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Research Article

Effect of *Coffea arabica* L on antibiotic-resistant *Pseudomonas aeruginosa*

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Abstract
Pseudomonas aeruginosa is considered an opportunistic bacteria that causes many risks and because of its resistance to antibiotics, the virulence of this bacteria has increased. To get rid of the problem of bacterial resistance to antibiotics, effective medicinal plants were used to get rid of pathogenic bacteria so during this study, 200 samples were collected from patients with wounds and burn from Al-Sadiq Teaching Hospital in Babylon Governorate, distributed to 130 burn samples and 70 wound samples. The samples were cultured with specific and differential media. Where 30(15%) samples of *P. aeruginosa* were obtained and the diagnosis was confirmed by microscopy and biochemical tests and confirmed by VITEK-2 Compact. The sensitivity test showed its resistance to many antibiotics piperacillin 15/30 (50%) , piperacillin - tazobactam (4/30(13.3%), ceftazidime and cefepimewere each one (25/30(83.3%), Aztreonam 8/30(26.6%), Imipenem5/30(16.6%), Gentamicin 12/ 30(40%) 30, Tobramycin9/30(30%), Amikacin 6/30(20%), ciprofloxacin 13/30(43.3%), ofloxacin 5/30(16.6%). Because of the role of alternative medicine in combating antibiotic-resistant bacteria, the effectiveness of the coffee plant was measured against antibiotic-resistant *P. aeruginosa* was sensitive to the coffee aqueous extract, *Coffea arabica Linn*. (Rubiaceae) .The properties of the active substances in coffee were determined using characteristic techniques UV-Visible Spectra analysis, X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The study aims to investigate the effectiveness of the Arabica coffee plant as a bacterial antibiotic to challenge the problem of pathogenic bacterial resistance to the used antibiotics.

Keywords: Alternative medicine, Antibiotic-resistant, *Coffea arabica L.*, *Psuedomonas. aeruginosa*, VITEK-2 Compact

INTRODUCTION

Pseudomonas aeruginosa, a Gram-negative bacterium widespread in various environments, is an opportunistic bacterium responsible for many serious infections and nosocomial outbreaks worldwide (Al Dawodeyah *et al.*, 2018). *P. aeruginosa* is characterized by its ability to resist antibiotics due to its possession of resistance genes carried on plasmids or as a result of certain genetic mutations. In both cases, the presence of the resistance property treating and eradicating it is very difficult (Streeter and Katouli, 2016). Since ancient times, humans have used herbs and plants to treat many injuries (Cowan, 2019). Some plants were highly able to eliminate and kill pathogenic microorganisms such as bacteria and fungi. Tea and coffee are one of the most popular drinks among all classes of society (Fatima *et al*., 2021) . Existing coffee, which humans use as a drink, is available in the market in the form of two types (ground and instant coffee), both of which have the same biological activity (Basri and Fan, 2015) as research has proven that coffee has a significant role in preventing cancer (Sagun *et al.*, 2016 ; Bushman, 2018) and many diseases of the heart, joints and multiple infections (Sesso *et al.*, 2019). This effectiveness is mainly due to its polyphenol contents (flavonoids, antioxidants, tannins, caffeine, etc(Yang and Landau, 2020 ; McKay and Blumberg, 2022).The present study aimed to determine the effect of *Coffea arabica* L on antibiotic-resistant *P. aeruginosa*.

MATERIALS AND METHODS

Collection of specimens

During the study period in 2022, 200 burn and wound samples were collected from Marjan Teaching Hospital

in Babylon Governorate, and distributed to 130 burn samples and 70 wound samples.

Isolation of bacteria

The clinical samples were cultured on Blood agar, Cetrimide agar and MacConkey agar (Pronadisa , British), and incubated for 24 hours at 37ºC. Cetrimide agar medium is a selective medium for bacteria, so it was used to isolate *P. aeruginosa* Cetrimide agar medium is a selective medium for bacteria, so it was used to isolate *P. aeruginosa.*

Bacterial identification

Depending on the shape of the colonies on the blood agar and the Maconkey and the Agar cetrimide. They were distinguished for their viscosity, color and strength, in addition to microscopic diagnosis using a light microscope, and the diagnosis was confirmed using the VITEK 2 compact system.

