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Evaluation of genetic variation, antioxidant and antibacterial activities of two Sidr varieties in Medina

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Abstract: Genetic variation of two *Ziziphus spina-christi* L. (Sidr) varieties was determined by Random Amplified Polymorphic DNA (RAPD). The activity of antioxidant enzymes (peroxidase and catalase) was also determined for the two varieties (Balady and Pakistani). Moreover, the antibacterial effects of different concentrations of aqueous and ethanolic extracts of leaves of the two varieties of *Ziziphus spina-christi* L. were assessed against Gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae, Proteus mirablis* and *Escherichia coli*). The similarity of RAPD profiles was found to be 85%. Our results suggest that *Ziziphus spina-christi* is an effective source of antioxidants and its leaf extract is a potential promising alternative antibacterial agent against different pathogenic bacteria.

Keywords: *Ziziphus spina-christi* L., genetic variation, RAPD, antioxidant, antibacterial

INTRODUCTION

Ziziphus spina-christi L. (Sidr) is a key medicinal plant in Medina, Saudi Arabia. It is commonly known as "Nabka" (Adzu *etal*., 2002), it is belonging to family Rhamnaceae and contains around 60 genera and more than 850 species. *Ziziphus* encloses around 100 species of the shrubs and deciduous trees in the world (Shahat *et al.,* 2001). It is a widely used tree that grows in warm areas, such as South Europe, East Asia, South America, Australia and the Middle East (Muller *et al.,* 2011).

In the last decade, many PCR-based molecular markers have been made used for biological research (Gostimsky *et al*., 2005). Random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeats (ISSR), are the most commonly used markers (Williams *et al*., 1990). RAPD markers are used to study similar patterns of genetic diversity (Isabel *et al*., 1995). However, RAPD tends to provide more diagnostic, race-, population- and species-specific markers. The RAPD has been successfully utilized to examine population genetic relationship or genetic structure for a number of rare endemic plant species (Palacios and Gonzalez-Candelas, 1997; Ayers and Ryan, 1999)

Ziziphus spina-christi L. has a great significance because it contains antioxidants (Ali *et al.,* 2018). Antioxidants are relevant to the work of clinicians and biologists due to its vital role in the protection against the damages caused by the reactive free radicals, as in the case of Parkinson's disease, Alzheimer's disease, ischemic heart disease, atherosclerosis, cancer and aging process (Aruoma, 2003). In addition, *Ziziphus spina-christi* L. is prescribed as an anti-microbial agent for relieving digestion disorders, obesity and urinary troubles, and for skin care (Kadioglu *et al.,* 2016). Its seeds are also used to decrease abdominal pain and pregnancy vomiting (Waggas and Al-Hasani, 2010), whereas its leaves are used in treating liver, asthma and fever (Elaloui *et al.,* 2107). It is also used as ahypotensive, hypoglycemic, antimicrobial, anti-inflammatory and liver protector and as an immune system stimulant (Shahat *et al*., 2001), whereas its twigs' powder can help to treat scorpion sting and rheumatism (El-Khalifa, 1999).

The genus *Zizyphus* has a medicinal significance, all parts of the plant are used in Saudia Arabia to help maintain a healthy life style. *Zizyphus* has been reported to have activity against bacterial and fungal pathogens. It is utilized extensively as a diuretic for the treatment of eye diseases, wounds, ulcers, bronchitis febrifuge and as anti-inflammatory agent for attenuating skin diseases, like atopic dermatitis. Similarly, different parts of the plant are utilized for various medicinal purposes among people. It is used for the treatment of urinary infections, stomach discomfort, burns and wounds (Soliman *et al*., 2017). The extracts of *Ziziphus* have shown significant anti-oxidant, antimicrobial activity and anti-nociceptive effects (Huang *et al.,* 2017).

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MATERIALS AND METHODS

Plant material

Zizyphus spina-christi L. fresh leaf tissues were collected from one-year old (Balady and Pakistani) Sidr plants from the study area (Abar Al-Mashi farm in Medina, Saudia Arabia).

Methods

Extraction and Purification of Genomic DNA

DNA was extracted from fresh leaves of *Ziziphus spina-christi* L., Balady and Pakistani varieties, using the Qiagen DNeasy (from Qiagen Santa Clara, CA) according to the manufacturer's instructions.

