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Sensitivity of multidrug-resistant pathogenic bacteria to ethanolic extract of *Ziziphus spina-christi L. (*Sidr*)* leaves

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Antibiotic overuse has resulted in a high prevalence of resistant bacterial strain infections and increased side effects. The plant Sidr was chosen for this study because it has a long history as a traditional remedy to treat various human diseases including infections. Therefore, the aim of this study is to evaluate the antibacterial efficacy of ethanol extracts of Sidr leaves against commonly encountered multidrug-resistant pathogenic bacteria. Susceptibility of seven multi-drug resistant (MDR) clinical bacterial isolates; *Escherichia coli, Klebsiella pneumoniae*, *Shigella sonnei, Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* (ATCC 27853) as a reference strain were evaluated to ethanol extracts of Sidr leaves using agar (well and disk) diffusion assays and micro broth dilutions assay. Minimum inhibitory concentrations (MIC), zone of inhibition (ZI), and minimum bactericidal concentration (MBC) were calculated. The Ethanolic extract of Sidr leaves demonstrated antibacterial activity against all the tested bacteria at all concentrations (10, 20, and 30 mg /ml) in both agar well diffusion assay and disk diffusion assay. The extract showed the highest antibacterial activity (24±0.57 mm against *Staphylococcus aureus* while the lowest activity (8.5±0.5) was observed against *Pseudomonas aeruginosa* at 30mg/ml concentrations. The MIC and MBC of the extract were recorded 1 mg/mL and 2mg/ml for *Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Staphylococcus aureus* respectively. The extract of Sidr leaves inhibited all of the bacteria tested. Furthermore, raising the concentration of Sidr increased the antimicrobial activity. Further study is needed to identify active ingredients responsible for antibacterial potential in the extract.

Keywords: ***Antibacterial activity, Saudi, Sidr, Ziziphus spina –Christi, Susceptibility, Multidrug-resistant, Pathogenic bacteria***

INTRODUCTION

Medicinal plants have indeed been gaining worldwide recognition as sources of therapeutic agents. They act as significant therapeutic agents in combating diseases and as raw active ingredients in producing various traditional and modern therapies (Tapsell et al. 2006, Bamola et al. 2018, Makhawi et al. 2020). Plants have an important role in our daily lives in many indigenous Arabian communities since they provide us with food, housing, clothes, fuel, ornamentals, flavor, and medication (Bukar et al. 2015). Herbal medicines used in traditional folk medicine provide an intriguing and largely unexplored source for the creation and development of potential new chemotherapy drugs that might assist in overcoming the growing problem of resistance to currently available commercial antibiotics as well as their toxicity (Ali et al. 2001).

According to ethnobotanical literature (Ara et al. 2012, Ebid 2015, Chandra et al. 2017, El-Ansary et al. 2018, Aati et al. 2019, Badr et al. 2020), wide range of medicinal plants are used to treat microbial infections, particularly in rural Saudi Arabia, where traditional folk medicine is still a significant source of treatment for diseases. *Ziziphus spina-christi L.* (Sidr) is a traditional medicinal plant commonly used in the Western region of Saudi Arabia (Badr, El-Sherif et al. 2020). It belongs to the Rhamnaceae family and has nearly 60 genera and over 850 species. It is generally known as "Nabka."(Adzu et al. 2002, Badr et al. 2020). It has some local Arabic name(s) including; Sidr, Nebeq, Elbb,Jabat, Zejzaj, Zefzoof, Ardeg(Badr et al. 2020). The Zizyphus genus has therapeutic value, and all parts of the plant are utilized in Saudi Arabia to promote a healthy lifestyle. It is also used to treat digestive problems, obesity, urinary problems, and skincare problems. Zizyphus has been shown to have antibacterial and antifungal properties (Kadioglu et al. 2016, Soliman et al. 2017, Badr et al. 2020).

