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Extraction, phytochemical screening and activity evaluation of *Eichhornia crassipes* leaves extracts against gram-negative and gram-positive antibiotic-resistance isolates

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Abstract

It has been observed that the leaves of *Eichhornia crassipes* have a significant capacity to absorb water from rivers, leading to water loss and potentially blocking the flow of rivers. However, previous studies provided that the *Eichhornia crassipes* has biological important in the environment. The objective of this study is to isolate and identify pathogenic bacterial species from patients at AS-Shatrah Hospital, to examine the antibacterial properties of *Eichhornia crassipes* leaf extracts against specific pathogenic bacterial species, including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, and *Escherichia coli*. Furthermore, the study seeks to investigate the metabolic activity of these pathogenic isolates in utilizing 13 different sugars and 10 amino acids. The plant leaves were collected and subjected to phytochemical examination in order to identify their individual components. The antibacterial properties of the ethanol extracts were evaluated using the Plate diffusion technique. The results suggest that ethanol extracts have a substantial effect on both bacterial and multidrug-resistant (MDR) bacterial strains, particularly when compared to the antibiotic used as a control. The study's findings indicated that the ethanol extract obtained from water hyacinth exhibits significant antibacterial and antioxidant activities. The observed effects can be attributed to the existence of numerous biologically active constituents, such as flavonoids, alkaloids, and phenols. Consequently, this research suggests that water hyacinth, despite being considered a causing harm aquatic herb, holds potential as an alternative source of compounds for therapeutic applications. It is employed as a means of controlling bacterial infections and minimizing the impact of infectious diseases, and reduces the consumption of antibiotics as well.

1. Introduction

Eichhornia crassipes, commonly referred to as water hyacinth, is an aquatic plant that grows quickly and is distinguished by its large fibrous root systems and significant biomass. It is abundant in different tropical and subtropical regions worldwide (**Gao and Bo, 2004**). Many plant species have the capacity to act as valuable reservoirs of important antibacterial properties. Consequently, the utilization of phytochemicals and plant extracts, which are acknowledged for their established antibacterial qualities, assumes an essential function in treatment strategies (**Sanaa, *et al.*, 2012**). The examination of alcoholic plant extract against bacterial species is being done because to the plant's essential qualities and structural features. *Staphylococcus aureus* is a bacterium that is spherical in shape and has a diameter of around 1µm. It is classified as Gram-positive. After being incubated for duration of 24 hours, the microorganism develops clusters resembling grapes. These colonies display a notable, as well, round, and glossy appearance on the growth medium. The optimum temperature for its growth is 37°C, and it flourishes within a pH range of 7.4-7.6. *Staphylococcus aureus* is therapeutically significant because it possesses a variety of virulence factors. These elements contribute to its capacity to invade host organisms, provoke pathogenicity, and acquire resistance to drugs. Notably, one such virulence factor is protein-A, which covalently attaches to the peptidoglycan layer (**Rayan and Ray 2004**). Certain strains possess a capsule consisting of glucosamineuronic acid, which is lost throughout the process of cultivating. Additionally, these strains exhibit the capacity to create enzymes such as Protease, Hyaluronidase, Nuclease, phosphatase, and Fibrinolysin coagulase (**Francois and Screzel 2008**) and (**Humphreys 1997**). *Pseudomonas aeruginosa,* a Gram-negative rod-shaped bacterium, is widely distributed in the environment. It is recognized as a significant pathogen that poses a serious threat to individuals with compromised immune systems, causing nosocomial infections, as well as persistent respiratory tract infections in patients with cystic fibrosis (CF) (**Lyczak, 2006**). The capacity to adapt many ecological settings and acquire resistance to conventional antibiotic treatments has engendered a new research attention with bacteriophages. Phage mixtures have been employed by many researchers for the treatment of