Measurement of DNA concentration

The extracted DNA was diluted 1:5 in distilled water. Then, the samples were electrophoresed in 1% agarose gel together with a DNA ruler, which covers a range of 95 ng and 11 ng DNA concentration. The concentration of DNA was estimated by comparing the fluorescence of the band with the DNA size ruler.

RAPD- PCR amplification and profiling of plant DNA

Ten primers were used for RAPD reactions (table 1). Total DNA was isolated from the two Sidr varieties (Balady and Pakistani). PCR was performed in a Bio-Rad thermal cycler (Bio-Rad, USA). The following conditions were used for the amplification of DNA from the two varieties: 2µl DNA, 3µl primer, 0.5µl dNTP, 5µl Taq polymerase buffer, 1.5µl $MgCl_2$, 0.3µl Taq polymerase and the reaction mixture was completed up to 25µl with water. The PCR amplification steps are: denaturation: 94ºC for 1 min, annealing: 36ºC for 1 min, extension: 72ºC for 1.5 min; for40cycles. A last extension step was carried at 72°C for 7 min. The amplified products were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide and viewed under a UV trans-illuminator. Variability was scored as presence or absence of a specific DNA amplification product and scored as 1 or 0 respectively (Klein-Lankhorst *et al*., 1991 and Deragon and Landry, 1992).

Determination of peroxidase and catalase enzyme activity in Ziziphus spina-christi* L. *varieties

Crude Leaf Extract for Antioxidant Enzymes Assays

About 200 mg of 1-year old *Ziziphus* fresh dry leaves tissue was ground to a fine powder, homogenized in 1.2 ml of 0.2M potassium phosphate buffer and were centrifuged at 15,000×g at 4°C for 30 min. The supernatant was discarded, and the pellet was re-suspended in 0.8ml of potassium phosphate buffer. The suspension was centrifuged at 15,000×g for 20min and stored at 4°C.

Peroxidase assay

Peroxidase enzymatic activity was determined using Pyrogallol as a substrate according to the following reaction:

$$H_2O_2 + \text{Pyrogallol (donor)} \xrightarrow{\text{Peroxidase}} 2\,H_2O + \text{Purpurgallin (oxidized donor)}$$

A total of 3.00 ml reaction mixture containing 0.04-0.07-unit peroxidase,14mM potassium phosphate, 0.027% (w/w) hydrogen peroxide, and 0.5% (w/v) pyrogallol. The mixture was equilibrated at 20°C in the spectrophotometer, A_{420} was monitored until stable. The phosphate buffer (0.1 ml) and peroxidase working solution (0.1ml) were used as Blank.

Catalase assay

The continuous spectrophotometric rate determination of Catalase reaction is depending on the following equation, where one unit of catalase is defined as: 0.01 decrease in absorbance at 240 nm /g /min.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The final assay concentrations were 50 mM potassium phosphate, 3% (w/w) hydrogen peroxide, and ~10 units of catalase. In a 3.00 ml reaction mix, hydrogen peroxide solution (2.9 ml) was pipetted in a cuvette containing Phosphate buffer and A_{240} was monitored until constant. Catalase Solution (100 µl) was added, mixed well by inversion and the time of decrease in absorbance was recorded until the reading was stable. Phosphate buffer was used as Blank (Böhmer *et al.,* 2011).

The assessment of the potential antibacterial activity of Ziziphus spina-christi* L. *extracts

Plant Extract Preparation

Fresh leaves of *Zizyphus spina-christi* L. were collected from Medina, Saudi Arabia. The leaves were washed, air dried and homogenized into a fine powder and stored in airtight bottles. To prepare an aqueous extract, twenty grams were extracted with 200 ml ultra-pure water at room temperature for 24 hrs. The extract was filtered by Millipore filter (diameter 25 µm and pore size 0.24 µm) and stored at 4°C. The aqueous extract was dissolved in Dimethyl Sulfoxide (DMSO) and different concentrations were prepared from the stock. To prepare an ethanol extract, twenty grams of leaves powder were extracted with absolute ethyl alcohol and filtered. The filtrate was evaporated at room temperature to dry and stored at 4 °C in sterile airtight containers until use.

