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Research Article

Biochemical profile, antioxidant capacity and allelopathic effects from five *Ziziphyus spina-christi* (L.) provenances growing wild in Saudi Arabia

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Abstract

The aim of this work was to evaluate phenolic profile, antioxidant capacity and allelopathic effects of six *Ziziphyus spina-christi* provenances in Saudi Arabia. Total phenols, flavonoids, tannins and antioxidant activity were evaluated. Total phenols, condensed tannins and total flavonoids were present at levels of 62.29 mg GAE/g DM, 16.69 mg CE/g DM and 15.45 µg mg QE/g DM, respectively. The high antioxidant activity (0.15 mg/ml) was noted in Mecca Road provenance. The highest germination, shoots and radicle lengths of tested species were observed in At-taif provenance. *Z. spina-christi* leaf extracts may be suggested in foods and pharmaceutical industries. Leaf extracts could also provide a natural herbicide with a positive impact on the environment. Provenance of Jizan, Northern Borders and Mecca Road were shown to be particularly effective in antioxidant capacity, while Provenance of Riyadh and Northern Borders shown to have the best allelopathic activity.

Keywords: allelopathic effects; antioxidant activity; provenance; Saudi Arabia; secondary metabolites; *Ziziphyus spina-christi*

Introduction

Ziziphyus spina-christi (*Z. spina-christi*), an evergreen tree, had been credited with many medicinal fields of applications, including immunity and nutrition (Jiang *et al.*, 2007). The fruit of *Z. spina-christi*, as known as 'Nbeg', tasted like a mixture of dates and apples and was usually eaten fresh or dried (Bukar *et al.*, 2015). Many glycoproteins, proteoglycans and glycopeptides had been identified from the *Z. spina-christi* honey (Seif Eldin *et al.*, 2015). In addition, the allelopathy activity, an important biological process in ecology, had been widely employed to protect crops and replace herbicides, fungicides and insecticides being harmful to the environment. The leaf extracts of *Z. spina-christi* presented allelopathic effects on the growth and the development of other plants or micro-organisms (Bhadoria *et al.*, 2011). *Ziziphyus spina-christi* (sidr) is a shrub, sometimes a tree, native to a vast area of Africa stretching from Mauritania to West Africa. In the Kingdom of Saudi Arabia, it is a medicinal plant for many diseases (Alansi *et al.*, 2016). The genus *Ziziphyus*

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belongs to the family of Rhamnaceae with about 85 species and *Ziziphyus* is the most common plants of economic importance in Saudi Arabia and are the major floral sources of high-valued expensive honeys (Alqarni *et al*., 2015; Alqarni, 2015). Z. *spina-christi* can grow either as a tree or as a shrub. The leaves are short, the flowers are pedunculated and the yellow or red fruits are edible (Chaudhary *et al*., 2001). *Ziziphyus jujuba*, a plant locally grown in Saudi Arabia and used extensively for its health benefits. The medicinal properties of *Ziziphyus* tree, also known as Nabq or Sidr, were also recognized and have been in use in Pharaonic carpentry, diet, and medicine (El-Ansary *et al*., 2018). Z. *spina-christi* is a medicinal plant and its leaf extract (peptide and cyclopeptide alkaloids) has neuroprotective and therapeutic roles against pentylenetetrazol convulsant effect (Waggas *et al*., 2010). The Quran mentions the sidr tree twice and accordingly it is highly respected by the Muslims throughout the Middle East (Farooqi, 1997). It is traditionally used in the Muslim countries to wash the bodies of dead Muslims prior to burial, with water in which *Ziziphyus* jujuba leaves had been soaked (Dafni *et al*., 2005. In Saudi Arabia, it is used for the treatment of many diseases like wounds, ulcers, etc. Mizrahi *et al*. (2002) reported that *Ziziphyus* species are cultivated in hot and arid regions. Some pharmacological screening studies indicated that Z. *spina-christi* leaves appear to be a safe alternative to lower the blood glucose level. Honeys originating from Sidr (*Ziziphyus spina-christi* L.) trees in Saudi Arabia exhibited substantial antimicrobial activity against pathogenic (Owayss *et al*., 2020). Leaves of *Ziziphyus jujuba* contain a compound known as *Ziziphyus* which demonstrates medicinal properties and is commonly used in traditional medicine. Among the medicinal properties of *Ziziphyus jujube* are sedative properties, anticancer, antioxidants, antimicrobial and anti-inflammatory effects (Li *et al*., 2005; Abdel-Zaher *et al*., 2014). In Saudi Arabia, *Ziziphyus spina-christi* is one of the most popular species of *Ziziphyus*. It is a wild and cultivated plant distributed in the Middle East, Pakistan and in the North and East of Africa (Alansi *et al*., 2016).