Antimicrobial resistance is a worldwide issue with significant economic, social, and political implications

(Sack et al. 1997, Zeighami et al. 2015). Antimicrobial resistance is a massive problem in poorer nations, where the burden of disease is severe and financial restrictions restrict the use of more expensive alternatives (Al-Masaudi et al. 2020). Without effective antimicrobial treatment, significant advances in contemporary medicine, such as major operations, organ transplants, and cancer treatments, are in danger (Cars et al. 2008). Antimicrobial resistance (AMR) occurs when a bacterium becomes resistant to an antimicrobial agent to which it was previously susceptible (WHO, 2011). Antibiotic resistance occurs naturally through random mutation, but it may also be manipulated by putting a population under evolutionary stress. When bacteria produced a resistance gene, they can use plasmid exchange to transfer the genetic information horizontally (between individuals) (Butler et al. 2006). A superbug, also known as a multi-resistant or pan-resistant bacteria, possesses many resistance genes (Butler et al. 2006). Recently some of the resistant strains were isolated from south Makkah region and is a great threat to the control of the infection (Al-johny and Alkhuzaee 2019). Indiscriminate antibiotic usage has many setbacks because it alters the microbial composition, increase the risk of the illness, cause secondary infections such as asthma, and obesity. It also encourages the spread of drug-resistant infections, necessitating the search for alternate medical treatments (Becattini et al. 2016).

Multidrug resistance (MDR) bacteria strains are quickly spreading due to their ability to develop and transmit exogenous genes via mobile genetic components such as transposons, R-plasmids, and introns linked to bacterial chromosomes (Ud-Din et al. 2013). Furthermore, it is noted that bacteria increase their adaptation to the resistance of antibiotics and chemicals (Hegazi et al. 2017). Thus, there is need for alternative compounds to antibiotics from plant natural products. Medicinal plants such as Sidr had been used for many centuries to treat many diseases (Badr et al. 2020). In addition, Sidr was also used in ancient times as an antibacterial agent (Gheith 2018, Badr et al. 2020). Sidr has an antibacterial activity that inhibits a wide range of microorganisms, including both gram-negative and gram-positive bacteria. Sidr has numerous hidden treasures in its components and characteristics. Much research around the world has tested Sidr's antibacterial efficacy using numerous in vitro tests against pathogenic bacteria.(Araet al. 2012, Chandra et al. 2017, Badr t al. 2020). However, there is a lack of evidence regarding the efficacy of Sidr against multidrug-resistant (MDR) bacteria.

Therefore, in this research, we have screened the antibacterial efficacy of ethanol extract of Sidr leaves collected from Huda Al-Sham, Saudi Arabia using agar (well and disk) diffusion assays against selected MDR bacteria. The Sidr sample was evaluated for minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) against bacteria by micro-broth dilutions assay in standard media; after the period of incubation, Resazurin dye was added to determine MIC.

MATERIALS AND METHODS

Plant material

Fresh leaves of Zizyphus spina-christi L. were obtained from Zizyphus spina-christi plants in the research region (Huda Al-Sham, Makkah Province, Saudia Arabia) in May 2021 (https://goo.gl/maps/xvc2MswjpE31RCm57) . The plant was authenticated by the Department of Biological Sciences, Faculty of Science, King Abdulaziz University. Sidr leaves were refluxed in running tap water before being washed with bi-distilled water. Following that, Sidr leaves were shade-dried for 14 days at room temperature in the open area before being ground into a powder with an electric blender (Gheith, 2018, Abubakar 2020, Makhawi et al. 2020).

Preparation of crude extracts:

Sidr leaves were dried and ground in an electric blender, then incubated for two days in a glass flask with 400 mL ethanol (80%), shaking (Shaker SHO 1-D) at 150 rpm for 48 hours at room temperature, and then filtered. Next, using a rotary evaporator (Buchi Rotavapor R-114 & Waterbath B-480), the solvent was evaporated after concentrating the extracts at 55°C under reduced pressure. After that, all dry extract was weighed (ADAM .0001g electronic balance), the yield obtained was calculated and stored in a dark glass bottle in the refrigerator at 4°C until needed (Kızıl et al. 2008, Abdul Qadir et al. 2017).