Some biochemical tests (oxidase, catalase and produce type B hemolysis on blood medium and It can grow on Cetrimide agar medium in the form of blue-green colonies under normal growth conditions ((at 37˚C for 24 hrs.) were performed for validation (Al Dawodeyah *et al*., 2018).

Susceptibility test of antibiotic

The susceptibility test of antibiotics (Gentamicin, Amikacin, Piperacilin, Meropenem, Ceftazidime, Ciprofloxacin, Imipenem, Cefepime, Ticarcillin, Colistin, Tobramycin and Ticarcillin/Clavulanic Acid from Mast Co., UK) was tested by using by spreading the isolates of *P. aeruginosa* on the agar(Toda et al, 2019).

Collection of coffee samples

Two instant coffee samples (CF) viz. CF A and CF B were obtained from the local market of Hilla City in Iraq.

Characterization of antibacterial compound in coffee

UV-Visible Spectra analysis

UV-vis spectroscopy was used for the determination of antibacterial compounds in coffee. Two ml of an aliquot of coffee was measured in a 1 cm path-length quartz cuvette and scanned at a medium scan rate 2 nm/ second, in the range of (200 – 800) nm. The absorbance at which the peak was formed was noted (Gangadoo *et al*., 2017).

X-ray diffraction

X-ray diffraction (XRD) was used to characterise coffee at the University of Kashan . The powder of coffee was used for the test. The coffee The sample was analyzed on a Bruker D8 Advance X-ray diffractometer after being discharged into a low-noise sample holder. X-ray diffraction was analyzed at a voltage of 40 kV, at a current of 40 mA and had a copper radiation of 1.54060 Å. Conducted at 0.02°/min with a time constant of 1.2 sec in the 2° range from 10° to 40° (Gangadoo *et al*., 2017 .

Fourier Transform Infrared Spectroscopy (FTIR)

By FT-IR Spectrophotometer the prepared formulations transmittance was accomplished, The data set was calculated on 64 scans in a spectral range of 400 cm^{-1}-4000cm^{-1} at a resolution of 2 cm^{-1}(Tugarova *et al*., 2018) .

Bactericidal activity of coffee

The effectiveness of coffee as a bacteria killer (Bactericidal) was measured using their different concentrations (5%, 10%,15%) by viable cell count method (Toda *et al.*, 2019)

RESULTS AND DISCUSSION

Isolation and identification of *P. aeruginosa*

Among 200 samples collected from burn to investigate *P. aeruginosa*, only 30 isolates of bacteria. Only 30 isolates (15%) were grown on Cetrimide agar medium. It was characterized by a smooth, mucous growth in a creamy color, flat edges, and a raised center, and it had a fruity smell. The results of the biochemical tests showed that *P. aeruginosa* was positive for catalase oxidase, growth on cetrimide agar in the form of blue-green colonies and production of hemolytic B on blood agar (at 37°C for 24 hours). All isolates were positive for oxidase, which is an important test for the diagnosis of *P. aeruginosa*. All isolates were positive for catalase due to their ability to produce the enzyme catalase (Shila *et al.*, 2023).

Antibiotic susceptibility test

The results showed that the bacteria were resistant to piperacillin 15/30 (50%) and to piperacillin - tazobactam (4/30(13.3%), ceftazidime and cefepimewere each one (25/30(83.3%), Aztreonam 8/30(26.6%), Imipenem5/30 (16.6%), Gentamicin 12/ 30(40%) 30, Tobramycin9/30 (30%), Amikacin 6/30(20%), ciprofloxacin 13/30 (43.3%), ofloxacin 5/30(16.6%) (Fig. 1).

The presented study agreed with (Othman *et al*., 2014) and also with (Abdu lammer, 2018) in proving that *P. aeruginosa* is multi-drug resistant to antibiotics, but the resistance rate with a higher percentage and the same for (Javiya *et al*., 2008 ; Nouri *et al*., 2016.)

This results of the study disagrees with some previous researchers who showed that the number and percentage of ciprofloxacin-resistant *P. aeruginosa* isolates were only 29% (17), 28.9% (18).