In this study, total phenolic, total flavonoid, condensed tannin contents and antioxidant activity of *Ziziphyus spina-christi* methanolic leaf extracts harvested from six Saudi Arabia provenances were evaluated and compared. *In vitro* allelopathic activities of aqueous extracts of the leaves were also tested on *Trigonella foenum-graecum* and *Lens culinaris* seeds.

Materials and Methods

Plant material

Leaves of Z. *spina-christi* were sampled from At-taif, Riyadh, Jizan, Northern Borders, Mecca Road, Jeddah (Figure 1). The plant species were collected (200-250 g per species) during the vegetative phase. The harvested provenances of Z. *spina-christi* were authenticated and named using existing literature (Cope, 1985; Chaudhary and Akram, 1987; Mighaid, 1996; Collenette, 1999; Chaudhary, 1999-2001; Chaudhary, 1999, 2000, 2001; Al-Hassan, 2006). The harvested plant samples were processed (powdered at around 100 mesh) then dried in the shade at room temperature for two weeks) and finally stored in in plastic bags in the dark until chemical analysis.

Preparation of the plant extracts

The aerial part of each provenance was finely powdered and used for the different biochemical assays. The solvent is used in order to obtain plant extracts solutions (1 g / 10 ml), which were macerated for 24h in shaking conditions (50 rpm) and used to assay the total content of polyphenols, the total content of flavonoids and the total antioxidant activity (DPPH test). Then extracts were pooled and concentrated under vacuum (Hammi *et al*., 2015).

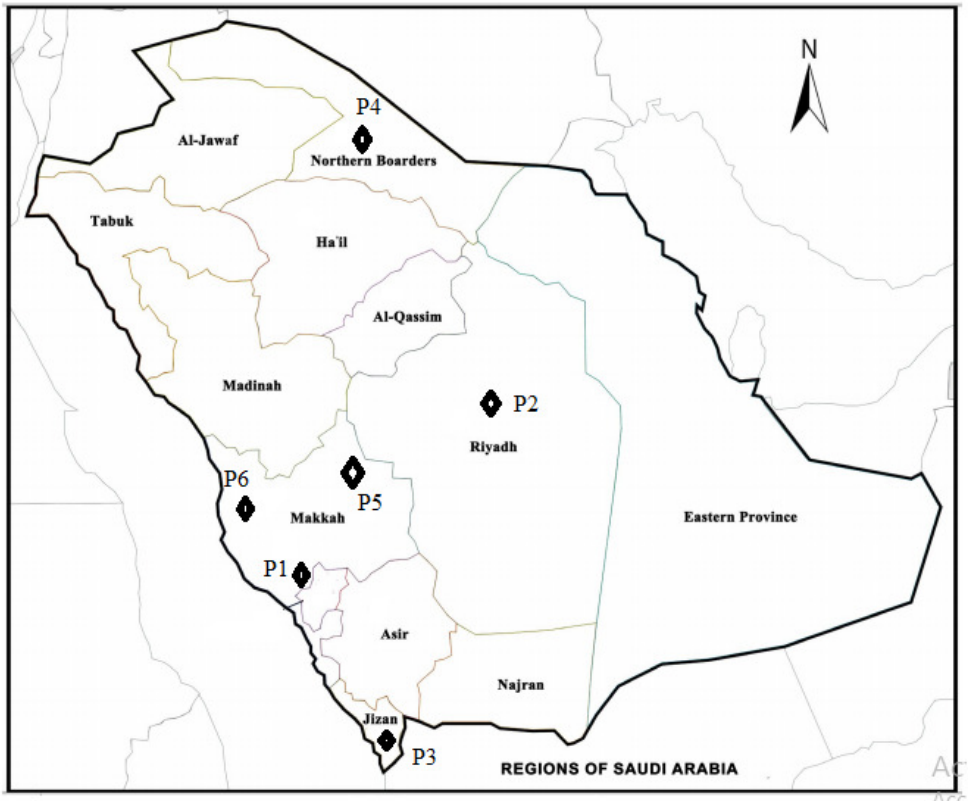

Figure 1. Localities of six *Ziziphus spina-christi* provenances in Saudi Arabia (P1: At-taif; P2: Riyadh; P3: Jizan; P4: Northern Borders; P5: Mecca Road; P6: Jeddah