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Pseudomonas aeruginosa infections; nonetheless, it is important to conduct clinical tests in order to assess their effectiveness and safety (**Kutateladze and Adamia 2008**). Several antibiotics have restricted penetration into biofilms (**Hoiby *et al*., 2010**). Hence, it has been observed that bacteria surviving in biofilms exhibit a remarkable ability to withstand antibiotic doses that are several orders higher greater than those tolerated by planktonic bacteria (**Ciofu *et al*., 2002**). Over a period of time, this phenomenon facilitates the emergence of antibiotic-resistant bacteria and leads to the prevalence of multi-drug-resistant (MDR) strains (**Hirsch and Tam 2010**). *Streptococcus pyogenes*, sometimes known as group A *Streptococcus*, is a frequently noted bacterium. *Streptococcus*, or GAS, is a type of Gram-positive cocci that commonly inhabits humans as its specific host. *Streptococcus* is a bacterial pathogen that causes a wide range of infections in humans, varying from mild conditions like sore throat and impetigo to more severe illnesses such as necrotizing fasciitis, glomerulonephritis, acute rheumatic fever, and streptococcal toxic shock syndrome (**Luca-Harari *et al*., 2009**) and (**Carapetis *et al*., 2005**). *Escherichia coli*, a member of the Enterobacteriaceae family, is a highly diverse and widespread group. Gram-negative facultative anaerobic bacilli are bacteria with dimensions ranging from around 0.3 to 1.0 µm in width and 1.0 to 6.0 µm in length. The bacteria possess peritrichous flagella, which enables them to move. The organism demonstrates its highest level of growth and metabolic activity when the temperature is between 25-35°C. It is capable of engaging in both respiratory and fermentative metabolic activities (**Public Health England, 2015**). During this study 10 swab samples were obtained from patients 15 isolates were isolated and diagnosed depending on biochemical tests and molecular technique, and undergone to alcoholic extract of *Eichhornia* leaves. The aim of this study is to isolate and identify disease-causing bacterial species from patients at AS-Shatrah Hospital. Additionally, we will investigate the antibacterial effects of Eichhornia crassipes leaf extracts on specific disease-causing bacterial species, such as *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*. Moreover, the study aims to examine the metabolic activity of these disease-causing samples in utilizing a total of 13 distinct sugars and 10 different amino acids.

2. Material and Methods

2.1 Materials

Eichhornia leaves were collected from the Tigris River/ AL-Shatrah city/ Iraq. Subsequently, they were subjected to drying process and afterwards ground until a fine powder. The resultant powder was then properly stored in a dry container. The chemicals were acquired from the laboratory involved with the college. The research was conducted at the organic chemistry laboratory in the Department of Pharmaceutical Chemistry Science.

2.2 Preliminary phytochemical screening

The ethanol extract was prepared by soaking 60 grams of powdered *Eichhornia Vulgaris* leaves in 300 mL of ethanol for a period of two weeks. The solution obtained from the extraction process was subjected to filtration, followed by the evaporation of ethanol using a rotary evaporator under vacuum conditions at a temperature of 450°C for duration of one to five hours. The filtrate was utilized for the purpose of conducting phytochemical screening, with the objective of confirming the presence of phytochemicals by carrying out of the tests described by (**Alyaseen, *et al*., 2018; Abdulridha, *et al*., 2019**).

2.3 Bacteria and media

In according to the procedure described by MacFadden (2000), specimens were procured, and swabs were carefully obtained from the site of infection. These swabs were then placed in tubes containing a medium for preservation, providing that the swabs remained moist during transportation to the laboratory. A total of 10 samples were obtained from patients at AS-Shatrah Hospital, which are represented 2 samples of throat, 3 samples of Urinary tract, 3 samples of burns, and skin wounds, and 2 samples of respiratory system. Swab specimens were transferred to the laboratory under aseptic conditions for analysis. All swab samples were cultured on blood agar, nutrient agar, and chocolate agar as well. Subsequently, the samples were divided into two groups based on the gram stain technique, which is gram positive and gram negative (**MacFadden 2000**).

2.3.1 Identification of Bacteria

While the samples of Gram negative isolates were cultured on MacConkey agar and Eosin methylene blue (EMB) (**Levine 2018**). The plates were incubated at 37°C for 24 hours. The Gram-positive specimens were subjected to culture on blood agar and mannitol saline agar (MSA), in order to investigate their hemolysis properties and to isolate *Staphylococcus*, respectively.

2.3.2 Chemical tests:

The pathogenic bacterial isolates were subjected to IMVIC, catalase, urease, oxidase, coagulase, blood hemolysis, and mannitol fermentation tests in order to achieve an accurate and unambiguous diagnosis.

2.3.2.1 Morphological and Microscopic examination

The morphology of colonies was examined using MacConkey agar, blood agar, EMB and MSA. After incubating at 37˚C for duration of 24 hours, many characteristics of the colonies were observed and recorded. These included the color, size, edge morphology, and the type of hemolysis on blood agar. Additionally, a single colony of each isolate was fixed onto a clean slide for the purpose of conducting a gram stain analysis under a light microscope (**Atlas *et al*., 1995**).