Bacterial Strains

Seven clinical isolates of *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Shigella sonnei* (SS), *Salmonella typhimurium* (ST), *Staphylococcus aureus* (SAU), *Staphylococcus epidermidis* (SE), and *Acinetobacter baumannii* (AB)were obtained from King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia).while *Pseudomonas Aeruginosa* (PA) (ATCC 27 853) was used as control. Re-identification of the isolates were done via morphological and biochemical characteristics. Final identification of organism was made by 16s rRNA using GN ID cards using macrogen system. The isolates were kept at -80^{o}C in Nutrient Broth (NB) containing 16% glycerol. A sterile stick was utilized to transfer culture in a glass tube containing 10ml of NB one day prior to the experiment. The culture was incubated for four h at 37^{o}C and moved to another fresh NB broth, then incubated at 37^{o}C without agitation for approximately 18h.

Susceptibility Testing

The antibacterial sensitivity test was carried out using the Kirby-Bauer disk diffusion method with the clinical laboratory standard institute (CLSI) Guidelines (Oxoid, Basingstoke, UK) (CLSI, 2010; Tanih et al. 2010).

Antibiotics such as Cefepime (CPM) (30µg), Amikacin (AK) (30µg), Tobramycin (TM) (10µg) , Piperacillin (PRL) (100µg), Gentamicin (GM) (10µg), Cephalothin (CEF) (30µg), Imipenem (IMI) (10µg), Cefoxitin (FOX) (30µg), Cefotaxime(CTX)(30µg), Ticarcillin (TC) (75µg), Ciprofloxacin(CIP) (5µg), and Norfloxacin (NOR) (10µg), were used.

Antibacterial activity

Agar well diffusion assay

Mueller Hinton Agar (MHA) plates inoculated with the tested bacteria at McFarland standard in duplicates (10^6 colony-forming units/mL). In each of the cultivation plates, five holes of 6 mm were punched using a sterile cock borer. An amount of 150 µL of Amoxicillin was used as the positive control, and SDW was used as a negative control; 120 to 150 µL of Ziziphus spina-christi extract were added in the remaining three holes using different concentrations 10, 20, and 30 mg/ml. The plates were incubated at 37 °C for 24 h. The zone of inhibition was recorded as the mean ± standard deviation (SD) of duplicate experiments.

Disk diffusion assay

Disk diffusion was performed on Mueller – Hinton Agar (MHA) using 6 mm filter paper disks. In this method, the bacterial isolate (107 colony-forming units/mL) is spread over an agar plate(MHA). Then paper discs impregnated with different concentrations (10,20,30 mg/ml) of Sidr ethanolic extract are placed and incubated at 37^{o}C overnight. Then, Sidr extract was allowed to diffuse into the medium for 30 min at room temperature. Amikacin (AK) (30µg), used as a positive control, and SDW was used as a negative control.

Estimation of Minimum inhibitory concentrations of Sidr leaves extract

Minimum inhibitory concentrations (MICs) are generally determined by micro-broth dilution assays using a 96-well microtiter plate. So, MICs were performed by micro-diluted broth Method as described by NCCLS to determine the MICs of the extracts against the tested bacteria (Wikler 2006). The incubation was performed for 24h at 37 ^{o}C. After incubation, 5 µl of Resazurin sodium salt dye solution (R7017 Sigma-Aldrich.) was added to each well. Column 12, which contains media, only confirms no contamination occurred in the plate while preparing the dish. Column 11 containing, a media with an extract that corresponds with the plates with complete inhibition. Column 1 is a negative control containing cultured strain only, while column 2 -10 is the serial dilution of the ethanol extract of Sidr from 32 to 0.25mg/ml with media. This technique helps to generate accurate MIC values while overcoming crucial difficulties relating to color and solubility that might interfere with growth measurements for several types of extracts (Elshikh et al. 2016).

Estimation of Minimal bactericidal concentrations (MBC)

The minimal bactericidal concentrations (MBC) are the lowest concentration of the Ziziphus spina-christi extract at which inoculated bacteria was killed. This was carried out by spreading 10 µl of medium from the MIC's microplates contents, which showed no bacterial growth on nutrient agar plates followed by incubation at 37°C for 24 hours (Tao et al. 2021).

Statistical analysis:

The IBM Statistical Package for Social Sciences software (SPSS 19.0) was used to analyze the data. First, the mean values of the inhibition zone of the sample and the MICs were calculated. The difference is considered significant when $P < 0.05$.

