Biological Effectiveness of Seed Extracts of Some Species From *Brassicaceae* Family Against Two Types of Pathogenic Bacteria

Afnan Esam Adnan [1*], Wisam Malik Dawood [1]

Abstract

Background: Cruciferous plants are particularly rich in bioactive compounds with potential therapeutic applications. The study aimed to assess their efficacy against Staphylococcus aureus and Escherichia coli. Methods: Extraction and antimicrobial testing occurred at the University of Diyala's College of Agriculture labs from March to September 2023. Two extraction methods and varying concentrations (100%, 150%, 200%) were used. Inhibition zones were measured via agar well diffusion, and biofilm formation was evaluated using microtiter plate assays. Results: Cress seed extract showed the highest S. aureus inhibition (21.99 mm), while Radish had the lowest (5.77 mm). Chloroform extraction yielded the highest inhibition (17.00 mm), with 200% concentration being most effective (19.77 mm). For E. coli, Cress extract had the highest inhibition (15.22 mm), and chloroform extraction at 200% concentration was most effective (13.11 mm). Cress seed extract inhibited biofilm formation, especially with chloroform, while Radish and Watercress showed moderate to strong inhibition. Conclusion: Cress seed extract, particularly with chloroform extraction at higher concentrations, exhibited potent antimicrobial activity against S. aureus and E. coli, inhibiting biofilm formation. Radish and Watercress extracts also showed moderate antimicrobial effects. These findings highlight the potential of Cruciferous seed extracts as natural antimicrobial agents, warranting further research for pharmaceutical and agricultural applications.

Keywords: Medicinal plants, Antimicrobial activity, Antibiotic resistance. Cruciferous

Significance | Cruciferous Plant-derived compounds showed diverse medicinal properties, combating antibiotic resistance, providing therapeutic alternatives, and inhibiting biofilm formation in pathogenic bacteria.

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Introduction

Plants have the ability to manufacture compounds as secondary metabolites found in seeds, leaves, and roots, as well as in fruits, including plants of the Cruciferous family known as Brassicaceae, which are characterized by containing active groups, including alkaloids, resins, phenols, flavonoids, saponins, glycosides, and volatile oils. These compounds are of great importance from a medical standpoint (Al-Qaisi, 2004).

It also contains many types of chemicals with important therapeutic properties that can be used for the treatment of diseases that affect humans, such as alternative (folk) medicine treatments, as an analgesic for joint pain, alleviation of respiratory diseases, coughs and colds, a regulator of high blood pressure, and the treatment of dermatitis diseases. Improving liver functions and treating dyspepsia problems and other diseases (Mazokopokis *et al.*, 2007).

The emergence of antibiotic-resistant bacteria is one of the most complex problems of microbial evolution in the last two decades. Doctors prescribe broad-spectrum antibiotics without

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susceptibility testing to those antibiotics. Therefore, plant-derived products can be tested to determine antibacterial activity that can be used to treat infectious diseases (Iqbal and Ashraf, 2019). Antibiotics have contributed to the treatment of many diseases, and occupied the forefront of resistance to pathogens. However, their widespread use led to the emergence of resistant strains of disease, in addition to their deadly effect on beneficial bacteria. Therefore, research in laboratories were directed to reduce their excessive use and provide natural medicinal therapeutic alternatives from plants, including plants under study, as they are available, health-wise, environmentally safe, and inexpensive, thus limiting the development of new resistant strains (Pulipati et al., 2017).

Medicinal plants nowadays play a key role in agricultural production due to their therapeutic importance, as they are used in medical cases in which it is difficult to use chemical medicines for fear of deteriorating the patient's condition and causing her/him serious side effects. Such plants are considered to be safe to use and easy to apply without the need for special skills and experience in preparing them for use and are available in most countries. The case which makes cheap and easy to be taken, when compared to expensive chemical medicines. Medical plants, on the other hand can be used to treat more than one disease at the same time as they contain many compounds as well as vitamins and minerals that are important in strengthening and preserving the patient's health as well as the psychological reassurance when used in treatment because they are natural (Sofowora *et al*., 2013).

Medicinal plants have been identified as plant preparations and a source of alternative medicine according to the World Health Organization (WHO, 2020), using techniques, as plant materials are introduced through processes such as extraction, fractionation, purification, concentration, and other physical processes that can essentially produce pharmaceutical plant products as drug treatments (Alo *et al*., 2012).

In the past, medicinal plants and herbs were used in many forms, including in the form of extracts. Seeds and leaves are used in this section. It may also be in the form of infusions using leaves by steeping them in the form of tea. Or, they may be found as tablets and capsules or oils for anointing and bandages. Herbs can be added to bathing water, as they can be used as required (Khalifa, 2009).

The increasing use of antibiotics has created resistance to these antibiotics and exacerbated disease conditions, in addition to the high cost of medications and their inefficiency due to the associated risks and resulting harmful side effects. This has led to the need to search for the use of safe alternatives (Karpagam and Manonmani, 2019).