The morphology and pigmentation of bacterial colonies were investigated. This was achieved by observing the shape and color of colonies on petri plates after they were cultivated and incubated overnight at a temperature of 37°C (**Arahal *et al*., 1996; Oren *et al*., 1997**). A sample of bacterial cultures, grown under optimal conditions, was collected and suspended in physiological saline solution. A small drop of this bacterial suspension was placed on a glass slide and gently mixed. The slide was then passed over a benzene flame and allowed to air dry for one minutes to facilitate the removal of excess moisture. Following this, a Gram stain (Crystal violet) was directly applied onto the bacteria and left for one minute. The slides were subsequently rinsed with tap water for five seconds to remove any unbound crystal violet. Next, Gram iodine was added and left for one minute before being washed with saline solution. A solution of 95% ethanol was applied for 20 second and washed. Safranin dye was added as a counter stain. Subsequently, the sample was cleaned and left to air dry. After that, the slides were examined by using a light microscope with an immersion lens and observed the Gram-negative and Gram-positive isolates (**Gram 1884; Sánchez-Porro *et al*., 2011**).

2.3.2.2 IMVIC

Bacterial growth was injected into test tubes with peptone water and cultivated at a temperature of 37°C for one night. Subsequently, two drops of kovag's reagent were added, and the appearance of a red or pink color was considered as a positive result for tryptophanase activity. The methyl red test involves inoculating glucose phosphate broth with bacterial growth and incubating it at 37°C for 48 hours. After incubation, 5 drops of methyl red are added, and the presence of a red hue indicates a positive result. The Voges-Proskauer test was conducted using the same procedure, but with the addition of 0.2 ml of 40% KOH and alpha-naphthol as the reagent. The positive result was a red color within one hour. The bacterial inoculum was cultured overnight in Simmon's citrate agar at a temperature of 37°C. Subsequently, a positive result was observed as the color of the agar changed to a deep blue hue (**Microbe Online, 2014**).

2.3.2.3 Urease, Oxidase, Catalase and Coagulase tests

Inoculation of the urea agar was performed within a test tube, followed by incubation for duration of 24 hours. The presence of urease enzyme was inferred by the change in color to pink in the urea agar test tubes, as reported by (**Bilgehan 2014; Abdulhusein 2023**). In the experimental procedure, the addition of tetra-dimethyl-para-phenylenediamine dihydrochloride onto the filter paper was followed by the smearing of bacterial colonies onto the same filter paper. Subsequently, the results were observed as the appearance of a blue-violet color within a time frame of 20 seconds (Abdulhusein 2023; Harley, and Prescott 2002). Two drops of hydrogen peroxide (H_2O_2) with a concentration of 3% was applied to the colonies grown on the growth medium. The colonies that exhibited a positive reaction produced gas bubbles, but the negative isolates did not demonstrate any bubble formation. The purpose of this experiment was to differentiate between *Staphylococcus* sp. and *Streptococcus* sp. by utilizing a specialized test that specifically targets aerobic and microaerophilic bacteria (**Abdulhusein 2023; Harley, and Prescott 2002**).. For coagulase, test tube contains rabbit plasma was inoculated with bacterial growth and incubated overnight at 37°C, the formation of clot considered as appositive result; whereas the absence of clot interpreted as a negative result (**Barrow and Feltham, 1993**).

2.3.2.4 Blood haemolysis

The isolates were grown on Blood agar, which consisted of a 5% human blood agar base. Subsequently, the Petri plates were placed in an incubator set at a temperature of 37°C for duration of 24 hours. The isolates were re-identified using the usual protocols outlined by Cowan (**Barrow and Feltham, 1993**). The isolates were classified based on the type of haemolysis observed around the developed colonies after the incubation period. The strain that showed a negative catalase test and exhibited beta haemolysis was identified as *Streptococcus pyogenes*.

