Synergistic Effect of Aloe Vera L. Extract Methanolic With Some Antibiotics Against S. Aureus, E-Coli and Klebslia Isolated From the Eye Affected by Inflammation

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ABSTRACT
The current study included the collection of (300) samples from (150) people from patients visiting Balad General Hospital and some private clinics in Balad District - Salah Al-Din Governorate, a sample was taken from both the right and left eyes using special dry and sterile cotton swabs that can be disposed of later, for the period ranging from November 2022 to January 2023, (235) positive samples for bacterial growth were obtained, constituting 78.3% of the total samples collected, positive growth samples were diagnosed depending on their morphological, farming and biochemical properties, and the diagnosis was confirmed for a number of them using the Vitek2 system technology. The results showed obtaining 140 isolates out of a total of (235) positive samples for the Gram stain by (59.6%), while the negative isolates of the Gram stain were (95) isolates and amounted to (40.4%) of the total isolates, S. aureus was 27.65% and E-coli 10.63%, while Klebsiella was 8.51% of the total bacterial isolates isolated from the eye, including (staphylococcus aureus, staphylococcus epidermidis, Bacillus, Streptococcus Viridians, Diphtheria, Pseudomonas aeruginosa, Klebsiella, E-coli, Neisseria). The results showed the synergistic effect of the alcoholic extract (methanol) of the aloe vera plant (Aloe Vera L.) with antibiotics a significant effect compared to methanol alcoholic extract alone, the results of the study also showed that the antibiotic Ciprofloxone synergistically with the alcoholic extract methanolic of the aloe vera plant (Aloe Vera L.) it had the highest effect already synergistically with most of the bacterial species under study.

1. Introduction

There are many causes of eye infection, but bacteria are the most common microbial cause, as infection usually begins with sensitivity to the treatments used, however, these antibiotics do not completely eliminate the infection, which means that there is another cause involved in the sustainability of bacterial infection, which is the bacteria without wall (CWDB), in addition to other pathogens such as viruses, mycoplasmas, chlamydia and fungi (Burns *et al*., 2019).

Excessive use of antibiotics leads to increased drug resistance or leads to normal flora changes (Egorov *et al* ., 2018), therefore, the researchers had to find alternatives to the antibiotics used, and one of these alternatives is the use of plant extracts or medicinal herbs, because they are major sources of production of medicines and medical drugs to treat various bacterial infections, including eye inflammation (Espín *et al*., 2024). Aloe vera L. is one of the plants that are currently widely used in the field of pharmaceuticals, Aloe vera gel is added in most cosmetics and with some nutrients, eye cosmetics were also used in the past to emphasize and highlight the eyes to this day and are still used by females of different age groups on a large scale of the world (Sanchez, 2020).

From the foregoing, it is clear the importance of studying the inhibitory activity of methanolic Aloe Vera L (Saadh *et al.,* 2024a). extract in synergy with antibiotics for the growth of negative and positive Gram stain pathogenic bacteria, because it contains many anti-bacterial growth compounds, hence the idea of this study began, which aimed to:

1-Isolation and diagnosis of some bacterial species that cause eye inflammation from patients attending Balad General Hospital and some private clinics.

2-Testing the sensitivity or resistance of bacterial isolates taken from the eye to different types and

concentrations of antibiotics.

3-Determine the synergistic effect of the methanolic extract of the aloe vera plant Aloe Vera L, with different antibiotics, thus determining which is better at inhibiting bacterial etiology.

2. Methodology

Preparation of Culture Media

Ready-made culture media were prepared according to the instructions of their manufacturers and fixed on the packaging of each medium, the pH was adjusted to 7.0 and sterilized according to the type of culture medium at 121°C and under pressure of 15 lb./ang2 for 15 minutes, after that, the planting media was incubated after pouring them into the dishes or tubes according to the requirements of the experiment at 37 m for 24 hours to ensure that there was no contamination, then kept in the refrigerator at a temperature of 4 ° C until use and attended the media according to what was stated in (Harley and Prescott, 2002).

Bacterial Culture of Samples

Bacterial culture of incubated samples with nutritious broth was carried out in the laboratory, where three bacterial culture media were prepared, namely the middle of blood agar, the center of the MacConkey agar and the medium of saline mannitol, then transfer part of the sample incubated with nutritious broth using a flame-sterilized germ carrier, in an amount filled with the carrier's loop to be planted on dishes containing the culture media, all the dishes were then incubated in the incubator at 37 pm for 24 hours, after which the growing colonies were examined and the required isolation and diagnostic tests were conducted.

