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Susceptibility testing of *Capparis spinose* against bacterial species isolated from environmental and clinical sources in Erbil city

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Abstract:

The current study aimed to susceptibility testing of *Capparis spinose* against bacterial species isolated from environmental and clinical sources. 260 samples were taken from indoor places (hospital and school). The environmental samples included air-condition system and air rooms of hospital (Rizgary Hospital and Erbil Teaching Hospital) and school buildings (Gwar-jara School, Shorsh School and Shaysta School) in Erbil city from January to April 2023. Out of 260 samples, 157 isolates (or 60.4%) showed good results for bacterial growth on the best cultured media, including blood agar, mannitol agar, and MacConkey agar. 103(39.6%) of 260 samples were appear as negative results for bacterial growth. *Staphylococcus aureus* was the most isolated, with 42(26.8%) isolates out of a total of 157 isolates, followed by *E. coli* as the number of its isolates reached 40(25.4%). While, *Enterobacter cloacae* the least isolated, as the number of its isolates reached 11(7.0%) out of a total of 157 isolates. The most sensitive was *E. faecalis* towards the *C. spinosa* aqueous extract, as the inhibition zone reached 24.2 mm, while *S. aureus* was resistant to the plant extract, as the inhibition zone reached 7.7 mm. while, the results shows the zone of inhibition of the ethanolic extract against the study bacteria, where the results show that *E. faecalis* was the most sensitive towards the ethanolic extract, as the inhibition zone reached 41.2 mm, while *S. aureus* was the least sensitive towards the ethanolic extract, as the inhibition zone reached 19.8 mm.

Keywords: *C. spinose*, HAIs, Gram negative bacteria, *E. coli*, *S aureus*

Introduction

These days, healthcare-associated infections (HAIs) are among the most important public health issues affecting both developed and developing nations, regardless of monthly income [1]. HAIs have a significant impact on a number of healthcare-related factors, including patient safety and financial burden [2]. In recent years, the importance of a contaminated hospital environment has become more attention [3]. Infections linked to healthcare facilities cause deadly diseases and high death rates. One form of bacterium is resistant to a broad range of antibiotics, which is why infections in healthcare facilities are characterized by furious "high virulence" and an uncommon capacity to resist medications numerous times at once [4-5].

Otherwise, a deeper comprehension of the ambient microbiome's involvement in environments and health is essential, as it is one of the fundamental elements of human exposures in built environments that greatly contributes to occupants' health [6]. This indicates that, aside from homes, schools are the places where teachers and students are exposed to the environment the most. According to a recent study on school's indoor pollution, schoolchildren's upper and lower respiratory tracts, eyes, and systemic illnesses were linked to higher exposures to particulate matter and volatile organic compounds in classrooms [7].

In light of the fact that synthetic drugs can have a wide range of negative side effects, using herbal medicine is one of the more promising solutions if it is supported by scientific research in regards to the ongoing rise in bacterial resistance to antibiotics, the emergence of multi-resistant strains, and the ensuing therapeutic issues. The beneficial effects of medicinal plants on health can now be valued and rationalized thanks to a number of facts that have recently been released in this direction [8-9]. *C. spinosa* L. (Caper) is a perennial spiny shrub of Capparidaceae family, it possesses wide antimicrobial spectrum including antibacterial and antifungal activity in addition to their antioxidant, along with their uses in the traditional medicine for controlling of many diseases [10-11].

Materials and methods

Study sites

The samples of the current study were collected from January to April 2023 from different locations, including hospitals (Rizgary Hospital and Erbil Teaching Hospital) and some schools (Gwarjara School, Shorsh School and Shaysta School).

Sample Collection

260 clinical and environmental samples were taken from clinical and environmental sources, including samples of blood, urine, sputum and wounds. The environmental samples included taking swabs from Surgical instruments, student clothes, student bags, sick beds and floors, hall air in hospitals and classroom air in schools Erbil at January to April 2023.

With regard to clinical samples, names, ages, gender and health status were taken. for volunteers in the study. As for the environmental samples, the date of construction, ventilation, lighting, room size, number of people per room, and temperature were recorded.

Collection of plant samples

The experiment's therapeutic plants were identified using a variety of sources, including relevant taxonomic literature. Roots are the components of *Capparis spinosa* that are employed. In May 2023, samples were gathered from

Diyala City. The plants were properly cleaned, cut into little pieces, shade-dried, and ground into a powder. A dry, clean separating funnel was used.

