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Allelopathic effects of *Tetraclinis articulata* on barley, lettuce, radish and tomato

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ABSTRACT

We evaluated the allelopathic effects of leaf leachates, aqueous and organic extracts from *Tetraclinis articulata* on seed germination and seedling growth of lettuce, barley, radish and tomato. Leaf powder from *T. articulata* added to soil and leaf leachate irrigated soil were evaluated in pot assays. The leaf extracts and the leachate significantly decreased the germination of the target plants. Total inhibition of lettuce, tomato and radish seedling growth was observed at 40g/l and 500g/l concentrations for the extract and the leaf leachate, respectively. All organic extracts were very toxic at 6000 ppm. Soil incorporation of residues at 100 g/kg significantly decreased the root and shoot length of target species. The irrigation with leaf aqueous extracts was harmful for tomato, radish and lettuce and leachate was harmful only to lettuce and tomato. Quantitative and qualitative analyses of the extracts were done by gas chromatography-mass spectrometry to identify the allelochemicals present in leaves.

Keys words: Allelochemicals, allelopathic effects, aqueous extracts, barley, chemical composition, GC-MS, leachates, lettuce, radish, seed germination, seedling growth, *Tetraclinis articulata*, tomato.

INTRODUCTION

The term 'allelopathy' refers to any process induced by secondary metabolites produced by bacteria, fungi, algae and plants that affects the growth and development of biological and agricultural systems (International Allelopathy Society 1996). Allelopathy is a natural phenomenon which occurs both in aquatic and terrestrial environments (27). From 1990's, allelopathic research shifted from merely laboratory work to pot culture and field studies. New biologically active substances, with allelopathic activity usually affects other living organisms (12). The most common effect observed is the growth inhibition of target species (27). Allelopathic substances may cause morphological and ultrastructural changes, generate reactive oxygen species and oxidative stress, inhibit photosystem II and affect photosynthesis, induces cell lysis and plant death (11). An equally promising way is to use allelopathy for weed control using the bioactive compounds of allelopathic plants as herbicides. Because such ecological herbicides are easily biodegradable and are much safer than present synthetic herbicides. Thus, better understanding of allelopathy can help in developing more sustainable agroforestry systems (12).

Tetraclinis articulata (also known as *Thuya articulata* or *Callitris quadrivalvis*, Cupressaceae family) is a monoic species that flowers in spring without leaf fall. It is endemic to North Africa, Malta, Spain Morocco, Algeria and Tunisia (5). In Tunisia, it is found in north-east region. It is included in the IUCN (International Union for Conservation of Nature) red list, and protected by law in several countries. Its parts are used in folk medicine mainly against respiratory, intestinal infections, gastric pains,

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diabetes, hypertension, antidiarrheal, antipyretic, diuretic, antirheumatic, oral hypoglycemic and to treat eyes inflammation (5). Its macerated leaves are used to prepare the herbal tea, the aqueous extract of its wood are used as natural biocides and the decoction of their leaves heals wounds and bruises (10). Previous studies reported that *T. articulata* exhibited antibacterial, antifungal, cytotoxic, anti-oxidant and anti-inflammatory properties (5).

Photograph of tree *Thuya articulate*

The *Lactuca sativa* (lettuce) is major leafy salad vegetable. It is most common indicator specie in most bioassay studies for Allelopathy. Radish is nutritious root vegetable used both as raw salads and in main recipes. It is also most common indicator specie in most bioassay studies for Allelopathy. Lettuce and radish were chosen for their high sensitivity to allelochemicals, and the other two species were chosen because they are very little studied. The tomato is major vegetable crop used in many cooking recipes or as fresh salads. Barley is major crop in dry land Agriculture in Tunisia and other countries. This study aimed to evaluate (i). the allelopathic potential of *T. articulata* on radish, lettuce, barley and tomato and (ii). to identify by GC/MS the chemical composition of organic extracts from its leaves for use in agriculture as natural pesticides.

MATERIALS AND METHODS

The fresh leaves of *Tetraclinis articulata* were collected in November 2010 from National Park, Dorsal Mountains, Zaghouan, Tunisia (Latitude: 36°23'59.41", longitude: 10°8'1.58", Elevation : 1295 m and 800 m above sea level. Annual Rainfall : 400-500 mm). Four kg leaves were washed with tap water and dried in shadow at room temperature for 10 days. The dried leaves were ground into a fine powder using pulverizer. Dry powder was stored in air tight plastic bags and kept in dark until use.