Plant Collection and Sample Preparation:

Aloe vera leaves of 30-60 cm in lengths were collected from a group of nurseries belonging to the city of Balad / Salah Al-Din, the plant was classified by Dr. Omar Khalil Jassim Al-Abbasi, a plant classification specialization in the research and development department of the State Company for Pharmaceutical Industry / Samarra, where the leaves were cleaned and washed with distilled water several times to clean them of dust and dust, and then left to dry in the air, they were left in the shade for several days until they dried and then cut into small pieces to be ready for extraction.

Alcoholic Extraction

80% methanolic alcoholic solvent was used in the preparation of the extract, mixing 100 g of dried and cut aloe leaves in a glass flask with 500 ml of the specified solvent, then leave the mixture at room temperature for 72 hours, shaking from time to time and at frequent intervals, after which the mixture was filtered using pieces of sterile gauze and then using filter paper Whatman, then take the filter and evaporate the rotary evaporator at a temperature of 55 ° C until the solvent is completely disposed of, the remaining material was then placed in airtight glass containers in the refrigerator until use **(Khanal *et al.*, 2021).**

Sterilization of Alcoholic Extracts of the Aloe Vera Plant:

Prepare the alcoholic extract by dissolving 1 g of dry alcoholic extract in 10ml of distilled water to obtain a concentration of 200%, the mixture was then sterilized by pasteurization at a temperature of 62.8 for 30 minutes, thus obtaining the standard concentration that was used to obtain the rest of the fear.

Synergistic Effect Test of Methanolic Alcoholic Extract with Antibiotics:

The synergistic effects of plant extracts with antibiotics were tested by tablet diffusion, plant extracts were mixed with antibiotics based on (Muhanna,2008) Where the plant extract was used in different concentrations by taking (1) milliliter of vegetable extract at a concentration of (200, 100, 75, 50, 25) milligrams / milliliter respectively and placed in a test tube (Test Tube), then the following

antibiotics were added to it the same volume (Chloramphenicol, Azithromycin, Cefraxone, Ceprofloxacin, Tetracycline, Gentamicin, Doxycycline, Tobramycin, Neomycin, Erythromycin, Trimethoprim), the concentration is as follows: (10, 15, 10, 10, 10, 10, 10, 10, 10) ug / ml and shake the tube well until the antibiotic mixes well with the plant extract, sterile filter paper tablets with a diameter of (6) mm of the type (Whatman No. 1) were prepared, and then these tablets were immersed in the mixture, as well as the tablets representing control were immersed in distilled water, then the tablets were fixed on the dishes containing the medium (Muller-Hinton Agar) and inoculated with diagnosed bacterial samples, then the dishes were incubated at a temperature of 37 ° C for 24 hours, after the completion of the incubation period, the diameter of the inhibition area around the disc was measured in mm, to observe whether there was a synergistic reaction between the antibiotic and the plant extract or not.

Statistical Analysis:

SAS (2004) was used in statistical analysis of data to study the effect of antibiotics, plant extracts and studied treatment concentrations on inhibition ratio, significant differences between averages were compared with the least significant difference (LCD) test, the Chi-square selection (x2 – Chi – Square) was also used to compare the significant differences between the studied ratios **(Levesque, 2007).**

3. Results and Discussion

Diagnosis of Isolates of Identification:

Isolates were diagnosed using culture and microscopic examinations as a primary diagnosis, depending on the shape, texture and shape of colonies, the results of microscopic diagnosis based on the Gram stain showed that 140 isolates out of a total of 235, which constitutes 59.6% of the bacterial isolate models, were positive for the Gram stain, while the negative bacterial isolates of Gram stain reached 95 isolates and constitute a percentage of 40.4% as shown in Table (1), this study was somewhat consistent with the study (**Hassan, 2019),** the percentage of bacterial isolates positive for Gram stain was 75%, while the percentage of bacterial isolates negative for Gram stain was 25%

Table (1) Numbers and Ratios of Gram-Positive and Negative stain Bacterial Isolates