The local study (46.96%) carried out by (Nader *et al*., 2017) was the same or very near to the results of the study about resistance against aminoglycosides (Amikacin, tobramycin and Gentamicin) in current

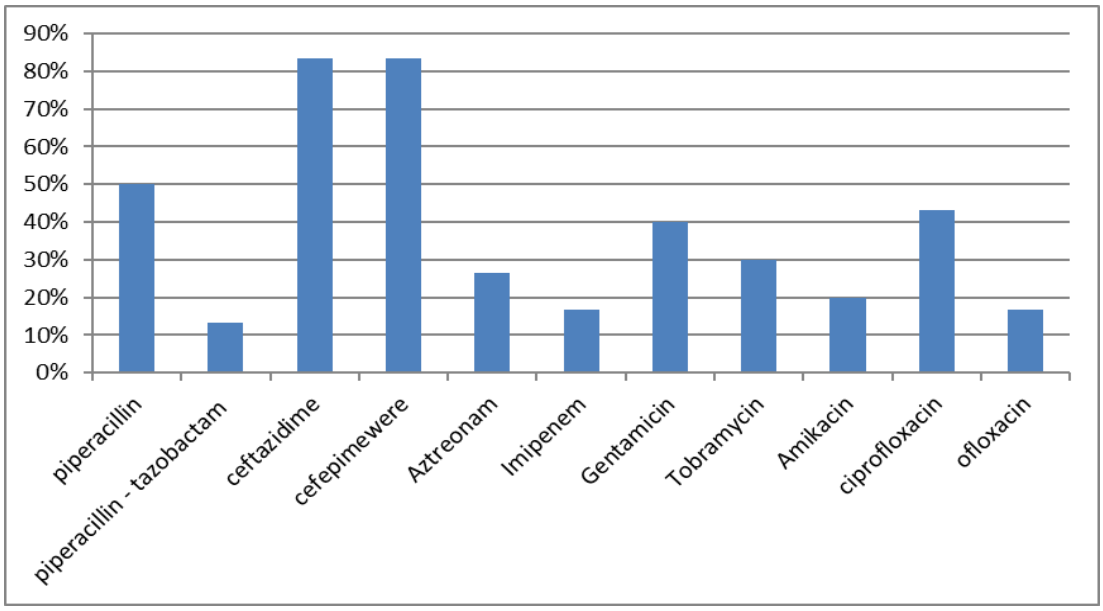

Fig. 1. *Multidrug resistance pattern of P. aeruginosa isolates*

study (40%).

The bacterial resistance to aminoglycoside is because aminoglycoside modifying enzymes and the production of βlactamase enzymes, which act to destroyed β-lactam (Al-Marjani, (2014); Boehr *et al.,* 2022).

Sensitivity of bacteria to coffee

Because of the role of alternative medicine in combating antibiotic-resistant bacteria and observing the effects of antibiotics on humans, the present study turned to the medicinal plant and studied its importance as an anti-bacterial .Coffee is one of the beverages people have used since ancient times, and it is still the most important beverage in our lives today. That is why we went to study its beneficial effects, especially its effect on *P. aeruginosa* antibiotic-resistant bacteria (Al-Dulami, 2017).

Bacteria were affected by different concentrations by measuring the diameter of inhibition in millimeters, where the effect increased the higher the concentration, as shown in Table 1.

The two coffee samples were approximately equally effective. The sensitivity of *P. aeruginosa* Isolates to coffee extract and its preparation was consistent with earlier studies of Toda *et al.* (2018) as well as its preparation corroborates earlier studies of (Mahajan and Arora, 2019`; Toda *et al*., 2015)

Chemically complex in composition, coffee contains 10 -13% protein substances, 15% sugars, “glucose, dextrin”, 10-13 fatty acids, consisting mainly of some acids. Among coffee's most important active substances are caffeine and tannin, to which the astringent effect is attributed (Belitz *et al*., 2019).

Greater resistance of Gram-negative bacteria to plant extracts has been reported (Toda *et al*., 2015) and this could be attributed to the differences in their cell wall structure. There may be differences between this study and other studies presented previously or recently due to strain variations, the sources and infusion strength of the various samples (coffee) (Toda *et al.*, 2015).