The test crops were *L. sativa* L. var. longifolia, *R. sativus* L. var. sativus, *S. lycopersicum* L. var. lycopersicum and *H. vulgare* L. var. nudum. Seeds were sterilized with 0.525 g/l sodium hypochlorite for 15 min, then rinsed four times with deionized water, imbibed in it at 22°C for 12 h and carefully dried (7).

Bioassays

(i). Leachates: Five hundred g dry leaves were soaked in 1000 ml distilled water at room temperature and kept in dark for 24 h. The leachate was filtered through Whattman N°1 paper and stored at 4°C in dark until use. In bioassays, the leaf leachate was tested at 5-concentrations :100, 200, 300, 400 and 500g/l on germination and seedling growth of test crops.

(ii). Aqueous extracts: Forty g leaves powder was soaked in 1000 ml distilled water at room temperature for 24 h to give a concentration of 40g/l (7). The extract was filtered through Whattman N°1 paper and stored at 4°C in the dark until use. The aqueous extract was tested at four concentrations (10, 20, 30 and 40g/l) in bioassays.

(iii). Organic extracts : Sequential extraction was done with organic solvents with increasing polarity: Petroleum ether, chloroform and acetone. One Hundred g dried powder of leaves was immersed in petroleum ether for 7 days at room temperature, the residue was subsequently extracted with chloroform for 7 days followed by acetone extraction (17). All recovered organic extracts were evaporated to dryness under reduced pressure at 45 - 50°C, using Rotavapor R-114 (Buchi, France). The residues yields were weighed. Dry fractions were stored at 4°C until use. In bioassays the extracts were tested at 2000, 4000 and 6000 ppm to find the concentrations inhibitory to test crops.

I. PETRI PLATES ASSAY

(i). Aqueous extracts and leachate

Leaves aqueous extracts and leachate were tested on radish, lettuce, barley and tomato. Twenty seeds of each target specie were separately placed on the filter paper in Petri dishes. Five ml of aqueous extracts or leachate were applied per petri dish for each treatment. Seeds of each test crop watered with distilled water served as control. The Petri plates were then placed in growth chamber [400 µmol photons m^{-2} s^{-1} photosynthetically active radiation at 24/22°C for 14/10 h light and dark periods]. Treatments were replicated thrice in completely randomized design.

The germination was recorded by counting the number of germinated seeds at 24 h intervals till first 7 days after incubation. Shoot and root length of receiver species were measured 7 days after sowing. The Germination index (GI) was calculated as under (6):

$$GI = (N_1) \times 1 + (N_2 - N_1) \times \tfrac{1}{2} + (N_3 - N_2) \times \tfrac{1}{3} + \ldots\ldots\ldots + (N_n - N_{n-1}) \times \tfrac{1}{n}$$

Where, N_1, N_2, N_3, ...N_n : Proportion of germinated seeds observed after 1, 2, 3, ..., n-1, n days.

This index represents the delay in germination induced by extract/leachate.

The inhibition / stimulation (%) was calculated as under (8):

$$[\text{Inhibition}(-) / \text{stimulation}(+)] (\%) = [(\text{extract} - \text{control})/\text{control}] \times 100$$

Where, Extract: Parameter measured in presence of extract/leachate and Control: Parameter measured in presence of distilled water.

(ii). **Tests with organic extracts**

Crude residues obtained from petroleum ether, chloroform and acetone were dissolved in methanol at 2000, 4000 and 6000 ppm doses. Two controls used: Distilled water and Methanol, to eliminate its eventual effect. Two filter papers placed in each Petri dish was moistened with distilled water, methanol or the different organic extracts. Solvents were evaporated for 24 h at 24 °C, then 5 ml distilled water was added and 20 soaked seeds were sown and germination was recorded till 7 days. Germination, shoot and root length of target species were determined as per aqueous extracts above. Treatments were replicated thrice in completely randomized design and data were transformed to % of control for analysis.