Type of isolates	Number	Percentage %
Positive for Gram stain	140	59.6
Negative for Gram stain	95	40.4
Total	235	100.0

The results showed the predominance of Staphylococcus aureus by 27.65% of the total isolates as their colonies appeared when developed on the medium of blood agar as they cause complete decomposition of red blood cells (β hemolysis) around the colony, the bacteria were able to grow on the solid Mannitol salt agar medium, which is a selective medium suitable for the initial isolation of the staphylococcus group, separating the Staphylococcus aureus from other species of this group, this medium is characterized by containing sodium chloride NaCl at a concentration of 7.5%, which works to inhibit other microorganisms that are intolerant to high salinity, staphylococcus aureus is also distinguished from other types of the genus Cocci by its ability to ferment mannitol sugar and transform the medium from pink to yellow due to the presence of a red methyl reagent in the medium (Hrycko *et al.*, 2022).

Pseudomonas aeruginosa isolates ranked second with 14.89% of the total isolates, pale colonies were selected, unfermented for the lactose sugar in the medium, smelling similar to that of fermented grapes on the hard MacConkey medium, and dark in color (Kumar *et al.*, 2024), mostly surrounded by a transparent halo on the blood acaron medium, evidence of blood analysis and hemolysine production, King A medium had adequate concentrations of growth-promoting potassium and magnesium salts, so most isolates were producing blue-green biocyanin (Lahij *et al.*, 2021).

Staphylococcus epidermidis isolates appeared in 14.89% as white colonies on central Mannitol salt agar, it has the advantage that it does not have the ability to ferment mannitol sugar (Namvar *et al.*, 2014). Microscopic examination has shown staphylococcus positive for the Gram stain (Hadi *et al*., 2024).

E.Coli isolates accounted for 10.63% of the total isolates and their colonies appeared pink on the medium of MacConkey as a result of their dry lactose fermentation of medium size, convex and regular on the medium of MacConkey and negative for oxidase testing, Neisseria gonorrhoeae isolates accounted for 6.38% of the total, as they appeared in the form of transparent and swollen colonies (Hsu *et al*., 2024), they appeared on the center of the Theyar Marten medium, which is considered one of the selective circles in the form of small swollen gray colonies, microscopy of the previous three genera was negative for the Gram stain. Table (2) shows the number and percentage of bacterial isolates.

Table (2) Bacterial isolates and their percentages

Isolated genders	Gram stain	Number	percentage
Staphylococcus aureus	+	65	27.65
Staphylococcus epidermidis	+	35	14.89
Bacillus	+	15	6.38
Streptococcus viridians	+	10	4.25
Diphtheria	+	15	6.38
Pseudomonas aeruginosa	-	35	14.89
Klebsiella	-	20	8.51
E. coli	-	25	10.63
Neisseria gonorrhoeae	-	15	6.38
Total		235	100%

Biochemical tests were relied upon for the purpose of species differentiation and confirmation of the initial diagnosis, as shown in Table (3).

The results of this study converged with what was indicated by (Abdel Wahab and **(Hussein, 2015),** it was indicated that, Staphylococcus aureus had predominance in conjunctival injury by 40%, while the percentage of aeruginosa was 10%, while the E.coli isolates were 14%, these results were somewhat consistent with(EwadhP *et.al*, 2014) who indicated that 119 of the 191 isolated isolates from eye injuries were positive for Gram stain while 75 isolates were negative for Gram stain. Staphylococcus aureus isolates ranked first with 38.6%.

This study also somewhat agreed with the study (Saadh *et al*., 2024b), which indicated that 67.4% of isolated isolates from eye injuries were positive for the Gram stain. Staphylococcus aureus isolates ranked first while Gram-negative isolates were 32.5%.

The different isolation rates of bacteria and their types in these studies may be due to several reasons, including the different seasons of sample collection, isolation times, different geographical location of the isolation, the number of samples, and other influencing factors, as well as the most important factor, which is antibiotics and their misuse (Behmagham *et al.*, 2024), whether the patient is taking drugs that prevent the growth of bacteria or those that are resisted by bacteria and grow, which contributed significantly to their spread (Ortiz *et al*., 2024).