Bacterial isolates

Five clinically relevant bacterial isolates (from human and animal origin) were used in the present study, three Gram-negative bacteria (*E. coli, K. pneumoniae* and *P. mirabilis*) and two Gram-positive bacteria (*S. pyogenes* and *S. aureus*). The bacterial isolates were identified using the phoenix automated microbiology system (BD Biosciences, sparks, MD) (Donay *et al*., 2004).

Antibacterial activity assays

The antibacterial activity of alcoholic and aqueous *Zizyphus spina-christi* L. extracts was evaluated by agar-

well diffusion assay (Perez *et al*., 1990). Small wells were created and filled with 0.1 ml of the extract aliquots. The plates were held for 2 hours for the extract diffusion into the agar. A loopful of the bacterial isolate was activated in nutrient broth at room temperature. Then an inoculum was streaked on the plates surface and incubated for 24 hrs at 37°C. The diameter of each inhibition zone was measured. The experiments were done in triplicate and the inhibition zone was calculated as an average for the three replicates.

STATISTICAL ANALYSIS

The averages of data were expressed as mean of 3 replicates +/-STDEV and were statistically analysed using Minitab 16. Significant values were determined according to the least significant difference (LSD) ($p<0.05$).

RESULTS

Morphological description

Ziziphus spina-christi L. varieties (Balady and Pakistani) Sidr are similar in most of the morphological characteristics except that Pakistani Sidr has a larger leaf and a smooth stem while Balady Sidr is characterized with its spiny stem and smaller leaf size than the Pakistani Sidr (fig. 1).

Fig. 1: Leaves of *Ziziphus spina-christi* L. varieties, Pakistani (P) and Baladi (B) Sidr (The plants were collected from Abar Al-Mashi farm in Medina, KSA).

RAPD Technique for Ziziphus spina-christi L

Randomly amplified polymorphic DNA was carried to assess the genetic diversity and polymorphism of the two studied *Ziziphus spina-christi* L. varieties (Balady and Pakistani) using similarity index. Ten arbitrary random primers were used to determine RAPD polymorphism of the two Sidr varieties (fig. 2).

The resulted amplified fragments and their densitometric analyses are illustrated in table 2 (a-j). A total number of 72 fragments were visualized across the two investigated genotypes, Primers produced many bands ranging from six (primers OP-C08, OP-D03, OP-D14 and OP-D16) to ten (primer OP-H13) across varieties. The size of amplified fragments ranged from 89 to 1500 bp. The number of polymorphic bands obtained using primer OP-Z09, was the highest, while the primers OP-D14, OP- F09 and OP-M18 gave the lowest number of polymorphic bands.

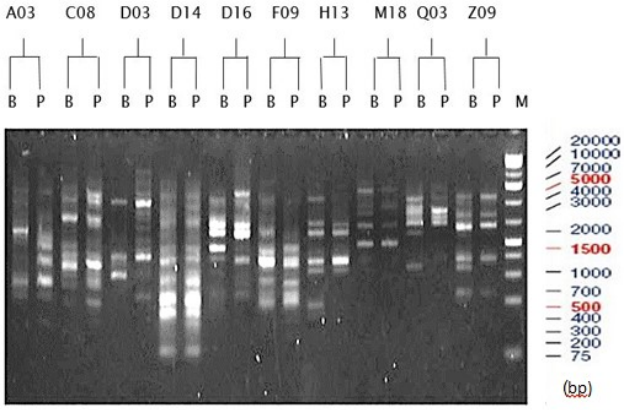

Fig. 2: DNA Polymorphism between *Ziziphus spina-christi* L. varieties (Balady and Pakistani) Sidr generated by RAPD using ten primers.