II. POT CULTURE

(i). Powdered biomass incorporation in soil

Plastic pots (10 cm dia) were filled with sandy soil at 250 g/pot. Powder of *T. articulata* leaves was thoroughly mixed with soil (at 50 g and 100 g/kg dry weight). Control soil was without *T. articulata* material. Then 10 target seeds (radish, lettuce, barley and tomato) were sown per pot at 1-2 cm depth, (i.e. seed rate of 2 kg lettuce seeds/ha, 4 kg tomato seeds/ha, 35 kg radish seeds/ha, 136 kg barley seeds/ha). Treatments were replicated thrice in completely randomized design. Pots were irrigated after seed sowing with 5 ml tap water daily to keep the soil moisture level at field capacity. The pots were placed in growth room (25°C, 12 h photoperiod) for 7 days and then transferred in open sunlight for 21 days. Plants were harvested 4 weeks after sowing and their root and shoot length were measured.

(ii). Irrigation with aqueous extracts and leachate

To determine, if the phytotoxic effects of *T. articulata* extracts and leachate would persist in soil, plastic pots (10 cm dia) were filled with sandy soil at 250 g/pot. Ten seeds (radish, lettuce, barley and tomato) were sown per pot at 1-2 cm depth, then sprayed with distilled water to moisten the soil. Treatments were replicated thrice in completely randomized design. Three days later, 5 ml leachate or extract of leaves at IC_{50} and MIC were added per pot as per treatment. The pots for aqueous extracts and leachates applied were kept separately. The control was irrigated with distilled water.

III. EXTRACTION with organic solvents of increasing polarity

One g of powdered *T. articulata* leaves were extracted with 5 ml of petroleum ether, chloroform and acetone, respectively in dark at room temperature for 24 h on a shaker (Eyela Model MMS-300, Tokyo Rikakikai Co., Ltd., Japan). Thereafter the solvents were evaporated by vacuum rotary evaporator (EYELA N1000, Japan). After filtration through 0.45 µm, all obtained extracts were transferred to vials and kept in dark at -20°C until use, to prevent changes in the chemical components.

Gas Chromatography-Coupled with Mass Spectrometry (GC-MS) analyses

Quantitative and qualitative analysis of the extracts were done by gas chromatography-mass spectrometry (GC-MS). GC-MS was performed in a Hewlett-Packard 5972 MSD System. An HP innowax capillary column (30 m x 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the mass spectrometry. The carrier gas was helium, with a flow rate of 1.2 ml/min. Oven temperature was programmed (50 °C for 1 min, then 50-250 °C at 5 °C/min) and subsequently held isothermal for 4 min. Injector port: 250 °C, detector: 280 °C, split ratio: 1:50. Volume injected: 1 µl of 1% solution (diluted in hexane); mass spectrometer: HP5972 recording at 70 eV; scan time: 1.5 s; mass range: 40-300 amu (atomic mass unit). Software adopted to handle mass spectra and chromatograms was ChemStation. The identification of the compounds was based on mass spectra (compared with Wiley 275.L, 6th edition mass spectral library). Further confirmation was done from Retention Index data generated from a series of alkanes retention indices (relatives to C8-C28 on the HP innowax column) (9).

Statistical analysis

The laboratory bioassays and pot culture data were statistically analysed with ANOVA and LSD tests with PASW Statistics 21, for Windows program, to analyze treatment differences. The means were separated on the basis of least significant differences at the 0.05 probability level.

RESULTS AND DISCUSSION

AQUEOUS EXTRACTS

Germination

Phytotoxicity of *T. articulata* leaves was evaluated on four crops (lettuce, radish, tomato and barley) by testing their aqueous and organic extracts, leachate, and leaves residues. Results showed that *T. articulata* had a significant allelopathic potential. The leaves aqueous extracts significantly delayed the germination of all target plants (Table 1). The most toxic effect was recorded at 30 g/l for lettuce (GI and germination % = 0), and at 40g/l for radish (GI=3.94%), tomato (4.52%) and barley (30.93%). For GI, the means were 1.3 and 47.9 for all target species, but the germination % varied between 25 and 65.4 (Table 1).