Total polyphenol content

Total phenolics were determined with Folin-Ciocalteu assay as per the Singleton *et al.* (1999) method which was slightly modified by Deawanto *et al.* (2002). The results are obtained using a UV/Vis Jenway-6300 spectrophotometer (United Kingdom) under 760 nm. The total phenolics content was expressed as milligrams of Gallic acid equivalents per gram of dry matter (mg GAE/g DM) through the calibration curve of Gallic acid. The sample was analysed in three replications (Rouz *et al.*, 2015).

Total flavonoid contents

Total flavonoid contents were determined using the Aluminium trichloride method (Rice-Evans et al. 1996; Popova *et al.*, 2004;). 1 ml of $AlCl_3$ (2%) was added to 1 ml of plant extract. The volume was adjusted to 25 ml with methanol and thoroughly mixed. The absorption was measured after 40 min by a Shimadzu UV-160 (Tokyo, Japan) spectrophotometer at 420 nm. Total flavonoids were expressed in milligrams quercetin equivalents per gram of dry matter (mg QE/g DM) through the calibration curve of quercetin. All measurements were repeated three times for accuracy and reliability (Elaloui *et al.*, 2016).

Condensed tannin contents

Condensed tannin levels were assayed following the Earp *et al.* (1981) method. 1 ml of vanillin (1%) mixed with 4 ml of HCl were added to 200 µl of leaf extracts and incubated 20 min in obscurity. Catechin was used as a standard (0-1250 µg/ml) and the results were expressed as microgram catechin equivalent per gram dry weight (µg CE/g DM). All measurements were performed in three replications. After agitation, the absorbance was read at 500 nm using a Jenway 6100 spectrophotometer.

Antioxidant activity

In test tubes 2.36 mg of DPPH, previously dissolved in 100 ml of ethanol, was mixed and incubated in obscurity. Different concentrations (0.75; 0.5; 0.25; 0.125 µg/ml) were prepared from 1 mg/ml of each leaf extracts. The control sample was done using ethanol and DPPH. The absorbance was measured at 490 nm after incubation for 30 min in dark. Measurements for each experiment were done in triplicate. Antioxidant activity expressed as inhibitory effect of the DPPH radical was calculated using this formula:

The percentage of inhibition = [(A0 – Ac) / A0] × 100

Where A0 was the absorbance of the control and Ac was the absorbance of the plant extract/ standard.

The IC50 value, the concentration (in µg/ml) of the compound required to scavenge DPPH radical by 50, were determined graphically by the linear regression (Basuny *et al*., 2013).

Allelopathic activity of Ziziphyus spina-christi leaf aqueous extracts

Extracts were obtained by macerating from the *Ziziphyus spina-christi provenances* leaf extracts as various concentrations: 5 g, 20 g, 40 g, 60 g and 100 g powdered plant in 1 litre of distilled water for 24 h. The solutions were filtered through double layers of muslin cloth followed by filter paper. *Trigonella foenum-graecum* and *Lens culinaris* seeds were sterilized with 0.3% calcium hypochlorite, rinsed in distilled water, and dried on filter paper in the laboratory at room temperature for 7 days. For germination, two layers of filter papers were placed in 90-mm-diameter glass petri dishes. Twenty seeds were placed in each petri dish, followed by 02 mL of plant extract. The control groups were each given 2 ml of deionized water and leave at room temperature (25 °C). The Petri dishes were sealed with plastic wrap to prevent the loss of moisture and avoid contamination. Starting from the first day after the experiment began, germinated seeds were counted and removed daily. The experimental design was carried out as a randomized complete block (RCB) with 5 replications. The germination percentage (GP) was calculated. For growth bioassays, the seeds of *Trigonella foenum-graecum* and *Lens culinaris* were germinated on filter paper in the dark at room temperature (25 °C) and a photoperiod of 16 hours. Fifteen germinated seeds were transferred to petri dishes, which were filed with 25 g of sterilized quartz sand, and 02 mL of tested extract was added. In addition, control sample was added to the experiment without any treatment. Shoot (SL) and root lengths (RL) of seedlings were measured 15 days after treatment (DAT).

