SUPPLEMENTARY MATERIAL

Phenolic profile, antioxidant capacity of five *Ziziphus spina-christi* (L.) Willd provenances and their allelopathic effects on *Trigonella foenum-graecum* L. and *Lens culinaris* L. seeds

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Abstract

The aim of this work was to evaluate some secondary metabolites, antioxidant activity of methanolic leaf extracts of five *Ziziphus spina-christi* provenances (INRGREF, Tozeur, Degueche, Nafta and Kebelli) and their allelopathic effects on *Trigonella foenum-graecum* and *Lens culinaris*. Leaves were collected during 2013 and 2014.

Total phenols, flavonoids, tannins and antioxidant activity were evaluated using the Folin ciocalteux, Aluminum trichloride, vanillin and scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical methods, respectively.

Total phenols, tannins and flavonoids were present at levels of 57.41 mg GAE /g DW, 31.98 mg RE /g DW and 14.68 µg CE/g DW; respectively. The high antioxidant activity (0.086 µg/ml) was noted in kebelli provenance (2013). The highest germination, plumule and radicle lengths of tested species were observed in INRGREF provenance.

Ziziphus 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.

Key words: Secondary metabolites; *Ziziphus spina-christi*; provenance; antioxidant activity; allelopathic effects.

3. Experimental

3.1. Plant material

Leaves of *Z. spina-christi* were sampled from Nafta, Dgueche, Tozeur, kebelli and INRGREF provenances during two successive years (2013 and 2014). The identification of the plant material (20 years -old) was done by Professor Mohamed Boussaid and a voucher specimen of the plant (N° Pn 959) was deposited at the Herbarium of INRGREF (Tunisia). The leaves were dried at room temperature during a half month in a dry and airy environment. Dried leaves were grounded by a mill equipped with a grid whose holes were 1.00 mm in diameter and stocked in plastic bags in the dark until chemical analysis (Figure S1).

3.2. Chemical Reagents

Folin-ciocalteu, DPPH, gallic acid, catechin Sodium carbonate, Hydrochloric acid and methanol were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used were of the highest purity.

3.3. Extraction

Leaf powders (1 g) were submitted to maceration with 10 ml of pure methanol for 30 min. The extracts were filtered through Whatman No.1 filter paper. Then extracts were pooled and concentrated under vacuum (Hammi et al. 2015).

3.4. Total phenol and flavonoid contents

Total phenol contents were estimated by the Folin-Ciocalteu method (Wong et al. 2006). From each sample, 0.5 ml of methanolic solution was added to 2.5 ml of Folin- Ciocalteu reagent and 2 ml of Sodium carbonate (75 g/l) solution. The reading of the absorbance was done at 765 nm using a Shimadzu 1600-UV spectrophotometer after incubation during 30 min. Total phenol contents of each fraction were expressed into mg GAE/g DW (Khoudali et al. 2014).

Total flavonoid contents were determined using the Aluminum trichloride method (Popova et al. 1996). 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, Japon) spectrophotometer at 420 nm. Flavonoid contents were expressed as mg RE /g DW. All samples were analyzed in three replications.

3.5. 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 DW). All measurements were performed in triplicate. After agitation, the absorbance was read at 500 nm using a Jenway 6100 spectrophotometer.

3.6. 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:

$$\text{The percentage of inhibition} = [(A_0 - A_c) / A_0] \times 100$$

where A_0 was the absorbance of the control and A_c 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).

3.7. Allelopathic activity of *Z. spina-christi* leaf aqueous extracts

Ziziphus leaf extracts were obtained by macerating 5 g; 20 g; 40 g; 60 g and 100 g powdered plant in 1 l of distilled water for 24 h. The macerate was centrifuged and the supernatant was filtered.

Trigonella foenum-graecum and *Lens culinaris* seeds were sterilized with 2% sodium hypochlorite for 2 min before sowing, then rinsed four times with distilled water. Seeds (25) were arranged in Petri dishes (9 cm diameter) lined with two discs of Whatman No.1 filter paper. 2 ml of aqueous extracts were added to each Petri dish. The control groups were each given 2 ml of deionized water. The Petri dishes were sealed with plastic wrap to prevent the loss of moisture and avoid contamination. The treatments were kept at a laboratory bench with 12 h supply of fluorescent light during the night. The germination percentages (GP), shoot (PL) and root lengths (RL) were recorded after seven days. Relative reduction or

stimulation of seeds germination, shoot and radical lengths as affected by the allelopathic substances were calculated. Three repetitions were done.

3.7. Statistical analysis

Results were statistically evaluated using STATISTICA. Data from three samples was reported as means $\pm$ standard deviation. Differences were tested for significance with the ANOVA procedure using the Duncan test with a significance level of $p < 0.05$.

References

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Table S1. Effects of different aqueous leaf extracts of *Z. spina-christi* on *Lens culinaris* and *Trigonella foenum-graecum* root lengths during 2013, 10 days after planting the seeds under laboratory conditions.

C (g/L)	Species	INRGREF	Kebilli	Dgueche	Nafta	Tozeur
0	L	5.6±0.1	4.25±0.05	4±0	3.9±0.1	2.95±0.05
	T	1.95±0.05	1.2±0.1	1.65±0.05	1.3±0.1	1.45±0.05
5	L	6.55±0.05	5.4±0.1	4.15±0.05	4.25±0.05	3.55±0.05
	T	2.05±0.05	1.8±0.1	2.05±0.05	2±0	2.3±0.1
20	L	6.45±0.05	5.85±0.15	4.5±0	4.05±0.05	4.1±0.1
	T	2.25±0.05	2.15±0.15	2.15±0.05	2.35±0.15	2.25±0.05
40	L	5.85±0.15	5.35±0.15	4.55±0.05	3.75±0.05	4.75±0.05
	T	2.15±0	1.95±0.15	2±0	1.65±0.15	2.2±0.1
60	L	2.25±0.05	2.05±0.05	2.05±0.05	1.4±0.1	1.1±0.1
	T	1.55±0.05	0.95±0.05	1.35±0.05	0.85±0.05	0.95±0.05
100	L	1.5±0.1	1.4±0.1	1.65±0.05	0.8±0.1	0.7±0
	T	1.05±0.05	0.55±0.05	0.95±0.05	0.45±0.05	0.45±0.05

Note :

1. L : *Lens culinaris* ; T : *Trigonella foenum-graecum*

2. The data are the mean values of three measurements ± SD (standard deviation)

a

b

Figure S1. Leaves (a) and powders (b) of five tunisian *Ziziphus spina-christi* provenances (Tozeur, Degueche, Nefta, Kebelli and INRGREF).