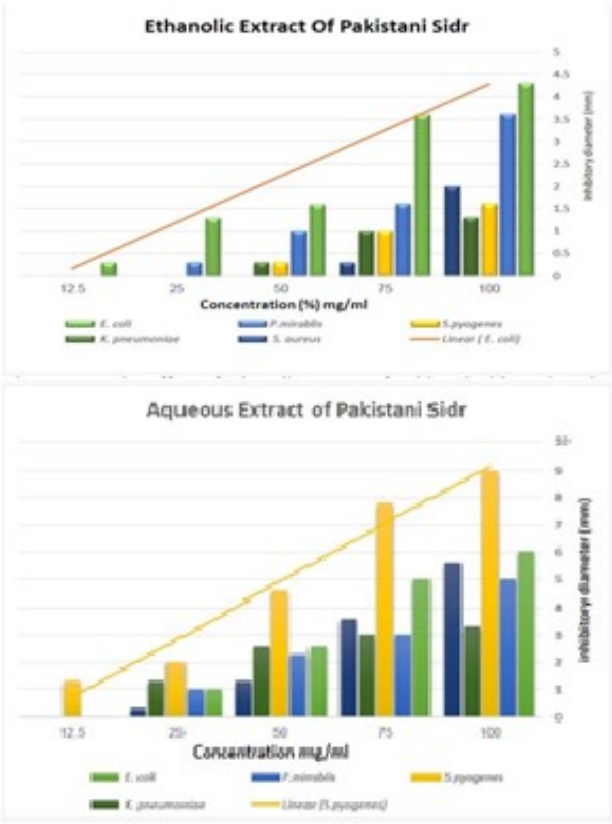

Fig. 3: The effect of aqueous and ethanolic extract of Pakistani Sidr against the growth of bacteria strains

Primer OP-A03 resulted in seven bands with sizes ranging from 141 to 1310 bp, the bands with sizes 141, 370 and 469 bp are polymorphic bands and were detected in Balady Sidr while the rest of the bands are monomorphic (table 2 a). Primer OP-C08 resulted in six DNA fragments

Table 1: Sequence of primers used in RAPD reactions

No.	Primer	Sequence 5'-3'
1	A-03	5'-AGTCAGCCAC-3'
2	C-08	5'-CCAAGCTTCC-3'
3	D-03	5'-GACGCCACAC-3'
4	D-14	5'-CACCATCCGT-3'
5	D-16	5'-GGTCACCTCA-3'
6	F-09	5'-AGGGCGTAAG-3'
7	H -13	5'-TGGACCGGTG-3'
8	M -18	5'-GTCGCCGTCA-3'
9	Q -03	5'-CTTCCCCAAG-3'
10	Z-09	5'-CACCCCAGTC-3'

ranging from 484 to 1310 bp, the bands with sizes 484 and 947 are polymorphic and were amplified in DNA from Pakistani Sidr and the rest of bands are monomorphic (table 2b). Primer OP-D03 caused the amplification of six bands with sizes ranging from 342 to 1094 bp, the bands with sizes 342 and 583 are polymorphic and were detected in Pakistani Sidr while the rest of bands are monomorphic (table 2c). Primer OP-D14 indicated the amplification of six bands with sizes ranging from 225 to 1045 bp, all the bands are monomorphic except the band with size 333 bp, which is polymorphic and was found in Pakistani Sidr (table 2d). Primer OP-D16 resulted in six DNA fragments ranging from 190 to 1094 bp, the bands with sizes 190 and 1094 bp are polymorphic and found in Pakistani Sidr while the rest of bands are monomorphic (table 2e). Primer OP-F09 indicated the amplification of seven bands with sizes ranging from 100 to 1145 bp, all the bands are monomorphic except the band with size 348 bp which is polymorphic and detected in Pakistani Sidr (table 2f). Primer OP-H13 resulted in ten DNA fragments with sizes ranging from 89 to 1145 bp, all the bands are monomorphic (table 2g). Primer OP-M18 resulted in seven DNA fragments ranging sizes from 218 to 1197 bp, all the bands are monomorphic except the band with size 454 bp found in Pakistani Sidr which is polymorphic (table 2h). Primer OP-Q03 indicated the amplification of eight bands with sizes ranging from149 to 1500 bp, the bands with sizes 583, 1046 and 1500 bp are polymorphic and were detected in Pakistani Sidr while the rest of the bands are monomorphic (table 2). Primer OP-Z09 indicated the amplification of nine bands with sizes ranging from 89 to 1166 bp, the bands with sizes 89, 200 and 380 bp are monomorphic while the rest of the bands in case of Balady Sidr are polymorphic (table 2J).

The observed data were organized using the cluster analysis in order to develop taxonomies. Similarity matrix was calculated using unweighted pair group method using arithmetic average (UPGMA). The results showed that the two varieties Balady and Pakistani, of *Z. spina-christi* L. share an 85% similarity as shown in table 3.