RESULTS

Identification of Bacterial Isolates

The identification of bacterial isolates was based on the 16s rRNA gene sequences received from macrogen (Korea) and analyzed with the BLAST tool available in the NCBI GenBank database (htt://www.ncbi.nlm.nih.gov) . These isolates were molecularly identified as *Escherichia coli*, *Klebsiella pneumoniae, Shigella sonnei, Salmonella typhimurium*, *Staphylococcus aureus, Staphylococcus epidermidis*, *and Acinetobacter baumannii*.

Antibacterial test using agar diffusion assay

The findings of this research indicated that the inhibition zones range from minimum 8.5±0.3 mm to maximum 24±0.57 mm against the tested bacteria. The extracts had variable inhibitory effect, depending upon the concentrations (30, 20, and 10 mg /ml) of extract. The extract had the highest activity (24±0.57 mm zone diameter) against *Staphylococcus aureus* at 30 mg/ml, while the lowest activity (8.5±0.5 mm) against *Pseudomonas aeruginosa* at 10 mg/ml concentration (Table 1). Disk diffusion assay carried out at similar concentration (30 mg/ml, 20 mg/ml, and 10 mg/ml), as used in agar well diffusion assay. The findings indicated that the higher inhibition zone (16.5±0.57 mm) was formed against *Acinetobacter baumannii* whereas the lowest inhibition zone (8.5±0.5 mm) against Pseudomonas *Aeruginosa* (Table 2, Figure 1).

Antibacterial test using MIC and MBC

The MIC of the ethanolic extract was recorded as 1 mg/mL against *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* whereas the MBC was 2mg/ml for the same three bacteria. On the other hand, *Escherichia Coli* and *Acinetobacter baumannii* were inhibited at MIC 4mg/ml while MBC was 8mg/ml . The *Staphylococcus epidermidis*, *Shigella Sonnei,* and *Salmonella typhimurium* were inhibited and killed at 2gm/ml and 4gm/ml, respectively(Table 3*)*.

Resistant profile of Bacterial strains

Sensitivity tests indicated that all the bacteria tested were susceptible to Imipenem (IMI) (10µg) with variation zone of inhibition from 27±0.5 mm to 38±0.5 mm, moderately sensitive to Gentamicin (GM) (10µg), Amikacin (AK) (30µg), Cefepime (CPM) (30µg) showed the inhibition zone among 13±0.6 mm to 22±0.6 mm. On the other hand, all bacteria were resistant at least to two remaining antibiotics (Ticarcillin, Piperacillin, Norfloxacin, Tobramycin, Cephalothin, Cefoxitin, Ciprofloxacin, and Cefotaxime), except *Pseudomonas aeruginosa* which was sensitive to all antibiotics used (Table 4).

Table 1 :The zone of inhibition in diameter mm of all tested bacteria with ethanol extract of *Zizyphus spina-christ*i leaves using well diffusion assay

Bacteria strain		Concentration mg/ml				
	Bacteria strain	10 mg/ml	20 mg/ml	30 mg/ml	Control +	Control -
1	*Escherichia Coli* (EC)	12.3±0.57	14.3±0.76	17.3±0.5	18.5±0.8	NI
2	*Klebsiella Pneumoniae* (KP)	13.5±0.8	14.3±0.5	16. 3±0.57	17.5±0.8	NI
3	*Pseudomonas Aeruginosa*(PA)	8.5±0.5	10.3±0.76	12.5±0.8	22.5±0.8	NI
4	*Staphylococcus Aureus* (SA)	20.3±0.57	22.5±0.57	24±0.57	20.3±0.5	NI
5	*Staphylococcus epidermidis* (SE)	15.5±0.8	18.5±0.8	22.5±0.8	22.3±0.5	NI
6	*Shigella Sonnei* (SS)	11.5±0.3	12.3±0.76	14.5±0.8	16. 5±0.5	NI
7	*Salmonella typhimurium* (ST)	10.5±0.3	13.3±0.76	16.5±0.8	19. 3±0.5	NI
8	*Acinetobacter baumannii*(AB)	10.3±0.3	12.5±0.76	16.5±0.57	26.5±0.8	NI