The development of bacterial resistance to antibiotics is multifactorial, such as the specific nature of the relationship between bacteria and antibiotics, the use of the antibacterial agent, host characteristics and environmental factors, which has forced scientists to search for new antimicrobial materials from various sources such as new antimicrobial chemotherapy agents. However, the cost of producing synthetic drugs is high and they result in harmful effects compared to plant-derived drugs. Therefore, since ancient times, herbs and plants have been used as a source of medicinal compounds because of their role in maintaining human health as a traditional herbal treatment for approximately 80% of the world's population, according to what was indicated by the World Health Organization, as more than 50% of all clinical medicines are originated from natural products. For example, phytochemicals such as vitamins (K, E, C, A), carotenoids, alkaloids, enzymes, pigments, minerals, and others have antimicrobial activity and antioxidants (Jouda, 2013).

The progress that has been made in securing new sources of natural products with antimicrobial activities and expanding the chemical diversity of antibiotics provides chemical agents for new drugs. There are many natural plant products that have antifungal and antibacterial activities that can be used either systemically or locally in the body of an organism. During the second half of the twentieth century, the development of microbial resistance to conventional antibiotics led researchers to investigate the antimicrobial activities of medicinal plants because they have tremendous therapeutic potential for infectious diseases while at the same time reducing many of the side effects that accompany synthetic antibiotics (Valle Jr *et al*., 2015).

Plants that have medicinal value are usually extracted from effective and active ingredients, however, they differ from one plant to another due to their high content of these ingedients, as scientific experiments confirm their pharmaceutical importance (Anibijuwon and Udeze, 2009). Interest in plant extracts has increased recently as a source of natural products that are always available, as the extracts possess protective and protective properties when used as therapeutic alternatives to many treatments against pathogenic microorganisms (Tepe *et al*., 2004).

Cruciferous family plants are characterized by containing volatile compounds and aromatic oils, as well as indole compounds, especially Indol-3-carbinol, chlorophyll, carotenoids, flavonoids, phenols, and sulfur glycosides, which are among the natural compounds that characterize the family and are responsible for the flavor and its own taste (Kassie 1999).

S. aureus is considered to be a gram-positive bacteria that grows on enriched culture media, such as blood agar medium, with a golden yellow color, and also grows on mantol differential media. Microscopically, it has single spherical shapes that take on several levels to form something similar to a bunch of grapes, hence the name "*Staphylococcus aureus*." It ranges from its diameter ranges between 0.5 - 1.5 micrometers, and it is the most common among the species of this genus (Al-Taweel, 2016). Because *S. aureus* possesses many virulence factors, extracellular enzymes, and toxins

that it can secrete and multiply rapidly. This makes it more pathogenic compared to its peers of the same sex, in conjunction with immunodeficiency in some patients, it causes these serious diseases. In addition to pneumonia, bone infection, wound infection, burns, middle ear infection, and food poisoning, which can occur as a result of its secretion of intestinal toxins (Tiwari, 2020).

S. aureus is isolated from multiple sources and is an opportunistic pathogen compared to other bacteria that can develop many types of resistance through diverse mechanisms (Dari, 2019). S. aureus is an opportunistic pathogen that causes skin and soft tissue infections and is common in burn patients, approximately 28-65% of deaths are due to burns worldwide. In an intensive care burn unit in South Africa, methicillin-resistant *S. aureus* (MRSA) was the third most common organism in blood cultures, as 17% of patients who had a positive blood culture result died (Amissah, 2017).

These bacteria can be spread by food handlers, hand contact surfaces, and food contact surfaces during processing and packaging. Therefore, *S. aureus* have been frequently discovered in a variety of foods, and here biofilms are considered part of the natural life cycle of *S. aureus* in the environment (Chen *et al.*, 2020). *E. coli* are gram negative bacilli which are considered to be one of the most common causes of urinary tract infections, especially in pregnant women, as the infection rate reaches about 10% (Dielubanza, 2011). The reason for the pathogenicity of bacteria is attributed to their possession of many virulence factors, such as the fact that their cell wall contains lipopolysaccharides. In addition to the two types of toxins secreted by bacteria, which are internal toxins of the heat-stable type and toxins that are not heat-stable, they also possess the hemolysin enzymes, which plays an important role in their pathogenesis (Allen, 2012). This study aimed to test the effectiveness of the type of extract in inhibiting the growth of two types of pathogenic bacteria. The effectiveness of these extracts was studied by diffusion method in holes at three concentrations: 100%, 150%, and 200% mg/ml on the growth of *S. aureus* and *E. coli*.

Materials and Methods

Sterilization Methods:

Several methods were used in this study to sterilize tools, glassware, materials, and agricultural media, according to what was stated in Jawetz *et al.* (2016), which are physical sterilization methods, including dry heat sterilization and moist heat sterilization, chemical sterilization methods, and mechanical sterilization methods, as the filtration method was used to sterilize materials. Heat-degraded solutions using Millipore filter paper with a diameter of 0.22 micrometers.