Bactericidal activity of coffee

The data obtained by Agar diffusion assay was supported by the viable cell count method. When the bacteria were incubated with aqueous extract prepared at a concentration of 5%, the incubation regressed, resulting in a steady decline of viable cell counts as indicated in Fig.2

As shown in Fig.2, the complete killing could not be obtained even after 24 h. However, in earlier studies (Lin *et al*., 2017), 100% killing was observed within 3-4 h, which might be attributed to the high concentrations (20–25%) of tea and coffee used.

Table 1. Comparative sensitivity of bacteria (in mm) to standard antibiotics and aqueous extract coffee

Coffee concentrate	Sensitivity of Bacteria to Coffee (Damping diameter in mm)		Sensitivity of Bacteria to Standard Antibiotics (Damping diameter in mm)	
	CF A	CF B	A	C
5%	20.4	21.4	—	16
10%	22.5	23.6		
15%	30.5	35.2		

A = Ampicillin, C = Chloramphenicol,

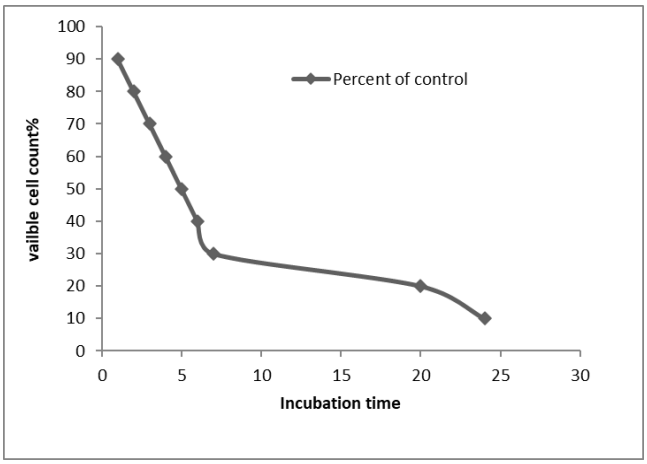

Fig. 2. *Viable cell count of different bacteria with 5% aqueous Coffee extract*

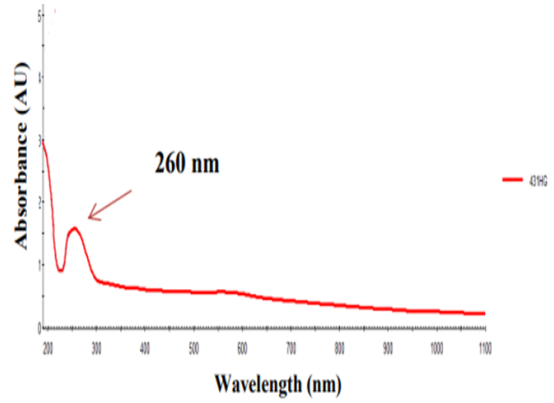

Fig. 3. *UV- Visible Spectophotometry of the coffee*

Characterization of the antibacterial compound in coffee

UV-Visible Spectroscopy

UV-Visible Spectrophotometer is a proven technology for the detection of active compounds in most bioactive compounds. After 24 hours of incubating the reaction mixture, it was observed that a color change in the reaction indicated the presence of active compounds. The biosynthesis of the active compounds can be confirmed by measuring the absorption band using UV spectroscopy in the region of 200-1100 nm. The single peak was at 260 nm (Fig. 3) .

There was a lot of study about coffee formation because various absorption peaks in UV-vis spectra indicate the presence of antibacterial compound from coffee (Hemalatha *et al*., 2014) . The peak appeared at nearly 362 nm and had a maximum absorption peak at 650 nm (Ullah *et al*., 2021) with strong absorption bands at 265 nm and 265.5 nm, respectively (Santanu *et al.,* 2015; Shubharani *et al*., 2019)**.**

X-ray diffraction

X-ray diffraction (XRD) analysis using Powder X-Ray Diffractomete) further characterised the formation of the antibacterial compoundr. The study showed a characteristic peak at 2θ value of 23.601, 29.896 and 44.015 Fig.4.