The superiority of positive bacteria compared to negative bacteria is due to the fact that some types of positive bacteria are coexisting with humans and form part of the natural flora of the skin (Kalanuria *et al.*, 2014). Positive bacteria are also characterized by containing a layer of peptidolycans in the form of your name from negative bacteria and techoiccid acid (Techoiccid) (Valverde *et al*., 2024), and some protein components that facilitate and help colonize the host tissues and inhibit the process of phagocytosis, as well as the activity of a number of toxins that break down host tissues, cause

pathological symptoms and gnaw tissues (Kojima *et al.*, 2022)

Studies confirm the presence of staphylococcus in the conjunctiva and eyelids because they are concentrated in wet areas, namely the whites of the eyes and eyelids, where the presence of enzymes and tears is concentrated **(Pourmand *et al.*, 2009).**

Table 3 Biochemical Test Results for Various Bacterial Isolates Under Study

Test Type	***Stre.viridans***	***Diphtheria***	***S.aureus***	***S.epi.***	***N.gon.***	***E.coli***	***P.auroginosa***	***Klebsiella***	***Bacillus***
Gram stain	-	+	+	+	-	-	-	_	+
Catalase enzyme test	+	+	+	+	+	+	+	+	+
Oxidase Test	+		-	-	+	-	+	_	+
Indole Production Test	-				+	-	_	_	_
Red Instance Test	-		/	/	-	+	+	_	+
Fox-Proskaur Test	-		/	/	/	-	_	_	+
Jacket consumption test	+		/	/	+	+	+	+	+
Motion Test	+		-	-	-	+	+	_	+
Urease examination	+	-	+	+	-	+	+	+	_
H2S output	-		-	-	-	+	_	_	_

(+) Positive for testing

(-) Negative for testing

(/) Failure to perform the examination

A=Acid K=Alkalain

Staphylococcus aureus has been isolated from people with keratitis, contact lenses and others, as these lenses cause keratitis and may lead to vision loss, staphylococcal bacteria are mainly concentrated in the conjunctiva, it is the first cause of conjunctivitis, as it is part of the normal flora of the upper respiratory tract, especially the nasal area, it is transmitted from the nose to the eye through the tear ducts and occurs inflammation of the inside of the eye and the eyelid area and occurs conjunctivitis. (Haas *et al.*, 2012; Chowdhary *et al*., 2024). As for the negative isolates, the isolates of the bacteria Pseudomonas aeruginosa ranked first because of their wide spread in nature and their ability to adapt, in addition to its ability to produce a number of enzymes, including the enzyme protease and collagenase, which cause conjunctivitis and secondary keratitis, the ability of aeruginosa isolates to form a biofilm is one of the reasons for their spread and prevalence (Al-Janabi, *et al.*, 2013).

Synergistic effect test between aloe vera plant methanolic extract and antibiotics

The study showed an increase in the inhibitory efficacy of aloe vera methanolic extract synergistic with antibiotics against the bacterial species under study with no significant difference between the third and fourth concentration already synergistic with antibiotics against S. aureus bacteria, a significant difference was observed between the rest of the concentrations, as the highest average inhibition of the synergistic act was between the antidote (DO) and the methanolic extract Table (4). The study agrees with the findings of (Chacón *et al.*, 2019) in their study that the use of aloe vera, either alone or in combination with synthetic antibiotics (Saadh *et al.*, 2024c), is effective in inhibiting and delaying the onset of resistance in strains S.aureus ATCC 29213 and S.aureus ATCC 43300, these results show that compounds of natural origin are a viable alternative to address the

current problem of bacterial resistance, antibiotics taken with aloe vera make it possible to counteract the problem of excessive use of synthetic antibiotics in the treatment of infections and bacterial infections.

Table 4: Synergistic effect between aloe vera plant methanolic extract and antibiotics against S. aureus bacteria

Type of antibiotic	Concentration in milligrams					Average synergistic effect
	1 25	2 50	3 75	4 100	5 200	
GEN	15	13	9	12	22	14.2 C
AZM	17	13	7	12	18	13.4 CD
CRO	17	17	17	17	21	17.8 B
CIP	17	14	6	12	20	13.8 CD
E	15	4	11	4	17	10.2 F
C	15	16	18	15	18	16.4 B
DO	20	20	19	19	19	19.4 A
N	15	10	5	6	20	11.2 EF
TOB	11	10	4	3	13	8.2 G
TE	8	13	11	11	17	12.0 DE
TMP	12	10	7	7	18	10.8 F
Average concentration	14.7 b	12.7 c	10.4 d	10.7 d	18.5 a	