Water extraction

In order to perform an aqueous extraction, two hours were spent boiling 20 grams of powdered roots in 150 milliliters of distilled water over low heat. The supernatant was then collected after it was filtered through eight layers of muslin cloth and centrifuged for ten minutes at 5000g. After six hours, the supernatant was collected every two hours, combined, and concentrated to produce a final amount that was one-fourth of the initial volume [12]. This process was repeated twice.

Ethanol extraction

Plant extracts were obtained by following Jameela *et al.*, [13] procedure, which involved using soxhlet apparatus to extract 20 grams of leaves and 20 grams of roots individually for 8 hours using 150 milliliters of 80% ethanol as a solvent. After filtering, the extract was placed in an oven set at 45 °C to evaporate. After being dissolved in dimethyl sulfoxide (DMSO), the dry extract was refrigerated for later use.

Identification of Morphological characteristics

The forms, colors, diameters, odors, and other properties of the bacterial isolate colonies that were grown on blood agar and MacConky media were characterized (Microscopical examination and biochemical testing) [14].

Well diffusion method assay

Obeidat *et al.* [15] state that an inoculum suspension was uniformly swabbed onto 20 mL of solidified Mueller-Hinton Agar (MHA) to enumerate bacteria, and the inoculum was then given five minutes to dry. Glass Pasteur pipettes were used to create 6 mm diameter holes in the seeded agar. After adding an aliquot of 20 µl of each plant's crude extract (200 mg/ml) to each well on the seeded medium, the wells were left to stand on the bench for one hour to allow for adequate diffusion, and then they were incubated for twenty-four hours at 37 °C. The millimeters (mm) of the ensuing inhibition zones were measured.

Results and discussion

Samples distribution

The current study included 260 samples including 146(56.2%) clinical samples and 114(43.8%) environment samples (table 1). The findings were found that 157(60.4%) of 260 samples were appear as positive results for bacterial growth that in optimal cultured media such as blood agar, mannitol agar, and MacConkey agar. 103(39.6%) of 260 samples were appear as negative results for bacterial growth. On the other hand, the positive results for bacterial growth of clinical samples (blood, urine, sputum and wounds) was 102(69.9%), while, the positive results for bacterial growth of environment samples (surgical instruments, student clothes, student bags, sick beds and floors, hall air) was 55(48.2%).

Table (1): Distributed of study samples according to sample sources

Groups	No. (%) +ve culture	No. (%) -ve culture	Total No.(%)
Clinical samples	102(69.9%)	44(30.1%)	146(56.2%)
Environment samples	55(48.2%)	59(51.8%)	114(43.8%)
Total	157(60.4%)	103(39.6%)	260(100%)

Table (2) shows the numbers and percentages of isolates for each type of bacteria that were isolated from clinical (blood, urine, sputum and wounds) and environmental (surgical instruments, student clothes, student bags, sick beds and floors, hall air) sources, where it is noted that *Staphylococcus aureus* was the most isolated, with 42(26.8%) isolates out of a total of 157 isolates, followed by *E. coli* as the number of its isolates reached 40(25.4%). While, *Enterobacter cloacae* the least isolated, as the number of its isolates reached 11(7.0%) out of a total of 157 isolates.

Table (2): Distributed of study samples according to sample sources

Groups	Clinical	Environment	Total No.(%)
<i>Escherichia coli</i>	23(57.5%)	17(42.5%)	40(25.4%)
<i>Klebsiella spp.</i>	16(59.3%)	11(40.7%)	27(17.2%)
<i>Pseudomonas aeruginosa</i>	14(66.7%)	7(33.3%)	21(13.4%)
<i>Enterococcus faecalis</i>	11(68.8%)	5(31.2%)	16(10.2%)
<i>Enterobacter cloacae</i>	9(81.8%)	2(18.2%)	11(7.0%)
<i>S. aureus</i>	29(69.1%)	13(30.9%)	42(26.8%)
Total	102(69.9%)	55(48.2%)	157(100%)