Preparation of Solutions and Media:

The physiological saline solution was prepared according to Forbes *et al.* (2007), autoclaved, and stored in the refrigerator until used. Ready-made Gram stain solutions were used by the Indian company Himedia, consisting of crystal violet dye solution, iodine solution, alcohol, and safranin dye. They were used to stain glass slides prepared from bacterial cultures to observe their properties and microscopic characteristics and classify them into positive and negative for that dye.

Preparation of Dye and Buffer Solutions:

The crystal violet dye solution was prepared based on Namasivayam *et al.* (2013), and was placed in a sterile dark bottle and kept in the refrigerator until use. It was used to detect the bacteria that form the biofilm. The saline phosphate buffer solution was prepared by dissolving 15 g of the buffer powder in 1000 ml of sterile distilled water. According to the instructions of the Indian manufacturer Himedia, Mueller-Hinton medium was prepared according to the instructions of the Indian manufacturer Himedia. After sterilizing it in an autoclave, it was poured into petri dishes and left until it cooled and solidified and was kept in the refrigerator until use (Stahil, 1969). Mayer's reagent was prepared based on Sousek et al. (1999), and ferric chloride solution (1%) according to Atlas *et al.* (1995).

Preparation of Plant Extracts:

Seeds of cruciferous family plants (Cress, *Lepidium sativum* L., Watercress Eruca L. sativa, Radish, L. Raphanus sativus) were collected and classified by a plant taxonomist, then washed well to remove dirt and dust, dried at room temperature for about three days, and then ground using an electric grinder. To obtain a fine powder, it was then stored in tightly sealed dry bags in the refrigerator until used for extraction.

Preparation of Biological Test Solutions:

The concentrations used in biological tests were prepared by dissolving 2 grams of plant extract powder in 10 ml of distilled water for the hot aqueous extract and the same weight in the solution Buffer phosphate for the alcoholic extract. It is considered the basic solution for storage and using the general dilution law C1V1 = C2V2. The concentrations were prepared (100, 150, (200 mg/ml and sterilized using a Millipore filter with holes 0.22 micrometers in diameter (Al-Awadi, 1993).

Bacterial Samples and Gram Staining:

Bacterial samples, isolated and identified by specialists were collected for both *E. coli* and *S. aureus*. A portion of the bacterial colony was taken using the Loop bacterial vector and spread on a glass slide. The slide was stained with Gram stain for microscopic examination under the oil lens of an optical microscope. The microscopic characteristics were studied through the interaction of the dye with the bacterial isolate to show the shape of the cells, their aggregation, and their receptiveness to the violet dye, which means that the bacteria from the positive group or not, which means that it is from the negative group (Gillet *et al.*, 2002).

Preparation of Bacterial Suspension:

Bacterial suspension was prepared using a swab or spore transfer to transfer a sufficient number of bacterial colonies from a pure culture grown on solid Mueller-Hinton culture medium incubated at 37°C for 18-24 hours into 3 ml of sterile physiological saline solution 4.5-5.0% NaCl and pH ranged between 4.5 - 7.0 in a transparent plastic test tube, then the turbidity of the suspension was compared with a standard constant turbidity solution, which gives an approximate number of cells of (1.5 x 108) cells/ml (Pincus, 2006).

Diffusion Method for Antibacterial Testing:

Diffusion method was used in drilling according to what was stated therein (Obaidat *et al*., 2012) for three repetitions for each culture of the bacterial species under study and for each solvent. This has been made by transferring bacterial colonies from the bacterial suspension after comparing it with the standard McFarland solution, in which the approximate number of bacterial cells is equal to 1.5 x 10 CFU/ml. After growing it on nutrient agar medium at a temperature of 37°C for 24 hours, spreading it on solid Mueller-Hinton medium and leaving it for a period of between (5-10) minutes to dry, then making 4 holes with a diameter of 5 mm using the cork drill, three holes for the concentrations to be used and one hole of sterile distilled water for aqueous extracts and phosphate buffer solution for alcoholic extracts was considered as control at a rate of 50 microliters for each hole.

Biofilm Formation Detection:

These holes were left for an hour to ensure proper spread and incubated at a temperature of 37°C for 24 hours. The inhibitory effectiveness of each of the concentrations of chloroform, acetone, and hot water extracts was determined. All readings of the inhibition zones for the three replicates were taken using a standard ruler, and the average was taken for each concentration for all plant extracts. The method of Almeida *et al.* (2013) was followed to detect the ability of isolates to form a biofilm. The isolates were grown on nutrient agar medium for 24 hours at 37°C, and 3-4 colonies were transferred to 2 ml of nutrient broth using a sterile carrier for comparison with a fixed solution. Standard turbidity (McFarland 0.5) to obtain an approximate cell count of approximately 1.5 x 108 CFU\ml, Then, 150 microliters of the culture was transferred to microtiter dishes, three holes for each isolate, and 150 microliters of sterile nutrient broth (free of bacteria) was added to three holes, which were considered a comparison treatment. The dishes were covered with a special cover to provide sterile conditions, and then They were incubated at 37 degrees C for 24 hours, then the holes were washed with distilled water using an Elisa Washer device at a rate of three washes and left to dry in room air for 15 minutes. 200 microliters of crystal violet dye (1%) was added to each hole and left for 15 minutes. Then the holes were washed several times with distilled water and left to dry in room air for 15 minutes. Then the remaining dye was dissolved by adding 200 microliters of 95% ethanol, and the results were read by an ELISA reader device at a wavelength of 630 nm.