Table 1. Effects of aqueous extracts and leachates of *Tetraclinis articulata* leaves on seed germination of *Lactuca sativa, Raphanus sativus, Hordeum vulgare* and *Solanum lycopersicum*

		Target species							
Treatments	Conc (g/l)	*L. sativa*		*R. sativus*		*H. vulgare*		*S. lycopersicum*	
		GI	Germination (%)	GI	Germination (%)	GI	Germination (%)	GI	Germination (%)
Aqueous extracts	Control	-	91.66^{b}	-	100^{d}	-	80^{b}	-	100^{c}
	10	4.2^{b}	6.66^{a}	59.71^{d}	90^{d}	52.66^{a}	55^{a}	64.19^{d}	96.66^{c}
	20	0.87^{ab}	1.66^{a}	35.09^{c}	63.33^{c}	55.18^{a}	55^{a}	45.85^{c}	80^{b}
	30	0^{a}	0^{a}	16.77^{b}	31.66^{b}	51.97^{a}	58.33^{a}	37.26^{b}	75^{b}
	40	0^{a}	0^{a}	3.94^{a}	6.66^{a}	30.93^{a}	40^{a}	4.52^{a}	10^{a}
Mean		1.3	25.0	33.5	47.9	47.9	52.1	38.0	65.4
Leachates	100	56.03^{c}	61.66^{c}	47.87^{c}	46.66^{c}	75.21^{b}	68^{ab}	25.13^{b}	36.66^{b}
	200	16.06^{b}	21.66^{b}	23.47^{b}	21.66^{b}	86.28^{b}	66.66^{b}	9.77^{ab}	10^{a}
	300	0^{a}	0^{a}	5.31^{a}	5^{a}	68.80^{b}	65^{ab}	0^{a}	0^{a}
	400	0^{a}	0^{a}	3.12^{a}	5^{a}	41.39^{a}	45^{a}	0^{a}	0^{a}
	500	0^{a}	0^{a}	0^{a}	0^{a}	28.63^{a}	45^{a}	0^{a}	0^{a}
Mean		14.4	16.7	16.0	15.7	60	57.9	7.0	9.3

GI : Germination Index % Control, Control: Distilled water

The same letters (a, b, c and d) in each row indicate that the means are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test). Values ($N = 3 \pm$ S.D.).

Leachate was also toxic to seeds germination of all target species. Lettuce and tomato were the most sensitive and their germination was completely inhibited at 300 g/l (GI=0%). For radish and barley, the most toxic effect was recorded at 500 g/l, the IG was 0 and 28.63%, respectively (Table 1). For GI, the means were between 7 and 60 for all target species, but germination varied between 9.3 and 57.9% (Table 1). Germination index (GI), measures retardation or acceleration in the germination process and it is more sensitive indicator of allelopathic effects (3). Leaves aqueous extracts and leachate delayed and reduced the germination for all target seeds. Some concentration of an allelochemical severely reduces the seed germination, while other only depress or delay it. Such inhibitory effects could be caused by allelochemicals interfering with physiological and biochemical processes in target species (23). The inhibition of germination may be the consequence of the inhibition of water uptake, increased abscisic acid content, and decreased indole-3- acetic acid and zeatin riboside contents (26). The inhibition of seed germination in *Pinus laricio* was attributed to disruption of the metabolic enzymes activity that are involved in glycolysis and the oxidative pentose phosphate pathway (15). It is caused also by disruption of mitochondrial respiration (19). Some allelopathic compounds interact with the mitochondrial membrane and directly impairs the mitochondrial respiration. Abrahim *et al.* (1) reported that α-pinene acts on *Zea mays* L. seedling growth by two mechanisms, uncoupling oxidative phosphorylation and inhibition of electron energy metabolism and inhibition of mitochondrial adenosine triphosphate (ATP) production and hence, alteration of other cell processes which are energy-demanding. The actions of α-pinene on isolated mitochondria was consequence of unspecific disturbances in the inner mitochondrial membrane. The exposing of seedlings to α-pinene inhibited the seedling growth due to oxidative damage in the root tissue (23). Moreover, the increased generation of reactive oxygen species after the exposure of *Cassia occidentalis* roots to α-pinene, enhanced the activity of anti-oxidant enzymes [superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, peroxidase and catalase (23)].

Seedling growth

Leaves aqueous extract reduced the seedling growth of target species and the inhibitory effect increased with increase in concentration i.e. was concentration dependent. Seedling growth of lettuce was most sensitive and showed significant reduction in root and shoot length (up to 100%) at all tested concentrations. Furthermore at 40 g/l, root and shoot growth revealed significant respective inhibitions of 95.5% and 98.67% for tomato, 85.87% and 76.1% in barley and 97.07% and 95.32% for radish (Fig 1).