***Antioxidant activity of Ziziphus spina-christi* L.**

The catalase and peroxidase activities of *Z. spina-christi* L. varieties Balady and Pakistani were determined using a spectrophotometer at wavelength 240 nm and 420 nm, respectively. Antioxidant enzyme activities were found to be higher in Balady Sidr than in Pakistani Sidr. Peroxidase activity was higher in Balady Sidr (0.078 U/g/min) compared to its activity in Pakistani Sidr (0.023 U/g/min). In addition, catalase activity was found to be higher in Balady Sidr (0.036 U/g/min) as compared to that found in Pakistani Sidr (0.031U/g/min) (table 4). Catalase and peroxidase enzyme activities of Balady and Pakistani Sidr were recorded at different time points, the rate of enzyme activity was recorded every 20 seconds for 3 minutes (table 4).

Antibacterial Activity of Ziziphus spina-christi L.

The antibacterial activity of different concentrations (12.5, 25, 50, 75 and 100mg/ml) of aqueous and ethanolic leaves extract of *Z. spina-christi* was evaluated against Gram-negative (*Klebsiella pneumoniae*, *Proteus mirablis* and *Escherichia coli*) and Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) pathogenic bacteria. The aqueous extracts of Balady Sidr leaves showed a remarkable inhibition zone of a 5.6 mm at a concentration of 100 mg/ml against *Streptococcus pyogenes*. Followed by *E. coli* and *Klebsiella pneumonia* of 4.6 mm. *Proteus mirablis* showed an inhibition zone of 4.3 mm. The least inhibition zone was exhibited around *Staphylococcus aureus*. The ethanolic extract of Balady Sidr leaves of 100 mg/ml concentration, showed the largest inhibitory zone (6.6 mm) against *Klebsiella pneumonia*, while, others showed smaller zones that ranged between 2.6 mm and 4.3 mm (table 6 and fig. 3).

On the other hand, the aqueous extract of Pakistani Sidr leaves showed a remarkable inhibition zone at a concentration of 100 mg/ml against *Streptococcus pyogenes* (9 mm), *E. coli* (6 mm) and *Staphylococcus aureus* (5.6 mm). The ethanolic extract showed smaller inhibition zones, while the concentration of 100 mg/ml produced the largest inhibition zone (4.3 mm) against *E. coli.* Followed by *Proteus mirablis* (3.6 mm) as shown in table 7 and fig. 3.

Table 2: DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the two *Zizyphus spina-christi* L. varieties (Baladi and Pakistani Sidr) with different primers

Primer Names																													
A03			C08			D03			D14			D16			F09			H13			M18			Q03			Z09		
(bp)	B	P	(bp)	B	P	(bp)	B	P	(bp)	B	P	(bp)	B	P	(bp)	B	P	(bp)	B	P	(bp)	B	P	(bp)	B	P	(bp)	B	P
1310	1	1	1310	1	1	1094	1	1	1045	1	1	1094	1	0	1145	1	1	1145	1	1	1197	1	1	1500	1	0	1166	0	1
565	1	1	947	1	0	857	1	1	803	1	1	679	1	1	1046	1	1	797	1	1	703	1	1	1046	1	0	700	0	1
469	0	1	823	1	1	823	1	1	640	1	1	583	1	1	723	1	1	700	1	1	619	1	1	583	1	0	426	0	1
370	0	1	772	1	1	700	1	1	380	1	1	360	1	1	348	1	0	413	1	1	454	1	0	413	1	1	380	2	1
275	1	1	658	1	1	583	1	0	333	1	0	330	1	1	275	1	1	370	1	1	370	1	1	360	1	1	370	0	1
245	1	1	484	1	0	342	1	0	225	1	1	190	1	0	196	1	1	275	1	1	227	1	1	333	1	1	275	0	1
141	0	1	.	.	.	.	.	.	.	.	.	.	.	.	100	1	1	214	1	1	218	1	1	275	1	1	267	0	1
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	212	1	1	.	.	.	149	1	1	200	1	1
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	163	1	1	.	.	.	.	.	.	89	1	1
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	89	1	1	.	.	.	.	.	.	.	.	.
[a]			[b]			[c]			[d]			[e]			[f]			[g]			[h]			[i]			[j]		

DISCUSSION

Randomly amplified polymorphic DNA (RAPD) analysis may be helpful to choose which ancestors should be crossed to obtain appropriate populations suitable for both genome mapping and breeding purposes. From the results of this study, we concluded that RAPD analysis was helpful for the evaluation of genetic diversity between two *Z. spina-christi* L. varieties. It may also help breeders in selecting parents for hybridization program and in studying the genetic variation between *Z. spina-christi* L. varieties.