Results and Discussion

Inhibitory activity of plant extracts:

It is noted from the results of Table (1) that there are significant differences between seed extracts of plant species, and that this difference is due to the type of bacteria, the nature of the extraction, and the type of solvent used, which is important in the quality and quantity of the active secondary metabolic compounds present in the plant and which affect the microscopic organisms (Adikala *et al*. (2017).

Table(1) shows the effect of plant species, extraction method, plant extract concentrations, and their interaction on the inhibitory diameter (mm) of the Gram-positive *S. aureus*. Cress seed extract excelled by recording the highest diameter of inhibition, reaching 21.99 mm, while Radish seed extracts recorded the lowest value. It reached 5.77 mm. The chloroform extraction method outperformed the other two methods significantly by achieving the highest inhibition diameter of 17.00 mm, while the two extraction methods with acetone and water did not differ from each other significantly. The results of this study agreed with the findings of Abreu et al (2011). It was shown that chloroform extract has a greater effect than other extracts in microbial inhibition of *S. aureus*. The difference in concentrations had a clear effect that reached the point of significance, as the 200% concentration was superior to the other two concentrations by recording the highest diameter of inhibition, which reached 19.77 mm, compared to the lowest diameter of inhibition for the 100% concentration, which reached 12.00 mm. That is, with increasing concentration, the diameters of inhibition increased.

Table (1) also shows the significant binary interaction between plant type and extraction method, and Cress seed extract with the three extraction methods (chloroform, acetone, and aqueous) and the Watercress seed extract with the chloroform method outperformed the rest of the significant binary interactions, recording the highest inhibition values of 22.66, 22, 21.33, and 19. 66 mm, respectively, compared to the lowest diameter of inhibition in the aqueous extract of radish seeds, which was 4.00 mm. The garden cress extract at a concentration of 200% excelled in achieving the highest inhibition diameter of 25.00 mm, compared to the lowest diameter of inhibition in the aqueous extract of radish seeds, as inhibition was completely absent.

The extraction methods (chloroform, acetone, and aqueous) with a concentration of 200% outperformed the rest of the interventions by achieving the highest diameters of inhibition of 20.00, 20.00, and 19.33 mm, respectively, compared to the lowest value for the aqueous extraction method with a concentration of 100%, which was 11.33 mm.

Table 1. The effect of plant type, extraction method, plant extract concentrations and their interaction on the inhibitory diameter (mm) of *S. aureus*

plant species	Extraction method	Plant extract concentrations(%)			Interaction between plant species & extraction method
		100	150	200	
Radish	Aqueous	0.00 f	0.00 f	12.00 e	d 4.00
	Acetone	0.00 f	0.00 f	14.00 d	4.66 d
	Chloroform	0.00 f	12.00 e	14.00 d	8.66 c
Watercress	Aqueous	15.00 d	17.00 c	22.00 b	18.00 b
	Acetone	15.00 d	18.00 bc	20.00 b	17.66 b
	Chloroform	19.00 b	19.00 b	21.00 b	19.66 ab
Cress	Aqueous	19.00 b	21.00 b	24.00 a	21.33 a
	Acetone	20.00 b	20.00 b	26.00 a	22.00 a
	Chloroform	20.00 b	23.00 ab	25.00 a	22.66 a
plant species	Radish	0.00 e	4.00 d	13.33 c	5.77 C
	Watercress	16.33 c	18.00 bc	21.00 b	18.44 B
	Cress	19.66 b	21.33 b	25.00 a	21.99 A
Extraction method	Aqueous	11.33 d	12.66 c	19.33 a	14.44 B
	Acetone	11.66 cd	12.66 c	20.00 a	14.77 B
	Chloroform	13.00 c	18.00 b	20.00 a	17.00 A
Effect of extract concentrations		12.00 C	14.33 B	19.77 A	

*Different letters indicate a significant difference at P≤ 0.05 according to Duncan's multiple range test

