 16 | .004        |       |      |
| Total                               | 4.858          | 19 |             |       |      |

4. Conclusions
The impact of certain plant extracts on Pyocyanin dye production from Pseudomonas aeruginosa bacteria has been identified here. Two different plant kinds, ginger and ginkgo, were used in this survey. The Sxolite device mined both plants in a waterway. The raw extract from these plants was
used with several levels of 20%, 40% and 80%, the measurement of the pyocyanin concentration also followed that. The researchers found that the decreased output of PIIocyanine dye in contrast with the control of the bacteria was significant after the introduction of those extracts. The decline in the output was centred on the dosage, with 80% improved results as compared to the rest of the therapies and regulation, the decrease in the development of teint. The results were higher.

5. References
[1] Dong L, Pang J, Wang X, Zhang Y, Li G, Hu X, Yang X, Lu C-D, Li C and You X 2020 Mechanism of pyocyanin abolishment caused by mvaT mvaU double knockout in Pseudomonas aeruginosa PAO1 Virulence 11 57-67
[2] Hossain M A, Sattenapally N, Parikh H I, Li W, Rumbaugh K P and German N A 2020 Design, synthesis, and evaluation of compounds capable of reducing Pseudomonas aeruginosa virulence European Journal of Medicinal Chemistry 185 111800
[3] Sood U, Singh D N, Hira P, Lee J-K, Kalia V C, Lal R and Shakarad M 2020 Rapid and solitary production of mono-rhamnolipid biosurfactant and biofilm inhibiting pyocyanin by a taxonomic outlier Pseudomonas aeruginosa strain CR1 Journal of Biotechnology 307 98-106
[4] Electrochemical I, Device PA, Torres MDT. Journal Pre-proof. 2020; (January)
[5] Maisetta G, Grassi L, Esin S, Kaya E, Morelli A, Puppi D, Piras M, Chellini F, Pifferi M and Batoni G 2019 Targeting Pseudomonas aeruginosa in the Sputum of Primary Ciliary Dyskinesia Patients with a Combinatorial Strategy Having Antibacterial and Anti-Virulence Potential International journal of molecular sciences 21
[6] Wei Q, Bhasme P, Wang Z, Wang L, Wang S, Zeng Y, Wang Y, Ma L Z and Li Y 2020 Chinese medicinal herb extract inhibits PQS-mediated quorum sensing system in Pseudomonas aeruginosa Journal of Ethnopharmacology 248 112272
[7] Mohamed F A, Shaker G H and Askoura M M 2020 Oxidative Stress Influences Pseudomonas aeruginosa Susceptibility to Antibiotics and Reduces Its Pathogenesis in Host Current Microbiology
[8] Parasuraman P, Devadatha B, Sarma V V, Ranganathan S, Ampasala D R and Siddhardha B 2020 Anti-quorum sensing and antibiofilm activities of Blastobotrys parvus PPR3 against Pseudomonas aeruginosa PAO1 Microbial Pathogenesis 138 103811
[9] Xi G, Zhao X, Wang S, Yang J and Sun J A Z 2020 Synergistic Effect Between Sulfate-reducing Bacteria and Pseudomonas Aeruginosa on Corrosion Behavior of Q235 Steel International Journal of Electrochemical Science 15 361-70
[10] Thierbach S, Sartor P, Onur Y, Fetnzer S. na of. 2019
[11] van Dongen M C, van Rossum E, Kessels A G, Sielhorst H J and Knipschild P G 2000 The efficacy of ginkgo for elderly people with dementia and age-associated memory impairment: new results of a randomized clinical trial Journal of the American Geriatrics Society 48 1183-94
[12] Ryu E Y, Park A J, Park S Y, Park S H, Eom H W, Kim Y H, Park G and Lee S J 2012 Inhibitory effects of Ginkgo biloba extract on inflammatory mediator production by Porphyromonas gingivalis lipopolysaccharide in murine macrophages via Nrf-2 mediated heme oxygenase-1 signaling pathways Inflammation 35 1477-86
[13] Taylor T N and Taylor E L 1993 The biology and evolution of fossil plants (NY, USA: Prentice Hall PTR)
[14] Mahadevan S and Park Y 2008 Multifaceted therapeutic benefits of Ginkgo biloba L.: chemistry, efficacy, safety, and uses Journal of food science 73 R14-9
[15] Marcocci L, Maguire J J, Droy-Lefaix M T and Packer L 1994 The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761 Biochemical and biophysical research communications 201 748-55
[16] Janssens D, Delaive E, Remacle J and Miichels C 2000 Protection by bilobalide of the ischaemia-induced alterations of the mitochondrial respiratory activity Fundamental & Clinical Pharmacology 14 193-201
[17] Jiratchariyakul W and Mahady G B 2013 Overview of Botanical Status in EU, USA, and Thailand Evidence-based complementary and alternative medicine: eCAM 2013 480128

[18] Sastre J, Lloret A, Borras C, Pereda J, Garcia-Sala D, Droy-Lefaix M T, Pallardo F V and Vina J 2002 Ginkgo biloba extract EGB 761 protects against mitochondrial aging in the brain and in the liver Cellular and molecular biology (Noisy-le-Grand, France) 48 685-92

[19] Zuercher A W, Imboden M A, Jampen S, Bosse D, Ulrich M, Chtioui H, Lauterburg B H and Lang A B 2005 Cellular immunity in healthy volunteers treated with an octavalent conjugate Pseudomonas aeruginosa vaccine Clin Exp Immunol 142 381-7