2.3.2.5 Mannitol Fermentation

Due to its 7.5% sodium chloride (NaCl) content, Mannitol agar has proven to be a selective medium for particular bacteria, such as *Staphylococcus* sp. The isolates cultivated on mannitol salt agar containing mannose and phenol red showed positive results for catalase activity and haemolysis after being incubated for 24 hours at 37°C. Yellow colonies observed on mannitol agar after the incubation time was considered as indicative of positive mannitol activity shown by *Staphylococcus aureus*. The change in color from red to pink was perceived as a favorable indication of mannitol action by other *Staphylococcus* sp. (**Santos *et al.*, 2015; Saab *et al.*, 2018**).

2.3.2.6 Utilization of amino acids and carbon sources by pathogenic bacterial isolates

The metabolic activity of pathogenic bacterial isolates was evaluated by using 10 amino acids and 13 carbohydrates. The amino acids including (L-valine, L-methione, L-phenylalanine, L-serine, L-alanine, Trans-4-hydroxy-L-proline, L-cystine, Glysin, L-tyrosine, and L-proline) The test tubes incubated at 35°C for 24 hours after had been inoculated with bacterial isolates separately (**Abdulhusein 2023; Harley, and Prescott 2002**). Using 1% (w/v) (D-(-)-ribose, D-(+)-galactose, Lactose, D-(+)-trehalose, Maltose, D- Sorbitol, D-(-)-Salicin, D-(+)-melibiose, D-(+)-xylose, Xylitol, D-(-)-fructose, N-acetylglucose amine, and Sucrose), the utilization of 13 sugar sources by tested isolates was examined separately. While production the purple color in the test tubes accepted as a positive result for utilization amino acids, the transformation color from red to yellow was interpreted as a positive result for carbohydrate utilization (**Abdulhusein 2023; Harley, and Prescott 2002**).

2.4 McFarland Standard

The McFarland Standards were employed in order to standardize the estimated quantity of bacteria present in a liquid suspension. This was achieved by comparing the level of visibility or turbidity seen in the test suspension with that of the McFarland Standard (**McFarland 1907**). The 0.5 McFarland Standards is the standard employed in clinical microbiology laboratories for antibiotic susceptibility testing and culture media performance testing. The turbidity of all suspensions containing pathogenic bacteria was adjusted to a concentration of 1×10^8 CFU/mL, according the directions of McFarland Standard No 0.5, using sterile saline solution.

2.5 Molecular diagnosis

By using specific extraction and purification kits (QIAamp DNA and QIAquick PCR Kits, respectively (Qiagen, Germany)), chromosomal DNA isolated and purified depending on instructions, then DNA amplified by using PCR technique and specific forward and reverse primers for *Staphylococcus aureus,* [F-(GTAGGTGGCAAGCGTTACC) R-(CGCACATCA GCGTCAG) (**Al-musawi *et al.*, 2014)],** for *streptococcus pyogenes* [F-(AAGAGAGACTAACGCATG TTAGTAAT) and R-(ATTTTCCACTCCCACCATCA) (**Kulkarni *et al.,* 2016**)], for *Pseudomonas aeroginosa* [F-(GGGGGATCTTCGGACCTCA) and R-(TCCTTAGAGTGCC CACCCG) (**Spilker *et al.,* 2004**)], and for *Escherichia coli* [F-(GACCTCGGTTTAGTTCACAGA) and R-(CACACGCTGACGCTGA CCA) (**Mamun *et al.*, 2016**)].

2.6 Evaluation the antibacterial activity of *Eichhornia crassipes* Leaves Extracts against pathogenic species

Approximately 10 µL of each bacterial suspension, with a concentration of 1×10^8 colony-forming units per milliliter, was applied onto the surface of Muller Hinton Agar (MHA) plates. The bacterial suspension was spread out using a hokey glass spreader and was allowed to settle for duration of 2 minutes. Subsequently, 3 µL of *Eichhornia crassipes* Leaves Extract was added to each agar plate. All agar plates were then incubated at a temperature of 37°C for the duration of one night. Following that, the measurement of inhibitory zones was conducted (**Rios *et al.*, 1988**).

3. Results

The result exhibited that from 10 swab specimens 15 isolates were isolated and depending on the results of Gram staining reaction these isolates divided into groups. The results of biochemical tests (Indole, Methyl red, citrate utilization, Vogues proskauer, blood haemolysis, urease, coagulase, catalase, oxidase and mannitol fermentation test) proved that all these 15 isolates belonging to four genus, which were including *Staphylococcus* (3 isolates), *Streptococcus* (2 isolates), *Escherichia* (7 isolates), and *Pseudomonas* (3 isolates), (Table 1). The four genuses were diagnosed as a Gram-positive was *Staphylococcus* and *Streptococcus* and as a Gram-negative were *Pseudomonas* and *Escherichia*, (Table 2).