Table 2. Effect of plant type, extraction method, concentrations of plant extracts, and their interaction on the inhibitory diameter (mm) of ***E. coli***

plant species	Extraction method	Plant extract concentrations(%)			Interaction between plant species & extraction method
		100	150	200	
Radish	Aqueous	0.00 e	0.00 e	0.00 e	0.00 e
	Acetone	0.00 e	0.00 e	12.00 c	4.00 d
	Chloroform	13.00 c	15.00 b	17.00 a	15.00 a
Watercress	Aqueous	12.00 c	14.00 b	14.00 b	13.33 b
	Acetone	13.00 c	12.00 c	12.00 c	12.33 b
	Chloroform	12.00 c	12.00 c	11.00 d	11.66 c
Cress	Aqueous	12.00 c	14.00 b	16.00 ab	14.00 ab
	Acetone	15.00 b	17.00 a	18.00 a	16.66 a
	Chloroform	13.00 c	14.00 b	18.00 a	15.00 a
plant species	Radish	4.33 d	5.00 d	9.66 c	6.33 C
	Watercress	12.33 b	12.66 b	12.33 b	12.44 B
	Cress	13.33 b	15.00 a	17.33 a	15.22 A
Extraction method	Aqueous	8.00 d	9.33 c	10.00 c	9.11 B
	Acetone	9.33 c	9.66 c	14.00 a	10.99 B
	Chloroform	12.66 b	13.66 b	15.33 a	13 .88 A
Effect of extract concentrations		10.00 B	10.88 B	13.11 A	

*Different letters indicate a significant difference at P≤ 0.05 according to Duncan's multiple range test

Table 3. Biofilm production as a result of plant species and extraction method for bacterial isolates

plant species	Extraction method	Type of bacteria	Biofilm production	
			Before adding the extract	After adding the extract
Radish	Aqueous	*S. aureus*	Strong(0.188)	Strong (0.150)
		E. coli	Strong(0.218)	Strong (0.123)
	Acetone	*S. aureus*	Strong(0.188)	Moderate(0.090)
		E. coli	Strong(0.218)	Strong (0.136)
	Chloroform	*S. aureus*	Strong(0.188)	Moderate(0.087)
		E. coli	Strong(0.218)	Moderate(0.108)
Watercress	Aqueous	*S. aureus*	Strong(0.188)	Negative(0.056)
		E. coli	Strong(0.218)	Moderate(0.063)
	Acetone	*S. aureus*	Strong(0.188)	Moderate(0.062)
		E. coli	Strong(0.218)	Moderate(0.083)
	Chloroform	*S. aureus*	Strong(0.188)	Moderate(0.075)
		E. coli	Strong(0.218)	Negative(0.047)
Cress	Aqueous	*S. aureus*	Strong(0.188)	Negative(0.047)
		E. coli	Strong(0.218)	Moderate(0.092)
	Acetone	*S. aureus*	Strong(0.188)	Negative(0.046)
		E. coli	Strong(0.218)	Negative(0.042)
	Chloroform	*S. aureus*	Strong(0.188)	Negative(0.056)
		E. coli	Strong(0.218)	Moderate(0.069)

Control = (0.056), Negative < = (0.056), Moderate > 0.056 – 0.112, Strong > 0.112

The three-way interaction between plant type, extraction method, and concentration was in line with the above results. The cress seed extract excelled in all the extraction methods used in this study (chloroform, acetone, and aqueous) at a concentration of 200%, and the cress seed extract using the chloroform method at a concentration of 150% outperformed the rest of the treatments by recording the highest diameters. The inhibition reached 25.00, 26.00, 24.00, and 23.00 mm, respectively.

It is noted from the results of Table (2) the effect of plant type, extraction method, plant extract concentrations and their interaction on the inhibitory diameter (mm) of E. coli bacteria. There are significant differences between the plant types used in the study, as the cress seed extract excelled by recording the highest average diameter of inhibition of 15.22 mm, while radish seed extracts recorded the lowest value, amounting to 6.33 mm.

As can also be seen from the same table that the chloroform extraction method was significantly superior to the other two methods by achieving the highest inhibition diameter of 13.00 mm. This is consistent with the findings of researcher Rashmi et al (2019), who showed that the chloroform extract had a high effect on E.coli bacteria, and the 200% concentration was superior to the other two concentrations by recording the highest diameter of inhibition of 13.11 mm compared to the lowest diameter of inhibition of the 100% concentration, which was 10.00 mm.

It appears from Table (2) that garden cress with the three extraction methods (chloroform, acetone, and aqueous) and radish with the chloroform method were significantly superior to the rest of the binary interventions, recording the highest values of inhibition amounting to 15.00, 16.66, 14.00, and 15.00 mm, respectively, compared to the lowest diameter of inhibition in the extract. The water level of radish seeds reached 0.00 mm. The cress seed extract at two concentrations of 200% and 150% excelled by achieving the highest diameters of inhibition, which were 17.33 and 15.00 mm, compared to the lowest diameter of inhibition for the aqueous extract of radish seeds, which reached 4.33 mm. The two extraction methods using chloroform and acetone with a concentration of 200% outperformed the rest of the interventions by achieving the highest diameters of inhibition that reached 15.33 and 14.00 mm, respectively, compared to the lowest average for the aqueous extraction method with a concentration of 100%, which reached 8.00 mm.

The triple interaction between the cress seed extract using the chloroform and aqueous method (chloroform, acetone and aqueous) at a concentration of 200%, the cress seed extract using the acetone method at a concentration of 150%, and the radish seed extract using the chloroform method at a concentration of 200% outperformed the rest of the interactions by recording the highest diameters of inhibition of 18.00, 18.00, 16.00 and 17.00 and 17.00 mm, respectively.