The current study also showed that the highest percentage of bacteria isolation from clinical and environmental samples was for Gram-positive bacteria, as *S. aureus* showed the highest isolation rate 26.8%, while *E. coli*, which is gram showed the highest isolation rate. The largest isolation percentage was 25.4% as shown in in Table (2). The results of this study agreed in terms of the proportion of *S. aureus* with the study of Kamel *et. al.*, [16], who isolated bacteria from pregnant vagina with a swab number of 43 and 13 swab samples from 6 delivery rooms. They indicated that the percentage of *S. aureus* was 34%, while the percentage of *Klebsiella spp.* 11.36%, and the percentage of *E. coli* isolates was 20.5%, while the percentage of *P. aeruginosa* was 27.27%.The current study differed with the above study in terms of the proportions of *P. aeruginosa* to the type of samples that were taken and the source of the samples, as among the most dangerous environmental sources was the Intensive Care Unit. ICUs are typically thought of as the epicenters of pathogens that are multidrug resistant (MDR). The most significant risk factors include the frequency of invasive device usage, the overuse of antibiotics, which selectively targets bacteria, and the proportion of immunosuppressed patient populations with serious underlying illnesses [17].

The isolation percentage in this investigation was consistent with many other studies that looked at the prevalence of *E. coli* in ambient samples from hospitals and clinical settings. In 210 environment samples in Hilla, 42(20%) *E. coli* isolates were found in a local investigation conducted by Al-Hilli [18], While the percentage of *E. coli* isolates in the current study was lower than that of Hadi [19] who found that *E.coli* was the most common (42.6%) organism isolated from patients with significant bacteriuria followed by *Klebsiella spp.*, While the percentage of *E. coli* isolates in the current study was much higher than that of AL-Amiedi [20] who noted the most isolate was *E. coli* (2.55%) every sterilized Hospital Environment of operation theater ,prematurity wards & emergency ward & kitchenat in Babylone Marternal &Children Hospital.

Table (3) showed that urine samples were the clinical samples that showed the most bacterial growth, amounting to 26(56.6%) out of a total of 46 isolates, the number of positive isolates from blood was 4 (8.7%), and the number of positive isolates from wounds was 14 (30.4%), while Sputum, which was collected from students, was the least in terms of bacterial growth, if the percentage of bacterial growth reached 2(4.3%) out of a total of 46 isolates.

Table (3): The number and percentages of bacterial isolates from hospital patients

Groups	Blood	Urine	Wounds	Sputum	Total No.(%)
<i>E. coli</i>	1(7.7%)	9(69.2%)	3(23.1%)	0(16.6%)	13(28.3%)
<i>Klebsiella spp.</i>	1(11.1%)	5(55.6%)	3(33.3%)	0(0.0%)	9(19.6%)
<i>P. aeruginosa</i>	1(14.3%)	3(42.9%)	2(28.5%)	1(14.3%)	7(15.2%)
<i>E. faecalis</i>	0(0.0%)	2(66.7%)	1(33.3%)	0(0.0%)	3(6.5%)
<i>Enterobacter cloacae</i>	0(0.0%)	4(66.7%)	2(33.3%)	0(0.0%)	6(13.0%)
<i>S. aureus</i>	1(12.5%)	3(37.5%)	3(37.5%)	1(12.5%)	8(17.4%)
Total	4(8.7%)	26(56.6%)	14(30.4%)	2(4.3%)	46(100%)

Table (4) shows that patients' beds are the most polluted, amounting to 10 (43.5%), while surgical instruments were the least polluted, amounting to 1 (4.3%) out of a total of 23 positive environmental isolates from hospitals. On the other hand, the number of isolates from hospital floors was 8 (34.8%), while the hall air, the number of isolates was 4 (17.4%).

Table (4): The number and percentages of bacterial isolates from hospitals

Groups	Surgical instruments	Sick beds	Floors	Air of rooms	Total No.(%)
<i>E. coli</i>	0(0.0%)	4(66.7%)	1(16.7%)	1(16.6%)	6(26.1%)
<i>Klebsiella spp.</i>	0(0.0%)	2(66.7%)	1(33.3%)	0(0.0%)	3(13.0%)
<i>P. aeruginosa</i>	0(0.0%)	1(50.0%)	1(50.0%)	0(0.0%)	2(8.7%)
<i>E. faecalis</i>	0(0.0%)	0(0.0%)	1(33.3%)	2(66.7%)	3(13.0%)
<i>Enterobacter cloacae</i>	0(0.0%)	1(50.0%)	1(50.0%)	0(0.0%)	2(8.7%)
<i>S. aureus</i>	1(14.3%)	2(28.6%)	3(42.8%)	1(14.3%)	7(30.5%)
Total	1(4.3%)	10(43.5%)	8(34.8%)	4(17.4%)	23(100.0%)