Table 1 The samples' codes and bacterial genus, and isolates' number

Isolates' codes	Bacterial genus	Isolates' number
SS1*	*Escherichia*	7
SS2	*Pseudomonas*	3
SS3	*Streptococcus*	2
SS4	*Staphylococcus*	3

*SS: Swab Sample

Table 2 Chemical tests of pathogenic isolates (The final diagnosis).

Isolates	(SSI4)	(SSI3)	(SSI2)	(SSI1)
Bacterial shape	Cocci/ clusters	Cocci/chins	Bacillus/rod shape	Rod
Gram stain	Positive	Positive	Negative	Negative
Catalase	Positive	Negative	Positive	Positive
Coagulase	Positive	Negative	Negative	Negative
Blood hemolysis	beta-hemolysis	beta-hemolysis	β-Hemolysis	none
Urease	Positive	Negative	Positive	Negative
Colonies shape	circular, convex	circular	low convex	Smooth
Colonies color	Yellow	white to gray	Greenish blue	Green metallic sheen
Motility	Non-motile	Non-Motile	Motile	Motile
Oxidase	Negative	Negative	Positive	Negative
Indole	Positive	Negative	Negative	Positive
Methyl red	Positive	Positive	Negative	Positive
Voges-Proskauer	Positive	Negative	Negative	Negative
Citrate	Positive	Negative	Positive	Negative

The result shows that the *Staphylococcus* sp. (isolate SS4), *Streptococcus* sp. (isolate SS3), and *Pseudomonas* sp. (Isolate SS2) produce transparent zones around colonies on blood agar, whereas *Escherichia* sp. (isolate SS1) does not produce blood haemolysis, (Table 2). While isolate (SS4) showed Gram-positive, catalase+, coagulase +, urease+, beta-hemolysis on blood agar, and oxidase negative. Isolate (SS3) showed Gram-positive, catalase-, coagulase-, urease-, beta-hemolysis on blood agar, and oxidase negative. The results of biochemical tests for isolates SSI and SS2 were Gram stain negative, catalase positive, and coagulase negative alike. While, isolate SSI exhibited urease and oxidase negative; isolate SS2 was positive for urease and oxidase alike. The result of molecular investigation by using specific 16S rRNA primers found that the isolates isolated from patients including *Staphylococcus aureus*, *Streptococcus pyogenes, Pseudomonas aeroginosa*, and *Escherichia coli*.

The results revealed that some of these isolates have ability to use carbohydrates as a carbon source and change the color of medium from red to yellow. Ribose, galactose, lactose, maltose, sorbitol, salicin, D-(+)-melibiose, xylose, fructose, N-acetylglucose amine, and sucrose were respectively utilized by 50%, 50%, 75%, 75%, 25%, 75%, 75%, 25%, 75%, and 75%. Although all isolates utilized trehalose 100%, none of the isolates used xylitol as a carbon source. Furthermore, all amino acids which used in this study were utilized by bacterial isolates with different percentages. While, methionine, phenylalanine, serine, alanine, and proline were utilized by all tested isolates, 50% of our isolates utilized valine, cystine, glysin and Trans-4-hydroxy-L-proline. Whereas, tyrosine utilized by *Staphylococcus*, *Pseudomonas*, and *Escherichia*; *Streptococcus* did not used tyrosine, (Table 3).

The results of phytochemical screening exhibited that the chemical structure of *Eichhornia* consist of alkaloids, flavonoids, carbohydrate, glycoside, tannin, and Terpenoids by using Dragendroof reagent and Wanger reagent, Shinoda test, Molish test, Fehling's test, $FeCl_3$, and Salkowski reaction, respectively (Table 4). This study aimed to analyze the presence of phytochemicals in the leaves of *Eichhornia* through ethanol extraction.

The findings of this study indicate that a significant impact of ethanol extracts on the tested bacteria (Table 5; Figure 2).