The seedling growth of lettuce, was totally inhibited at all tested concentrations. The seedling growth in tomato, barley and radish was inhibited, which increased with increase in concentration of leaf aqueous extract. Reduction of seedlings length may be attributed to the reduced cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts (13). The exposure to α-pinene and camphor inhibited the root growth of *Brassica campestris* by inhibiting the cell proliferation in root apical meristems and decreased mitotic index (24). Beside α-pinene disrupts the membrane permeability resulting in solute leakage and bio-energetic failure which induces cell death by apoptosis and necrosis (22).

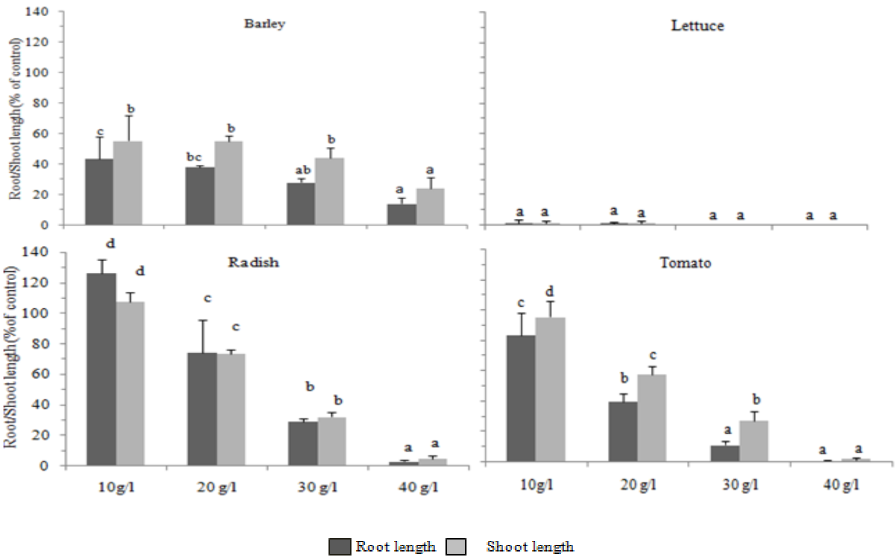

Figure 1. Root and shoot length (% of control) of target species, 7 days after germination, in the presence of different concentrations of *Tetraclinis articulata* leaves aqueous extract. The bars on each column show standard error. Value (N = 3 ± S.D.). Different letters on columns indicate significant differences among concentrations at $P < 0.05$ (Duncan test).

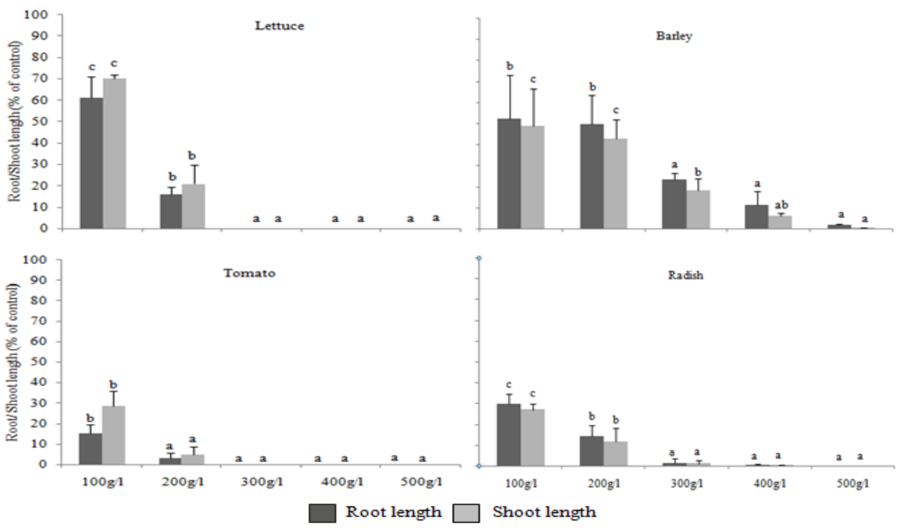

Figure 2. Root and shoot length (% control) of target species, 7 days after germination, in the presence of different concentrations of *Tetraclinis articulata* leaves leachate. The bars on each column show standard error. Value (N = 3 ± S.D.). Different letters on columns indicate significant differences among concentrations at $P < 0.05$ (Duncan test).