It can be concluded from the above results of tables (1) and (2) that the cress seed extracts had a greater effect on the two types of bacteria used in this study, as they inhibited their growth compared to the watercress and radish seed extracts. The reason may be due to the quality and proportions of the active substances in Garden cress, and the extraction method using chloroform was more efficient in extracting the active substances compared to the rest of the methods, as evidenced by its recording the highest diameter of inhibition for both types of bacteria. We also note that the higher the concentration used, the greater the diameter of inhibition, and this prompts the use of higher concentrations and within safety limits.

The results in Table (3) regarding the aqueous extract of radish seeds show that biofilm formation was strong in *S. aureus* at a rate of 0.150 and in E. coli at a rate of 0.123 compared to the control treatment, which reached 0.188 for *S. aureus* and 0.218 for *E. coli.* The result was consistent with the findings of Tajbakhsh (2016), which is that the rate of biofilm formation is strong in this bacteria. As for radish seed extract with acetone, the biofilm formation was moderate in *S. aureus* bacteria at a rate of 0.090, and the biofilm formation rate was 0.136 in *E. coli* bacteria, compared to the control treatment, which was 0.188 for *S. aureus* and 0.218 for *E. coli*. This percentage is close to what Ahmed (2016) found.

While in radish seed extract with chloroform, the biofilm formation was moderate in both S. aureus at a rate of 0.087 and 0.108 in E. coli bacteria, compared to the control treatment, which was 0.188 for *S. aureus* and 0.218 for *E. coli*. This result agreed with the findings of Al-Otbi (2013).

The results of the aqueous extract of garden cress seeds shown in Table (4-13) show that there was no biofilm formation in *S. aureus*, which was 0.056, while the average biofilm formation in E. coli was 0.063 compared to the control treatment, which was 0.188 for *S. aureus* and 0.218 for E. coli. These results are close to those reached by Jiyad (2016).

Biofilm composition for *S. aureus* was moderate (0.062%) and *E. coli* bacteria was 0.083 for cress seed extract with acetone compared to the control treatment, which was 0.188 for *S. aureus* and 0.218 for *E. coli.* This result was close to the findings of Zidane et al (2014), where the biofilm production rate was moderate.

Concerning Cress seed extract with chloroform, the biofilm formation rate was moderate (0.075) in *S. aureus*, and there was no membrane formation in *E. coli*, and the rate was 0.047, compared to the control treatment, which was 0.188 for *S. aureus* and 0.218 *for E. coli*. This result is consistent with the results of Al-Khalidi's study (2016).

The results of the Watercress extract shown in Table (3) show that there was no formation of a biofilm in *S. aureus*, which was 0.047, while the membrane was moderate in *E. coli*, reaching 0.092 for the same extract when compared with the control, which was 0.188 for

S. aureus and 0.218 for *E. coli*. This result agreed with the findings of Moteeb (2018).

Watercress seed extract with acetone did not form a biofilm on both *S. aureus*, the percentage was 0.046, and 0.042 for *E. coli*, compared to the control, which was 0.188 for *S. aureus* and 0.218 for *E. coli*. This result is consistent with the findings of Sabah (2018) .

As for Watercress extract with chloroform, there was no biofilm formation in *S. aureus*, which amounted to 0.056, while the membrane formation was modirate in *E. coli*, at a rate of 0.069, compared to the control, which was 0.188 for *S. aureus* and 0.218 for *E. coli*. This study agreed with the findings of Al-Sura Mary (2014).

Conclusion

The results of this study showed the critical role of the described method in assessing biofilm formation and categorizing its productivity as high, medium, or low. The extracts that inhibited biofilm formation or resulted in moderate biofilm development indicate the presence of effective substances within the extracts that hindered bacterial activity. This finding aligns with the conclusions of Al-Jumaili (2017), demonstrating the potential of certain extracts to prevent biofilm formation.

Conversely, the bacteria that formed strong biofilms exhibited robust virulence factors, remaining unaffected by the extracts' effectiveness. This observation is consistent with the findings of Tajbakhsh (2016), which suggest a strong relationship between biofilm production capability and pathogenicity. Bacteria that produce strong biofilms tend to be more resistant to antibiotics and phagocytosis and can withstand various environmental conditions. The protective nature of biofilms shields bacteria from the host's immune response, preventing penetration and destruction by the host (Khan et al., 2017). This study underscores the importance of biofilm formation in bacterial pathogenicity and the potential for targeted extracts to inhibit biofilm development, thereby mitigating bacterial virulence and resistance.

Author contributions

A.E.A., W.M.D. conceptualized, analyzed data, edited and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Abreu, A.C., McBain, A.J., and Simoes, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. Natural product reports, 29(9), 1007-1021.

Adikala, G. Raj ; Jayaraman, M. ; Krishnamoorthy , S. ; Chandrasekaran, M. and Venkatesalu, V.(2017). Screening of Different Extracts of Marine Macro Green Algae for Larvicidal Activity against Dengue Fever Mosquito, Aedes aegypti (Diptera: Culiadae). International Letters of Natural Sciences, Vol. 62, 44-51.