Table 3 Utilization of amino acids and carbohydrates by bacterial isolates

Isolate numbers	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Pseudomonas aeroginosa*	*Escherichia coli*	Total number	Positive %
L-valine	+	-	-	+	2	50
L-methionine	+	+	+	+	4	100
L-phenylalanine	+	+	+	+	4	100
L-serine	+	+	+	+	4	100
L-alanine	+	+	+	+	4	100
Trans-4-hydroxy-L-proline	-	-	+	+	2	50
L-cystine	-	+	+	-	2	50
Glysin	-	-	+	+	2	50
L-tyrosine	+	-	+	+	3	75
L-proline	+	+	+	+	4	100
D-(-)-ribose	+	-	-	+	2	50
D-(+)-galactose	+	+	+	+	2	50
Lactose	+	+	-	+	3	75
D-(+)-trehalose	+	+	+	+	4	100
Maltose	+	+	-	+	3	75
D- Sorbitol	-	-	-	+	1	25
D-(-)-Salicin	-	+	+	+	3	75
D-(+)-melibiose	+	-	+	+	3	75
D-(+)-xylose	-	-	-	+	1	25
Xylitol	-	-	-	-	0	0
D-(-)-fructose	+	+	-	+	3	75
Sucrose	+	+	-	+	3	75
N-acetylglucose amine	+	-	+	+	3	75

Table 4 Phytochemical screening, chemical structure, chemical test, test result of Thyme Leaves and chemical notes

Code No	Chemical structure	Chemical test	Test result of Thyme leaves	Chemical note
1	Alkaloids	Dragendroff reagent Wagner reagent	+ve +ve	formation Orange Brown ppt
2	Flavonoids	Shinoda test	+ve	formation of pinkish violet color
3	Carbohydrate	Molish test	+ve	Violet color ring formation
4	Glycoside	Fehling's test	+ve	Blue color formation
5	Tannin	FeCl3	+ve	Bluish black color Formation
6	Saponin	Shaken of the extraction	-ve	No Formation of foam
7	Sterols	Liebermann burchard	-ve	Formation green-blue color
8	Coumarine	Filter paper soaked by diluted NaoH	-ve	No formation of yellowish green color on filter paper
9	Terpenoids	Salkowski reaction	+ve	Formation of reddish brown color
10	Resins	Ethanol 95% +boiling + 4% hcl	-ve	No formation of turbidity
11	Anthraquinons	Borntrager's test	-ve	No Formation of red rose color

Table 5 Show the effects of Ethanol extracts of *Eichhornia vulgaris* leaves on Gr-ve and Gr+ bacteria

No	Isolates	Gram reaction	Ethanol extracts of *Eichhornia vulgaris* leaves
			Inhibition zone
1	*Staphylococcus aureus*	Gr+	40 mm
2	*Streptococcus pyogenes*	Gr+	45 mm
3	*Escherichia coli*	Gr-	45 mm
4	*Pseudomonas aeroginosa*	Gr-	50 mm