Table 3: Similarity matrix between two varieties Balady and Pakistani of *Ziziphus spina-christi* L.

Similarity matrix		
Variety	Balady	Pakistani
Balady	100	85
Pakistani	85	100

Table 4: Catalase and peroxidase enzymes activities of (Balady and Pakistani) Sidr.

Sidr varieties Antioxidant Enzyme Activity	Pakistani	Balady
Catalase activity	0.031 U/g/min	0.036 U/g/min
Peroxidase activity	0.023 U/g/min	0.078 U/g/min

The genetic relationships between different *Zizyphus* species have previously been studied by RAPD. The genetic relationships among *Z. spinosa* and *Z. jujuba* populations were reported. A number of 275 loci were obtained by 22 primers. Among them, 249 loci were polymorphic. The polymorphic loci percentage was 89% between *Z. spinosa* population while that of *Z. jujuba* population was 56%. Thirty-one specific RAPD markers were detected on fifteen *Z. spinosa* forms and three *Z. jujuba* varieties (Ping *et al*., 2005).

The genomic DNA polymorphisms of eleven varieties of *Z. jujube,* fourteen species of Chinese *Ziziphus* and one outgroup were analysed and studied using random amplified polymorphic DNA (RAPD). The random primers produced a total of 921 RAPD bands, among which 919 (99.78%) were polymorphic. The data of 921 RAPD bands were utilized to generate Dice's similarity coefficients and to construct a dendrogram using UPGMA in the NTSYS-pc program. It was concluded that all samples can be classified into six kinds with a genetic similarity of 0.26 (Su *et al*., 2003).

Exposure to various organic compounds containing drugs and environmental pollutants can cause damages of the cells due to the metabolic activation by reactive substances as reactive oxygen species (ROS). The use of plant antioxidants can be an effective way to fight the

Table 5: Mean +/- STED of catalase and peroxidase enzyme activities of Baladi and Pakistani Sidr at different time points (rates are recorded every 20 seconds for 3 minutes).

Time/Sec	Catalase activity ulg/min				Peroxidase activity ulg/min			
	Control	Pakistani Sidr	Control	Balady Sidr	Control	Pakistani Sidr	Control	Balady Sidr
20	0.044±0.0012	0.0621±0.0017	0.0415±0.0010	0.0665±0.0021	0.0115±0.0108	0.0216±0.0198	0.0423±0.0011	0.0723±0.0016
40	0.0407±0.0011	0.0607±0.0015	0.0401±0.0010	0.0641±0.0019	0.0117±0.0106	0.0218±0.0186	0.0429±0.0011	0.0729±0.0016
60	0.0399±0.0013	0.0599±0.0014	0.0391±0.0011	0.0621±0.0017	0.0131±0.0104	0.0231±0.0184	0.0437±0.0011	0.0735±0.0016
80	0.0371±0.0012	0.0576±0.0012	0.0384±0.0011	0.0608±0.015	0.0122±0.0104	0.0232±0.0184	0.0442±0.0011	0.0741±0.0017
100	0.0366±0.0011	0.0563±0.0011	0.0379±0.0012	0.0594±0.014	0.0123±0.0103	0.0233±0.0183	0.0347±0.0010	0.0745±0.0017
120	0.0321±0.0010	0.0529±0.0010	0.0357±0.0010	0.0575±0.012	0.0123±0.0101	0.0233±0.0181	0.0458±0.0010	0.0750±0.0017
140	0.0291±0.0011	0.0511±0.0009	0.0346±0.0010	0.0547±0.011	0.0122±0.0102	0.0233±0.0182	0.0459±0.0009	0.0755±0.0017
160	0.0290±0.0011	0.0505±0.0008	0.0323±0.0009	0.0515±0.009	0.0123±0.0101	0.0233±0.0181	0.0460±0.0010	0.0759±0.0017
180	0.0289±0.0010	0.0499±0.0007	0.0319±0.0008	0.0489±0.008	0.0124±0.0103	0.0234±0.0180	0.0464±0.0011	0.0762±0.0017