In study of Miller [21] referred that the distribution of bacteria throughout the yards of the three hospitals may be related to the type of patients in each yard and/ or the type of treatment provided in that yard. Staphylococcus species localized almost in all yards because it is considered as global first suspect in causing nosocomial infection even the commensal species like Staphylococcus spp., this explains the results of the current study in terms of the presence of *S. aureus* in all types of environmental samples, which included Surgical instruments, which amounted to 1 (14.3%), Sick beds, which amounted to 2 (28.6%), and floors, which amounted to 3 (42.8%). Also, isolates positive for *S. aureus* were collected. *S. aureus* amounted to 1 (14.3%). Other types of bacteria like *Klebsiella pneumonia* and *Pseudomonas aeruginosa* located in RCU mostly due to the in-patients who carry these bacteria and contaminating the ventilators or aspiration systems, such result is in agreement with Akash et al [22] and also established as a fact [23], this explains the results of the current study in terms of the presence of *Klebsiella spp.* On hospital floors it amounted to 1(33.3%) and patient beds 2(66.7%). On the other hand, the *E. coli* which are members of Enterobacteriaceae are considered as major causative bacteria for NIs in abdominal operation rooms [24].

On the other hand, the *E. coli* which are members of Enterobacteriaceae are considered as major causative bacteria for NIs in abdominal operation rooms [24]. This is also documented in this study, whereas these bacteria were isolated from the air of the hospital rooms, where the percentage of *E. coli* was 1(16.6%).

Table (5) shows the numbers and percentages of each type of bacteria that were isolated from clinical sources (blood, urine, sputum and wounds) in the schools that were accredited in the current study. Where it is noted that *E. coli* was the most isolated among the other species, amounting to 15(26.8%) of the total 56 clinical isolates, while *Enterobacter cloacae* was the least isolated type of bacteria, amounting to 2(3.6%). The results also show that Gwarjara School was the most polluted with a percentage of 20 (35.7%) compared to the rest of the schools, which included Shaysta School with a percentage of 19 (33.9%) and Shorsh School with a percentage of 17 (30.4%).

Table (5): The number and percentages of bacterial isolates from schools with clinical source

Groups	Gwar-jara School	Shorsh School	Shaysta School	Total No.(%)
<i>E. coli</i>	5(33.3%)	6(40.0%)	4(26.7%)	15(26.8%)
<i>Klebsiella spp.</i>	3(37.5%)	3(37.5%)	2(25.0%)	8(14.2%)
<i>P. aeruginosa</i>	4(44.5%)	2(22.2%)	3(33.3%)	9(16.1%)
<i>E. faecalis</i>	3(37.5%)	2(25.0%)	3(37.5%)	8(14.2%)
<i>Enterobacter cloacae</i>	1(50.0%)	0(0.0%)	1(50.0%)	2(3.6%)
<i>S. aureus</i>	4(28.6%)	4(28.6%)	6(42.8%)	14(25.0%)
Total	20(35.7%)	17(30.4%)	19(33.9%)	56(100.0%)

Table (6) showed that urine samples were the school students that showed the most bacterial growth, amounting to 29 (51.8%) out of a total of 56 isolates, the number of positive isolates from blood was 5(8.9%), and the number of positive isolates from wounds was 18(32.1%), while Sputum, which was collected from students, was the least in terms of bacterial growth, if the percentage of bacterial growth reached 4 (7.1%) out of a total of 56 isolates.

Table (6): The number and percentages of bacterial isolates from school students

Groups	Blood	Urine	Wounds	Sputum	Total No.(%)
<i>E. coli</i>	2(13.3%)	9(60.0%)	3(20.0%)	1(6.7%)	15(26.8%)
<i>Klebsiella spp.</i>	1(12.5%)	5(62.5%)	2(25.0%)	0(0.0%)	8(14.2%)
<i>P. aeruginosa</i>	0(0.0%)	4(44.4%)	4(44.4%)	1(11.2%)	9(16.1%)
<i>E. faecalis</i>	1(12.5%)	5(62.5%)	2(25.0%)	0(0.0%)	8(14.2%)
<i>Enterobacter cloacae</i>	0(50.0%)	1(50.0%)	1(50.0%)	0(0.0%)	2(3.6%)
<i>S. aureus</i>	1(7.1%)	5(35.7%)	6(42.9%)	2(14.3%)	14(25.0%)
Total	5(8.9%)	29(51.8%)	18(32.1%)	4(7.1%)	56(100.0%)

Regarding the number of clinical samples that were taken from students in schools in the current study, it was found that *E. coli* bacteria is the most common, amounting to 15 (26.8%) out of the total of 56 positive samples collected from students of current study schools. In a study conducted by Mohammad *et al.* [25] on school students, where 400 urine samples were taken from students, 164 (41%) urine samples showed a positive culture growth, while 236 (59%) samples had no growth. In positive culture growth, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were 73.2%,20.7%,2.4%, respectively, the results of the study were close to the results of the current study, as it was found that 29 (51.8%) of the urine samples showed positive growth of Gram-positive and Gram-negative bacteria.