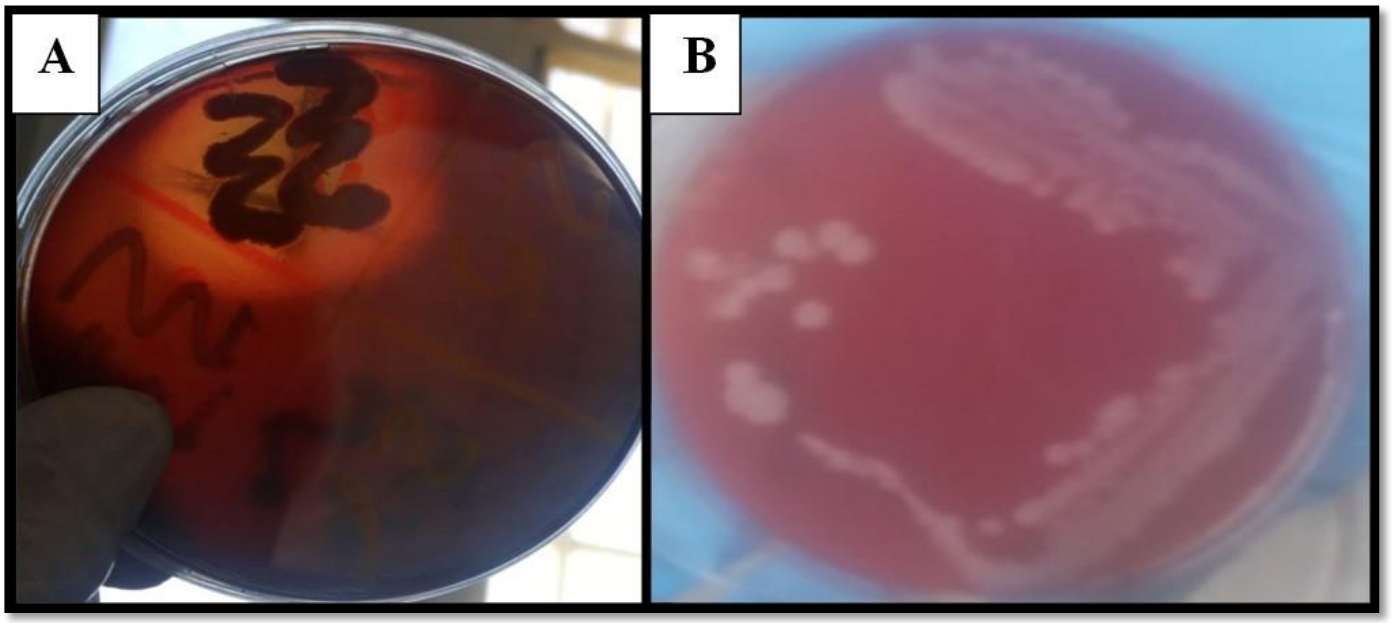


Figure 1 (A) Blood haemolysis by *Staphylococcus aureus* blood agar; (B) The colonies of *Staphylococcus aureus*