XRD results showed that synthetic antibacterial compound are crystalline, a natural form (Shubharani *et al.,* 2019). However, other experiments showed that the antibacterial compound had no specific crystalline form, and amorphous was seen (Shakibaie *et al.,* 2018; Borah *et al.,* 2021). The XRD analysis for the antibacterial compound from Coffee indicated three intense peaks in the whole spectrum of 2θ values ranging from 5 to 80. The diffractions peak at 2θ value of 23.780, 29.797 and 43.878 can be indexed to the 100, 101 and 102 planes of the antibacterial compound (Singh *et al.,* 2019)

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to obtain information about chemical compounds involved in the reduction and stabilization of antibacterial compounds, as shown in Fig. 5.

These results indicate the presence of various functional groups as biomolecules, such as hydroxyl groups in polyphenols and amide groups in proteins, having a key role in the reduction of selenium ions to their element and in the stabilization of the formed antibacterial compound (Fardsadegh *et al.,* 2019) . With the overall observations, it can be concluded that the proteins might have formed a capping agent over the antibacterial compound, which may respond to their stabilization (Sonkusre *et al.,* 2014).

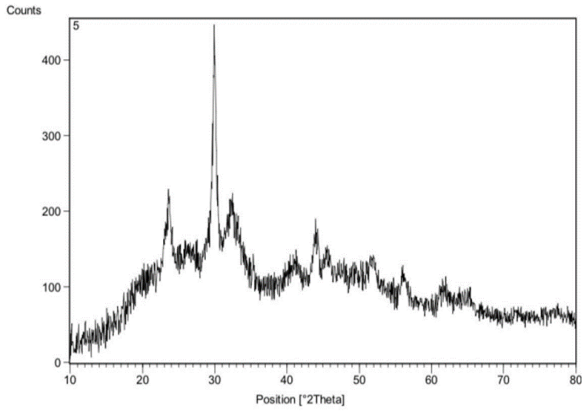

Fig. 4. *XRD pattern of antibacterial compound in coffee*

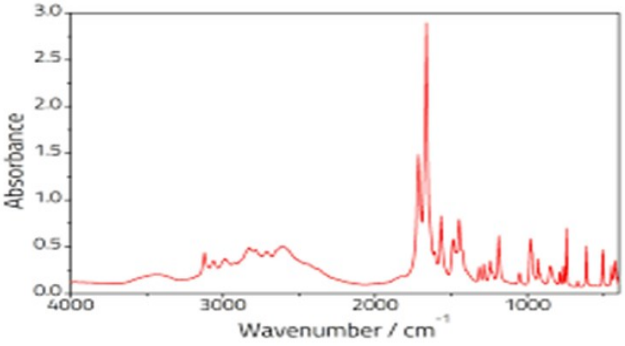

Fig. 5. *FTIR of antibacterial compound*

Conclusion

The Arabica coffee plant is one of the medicinal plants with high efficiency in treating and resisting many infections caused by *P. aeruginosa*. Diagnosis with different techniques showed that they contained many biologically active chemical compounds. Coffee showed great efficacy as an antibacterial against antibiotic-resistant bacteria isolated from pathological infections.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abdulammer, H. (2018). Detection of quinolones chromosomal resistance in Pseudomonas aeruginosa isolated from wounds infection. MSc. thesis in microbiology. Biology department. College of Science. Baghdad .
2. Al Dawodeyah, H. Y.; Obeidat, N.; AbuQatouseh, L. F. & Shehabi, A. A. (2018). Antimicrobial resistance and putative virulence genes of Pseudomonas aeruginosa isolates from patients with respiratory tract infection. *Germs.* 8, 31
3. Al-Dulami, H.F.L. (2017). Assessment the Effect of Quorum Sensing genes (lasI, rhlI) and Some Plant Extracts on Some Virulence Factors of Pseudomonas aeruginosa Isolated from Different Clinical Sources. MSc. Thesis. Department of Biology. College of Science. University of Anbar, Iraq.
4. Al-Marjani, M. F. (2014). Presence of qnr gene in environmental and clinical Pseudomonas aeruginosa isolates in Baghdad. *International J Microbiology and Applied Sci Issn.* 3: 853-857
5. Basri, D.F.and Fan, S.H. (2005). The potential of aqueous and acetone extracts of galls of Quercus infectoria as antibacterial agents. *Indian Journal of Pharmacology*. 37, 26–29 .
6. Belitz HD, Grosch W & Schieberle P. (2019). Coffee, tea, cocoa. *Food Chemistry. 4th ed*.:938-970
7. Boehr, D. D.; Jenkins, S. I. & Wright, G. D. (2022). The Molecular Basis of the Expansive Substrate Specificity of the Antibiotic Resistance Enzyme Aminoglycoside Acetyltransferase-6′- Aminoglycoside Phosphotransferase-2" The Role of ASP-99 as an Active Site Base Important for Acetyl Transfer. *Journal of Biological Chemistry*. 278: 12873-12880