*The values represent mean ± standard deviation of two replicates, NI; No inhibition

Table 2: The zone of inhibition in diameter mm of all tested bacteria with ethanol extract of *Zizyphus spina-christi* leaves using disk diffusion method

		Concentration mg/ml				
	Bacteria strain	10 mg/ml	20 mg/ml	30 mg/ml	Control +	Control -
1	***Escherichia Coli* (EC)**	10.3±0.57	11.3±0.76	13.3±0.5	10.5±0.5	NI
2	***Klebsiella Pneumoniae* (KP)**	9.5±0.8	10.3±0.5	11. 3±0.57	19. 3±0.5	NI
3	***Pseudomonas Aeruginosa*(PA)**	8.5±0.5	10.3±0.5	15. 3±0.57	21. 3±0.5	NI
4	***Staphylococcus Aureus* (SA)**	9.3±0.57	12.5±0.57	15.5±0.57	19.3±0.5	NI
5	***Staphylococcus epidermidis* (SE)**	9.5±0.8	11.5±0.8	12.9±0.8	18.5±0.8	NI
6	***Shigella Sonnei* (SS)**	9.5±0.3	11.3±0.76	12.5±0.8	9.5±0.8	NI
7	***Salmonella typhimurium* (ST)**	9.3±0.3	10.5±0.76	14.5±0.57	22.5±0.8	NI
8	***Acinetobacter baumannii* (AB)**	10.3±0.3	12.5±0.76	16.5±0.57	26.5±0.8	NI

*The values represent the mean ± standard deviation of two replicates.

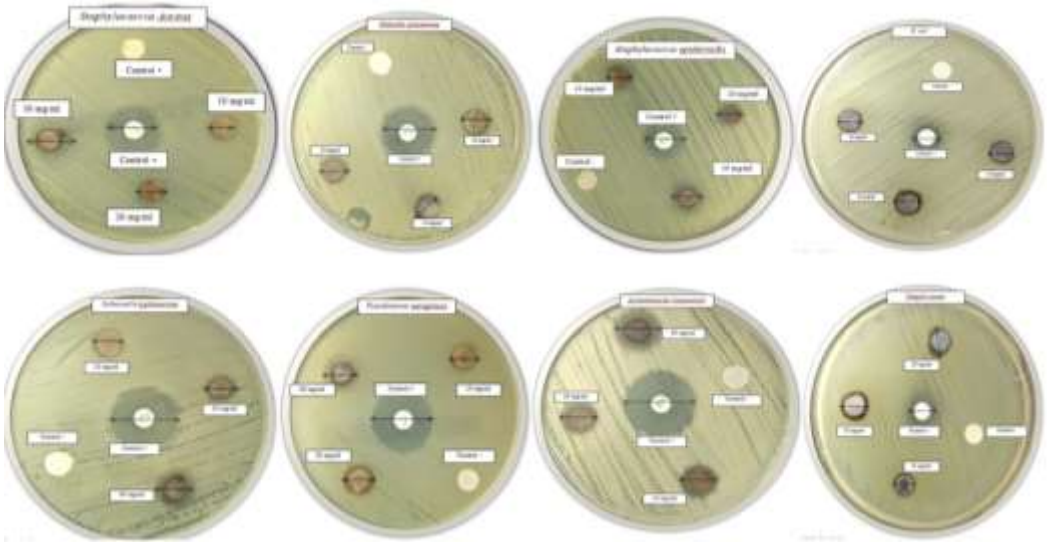

Figure 1:Zone of inhibition (mm) of *Zizyphus spina-christi* leaves extract at 30, 20, and 10mg/ml by agar disk

diffusion assay against all bacteria tested.