Statistical analysis

A descriptive analysis was performed to describe the entire results within each kind of test. Concerning the biochemical composition analysis and antioxidant activity a one-way analysis of variance (ANOVA one-way) followed by Duncan test was performed to test possible significant differences among mean values from different provenances. The level of significance was set at $P<0.05$ for all analyses. Statistical analyses were performed using PSS v.23 software.

Results

Analysis of secondary metabolites and antioxidant properties

Total polyphenol, total flavonoid and tannins contents

Total polyphenol levels varied from 23.65 mg GAE/g DM of the At-taif provenance to 62.29 mg GAE/g DM of the Mecca Road provenance (Table 1). The Jizan provenance has an important total polyphenol level (50.63 GAE/g DM). The relative values of total flavonoids (Table 1) oscillated between 6.35 (At-taif) and 15.45 mg QE/g DM (Jeddah). The Riyadh provenance has an important total polyphenol level (12.68 QE g/g DM). As we see in these results, the tree of *Ziziphyus spina-christi* in At-taif was the most stressed tree provenance. Leaf extracts of Jizan Z. *spina-christi* found to contain higher amounts of tannins (16.69 mg CE/g

DM) as compared to provenances of At-taif (12.36 mg CE/g DM), Riyadh (9.25 mg CE/g DM) Northern Borders (11.26 mg CE/g DM) Mecca Road (7.16 mg CE/g DM) and Jeddah (14.39 mg CE/g DM).

Antioxidant activity

The antioxidant activity ranged between 0.15 mg/mL and 0.06 mg/mL, respectively (Table 2). The provenance of At-taif, Riyadh and Jeddah provenances have a lower value of IC50 compared with Jizan, Northern Borders and Mecca Road provenances.

Table 1. Phytochemical composition extracts of six *Ziziphus spina-christi provenances*

Provenances	Total polyphenol contents (mg GAE/g DM)	Total flavonoid content (mg QE/g DM)	Condensed tannins (mg CE/g DM)
At-taif	23.65 ± 0.35^{f}	6.35 ± 0.32^{e}	12.36 ± 0.48^{c}
Riyadh	36.65 ± 0.51^{e}	12.68 ± 0.26^{b}	9.25 ± 0.13^{e}
Jizan	50.63 ± 0.26^{b}	9.45 ± 0.27^{c}	16.69 ± 0.42^{a}
Northern Borders	43.57 ± 0.034^{c}	7.68 ± 0.49^{f}	11.26 ± 0.11^{d}
Mecca Road	62.29 ± 0.28^{a}	8.59 ± 0.42^{d}	7.16 ± 0.92^{f}
Jeddah	42.08 ± 0.38^{d}	15.45 ± 0.76^{a}	14.39 ± 0.22^{b}

Data are presented as mean values ± standard deviation (n = 3). Statistical analysis: ANOVA test and DUNCAN test. The different letters above the values in the same column indicate significant differences ($p<0.05$). Values with the same superscript letters in the same column are not significant.

Table 2. Antioxidant activity of plant extracts

Provenances	DPPH (IC 50, mg/ml)
At-taif	0.06 ± 0.005^{f}
Riyadh	0.08 ± 0.015^{d}
Jizan	0.13 ± 0.036^{b}
Northern Borders	0.10 ± 0.003^{c}
Mecca Road	0.15 ± 0.05^{a}
Jeddah	0.07 ± 0.012^{c}

Data are presented as mean values ± Standard deviation (n=3). Statistical analysis: ANOVA test and Duncan test ($p<0.05$). Values with the same superscript letters in the same column are not significant.

Effects of aqueous Z. spina-christi leaf extracts on L. culinaris and T. foenum-graecum

Effects on seed germination

T. foenum-graecum seeds were more sensitive than those of *L. culinaris* seeds after treatment by aqueous leaf extracts with concentrations of 60 and 100 g/l (Tables 3 and 4). All extract of provenances of *Ziziphyus* have a negative effect on germination of *T. foenum-graecum* and *L. culinaris* seeds. The extract of Riyadh and Northern Borders provenance have a very remarkable influence on the germination of *T. foenum-graecum* (3 and 4% at 100 g/l respectively) and the germination of *L. culinaris* (9% and 8% respectively at 100 g/l).