In E. coli bacteria, the results showed that there was no significant difference between the averages of the first and second concentrations already synergistically with the antibiotics under study, the rate of inhibition was already synergistic with the anti-CRO with an inhibition diameter of (23.8) mm. Table (5), the natural bioactive ingredients found in parts of plants are deterrent to pathogenic agents. Aloe vera leaf extracts in synergy with potential antimicrobials showed activities for both S. aureus and E. Coli in places different concentrations of plant extracts, the extracts had a greater synergistic antimicrobial activity than when used separately against pathogenic microbes, thus, the results of the current study indicate that these plant extracts can be used against antibiotic-resistant bacteria to improve the performance of these antibiotics (FataloFalaro and Sitota, 2020)

Table 5: Synergistic effect between aloe vera plant methanol extract and antibiotics against E.coli bacteria

Type of antibiotic	Concentration in milligrams					Average synergistic effect
	1 25	2 50	3 75	4 100	5 200	
GEN	**18**	**15**	**17**	**16**	**23**	**17.8 CD**
AZM	**17**	**12**	**4**	**15**	**15**	**12.6**
CRO	**22**	**23**	**21**	**30**	**23**	**23.8 A**
CIP	**17**	**17**	**10**	**17**	**25**	**17.2 D**
E	**17**	**13**	**13**	**12**	**15**	**14.0 E**
C	**20**	**21**	**18**	**15**	**21**	**19.0 BC**
DO	**20**	**20**	**17**	**22**	**23**	**20.4 B**
N	**16**	**17**	**16**	**9**	**15**	**14.6 E**
TOB	**15**	**8**	**1**	**6**	**20**	**10.0 G**
TE	**15**	**17**	**20**	**14**	**16**	**16.4 D**
TMP	**11**	**16**	**7**	**10**	**16**	**12.0 F**
Average concentration	**17.1 b**	**16.3 by**	**13.1 d**	**15.1 c**	**19.3 a**	

As for Klebsiella bacteria, the results showed the highest synergistic effect between the average antibody (CRO) and the methanolic extract, the retarding diameter was (19.2) mm while the lowest inhibition diameter was synergistically with the anti-TOB with the retarding diameter (7.0) mm in Table (6).

Table 6: Synergistic effect between aloe vera plant methanolic extract and antibiotics against Klebsiella bacteria

Type of antibiotic	Concentration in milligrams					Average synergistic effect
	1 25	2 50	3 75	4 100	5 200	
GEN	12	12	22	15	16	15.4 B
AZM	12	5	7	13	20	11.4 D
CRO	22	17	20	16	21	19.2 A
CIP	23	7	12	13	17	14.4 BC
E	15	2	12	2	17	9.6 E

C	20	17	18	16	18	17.8 A
DO	21	20	17	18	18	18.8 A
N	16	7	20	5	17	13.0 C
TOB	10	2	5	5	13	7.0 F
TE	15	15	12	7	17	13.2 C
TMP	11	5	5	5	21	9.4 E
Average concentration	16.1 a	9.9 c	13.6 b	10.5 c	17.7 a	

The aloe vera plant used in the study was known for its medicinal importance, the current study examined the effect of the antibacterial extract of the aloe vera plant alone and in synergy with antibiotics against the bacterial genera under study (Jameel *et al*., 2024), antibacterial testing showed that to varying degrees of concentrations all extracts have antibacterial activity and have shown inhibition of bacterial growth that is more active at high concentrations and decreased activity at low concentrations, the study suggests that inhibition of bacterial growth activity depends on the dose of the extract and antibiotic (Saadh *et al*., 2024d ; Laylani *et al.,* 2024) .

Due to the side effects of synthetic drugs, the trend towards the use of herbal medicines has recently been considered (Karimi *et al*., 2018). Aloe vera is an antibacterial, antiviral, antifungal and anti-inflammatory herbal compound, this herbal compound has an inhibitory effect on many pathogens due to its phenolic anthraquinone compounds (Karimi *et al*., 2018 ; Mahmoud *et al*., 2024). In one study on aloe vera, one researcher indicated that it had anti-biosynthesis activity and thus reduced the virulence of many bacterial pathogens (Ghasemi *et al.,* 2020 ; Mejía *et al.,* 2024).