Note: Each Value is the average of three replicates

harmful effects of oxidative stress. Botanical antioxidants contain high concentration of ascorbic acid, flavonoids, phenols and antioxidant enzymes such as peroxidase and catalase (Granato *et al.,* 2015). The antioxidant properties of various plant extracts reveal their stimulating effect on anti-oxidative enzymes. Catalase and peroxidase rich plant extracts can efficiently serve as potential antioxidant sources in human diet which helps preventing oxidative damage by ROS. It is known that these enzymes represent an effective mechanism for counteracting the negative effects of ROS. (Nagata *et al.*, 1999; Amin and Ghoneim, 2009; Sreelatha *et al*., 2009).

Previous studies have tested catalase activity of *Z. spina christi* L. alcoholic extract, the hepatoprotective and anti-schistosomal effects of its roots were also tested. The results showed that infection with *Schistosoma mansoni* increased lipid peroxides and decreased all antioxidant levels. It was found that the value of the catalase enzyme activity in *Z. spina-christi* L. was 35.55µmol/mg protein. It was also found that the treatment with *Z. spina-christi* L. reversed the effect of disturbed lipid peroxides and increased the antioxidant enzymes to the control values, thus improving its effect against liver damage caused by parasitic infection. The study confirms the use of plants for the treatment of liver diseases as an alternative to the chemotherapeutic agents, which resulted in a low incidence of side effects. Latest trends have shown increasing demand of some medicinal herbs that have proven to have a hepatoprotective potential (Amin and Ghoneim, 2009).

Abdel-Wahhab (2007) studied the effect of aqueous *Z. spina-christi* L. leaf extract on hepatic damage. The results showed that oral administration of oxidative stress cause a significant decrease in hepatic catalase and superoxide dismutase activities. The obtained value of the catalase enzyme activity (17.94 U/g of liver homogenate) indicates that aqueous leaf extracts of *Z. spina-christi* L. has a good hepatoprotective activity. These findings confirmed that *Z. spina-christi* L. aqueous leaf extracts can decrease reactive free radicals which lower the tissues oxidative damage and thus increase and improve the hepatic antioxidant enzyme activity. In addition, results reported by Amin and Ghoneim, (2009) agree with the results reported by Abdel-Wahhab (2012) and confirmed the control activities of endogenous antioxidant enzyme activity like peroxidase and catalase in liver. Glombitza *et al*., (1994) reported the effect of *Z. spina-christi* L. leaf butanol extract on antioxidant enzymes, which resulted in decreasing the serum glucose level and liver phosphorylase.

Habib *et al*., (2014), studied the phenolic compounds in some types of honeys including *Z. spina-christi* L.Sidr honey which was found to contain the highest amount of total phenolics. *Z. spina-christi* L. samples were obtained

Table 6: The effect of aqueous and ethanolic extract of Balady Sidr against the growth of different bacterial strains

Bacterial species	The extract concentration (mg/ml)									
	12.5 mg/ml		25 mg/ml		50 mg/ml		75 mg/ml		100 mg/ml	
	aqueous	ethanolic	aqueous	ethanolic	aqueous	ethanolic	aqueous	ethanolic	aqueous	ethanolic
Staphylococcus aureus	0*	0	0.6	0	1.3	0	2	1	3.6	3.3
Streptococcus pyogenes	0.3	0	0.6	0.6	2	0.9	3	1.6	5.6	2.6
Proteus mirablis	0	0	0.3	0.3	1.3	1.3	3.6	2.3	4.3	4.3
Klebsiella pneumoniae	0	0	0.6	1	2.3	2.3	3.6	5	4.6	6.6
Escherichia coli	0	0	1	0.3	1.3	1.6	3.2	2.3	4.6	3.6

Table 7: The effect of aqueous and ethanolic extract of Pakistani Sidr against the growth of bacterial strains