Effects on root and shoot lengths

Root lengths are very sensitive to aqueous Z. *spina-christi* leaf extracts (Tables 3 and 4). Compared with other provenances, the two provenances which have a great effect on the root length of *Trigonella foenum-graecum* L. are those of Riyadh (10 and 16 mm at 20 and 100 g / l respectively) and Northern Borders (15 and 2 mm at 60 and 100 g / l respectively). These two provenances also have the greatest effect on the root length of *Lens culinaris* L.: 13 and 9 mm at 60 and 100 g / l respectively for Riyadh provenances and 14 and 8 mm at 60 and 100 g / l for Northern Borders provenances (Tables 3 and 4). Shoot lengths are very sensitive to aqueous Z. *spina-christi* leaf extracts (Tables 3 and 4). Riyadh Northern Borders provenance have a very negative effect on seedling shoots of *Trigonella foenum-graecum* L. compared with other provenances with 23 and 8 mm at 60 and 100 g / l respectively and 18 and 6 mm at 60 and 100 g / l respectively. Riyadh Northern Borders

provenance have a very negative effect on seedling shoots of *Lens culinaris* L. compared with other provenances with 15 and 8 mm at 60 and 100 g / l respectively and 24 and 13 mm at 60 and 100 g / l respectively (Tables 3 and 4).

Table 3. Effects of different aqueous leaf extracts of six provenances of *Z. spina-christi* on the growth of *Trigonella foenum-graecum* L.

Parameters	Aqueous extract concentration (g/l)	At-taif provenance	Riyadh provenance	Jizan provenance	Northern Borders provenance	Mecca Road provenance	Jeddah provenance
Germination capacity (%) at 7 DAT	0	75±2.36^{a}	92±3.21^{a}	85±4.21^{a}	87±1.26^{a}	77±3.26^{a}	84±2.41^{a}
	5	60±1.25^{b}	52±2.36^{b}	57±4.15^{b}	53±2.15^{b}	50±2.35^{b}	52±3.97^{b}
	20	42±2.35^{c}	38±1.24^{c}	33±3.45^{c}	31±3.40^{c}	31±1.06^{c}	37±1.23^{c}
	40	23±1.12^{d}	26±1.98^{d}	21±2.15^{d}	28±1.02^{d}	22±0.68^{d}	26±3.15^{d}
	60	15±3.26^{e}	10±0.98^{e}	18±1.02^{e}	12±1.29^{e}	17±1.03^{e}	21±1.02^{e}
	100	08±3.26^{f}	04±4.16^{f}	12±5.14^{f}	02±1.24^{f}	11±2.49^{f}	10±0.26^{f}
Seedling root length (mm) at 15 DAT	0	52.15±1.20^{a}	65.15±2.15^{a}	49.25±1.64^{a}	54.12±2.36^{a}	56.34±2.10^{a}	53.12±1.35^{a}
	5	42.35±2.13^{b}	54.12±3.15^{b}	46.35±3.16^{b}	48.26±3.12^{b}	51.42±0.96^{b}	50.14±2.34^{b}
	20	33.25±1.23^{c}	43.12±0.95^{c}	42.15±2.15^{bc}	34.16±0.95^{c}	45.12±2.67^{c}	42.15±0.95^{bc}
	40	24.15±2.15^{dc}	33.15±2.15^{dc}	33.15±1.42^{d}	23.15±2.13^{d}	32.15±0.52^{d}	39.45±3.15^{d}
	60	18.65±3.26^{e}	16.54±3.21^{e}	25.35±0.92^{e}	15.24±2.57^{e}	23.15±1.04^{e}	32.65±2.48^{e}
	100	15.24±1.05^{f}	8.65±1.05^{f}	14.25±1.37^{f}	4.36±4.12^{f}	15.64±2.67^{f}	16.84±1.42^{f}
Seedling shoot lenght (mm) at 15 DAT	0	65.45±0.96^{a}	64.58±0.95^{a}	75.12±2.42^{a}	68.45±2.10^{a}	69.48±2.35^{a}	66.25±2.39^{a}
	5	48.25±2.15^{b}	53.12±4.35^{b}	59.85±0.75^{b}	63.25±3.15^{b}	64.12±3.45^{b}	52.46±2.38^{b}
	20	36.45±3.15^{c}	41.26±2.09^{bc}	45.16±3.15^{c}	54.12±1.03^{c}	54.13±2.03^{c}	41.36±2.98^{c}
	40	23.54±0.67^{d}	33.16±3.45^{d}	41.13±2.37^{dc}	33.15±2.35^{d}	34.15±2.34^{d}	34.95±3.45^{d}
	60	15.24±2.17^{e}	23.45±2.03^{e}	33.12±0.93^{e}	18.46±0.68^{e}	21.23±1.06^{e}	19.65±2.34^{e}
	100	12.25±1.37^{f}	8.46±2.15^{f}	19.35±3.15^{f}	6.45±1.35^{f}	12.45±.67^{f}	10.39±1.06^{f}

Data are presented as mean values ± Standard deviation (n = 3). Statistical analysis: ANOVA test and DUNCAN test ($p<0.05$). Values with the same superscript letters in the same column are not significant.