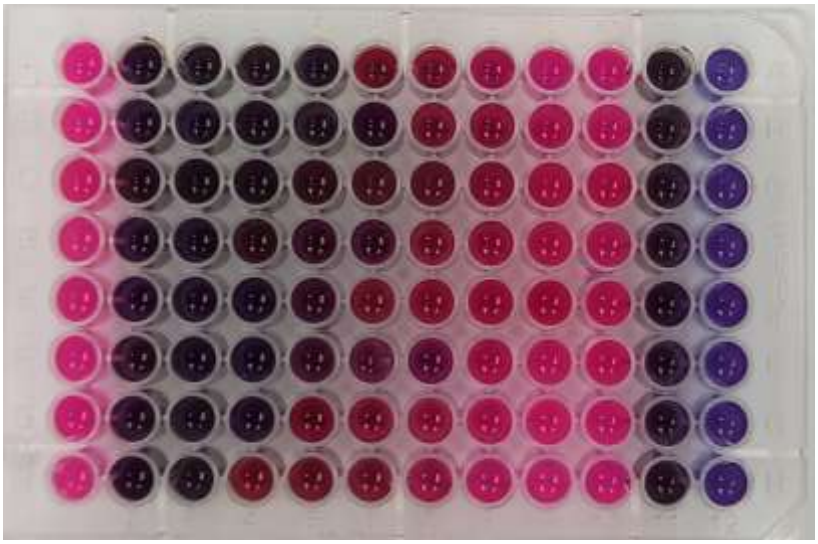

Figure 2: Determination of MIC for ethanol extract of *Zizyphus spina-christi* leaves against eight pathogenic bacteria using Resazurin dye. The rows A – H each represent a pathogenic bacterium(a-*Escherichia Coli* (EC) ,b-*Klebsiella Pneumoniae* (KP), c- *Pseudomonas Aeruginosa*(PA), d- *Staphylococcus Aureus* (SA) , e-*Staphylococcus epidermidis* (SE), f- *Shigella Sonnei* (SS), g- *Salmonella typhimurium* (ST), h- *Acinetobacter baumannii*(AB).

Table 3: Minimum inhibitory concentration (MIC and MBC) of *Zizyphus spina-christi* to pathogenic bacteria tested.

Bacteria strain	EC	KP	PA	SA	SE	SS	ST	AB
32 mg/ml	-	-	-	-	-	-	-	-
16 mg/ml	-	-	-	-	-	-	-	-
8 mg/ml	MBC	-	-	-	-	-	-	MBC
4 mg/ml	MIC	-	-	-	MBC	MBC	MBC	MIC
2 mg/ml	+	MBC	MBC	MBC	MIC	MIC	MIC	+
1 mg/ml	+	MIC	MIC	MIC	+	+	+	+
0.5 mg/ml	+	+	+	+	+	+	+	+
0.25 mg/ml	+	+	+	+	+	+	+	+

(a-*Escherichia Coli* (EC) ,b- *Klebsiella Pneumoniae* (KP), c- *Pseudomonas Aeruginosa*(PA), d- *Staphylococcus Aureus* (SA) , e- *Staphylococcus epidermidis* (SE), f- *Shigella Sonnei* (SS), g- *Salmonella typhimurium* (ST), h- *Acinetobacter baumannii*(AB).

Table 4: Phenotypic antimicrobial susceptibility profile of tested bacteria

No	Bacteria	AK	GM	TM	CPM	CTX	CEF	FOX	TC	PRL	IMI	NOR	CIP
1	*Escherichia coli*	21±0.57	22±0.5	10±0.5	22±0.5	15±0.4	9±0.5	9±0.5	R	R	30±0.55	20±0.54	28±0.6
2	*Klebsiella pneumoniae*	19±0.6	21±0.5	R	14 ±0.5	10±0.6	R	9±0.55	15±0.6	19±0.5	39±0.5	R	R
3	*Pseudomonas aeruginosa*	18±0.5	20±0.6	9±0.6	20±0.6	17±0.5	14±0.5	19±0.5	16±0.6	17±0.6	27±0.6	19±0.5	17±0.5
4	*Shigella sonnei*	20±0.6	20±0.6	R	18±0.5	R	R	12±0.5	R	R	31±0.6	R	R
5	*Salmonella Typhimurium*	22±0.6	18±0.6	14±0.5	22±0.6	12±0.5	R	R	R	13±0.5	26±0.5	21±0.6	R
6	*Staphylococcus aureus*	20±0.6	21±0.5	12±0.5	16±0.5	18±0.5	R	9±0.6	R	20±0.5	28±0.5	R	19±0.5
7	*Staphylococcus epidermidis*	13±0.6	19±0.6	12±0.6	22±0.5	17±0.5	R	19±0.5	R	25±0.5	28±0.6	R	13±0.5
8	*Acinetobacter baumannii*	19 ±0.6	17±0.6	21±0.5	19±0.6	13±0.5	18±0.5	R	R	R	27±0.5	12±0.6	R