Ahmed, Ali Fadel, (2016). A molecular study of a number of Escherichia coli bacterial adhesion factors isolated from urine samples from children under five years old. Master's thesis, Ibn al-Haytham College of Education for Pure Sciences, University of Baghdad.

Al-Awadi, Salwa Saber. (1993). Study of antibacterial activity. Master's thesis/College of Veterinary Medicine - University of Baghdad.

Al-Jumaili, Hanan Thamer Najm Abdul Ali Al-Jumaili (2017) A comparative study of the virulence factors of bacteria Isolated from urinary tract infections of cardiac intensive care patients. Master's thesis, college Education for pure sciences, University of Diyala.

Allen, W. J.; Phan, G. and Waksman, G. (2012). Pilus biogenesis at the outer membrane of Gram-negative bacterial pathogens. Current opinion in structural Biolog., 22:500-506.

Almeida, C., Azevedo, N. F., Santos, S., Keevil, C. and Vieira, M. (2013). Correction: Discriminating Multi-Species Populations in Biofilms with Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA FISH) . PLoS ONE, 8(6): 101-371.

Alo, M.; Eze, U. G. and Anyim, C. (2012). In vitro Antimicrobial activities of Extracts of Magnifera indica, Caricapapaya and Psidium guajava Leaves on Samonella typhi isolates. World J. of pub.Heal.Scie.,1:1-6.

Al-Otbi, Duaa Adnan Kazem (2013). A bacteriological study of some species of Enterobacteriaceae isolated from the halls of the maternity hospital in the city of Baqubah. Master's thesis, University of Diyala, College of Education.

Al-Qaisi, Hassan (2004), Dictionary of Medicinal Herbs and Plants, Dar Al-Kutub Al-Ilmiyyah, Beirut, 6th edition.

Al-Sura Mary, Lara Mahmoud Shafiq (2014). Study of serotypes and virulence factors of bacteria Escherichia coli isolated from human urinary tract infections in Diyala Governorate Education for pure sciences.

Al-Taweel, Rana Saleh. (2016). Drug sensitivity to Staphylococcus aureus isolated from women of reproductive age. Al-Qadisiyah Journal of Pure Sciences 1(21), 50-57.

Amissah, N. A., van Dam, L., Ablordey, A., Ampomah, O. W., Prah, I., Tetteh, C. S., ... and Stienstra, Y. (2017). Epidemiology of Staphylococcus aureus in a burn unit of a tertiary care center in Ghana. PloS one, 12(7), e0181072.

Anibijuwon, I.I, and Udeze, OA. (2009), Antimicrobial Activity of Carica papaya (pawpaw Leaf) on Some Pathogenic Organisms of Clinical Origin from south –Western Nigeria. Ethnobotanical Leaflets 13,850 – 64.

Atlas, R. M., Parks, L. C. and Brown, A. E. (1995). Laboratory Manual of Experimental Microbiology. 1st ed. Mosby, Inc. Missouri.

Chen, Q., Xie, S., Lou, X., Cheng, S., Liu, X., Zheng, W., ... and Wang, H. (2020). Biofilm formation and prevalence of adhesion genes among Staphylococcus aureus isolates from different food sources. Microbiologyopen, 9(1), e00946.

Dari, W. A. (2019). Incidence and Antibiotics Susceptibility Pattern of Coagulase Positive and negative Staphylococci amongst (UTI) children in Children Welfare

Hospital/Baghdad/IRAQ. Indian Journal of Public Health Research & Development, 10(1), 1067-1072.

Dielubauza, E. J. (2011). Urinary tract infection in women .Med. Clin. Of North America, 95(1):27-41.

Forbes, B. A., Sahm, D. F., and Weissfeld, A. S. (2007). Diagnostic microbiology. St Louis: Mosby.

Gillet, Y., Issartel, B., Vanhems, P., Fournet, J. C., Lina, G., Bes, M., ... and Etienne, J. (2002). Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. The Lancet, 359(9308), 753-759.

Iqbal, M. N., and Ashraf, A. (2019). Withania somnifera: Can it be a Therapeutic Alternative for Microbial Diseases in an Era of Progressive Antibiotic Resistance?. International Journal of Nanotechnology and Allied Sciences, 3(1), 16-18.

Jawetz , M and Adelberg's E.A. (2016). Midical Microbiology .27th ed ., The McGraw-Hill Companies ,Inc.New Yourk, USA.

Jiyad, Asmaa Ahmed (2016) The mixed effect of antibiotics on Staphylococcus SPP bacteria isolated from different clinical infections. Master's thesis, College of Education for Pure Sciences, University of Diyala.

Jouda, M. M. (2013). The antibacterial effect of some medicinal plant extracts and their synergistic effect with antibiotic and non-antibiotic drugs. Faculty of Science-Islamic University-Gaza.

Karpagam, P., and Manonmani, G. (2019). Antagonistic Property of Selected Herbal Combination for Substitute of Synthetic Antimicrobial Agents. Journal of Drug Delivery and herapeutics, 9(4-A), 53-56.