4. Conclusion

The current study concluded that the methanolic extract of Aloe Vera (Aloe Vera L.) exhibited significant inhibitory effects on the growth of certain bacterial species causing eye inflammation, particularly when used synergistically with antibiotics. This suggests that Aloe Vera extract can be an effective adjunct to traditional antibiotic treatments, enhancing their efficacy against bacterial infections in the eye.

Reference

[1] Abdulwahab, Hala Emad & Hussein, Taleb Abdullah (2015). Study of the effect of alcoholic and cold-water extract of fruits and leaves of the Sidr plant Ziziphus spina Christi against bacteria isolated from eye injuries (conjunctivitis) outside and inside the body, Baghdad Journal of Science, Vol. 12(1).

[2] Al-Janabi, A. O. F., Sarab, F., and Sarah, I. (2013). "Biofilms Formation on Contact Lenses: Clinical and Bacteriological Study." Diy. JM 5(2):11–18.

[3] Al-Shibli, Majid Kadhim, Al-Nashi, Ali Abdul Rahim, Al-Khafaji, Zahraa Khudair Abbas (2012). Isolation and diagnosis of bacteria that cause eye injuries Journal of Al-Qadisiya for Pure Science (quarterly) Al-Qadisiya Journal of Pure Sciences (quarterly), Year: 2012 Volume: 17 Issue: 3 Pages: 1-12.

[4] Behmagham, F., Mustafa, M. A., Saraswat, S. K., Khalaf, K. A., Kaur, M., Ghildiyal, P., & Vessally, E. (2024). Recent investigations into deborylative (thio-/seleno-) cyanation of aryl boronic acids. RSC advances, 14(13), 9184-9199.

[5] Burns, S. A., Elsner, A. E., Sapoznik, K. A., Warner, R. L., and Gast, T. J. (2019). Adaptive optics imaging of the human retina. Progress in retinal and eye research, 68, 1-30.

[6] Chacón, O., Natalia, F., Lissette, L., Rubén, M., Marcela, F., and Betty, S. M. (2019). "Effect of Aloe Barbadensis Miller

(Aloe Vera) Associated with Beta-Lactam Antibiotics on the Occurrence of Resistance in Strains of Staphylococcus aureus and Streptococcus uberis." European Journal of Integrative Medicine 32: 100996.

[7] CHOWDHARY, H., CHAUDHARY, D. N. K., HARAHSHEH, F. A. H., MUSTAFA, M. A., RAJAK, D. M., & TOMAR, R. K. (2024). TECHNICAL ANALYSIS OF INTERNET SHUTDOWNS: ECONOMIC AND CYBERSECURITY DIMENSIONS IN INDIA AND INTERNATIONAL CONTEXT. Journal of Theoretical and Applied Information Technology, 102(4).

[8] Espín, C. G. S., Morocho, W. M. B., Cordero, A. Á. S., Chandra, S., Bansal, P., Kaur, H., ... & Lasisi, A. (2024). Theoretical and experimental study of flower-like NiMoS/NiO/NF with interface layer as a novel highly efficient bifunctional-electrode toward supercapacitor and HER. Journal of Electroanalytical Chemistry, 960, 118163.

[9] EwadhP, R. M., Wathiq, A., Aldraghi, P., and Abdulnabi, J. A. (2014). "Genetic Study of The Etiology of Some Bacterial Pathogens in People with Inflammation of The Eye and to Investigate The Prevalence of The SEA Gene." Iraqi Journal of Biotechnology 13(2):23–34.

[10] FataloFalaro, T., and Sitota, T. T. (2020). "Antibacterial Efficacy of Aloe Vera L , Guava and Their Combined Leaf Extracts against Staphylococcus aureus and Escherichia coli Isolated from Mastitic Dairy Cows. An in-Vitro Study. " European Journal of Biological Sciences 12(1):1-9.

[11] Ghasemi, N., Mahsa, B., Mohammad , A., Elham, Z., and Hossein,S. K. (2020). "Antibacterial Properties of Aloe Vera on Intracanal Medicaments against Enterococcus faecalis Biofilm at Different Stages of Development" edited by B. Kukiattrakoon. International Journal of Dentistry 2020:8855277. doi: 10.1155/2020/8855277.