Bacterial species	The extract concentration (mg/ml)									
	12.5 mg/ml		25 mg/ml		50 mg/ml		75 mg/ml		100 mg/ml	
	aqueous	ethanolic	aqueous	ethanolic	aqueous	ethanolic	aqueous	ethanolic	aqueous	ethanolic
Staphylococcus aureus	0	0	0.3	0	1.3	0	3.6	0.3	5.6	2
Streptococcus pyogenes	1.3	0	2	0	4.6	0.3	7.8	1	9	1.6
Proteus mirablis	0	0	1	0.3	2.3	1	3	1.6	5	3.6
Klebsiella pneumoniae	0	0	1.3	0	2.6	0.3	3	1	3.3	1.3
Escherichia coli	0	0.3	1	1.3	2.6	1.6	5	3.6	6	4.3

*Inhibition zone (mm)

from two different regions in Yemen. The flavonoids in honey make it a good source of antioxidants. In addition, they have an antibacterial effect thus increasing its potential therapeutic activity (Beitlich *et al.,* 2016).

Experiments by Thirugnanasampandan *et al.,* (2017) revealed that fruits of some plants including two species of *Ziziphus* (*Ziziphus mauritiana* and *Ziziphus oenoplia*) are rich in antioxidants. They also assessed the enzymatic activity of peroxidase and catalase enzymes. It was found that the value of peroxidase in *Z. mauritiana* was 0.2100 (OD^{-1} min^{-1} gm^{-1} tissue wt.) and that of catalase was 1.8×10^4 (I.E.U. in 1gm fresh wt. tissue), it was also found that the value of peroxidase activity in *Z. oenoplia* was 0.0900 (OD^{-1} min^{-1} gm^{-1} tissue wt.) and catalase was 2.85×10^4 (I.E.U. in 1gm fresh wt. tissue). These studies illustrate the importance of *Ziziphus* plant which contains antioxidant enzymes that help the human body to fight against diseases by curbing the free radicals produced by secondary metabolic processes

The antibiotic resistance has emerged as a serious clinical problem (Rossolini *et al*., 2014), requiring new efficient antibacterial agents. Our results might indicate that Sidr leaf extract has an antibacterial activity and these findings might confirm the fact that the leaves include some special constituents like glycosides, saponin, alkaloids and flavonoids which are related to the antimicrobial activity. These compounds are familiar to have curative properties against several pathogens. This, hence, explains its possible utilization in the treatment of a number of ailments traditionally (Dkhil *et al*., 2018).

In the current study, much lower concentrations of leaf aqueous extract of Pakistani Sidr (25, 50, 75 and 100 mg/ml) inhibited the growth of *Staphylococcus aureus* by an inhibition zone of 0.3, 1.3, 3.6 and 5.6 mm respectively. *Staphylococcus aureus* is known to play an important role in causing superficial and deep follicular skin lesions (Brooks *et al*., 2002). Kalayou *et al.,* (2012) studied the effects of the aqueous extract of *Ziziphus* leaves on *S. aureus* and found that the concentration of 100 and 250mg/ml had no effect on the growth of *S. aureus*, while higher concentrations (500 and 750 mg/ml) inhibit its growth by an inhibition zone of about 9.00 and 12.00 mm respectively. These high antibacterial efficiencies of leaves extracts might be returning to genetic variations and differences in the environmental conditions or due to the efficiency of the extraction or could be due to the variation in the quality or compositions (installation) of the same plant species (Srinivasan *et al*., 2001).

CONCLUSION

In the present study, *Escherichia coli* and *Streptococcus pyogenes* were found to be susceptible to the ethanolic extracts of Balady Sidr leaves at a concentration of 100 mg/ml, and their inhibition zone were 3.6 and 2.6 mm respectively. *Escherichia coli* is the common cause of urinary tract infection and accounts for approximately 90% of most urinary tract infections among young women (Brooks *et al*., 2002). These results are concordant with the results of Kalayou *et al.,* (2012) who found that the ethanolic extract of *Z. spina-christi* L. was effective against *E. coli* and they relate this antimicrobial activity to the unsaturated fatty acids which represent the main components (83.5 %) of the ethanolic extract. Our results are in contrast with previous studies which showed that

the concentrations of 50, 100 and 200 mg/ml methanolic and ethanolic extracts from *Zizyphus spina-christi* L. had no effects on the growth of *E. coli* (Ali *et al*., 2001). These differences may be explained based on genotypic differences in cultivars grown under different environmental conditions or be due to different strains of *E. coli*.

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