Kassie , F; Pool- Zobel, B; Parzefall , W. and Knasmuller, S. 1999. Genotoxic effects of benzyl isothiocyanates, a natural chemopreventive agent. Mutagenesis 14:595-604 .

Khalifa, Hassan (2009), The Book of Herbal Paradise, first edition, Dar Al-Israa for Publishing and Distribution - Amman.

Khan, F.; Shukla, I.; Rizvi, M.; Mansoor, T. and Sharma, S. C. (2017). Detection of biofilm formation in Staphylococcus aureus .Does it have a role in treatment of MRSA Infection. Trends in Med. Res. Academic J., 6(2): 116-123.

Mazokopokis EE,papadakis J. and Apapadomanoloki MG, (2007), Effects of12 months treatment with L-seleno methionine on seruman anti Tpo levels in patients with it ashimotos thyroiditis.Thyroid17:609-612.

Moteeb, Sh. H.(2018). Quantitative and Qualitative Assayes OF Bacterial Biofilm Produced By Pseudomonas aeruginosa AND Klebsiella spp. J. of al-anbar university for pure science : Vol.2: No.3.

Namasivayam, S. K. R., Christo, B. B., Arasu, S. K., Kumar, K. A. M., and Deepak, K. (2013). Anti-biofilm effect of biogenic silver nanoparticles coated medical devices against biofilm of clinical isolate of Staphylococcus aureus. Global Journal of Medical Research, 13(3), 1-7.

Obeidat, M., Shatnawi, M., Al-Alawi, M., Al-Zubi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudah, J. and Qtri, I. (2012). Antimicrobial Activity of Crude Extracts of Same Plant Leaves. Res. J. of Microbiology ., 7:59-67.

Pincus, D. H. (2006). Microbial identification using the bioMérieux Vitek® 2 system. Encyclopedia of rapid microbiological methods, 1, 1-32.

Pulipatis,S ., Babu , P.S.,Narasu, M.L.,and Anush , N. (2017) An over view on urinary traet infection J. Med. Plant, 5 , 50-56 .

Rashmi, D.; Pathak, V. and Kumar, R. (2019). Effect of Ricinus communis L. on microorganisms: Advantages and dis advantages Int. J. Curr. Microbiol. App. Sci., 8(4): 878-884.

Sabah, Zaman Atiya Sabah. (2018). Bacteriological and molecular study of Escherichia coli bacteria Isolated from urinary tract infections in displaced persons camps and investigating their bacteriophages. Master's thesis, College of Science, University of Diyala.

Sofowora, A.; Ogunbodede, E. and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. Afr. J. Tradit. Complement. Altern. Med., 10(5) :210-229.

Stahil, R. (1969). Thin layer chromatoghraphy: A laboratory hand book. 2nd edition. Traslated by ashworth, M. R. Springer. Berlin, Heidelbery . N. Y.

Tajbakhsh, E.; Ahmadi, P. Abedpour-Dehkordi, E.; Arbab-Soleimani, N. and Khamesipour, F. (2016). Biofilm Formation, Antimicrobial Susceptibility, Serogroups and Virulence Genes of Uropathogenic E. coli Isolated from Clinical Samples in Iran. Antimicrob Resist Infect Control. 5(11): 1-8.

Tepe , B. ; Donmez , E. ; Unlu , M. ; Candan , F. ; Daferera , D. ; Vardar – Unlu , G. ; Polissiou , M. and Sokmen , A. (2004), Antimicrobial and antioxidative activities of the essential oils and methanol extracts of the Salvia cryptantha (Monthbret et Aucher ex Benth.) and Salvia multicaulis (Vol1), Food Chemistry. 84(4) : 519 – 525.

Tiwari, N., López-Redondo, M., Miguel-Romero, L., Kulhankova, K., Cahill, M. P., Tran, P. M., ... and Tuffs, S. W. (2020). The SrrAB two-component system regulates Staphylococcus aureus pathogenicity through redox sensitive cysteines. Proceedings of the National Academy of Sciences, 117(20), 10989-10999.

Valle Jr, D. L., Andrade, J. I., Puzon, J. J. M., Cabrera, E. C., and Rivera, W. L. (2015). Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. Asian pacific journal of tropical biomedicine, 5(7), 532-540.

World Health Organization (WHO). (2020). World health statistics 2020: monitoring health for the SDGs, sustainable development goals ISBN 978-92-4- 000510-5 (electronic version).

Zidane, Israa Ali Al-Qazzaz, Abdul Karim Abdul Razzaq, and Muhammad Ali Sadiq. (2014). The effect of the combination Antibiotics on clinically isolated Staphylococcus aureus bacteria. University Journal Baghdad Science, Department of Biotechnology, College of Science, University of Baghdad, 6 (4). Al-Khalidi, Zuwaida Kazem Khudair. (2016). Technological study of some Gram-negative Bacterial species isolated from vaginal infections. Master's thesis, College of Education for Pure Sciences, University of Diyala.