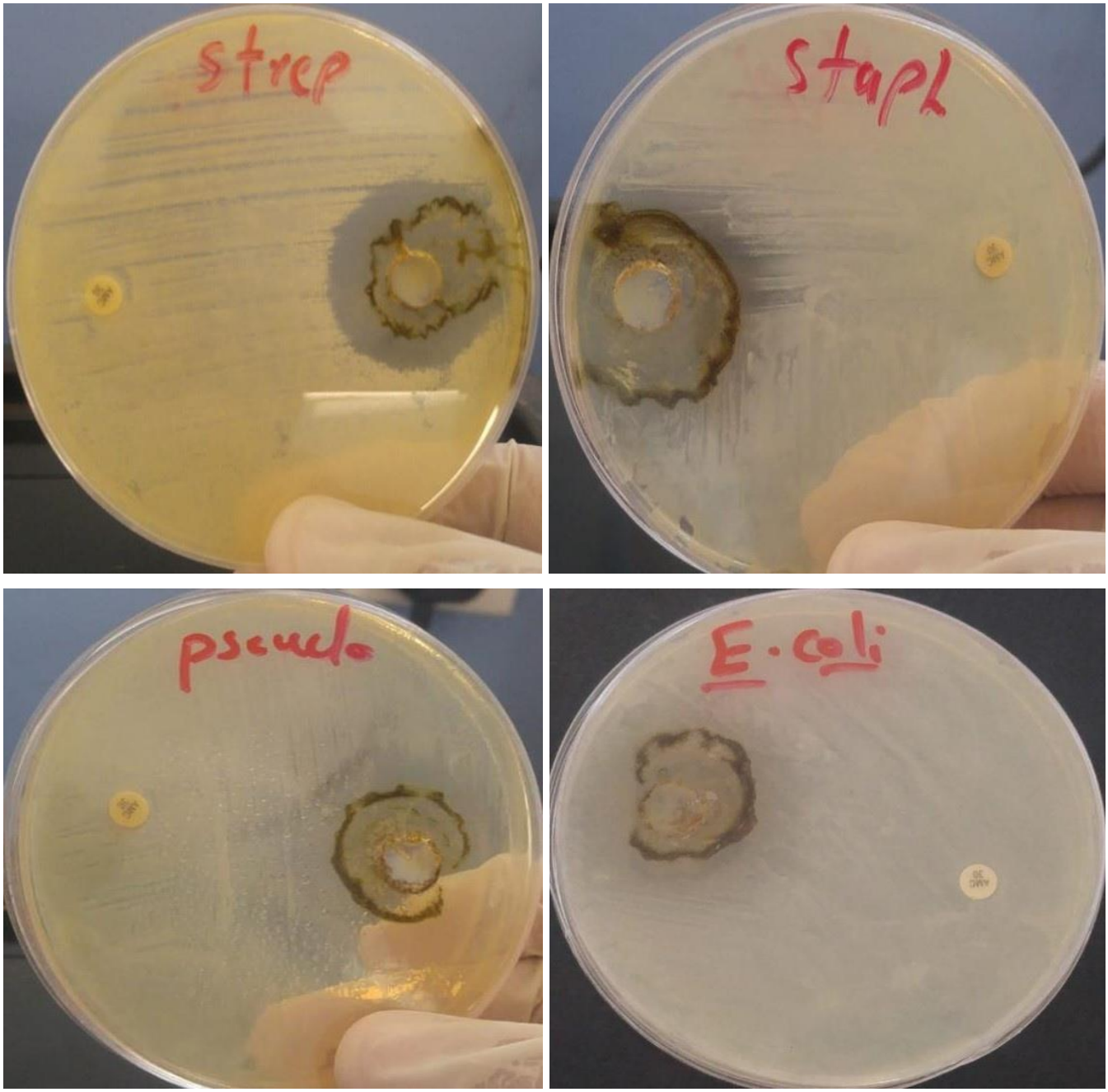


Figure 2 Show the inhibition zone around the extract that affected against bacterial species on Muller Hinton agar

4. Discussion

Earlier study found that the *Escherichia coli* utilize different kinds of amino acids in different percentages such as phenylalanine (**Liu *et al*., 2018**), serine (**Zhang *et al*., 2019**), alanine (**Xu *et al*., 2021**) and phenylalanine (**Satoh *et al*., 2023**). Our observations differ from findings reported by Baral and Vaidya in 2011, who found that the aqueous extract exhibited weak antibacterial activity, compared to other extracts, but demonstrated better efficacy as an antifungal agent. This phenomenon can be attributed to the bacteria's lack of prior exposure to these extracts, resulting in their absence of resistance. The findings of this study match with a previous study conducted by Sanaa M. *et al*. in 2010, as well as with the results reported by Hiba H. Hamid *et al*. in 2013 in Iraq. Bacterial cells and appropriate vectors are employed to introduce chemicals into the cell, with the aim of inhibiting the activity of coenzymes and their biologically active compounds (**Harbornne 1984; Mitscher 1992**).

These findings elucidate the mechanism underlying the capacity of multidrug-resistant (MDR) microorganisms to withstand the effects of antibiotics. The World Health Organization's yearly reports in 2016 indicate a significant increase in the prevalence of antimicrobial-resistant bacteria worldwide. Bacteria possess the capacity to proliferate despite the application of antibiotics within the leather industry (**Berber 2020**). In recent decades, the phenomenon of antibiotic resistance has become increasingly prevalent as a result of the uncontrolled and excessive utilization of antimicrobial drugs. Animal products, poultry, bird feces, and corpses have been identified as significant sources of multidrug-resistant (MDR) pathogenic bioagents. Multi-drug resistance (MDR) has emerged as a significant and serious problem in recent times, impacting both

humans and animals alike (**Abdulhusein, and Caglayan 2022)** The success of the sensitivity test is influenced by various factors, including the quantity and type of medium employed in the experimental procedure. In this particular study, Mueller Hinton medium was utilized, which is known for its ability to support the growth of the bacteria being investigated. Additionally, the pH constant value plays a significant role in facilitating the effectiveness of the antibiotic being tested (**Barry 1976**). It is important to note that other factors, such as the size of the inoculum used in the experiment and the incubation conditions, also contribute to the success of the sensitivity test. The findings of this study indicate that the bacteria exhibit a notable sensitivity to the group of organic plant extracts, which could possibly exceed their sensitivity to certain antibiotics, as evidenced by the obtained results.

5. Conclusion

Over a period of time, many kinds of antibiotics have been discovered. However, due to their uncontrolled usage, several bacteria and fungi have developed resistance against these antibiotics. Consequently, it is necessary that alternative approaches be employed in our continuing struggle against microorganisms. Over the past decade, there has been a significant increase of *Eichhornia crassipes* in Iraq, particularly in the southern regions of the Tigris River. This occurrence could potentially elucidate the lack of attention given to river cleaning efforts and the subsequent decrease in water levels. However, it is worth considering the potential utilization of alcoholic extracts of leaves from this plant as cost-effective alternative medications for combating certain multidrug-resistant Gram-negative and Gram-positive bacteria in controlled laboratory environments, similar to findings from previous studies. Additionally, an alternative explanation suggests that the extracted materials possess a chemical affinity for interacting with the components of the bacterial cell, facilitated by the presence of receptors on the bacterial cell wall.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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