8. Borah, S. N., Goswami, L., Sen, S., Sachan, D., Sarma, H., Montes, M., Peralta-Videa, J.R., Pakshirajan, K., & Narayan, M. (2021). Selenite bioreduction and biosynthesis of selenium nanoparticles by Bacillus paramycoides SP3 isolated from coal mine overburden leachate. *Environmental Pollution.* 285, 117519.
9. Bushman, J. (2018). Green tea and cancer in humans: a review of the literature. *Nutrition and Cancer*. 31, 151–159 .
10. Cowan, M.M.(1999). Plant products as antimicrobial agents. *Clinical and Microbiological Reviews*, 12, 564–582 .
11. Fardsadegh, B., Vaghari, H., Mohammad-Jafari, R., Najian, Y., & Jafarizadeh-Malmiri, H. (2019). Biosynthesis, characterization and antimicrobial activities assessment of fabricated selenium nanoparticles using Pelargonium zonale leaf extract. *Green Processing and Synthesis*, 8(1), 191-198.
12. Fatima, S.; Farooqui, A.H.A.; Kumar, R.; Kumar, T.R.S. & Khanuja, S.P.S. (2021) . Antibacterial activity possessed by medicinal plants used in tooth powders. *Journal of Medicinal and Plant Sciences*, 22, 187–189 .
13. Gangadoo, S., Stanley, D., Hughes, R. J., Moore, R. J., & Chapman, J. (2017). The synthesis and characterisation of highly stable and reproducible selenium nanoparticles. *Inorganic and Nano-Metal Chemistry*, 47(11), 1568-1576.
14. Hemalatha, T., Krithiga, G., Santhosh Kumar, B., & Sastry, T. P. (2014). Preparation and characterization of hydroxyapatite-coated selenium nanoparticles and their interaction with osteosarcoma (SaOS-2) cells. *Acta Metallurgica Sinica (English Letters)*, 27(6), 1152-1158.
15. Javiya, V. A., Ghatak, S. B, Patel, K. R. & Patel, J. A. (2018). Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. *Indian journal of pharmacology*. 40: 230 .
16. Lin, J., Opaku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., & Jager, A.K. (2017). Preliminary screening of some traditional Zulu medicinal plants for anti -inflammatory and antimicrobial activities. *Journal of Ethnopharmacology,* 68, 267–274.
17. Mahajan, V., Arora, D.S. & Sabharwal, U.(2019). Antibacterial activity of some tea samples. *Indian Journal of Microbiology*, 31, 443–445 .
18. McKay, D.L. & Blumberg, J.B. (2021). The role of tea in human health: an update. *Journal of American College of Nutrition*, 21, 1–13.
19. Nader, M. I., Kareem, A. A., Rasheed, M. N. & Issa, M. A. (2017). Biofilm Formation and Detection of pslÁ Gene in Multidrug Resistant Pseudomonas aeruginosa Isolated from Thi-Qar, Iraq. *Iraqi Journal of Biotechnology*. 16, 89-103 .
20. Nouri, R., Rezaee, M. A., Hasani, A., Aghazadeh, M. & Asgharzadeh, M. (2016). The role of gyrA and parC mutations in fluoroquinolones-resistant Pseudomonas aeruginosa isolates from Iran. *Brazilian journal of microbiology.* 47: 925-930.
21. Othman, N., Babakir-Mina, M., Noori, C. K. *&* Rashid, P. Y. (2014). Pseudomonas aeruginosa infection in burn patients in Sulaimaniyah, Iraq: risk factors and antibiotic resistance rates. *The Journal of Infection in Developing Countries*. 8: 1498-1502 .
22. Sagun, E., Durmaz, H., Tarakei, Z & Sagdic O. (2016). Antibacterial activities of the extracts of some herbs used in Turkish herby cheese against Listeria monocytogenes serovars. *International Journal of Food Properties*, 9, 255–260 .
23. Santanu, S., Sowmiya, R., & Balakrishnaraja, R. (2015). Biosynthesis of selenium nanoparticles using citrus reticulata peel extract. *World Journal of Pharmaceutical Research,* 4(1), 1322-1330
24. Sesso, H. Gaziano, J. Buring, J. & Hennekens, C. (2019). Coffee and tea intake and the risk of myocardial infarction. *American Journal of Epidemiology*, 149, 162–167 .
25. Shakibaie, M., Jafari, M., Ameri, A., Rahimi, H. R. & Forootanfar, H. (2018). Biosynthesis and physicochemical