[12] Haas, W., Lynne, S. G., Christine, K. H., Christine, M. S., and Timothy, W. M. (2012). "Microbiological Etiology and Susceptibility of Bacterial Conjunctivitis Isolates from Clinical Trials with Ophthalmic, Twice - Daily Besifloxacin." Advances in Therapy 29:442–55.

[13] Hadi, E. F., Baharuddin, M. Z. B., Zuhdi, A. W. M., Ghadir, G. K., Al-Tmimi, H. M., & Mustafa, M. A. (2024). Enhancing Remaining Useful Life Predictions in Predictive Maintenance of MOSFETs: The Efficacy of Integrated Particle Filter-Gaussian Process Regression Models. International Journal of Safety & Security Engineering, 14(2).

[14] Harley, J. P. and Prescott, L. M. (2002): Laboratory Exercises in Microbiology, 5th ed. McGraw-Hill Companies, Inc., New York: 292- 293.

[15] Hassan, Sarah Abdel Hamid (2019). Determining the Activity of Some Plant Extracts against Some Types of Tekeria Isolated from the Eyes of Students Using Contact Lenses and Perfection at Tikrit University, Master's Thesis, College of Education for Pure Sciences, Tikrit University.

[16] Hrycko, A., Pedro, M-G., Courtney, C., Rebecca, L –W., and Benjamin, E. (2022). "Severe Bacterial Infections in People Who Inject Drugs: The Role of Injection-Related Tissue Damage." Harm Reduction Journal 19(1):1–13. doi: 10.1186/s12954-022-00624-6.

[17] Hsu, C. Y., Mustafa, M. A., Kumar, A., Pramanik, A., Sharma, R., Mohammed, F., ... & Abosaoda, M. K. (2024). Exploiting the immune system in hepatic tumor targeting: unleashing the potential of drugs, natural products, and nanoparticles. Pathology-Research and Practice, 155266.

[18] Jameel, M. K., Mustafa, M. A., Ahmed, H. S., jassim Mohammed, A., Ghazy, H., Shakir, M. N., ... & Kianfar, E. (2024). Biogas: Production, properties, applications, economic and challenges: A review. Results in Chemistry, 101549.

[19] Kalanuria, A. A., Wendy, Z., and Marek, M. (2014). "Ventilator-Associated Pneumonia in the ICU." Critical Care (London, England) 18(2):208. doi: 10.1186/cc13775.

[20] Karimi, N., Babak, G., Hamed, H., Bahareh ,M., and Hossein, S. K. (2018). "Antioxidant, Antimicrobial and Physicochemical Properties of

[21] Khanal , M. , Lamichhane , S. , Bhattarai , A. ; Kayastha , B. L. and Labh , S. N.(2021). Extract of AloeVera (Aloe barbadensis Miller) Enhance the Growth , Protein Contents and Gastrosomatic. Index(GaSI) of common carp cyprinus capio. Journal of Nutrition and Metabolism , 2021.

[22] Kojima, N., Shohei ,K., Shin, H., Yoshiki, O., Daisuke, Z., Yuta ,T., Emi, O., Shin, I. Y., Masato, N., and Yasuhiro, K. (2022). "Wall Teichoic Acid-Dependent Phagocytosis of Intact Cell Walls of Lactiplantibacillus Plantarum Elicits IL-12 Secretion from Macrophages." Frontiers in Microbiology 13(August):1–18. doi: 10.3389/fmicb.2022.986396.

[23] Kumar, A., Mustafa, M. A., Fouly, A., Bains, P. S., Sharma, R., Bisht, Y. S., ... & Singh, P. (2024). NiO x/PANI nanocomposite doped carbon paste as electrode for long-term stable and highly efficient perovskite solar cells. RSC advances, 14(19), 13374-13383.

[24] Lahij, H. F., Abdulla, H. A., Muthanna, H. H., and Luma, A. Y. (2021). "The Effect of Qourum Sensing Genes (LasI, RhlI) in Some Virulence Factors of Pseudomonas aeruginosa Isolated From Different Clinical Sources."Medico-Legal Update 21(1):303–8. doi: 10.37506/mlu.v21i1.2325.