Inhibition zone (mm) (average ± standard deviation, n = 3), Antibiotics ; Gentamicin (**GM**) (10µg), Amikacin (**AK**) (30µg), Ticarcillin (**TC**) (75µg), Cefepime (**CPM**) (30µg), Norfloxacin (**NOR**) (10µg), Piperacillin (**PRL**) (100µg), Cephalothin (**CPF**) (30µg), Imipenem (**IMI**) (10µg), Cefoxitin (**FOX**) (30µg) ,Ciprofloxacin(**CIP**) (5µg), Tobramycin (**TM**) (10µg), and Cefotaxim(**CTX**)(30µg)**,R**; Resistant.

DISCUSSION

Saudi Arabia is the largest country in the Arabian Peninsula, with rich plants due to the climate diversity across the country. Some of these plants are used as traditional medicine to treat various diseases (Aati et al. 2019).

Antibiotic misuse results in the generation of multidrug-resistant bacteria (Al-Masaudi and Al-Maaqar 2020). Botanical medications are currently regarded to be safe alternatives to synthetic medicines. Plant crude extract of some plant species such as basil, ginger, cinnamon, Balsam and garlic show promising activity against different pathogenic bacteria (Alzoreky and Nakahara 2003, Castro et al. 2008, Al Johny 2019).

The present study demonstrated that ethanolic leaves extract of Sidr have important inhibitory effects against all microbial strains tested, namely, *Escherichia coli* (EC), *Klebsiella Pneumoniae* (KP), *Staphylococcus Aureus* (SAU), *Shigella sonnei*(SS), *Salmonella Typhimurium*(ST), *Staphylococcus epidermidis* (SE), *Pseudomonas Aeruginosa* (PA), and *Acinetobacter baumannii* (AB).

The study carried out by Motamedi et al. (2014) showed that the *Staphylococcus aureus* was sensitive to ethanol extract of *Ziziphus spina-christi* leaves. In our study, the zone of inhibition was 15.5mm that is identical to Motamedi's research, while MIC and MBC for his results were higher as compared to our result(Motamedi et al. 2014). The study conducted by Al-Mutairi et al. (2016) are in accordance with our result; it was found that *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella sp. Staphylococcus aureus*, and *Pseudomonas aeruginosa* were sensitive to ethanol extract of *Ziziphus spina-christi* (Al-Mutairi et al. 2016). A number of previous studies have reported that the extracts of *Ziziphus spina-christi* were active against these pathogens (Motamedi, et al. 2014, Ali et al. 2015, Bukar, Kyari et al. 2015, Halawani 2016, Saaty* 2019, Makhawi et al. 2020).

The ethanol extract showed high activity against *Acinetobacter baumanii*., as shown in a study by Halawani et al. (2016). They found that silver nanoparticles using *Ziziphus spina christi* leaf extract have inhibitory effects against *Acinetobacter* spp., *Pseudomonas aeruginosa* and *Escherichia coli* (Halawani 2016). Regarding *Shigella spp.,* our result agrees with the study done by bukar (2015), who found that *Ziziphus spina-christi L.* was active against *Shigella spp.,*(Bukar et al. 2015).

Finally, Plants are good candidates for exploring novel antibacterial medicines because they produce various compounds with antimicrobial characteristics such as antibacterial activity.

CONCLUSION

The Ethanol extracts of *Ziziphus spina-christi* (Sidr) has demonstrated good results activities against the tested bacterial organisms that were used. Further studies are needed to evaluate the efficacy of Sidr against antibiotic-resistant microorganisms. In addition, it requires more in-depth research to identify the active ingredients in Sidr responsible for this antibacterial activity.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SMA designed and performed the experiments and wrote the first draft of the manuscript. MA, MBH,FA and NA conceived the idea, plan and designed the experiments. BOA and SMA analyzed the data. BOA and MA reviewed the manuscript. All authors read and approved the final version.

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