characterization, and cytotoxic evaluation of selenium nanoparticles produced by streptomyces lavendulae FSHJ9 against MCF-7 cell line. Journal of Rafsanjan University of Medical Sciences, 17(7), 625-638.

26. Shila, S.J., Ahmed, N.N. & Dastogeer, K.M.G. (2022). Detection of Pseudomonas syringae pv. lachrymans associated with the seeds of cucurbits. *Universal Journal of Agricultural Research*. 1, 1-8
27. Shubharani, R., Mahesh, M., & Yogananda Murthy, V. (2019). Biosynthesis and characterization, antioxidant and antimicrobial activities of selenium nanoparticles from ethanol extract of Bee Propolis. *Journal of Nanomedicine and Nanotechnology,* 10:522, 1-7.
28. Singh, V., Basu, A., Shivapriya, P. M., Varadwaj, P. K., Samanta, S. K., & Sahoo, A. K. (2019). Exploration of interactions of 'bloodnano interface'of carbon-based nanomaterials for biomedical applications. *Journal of Materials Research,* 34(11), 1950-1964.
29. Sonkusre, P., Nanduri, R., Gupta, P., & Cameotra, S. S. (2014). Improved extraction of intracellular biogenic selenium nanoparticles and their specificity for cancer chemoprevention. *Journal of Nanomedicine and Nanotechnology*, 5(2), 194.
30. Streeter, K. & Katouli, M. (2016). Pseudomonas aeruginosa: A review of their Pathogenesis and Prevalence in Clinical Settings and the Environment
31. Toda, M., Okubo, S., Hara, Y., . Shimamura, T. (2015). Antibacterial and bactericidal activities of tea extracts and catechins against methicillin resistant Staphylococus aureus. *Japanese Journal of Bacteriology*, 46, 839–845.
32. Toda, M., Okubo, S., Hiyoshi, R. & Shimamura, T. (2019). The bactericidal activity of tea and coffee. *Letters in Applied Microbiology*, 8, 123–125
33. Toda, M., Okubo, S., Hiyoshi, R. & Shimamura, T.(2018). The bactericidal activity of tea and coffee. *Letters in Applied Microbiology*, 8, 123–125 .
34. Tugarova, A. V., Mamchenkova, P. V., Dyatlova, Y. A., & Kamnev, A. A. (2018). FTIR and Raman spectroscopic studies of selenium nanoparticles synthesised by the bacterium Azospirillum thiophilum. Spectrochimica Acta Part A: *Molecular and Biomolecular Spectroscopy*, 192, 458-463.
35. Ullah, A., Yin, X., Wang, F., Xu, B., Mirani, Z. A., Xu, B., Chan, M.W.H., Ali, A., Usman, M., Ali, N., & Naveed, M. (2021). Biosynthesis of selenium nanoparticles (via Bacillus subtilis bsn313), and their isolation, characterization, and bioactivities. *Molecules*, 26(18), 26185559
36. Yang, C. & Landau, J. (2020). Effects of tea consumption on nutrition and health. *Journal of Nutrition*, 130, 2409–2412 .