Table (7) shows that the highest percentage of pollution from environmental samples collected from schools was students' clothes, which amounted to 12 (37.5%) out of a total of 32, followed by contamination of students' bags, which amounted to 11 (34.3%), while classroom air pollution was It is the lowest and reached 3 (9.4%). The percentage of classroom floor contamination was 6 (18.8%).

Table (7): The number and percentages of bacterial isolates

Groups	Student clothes	Student bags	Floors	Air of class	Total No.(%)
<i>E. coli</i>	3(42.9%)	2(28.5%)	1(14.3%)	1(14.3%)	7(21.9%)
<i>Klebsiella spp.</i>	1(20.0%)	2(40.0%)	2(40.0%)	0(0.0%)	5(15.6%)
<i>P. aeruginosa</i>	0(0.0%)	2(50.0%)	1(25.0%)	1(25.0%)	4(12.5%)
<i>E. faecalis</i>	2(66.7%)	1(33.3%)	0(0.0%)	0(0.0%)	3(9.3%)
<i>Enterobacter cloacae</i>	1(50.0%)	1(50.0%)	0(0.0%)	0(0.0%)	2(6.3%)
<i>S. aureus</i>	5(45.4%)	3(27.3%)	2(18.2%)	1(9.1%)	11(34.4%)
Total	12(37.5%)	11(34.3%)	6(18.8%)	3(9.4%)	32(100%)

Environmental samples from various sites in the primary schools under investigation revealed a higher prevalence of Gram negative bacteria than Gram positive bacteria. These findings aligned with the results of a recent study, but they did not support the findings of Jomha *et al.* [26], which found that hand contamination of desks and other classroom objects was a significant predictor of the risk of diarrhea. It is necessary to investigate ways to lessen these contaminations, such as emphasizing hygiene education and keeping track of hand washing [27], concluded that during outbreaks of diarrhea, hands and classroom objects play a role in the transmission of diarrhea in day care centers with regard to Gram-positive bacteria, the current study showed that the number of *S. aureus* isolates was 9 (27.3%) out of a total of 32 isolates.

According to Meadow *et al.* [28], Staphylococcus species were also found on school chairs, which come into constant contact with human skin. The human skin and mucous membranes are frequently colonized by Staphylococcus species, which are an essential component of the body's natural microbiota. Its capacity to attach itself to the surface of biomaterials and form biofilms is a significant virulence factor and the primary pathogenic mechanism of staphylococcal infection [29].

Study of the effectiveness of *C. spinose* extract against bacteria

Bioactive compounds in *C. spinose* extract

General chemical assays conducted on capers extract showed the presence of a number of biologically important compounds, including: tannins using ferric chloride reagent and lead reagent [30], resins and saponins [31], flavonoids, alkaloids, steroids, and phenols, glycosides and carbohydrates, as shown in table (8). These results with regard to the active compounds in the caper plant were consistent with the results of [32-33].

Table (8): The results of the chemical reagents on the types of compounds present in *C. spinosa*

Types	Reagent	Results	Leave	Root
Tannins	1% Ferric Chloride	Bluish green color	+	+
Flavonoids	ammonia solution	Yellow color	+	+
Resins	4% hydrochloric acid	Turbid	+	+
Saponins	1% mercuric chloride	white precipitate	+	+
Alkaloids	1% tannic acid	A brownish white precipitate	+	+
Phenols	Potassium fricyanide	Dark bluish green	+	+
Steroids	(Sulfuric/ acetic) concentrated	Violet-blue-green	+	+
Glycosides	Benedict detector	red precipitate	+	+
Carbohydrates	Alpha - naphthol	Purple color	+	+

Antibacterial activity of *C. spinosa* extracts

Table (9) shows the inhibition zone of the aqueous extract against the study bacteria, as the results show that *E. faecalis* The most sensitive was *C. spinosa* towards the aqueous extract, as the inhibition zone reached 24.2 mm, while *S. aureus* was resistant to the plant extract, as the inhibition zone reached 7.7 mm.