Table 4. Effects of different aqueous leaf extracts of six provenances of *Z. spina-christi* on the growth of *Lens culinaris* L.

Parameters	Aqueous extract concentration (g/l)	At-taif provenance	Riyadh provenance	Jizan provenance	Northern Borders provenance	Mecca Road provenance	Jeddah provenance
Germination capacity (%) at 7 DAT	0	89±3.16^{a}	82±2.19^{a}	78±4.16^{a}	85±1.18^{a}	72±5.48^{a}	91±1.46^{a}
	5	75±2.31^{ab}	79±2.35^{ab}	69±6.35^{ab}	72±0.93^{ab}	70±2.35^{b}	85±2.13^{ab}
	20	60±2.03^{b}	63±3.21^{c}	65±3.45^{c}	62±5.15^{c}	63±0.74^{c}	61±0.85^{c}
	40	36±2.15^{c}	41±2.45^{d}	31±2.36^{d}	24±1.26^{dc}	34±3.15^{d}	36±3.21^{d}
	60	23±1.06^{cd}	35±3.45^{e}	19±0.95^{e}	33±1.07^{e}	21±2.15^{e}	15±2.35^{e}
	100	14±1.68^{d}	9±0.19^{f}	15±3.12^{f}	8±2.85^{f}	19±2.06^{f}	17±0.69^{f}
Seedling root length (mm) at 15 DAT	0	42.35±0.68^{a}	38.68±2.36^{a}	44.25±2.18^{a}	41.26±1.04^{a}	44.68±0.95^{a}	45.69±1.25^{a}
	5	35.25±2.15^{b}	32.15±1.02^{b}	30.25±3.49^{b}	33.15±3.25^{b}	30.78±1.02^{b}	36.45±2.04^{b}
	20	28.15±2.06^{bc}	29.65±2.18^{c}	21.25±1.42^{c}	25.64±2.38^{c}	29.58±1.35^{bc}	30.42±1.52^{bc}
	40	24.15±0.24^{dc}	23.54±0.58^{d}	18.68±2.15^{d}	21.25±1.45^{d}	19.65±2.04^{d}	29.25±3.16^{d}
	60	16.42±2.35^{e}	13.67±2.48^{e}	17.25±3.45^{e}	14.25±0.93^{e}	17.27±3.12^{e}	23.15±2.45^{e}
	100	10.26±1.29^{f}	9.36±1.09^{f}	14.16±2.45^{f}	8.16±1.04^{f}	15.14±0.73^{f}	18.15±3.48^{f}
Seedling shoot lenght (mm) at 15 DAT	0	32.65±3.21^{a}	33.25±1.23a	37.25±0.42^{a}	34.68±0.68^{a}	36.58±1.09^{a}	35.18±3.21^{a}
	5	31.15±1.45^{ab}	29.65±0.83^{ab}	28.15±1.04^{bc}	28.45±2.36^{bc}	27.15±2.34^{b}	34.35±0.84^{ab}
	20	30.12±1.42^{c}	27.15±1.58^{c}	22.35±2.03^{c}	26.35±3.15^{c}	22.15±3.16^{c}	33.25±0.28^{c}
	40	18.26±2.03^{d}	24.13±2.15^{d}	20.15±0.59^{d}	23.45±1.03^{de}	20.14±2.15^{cd}	31.25±1.28^{d}
	60	12.35±1.78^{e}	15.54±3.25^{e}	19.45±2.15^{e}	24.15±0.19^{e}	19.65±1.42^{e}	25.54±2.13^{e}
	100	14.24±3.15^{f}	8.15±0.62^{f}	18.26±1.03^{f}	13.26±2.35^{f}	18.56±3.26^{f}	21.15±1.25^{f}

Data are presented as mean values ± Standard deviation (n = 3). Statistical analysis: ANOVA test and Duncan test ($p<0.05$). Values with the same superscript letters in the same column are not significant.