[25] Laylani, L. A. A. S. S., Al-Dolaimy, F., Altharawi, A., Sulaman, G. M., Mustafa, M. A., Alkhafaji, A. T., & Alkhatami, A. G. (2024). Electrochemical DNA-nano biosensor for the detection of Goserelin as anticancer drug using modified pencil graphite electrode. Frontiers in Oncology, 14.

[26] Levesque. R.(2007). SPPS Programming and Data Management ,4th ed ., shicago, PP:522 .

[27] Mahmoud, Z. H., Ghadir, G. K., Al-Tmimi, H. M., Al-Shuwaili, S. J., Ami, A. A., Radi, U. K., ... & Mustafa, M. A. (2024). Polyaniline/TiO2 nanocomposite for high performance supercapacitor. Bulletin of the Chemical Society of Ethiopia, 38(4), 1177-1188.

[28] Mejía, N., Mustafa, M. A., Kumar, A., Kumar, A., Ghildiyal, P., Malik, A., ... & Wei, Q. (2024). Potential of Nanocages as Effective Catalysts for Oxygen Reduction Reaction. Silicon, 1-8.

[29] Muhanna, Muhammad Lafi Abdullah (2008). Efectos sinérgicos de algunos extractos de plantas y antibióticos sobre estafilococos aislados de muestras patológicas. Universidad Nacional An-Najah, Nablus - Palestina.

[30] Namvar, A. E., Sara, B., Niloufar, A., Ghazaleh, S. G., Sara, F., Parastoo ,A., Mahsa ,H., Sholeh, Z. B., Zahra, J., and Sara, G. Ch. (2014). "Clinical Characteristics of Staphylococcus epidermidis: A Systematic Review." GMS Hygiene and Infection Control 9(3):Doc23. doi: 10.3205/dgkh000243.

[31] Ortiz, D. T. C., Ghadir, G. K., Mustafa, M. A., Chandra, S., Kaur, I., Saadh, M. J., ... & Elmasry, Y. (2024). Exploring the photovoltaic performance of boron carbide quantum dots doped with heteroatoms: A DFT analysis. Diamond and Related Materials, 110933.

[32] Pourmand, M. R., Mojtaba, M., Mostafa, H., and Sahar, B. Y. (2009). "High Prevalence of Sea Gene among Clinical Isolates of Staphylococcus aureus in Tehran." Acta Medica Iranica 357–61.

[33] Saadh, M. J., Ajaj, Y., Mustafa, M. A., Kattab, N. O., Osman, S. M., Ahmad, H., ... & Elawady, A. (2024). Iridium (Ir) decorated silicon Carbide (SiC) nanosheet as a promising sensitive material for detection of γ-Hydroxybutyric acid drug based on the DFT approach. Molecular Physics, e2356754.

[34] Saadh, M. J., Lagum, A. A., Ajaj, Y., Saraswat, S. K., Dawood, A. A. A. S., Mustafa, M. A., ... & Elmasry, Y. (2024). Adsorption behavior of Rh-doped graphdiyne monolayer towards various gases: A quantum mechanical analysis. Inorganic Chemistry Communications, 160, 111928.

[35] Saadh, M. J., Morocho, W. M. B., Ajaj, Y., Yadav, A., Cabezas, N. T. M., Bansal, P., ... & Muzammil, K. (2024). Direct CO2 disassociation and HA activation mechanisms on Fe-doped graphdiyne for enhanced catalyst design. Sustainable Chemistry and Pharmacy, 38, 101487.

[36] Saadh, M. J., Mustafa, M. A., Batoo, K. M., Chandra, S., Kaur, M., Hussain, S., ... & Su, G. (2024). Performances of nanotubes and nanocages as anodes in Na-ion battery, K-ion battery, and Mg-ion battery. Ionics, 1-8.

[37] Sánchez, M., González-Burgos, E., Iglesias, I., Gómez-Serranillos, M.P.(2020). Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. Molecules. Mar 13;25(6):1324. doi: 10.3390/ molecules25061324. PMID: 32183224; PMCID: PMC7144722.

[38] Valverde, V., Ortiz, D. T. C., Mustafa, M. A., Kumar, A., Kaur, I., Karim, M. M., ... & Lasisi, A. (2024). Design gas sensor based on transition metal doped graphene like nanosheets: A quantum chemical study. Diamond and Related Materials, 110895.