Table (9): inhibition zone of the ethanol extract against the studied bacteria

Antibiotics	<i>E. coli</i>	<i>Klebsiella spp.</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>	<i>Enterobacter cloacae</i>	<i>S. aureus</i>
P1	19	22	16	25	18	10
P2	26	20	20	27	20	0
P3	21	18	18	21	29	11
P4	28	21	20	29	24	7
P5	20	23	17	26	18	9
P6	29	21	19	21	26	8
P7	21	20	16	22	19	8
P8	19	24	25	26	18	7
P9	30	21	21	20	24	8
P10	25	17	20	25	27	9
Average	23.8	20.7	19.2	24.2	22.3	7.7

Table (10) shows the zone of inhibition of the ethanolic extract against the study bacteria, where the results show that *E. faecalis* was the most sensitive towards the ethanolic extract, as the inhibition zone reached 41.2 mm, while *S. aureus* was the least sensitive towards the ethanolic extract, as the inhibition zone reached 19.8 mm.

Table (10): inhibition zone of the aqueous extract against the studied bacteria

Antibiotics	<i>E. coli</i>	<i>Klebsiella spp.</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>	<i>Enterobacter cloacae</i>	<i>S. aureus</i>
P1	32	29	35	43	41	22
P2	39	35	31	41	35	19
P3	40	33	28	39	31	17
P4	35	37	29	42	37	21
P5	41	35	33	38	42	23
P6	33	37	31	44	40	22
P7	31	36	36	40	38	16
P8	39	40	33	45	39	18
P9	38	41	33	41	38	19
P10	40	38	37	39	31	21
Average	36.8	36.1	32.6	41.2	37.2	19.8

In our study, the growth of *S. aureus*, *P. aeruginosa*, *Kelibsella spp.*, and *E. faecalis*—all of which were found to be resistant to many standard antibiotics—was remarkably inhibited by the alkaloids compounds. This indicates the potential of these compounds to control the growth of drug resistance microbes. Bacterial resistance to antibiotics is still a serious public health concern because it is a widespread nosocomial pathogen with immune system weakness due to disease or genetic disposition. Additionally, humans are a natural reservoir for this organism [34]. Furthermore, infections caused by multi-drug resistant bacterial species are among the most difficult to treat with conventional antibiotics. The alkaloids were removed and separated for the reasons mentioned above since it is widely known that alkaloids have potent biological and antimicrobial qualities [35].

Among the results, we note that the ethanol extracts had a higher inhibitory effect than the aqueous extracts against Gram-negative and positive bacteria, and this is what Parekh and Chanda [36] found in the fruits of *C. spinosa*, that the ethanol extract of the fruits had the most effect on bacteria compared to the aqueous extract. It may be due to the effectiveness of the ethanol extract in inhibiting bacteria, or to the fact that the active compounds may have solubility in organic solvents. It also agrees with Todar [37] who indicated that ethanol extracts of *C. spinosa* fruits showed high inhibitory activity on test organisms, and this can be deduced from the ability of ethanol to extract more base oils and secondary metabolites that are believed to be It exerts bacteriostatic activity on live test organisms [38].

Gram negative bacteria were more sensitive to extracts than Gram positive bacteria, according to the study's results, which were obtained using ethanol and aqueous extracts. This difference in sensitivity may have resulted from the complex multilayer structure of Gram-positive cell walls [39] and the function of secreted exoenzyme in protecting bacteria against plant extracts [40].

Our findings corroborated those of Mahasneh [41], who reported that *C. spinosa* aerial parts exhibited antibacterial activity against both Gram positive and Gram negative bacteria when dissolved in water, ethanol, and beutanol. This discrepancy may have resulted from the use of distinct solvents, strain types, isolation conditions, and assay techniques. Additionally, our findings corroborated those of Ali-Shtayeh *et al.* [42], who reported antibacterial activity of *C. spinosa* aerial parts against Gram negative bacteria, while Bonjar *et al.* [43] reported no inhibitory effects of *C. spinosa* aerial parts against Gram positive and negative bacteria.

Conclusions

Based on the results of the current study, it was found that the alcoholic and aqueous extract of *C. spinose* was effective against Gram-positive and Gram-negative bacteria.

Author Contributions Statement

All research stage: laboratory work, collection and analysis of data, research write were done by the author.

Declaration of competing interest

The author confirms that there was no competing interest with others.

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