Discussion

Analysis of secondary metabolites and antioxidant properties

The total polyphenol content varies from one species to another. The leaves of the *Ziziphyus* of Mecca Road presented the highest total polyphenol (62.29 mg GAE/g DM) while *Ziziphyus of At-Taif* is the lowest one (23.65 mg GAE/g DM). The variations are probably due to the genetic characteristics of the species and by the environmental conditions in which the plants are found. These results corroborate with those obtained by Bettaieb *et al.* (2012), Hadacek (2002), Miliauskas *et al.* (2004), Ebrahimi *et al.* (2008), Andarwulan *et al.* (2010). Similar results were reported by Rouz *et al.* (2015). Our results were higher than those obtained for the *Ziziphyus spina-christi* Tunisian provenances (Elaloui *et al.*, 2016a; 2016b) where trees are from a young age than ours. This variability the total polyphenol levels could reflect the influence of the geographic conditions (humidity, temperature, altitude), the soil-growth conditions, and the origin and the period of leaves harvest (Brito *et al.*, 2015). The methanol was found to be the efficient solvent to extract *Ziziphyus* leaves. This idea was also confirmed by Medini *et al.* (2014). Indeed, the increase in the biosynthesis and accumulation of phenolic compounds occurs frequently in plant tissue in response to biotic and abiotic stresses. These compounds are involved in the defence against reactive oxygen species (ROS), which are inevitably produced when the aerobic metabolism or photosynthetic is weakened by environmental stress. Genetic factors and growth conditions of the plant can also play an important role in the formation of secondary metabolites, including phenolic acids (Islam *et al.*, 2003; Hashempour *et al.*, 2010). Other factors especially the extraction method and the quantification method may also influence the estimate of the content of total phenols (Lee *et al.*, 2003). The polyphenols played an important role in disease resistance by exerting free-radical scavenging, modulation of enzymatic activity and alteration of signal transduction pathways (Elaloui *et al.*, 2016a). This study revealed a strong influence of the localities on the constituent of the leaf's extracts. This composition could also be influenced by the extraction time and the ratio of aqueous methanol (Elaloui *et al.*, 2016c). As well, this variability could be explained by many factors including the origin, the period of harvest, the age and the stage of plant development. Other environmental factors (temperature, altitude, sunshine, animal aggression and diseases) could also influenced this variability (Elaloui *et al.*, 2017). The amount of flavonoids of *Z. spina-christi* significant varied ($P < 0.01$) between 6.35 (At-taif provenance) and 15.45 mg QE/g DM for Jeddah provenance. With the same method, Z. s*pina-christi* leaves for our provenance had more important flavonoid levels than those obtained for Z. *joazeiro* (Elaloui *et al.*, 2016a; 2016b; Adzu and Haruna, 2007). Similar results were reported by Rouz *et al.* (2015). It has been proven that the contents of total phenols and flavonoids are high. The difference in the phenolic content including flavonoids described in the literature can be attributed to several factors such as the extraction method and the quantification method. Moreover, variables such as temperature, humidity and light intensity, added to the effects of the biota and the physiochemical structure of the soil can affect not only the production of secondary metabolites but also the chemical structure of substances (Reigosa *et al.*, 2013). It has been proved that the levels of total phenols and flavonoids were high when the environment conditions of the plant were not adequate. In this case, the plant promoted the synthesis of secondary metabolites in order to adapt and survive Tim and Lamb (2005). A few papers reported the amount of phenolics in some of plants; recently, in a review about the biochemical composition of different parts of Z. *lotus*, Azize (2016) reported around 7 mg/g of phenolics to be present in the leaves, almost 5 times less than the amount measured in Tlili *et al.* (2019). Moreover, in the same review, the tannin content observed in the leaves was the same that Tlili *et al.* (2019) measured (around 3.0 mg/g). Conversely, the content of phenolics in the leaves of *T. hirsuta* and R. *tripartitum*, including flavonoids, and the DPPH values found by Akrout *et al.* (2011) and Itidel *et al.* (2013) were in line with those measured by Tlili *et al.* (2019). 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 from two different regions in Yemen. Ours results were in consistence with those obtained in *Z. mauritiana* (IC50 = 0.11 mg/mL) (Ashraf *et al.*, 2015). Ours studies showed that the

antioxidant activity augmented by the increase in total phenol levels. Adzu and Haruna (2007) also confirmed this idea. For *Ziziphyus lotus* (L.) Tlili *et al.* (2019) found the same results as we found in our study. When Alghazeer *et al.* (2012) studied the antioxidant activity of some plants growing in Libya, he found two times higher the amount of polyphenols in *Hyoscyamus albus* than we found in this study. The flavonoids in honey make it a good source of antioxidants (Bader *et al.*, 2020).

Effects of aqueous Z. spina-christi leaf extracts on L. culinaris and T. foenum-graecum

Effects on seed germination

For both target species (*L. culinaris* and T. *foenum-graecum*), the highest germination was obtained for the control and inhibition of germination, which increases by elevating the concentration of aqueous extracts. The concentration of 100 g/l gives the lowest germination for both species with a respective *L. culinaris* and *T. foenum-graecum* of 8 at 19% and 2 at 12%. Similar results were reported by Rouz *et al.* (2015). Inhibitory effect on germination and seedling development were obtained especially at higher concentrations (60 and 100 g/l). Similar findings were also recorded by many researchers (Arora *et al.*, 2015; Prasad *et al.*, 2016; Turker *et al.*, 2008). The degree of inhibition was largely dependent on the concentration of the aqueous extracts (Turk *et al.*, 2002). These results confirm the herbicidal properties of this medicinal specie of Z. *spina-christi* containing natural molecules like juglone and artemisin that affect membrane permeability, water balance and osmotic regulation (Sanchez-Moreiras *et al.*, 2008).

Effects on root and shoot lengths

Ours results showed that interaction between level of concentration of aqueous extracts and species decrease significantly the roots growth. Similar results were reported by Rouz *et al.* (2015). With the concentrations of 100 g/l the length of the roots of *L. culinaris* varied between 8.16 and 18.15 mm and the shoot varied between 8.15 and 21.15 mm. With same concentration the length of the roots of *T. foenum-graecum* varied between 4.36 and 16.84 mm and the shoot varied between 6.45 and 19.35 mm. The root lengths were more inhibited than shoot lengths. These results are in agreement with those of Patil and Kore (2016). These results show that the aqueous extracts of the six provenances of *Ziziphyus* studied have allelopathic potential and significantly inhibit the germination and growth of *T. foenum-graecum* and *L. culinaris*. These excerpts are provided with a potent cytotoxic activity. Root growth is more sensitive to different concentrations of aqueous extracts of six provenances of this medicinal specie than germination. Turk and Tawaha (2002) concluded that the aqueous extracts of allelopathic plants generally act on root growth rather than growth of the hypocotyl. This was explained by the fall, which made the roots in contact with the first allelochemicals inhibit cell division in meristematic tissues (Ashrafi *et al.*, 2008). Mitosis is the most important event in the development of the plant and many enzymes and regulatory factors of these phenomena are affected by allelochemicals via a chromosome aberration (Turker *et al.*, 2008). Thus, the aqueous extract can affect the absorption of water by the seed and delay the mobilization of reserves (Kato-Noguchi and Macias, 2006). Even if the seed happens to germinate, we recorded a blocking root growth and / or the aerial part of plants. Tucker *et al.* (2008) reports that the growth phenomenon is more sensitive to the phytotoxic allelochemicals than germination. So, the exact identity of the compounds responsible for this apparent phytotoxic activity, and quantified with certainty needs to be known. Overall, the observed results could form the basis for further investigations of the biological properties of aqueous extracts of six provenances of this medicinal specie. In addition, the use of these aqueous extracts as a biopesticide is a promising path towards recovery of this fraction.

Conclusions

This study demonstrated that allelopathic potential of aqueous extracts of six provenances of medicinal specie (*Ziziphyus*) and invasive grass by influencing either the germination or growth are the two phenomena

that target both species. This indicates that these extracts are rich in natural bioactive substances, suggesting that their importance in the development of natural bioherbicides as an alternative to minimize the release of synthetic non-degradable molecules in crop production. Further studies are needed to understand the mode of action of these allelochemicals. Also, a separation, purification and identification of bioactive molecules that could potentially be used as herbicides or as plant growth regulators would be need to be recorded for further study. Allelopathy, used as herbicides, had an economic benefit to farmers and also reduced negative impacts on the environment and on the health of the human being. In conclusion, all the data confirm the importance of the Saudi Arabia local vegetation as a potential source of various bioactive phytochemical compounds; the investigation is based on the need for different biological agents from natural sources with potent activity and lesser side effects as substitutes for chemical therapeutics.

Authors' Contributions

Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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