Similar to aqueous extracts, the leachates also showed significant toxicity, which increased with increase in its concentration. It inhibited growth in radish, lettuce and tomato seedlings at 300 g/l and in barley at 500 g/l (Fig 2). Like the aqueous extracts, the phytotoxicity of leachate of *T. articulata* leaves, was also directly proportional to the concentration.

ORGANIC EXTRACTS

Based on dry matter, the chloroform extract gave higher yield (3.76%) than petroleum ether (2.71%) and the acetone extracts (2.27%).

Seed germination

Leaf organic extracts generally did not affect the seed germination of lettuce, but delayed the germination of the remaining target species, with a greater sensitivity at 6000 ppm especially for radish and barley (Table 2). Indeed, at this concentration, GI varied between 17.3% and 46.6% for radish and between 23% and 47.9% for barley.

Table 2. Effects of organic extracts of *Tetraclinis articulata* leaves on seed germination of *Lactuca sativa, Raphanus sativus, Hordeum vulgare* and *Solanum lycopersicum*

Extracts of different polarity	Extract Conc. (ppm)	Target species							
		L. sativa		*R. sativus*		*H. vulgare*		*S. lycopersicum*	
		GI	Germination (%)	GI	Germination (%)	GI	Germination (%)	GI	Germination (%)
Petroleum ether	Control	-	95^{b}	-	96.66^{b}	-	60^{b}	-	91.66^{a}
	2000	74.7^{ab}	90^{b}	70.26^{a}	81.66^{ab}	44.18^{a}	28.33^{a}	80.75^{a}	76.66^{a}
	4000	88.04^{b}	95^{b}	53.53^{a}	71.66^{ab}	40.69^{a}	25^{a}	82.32^{a}	76.66^{a}
	6000	64.53^{a}	80^{a}	46.63^{a}	58.33^{a}	31.69^{a}	23.33^{a}	73.33^{a}	80^{a}
Mean		75.8	88.3	56.8	70.6	38.8	26.5	78.8	77.8
Chloroform	2000	83.03^{a}	81.66^{bc}	55.36^{b}	61.66^{b}	57.67^{a}	40^{a}	108.96^{b}	85^{bc}
	4000	68.86^{a}	75^{ab}	25.85^{a}	35^{a}	43.95^{a}	30^{a}	61.96^{a}	63.33^{a}
	6000	61.29^{a}	65^{a}	23.57^{a}	28.33^{a}	47.91^{a}	28^{a}	73.4^{ab}	66.66^{ab}
Mean		71.1	73.9	34.9	41.7	49.8	32.7	81.4	71.7
Acetone	2000	77.54^{a}	81.66^{a}	26.53^{a}	36.66^{ab}	30.68^{a}	25^{a}	94.49^{c}	91.66^{b}
	4000	74.32^{a}	81.66^{ab}	33.12^{a}	30^{b}	28.87^{a}	18.33^{a}	52.34^{b}	55^{a}
	6000	70.05^{a}	75^{a}	17.32^{a}	18.33^{a}	22.97^{a}	15^{a}	33.33^{a}	40^{a}
Mean		74.0	79.9	25.6	28.3	27.6	19.4	60.0	59.2

The same letters in each row indicate that the means are not significantly different at $P < 0.05$ (Duncan's Multiple Range Test). Values (N = 3 ± S.D.).

Acetone extract showed more toxic effect than other extracts. For tomato, the maximum germination index (GI = 33.3%) was observed with acetone extract at 6000 ppm. However, lettuce was less sensitive than others, and the most toxic effect was registered with chloroform extract at 6000 ppm, where GI was 61.29% and germination was 65%. For lettuce with chloroform, the mean of IG was 71.1 and germination was 73.9 % (Table 2). For organic solvents, leaf extracts significantly delayed the seed germination proportionately with concentration. It was reported that organic extracts contain different types of allelochemicals which exerts their differential effects (18). Often the phytotoxic effect is not observed in the final germination (%), but rather in the speed of germination, this fact can provide indications of the allelochemicals presence. The delay in seed germination can have important biological and ecological implications, because it can affect the ability of the seedling to establish themselves in natural conditions.

Seedling growth

For radish, a strong inhibition was registered with the three fractions especially at 6000 ppm. The most toxic effect was recorded with acetone extract at 6000 ppm. Thus, the inhibition percentage was 98.92% for root and 98.8% for shoot length (Fig. 3).

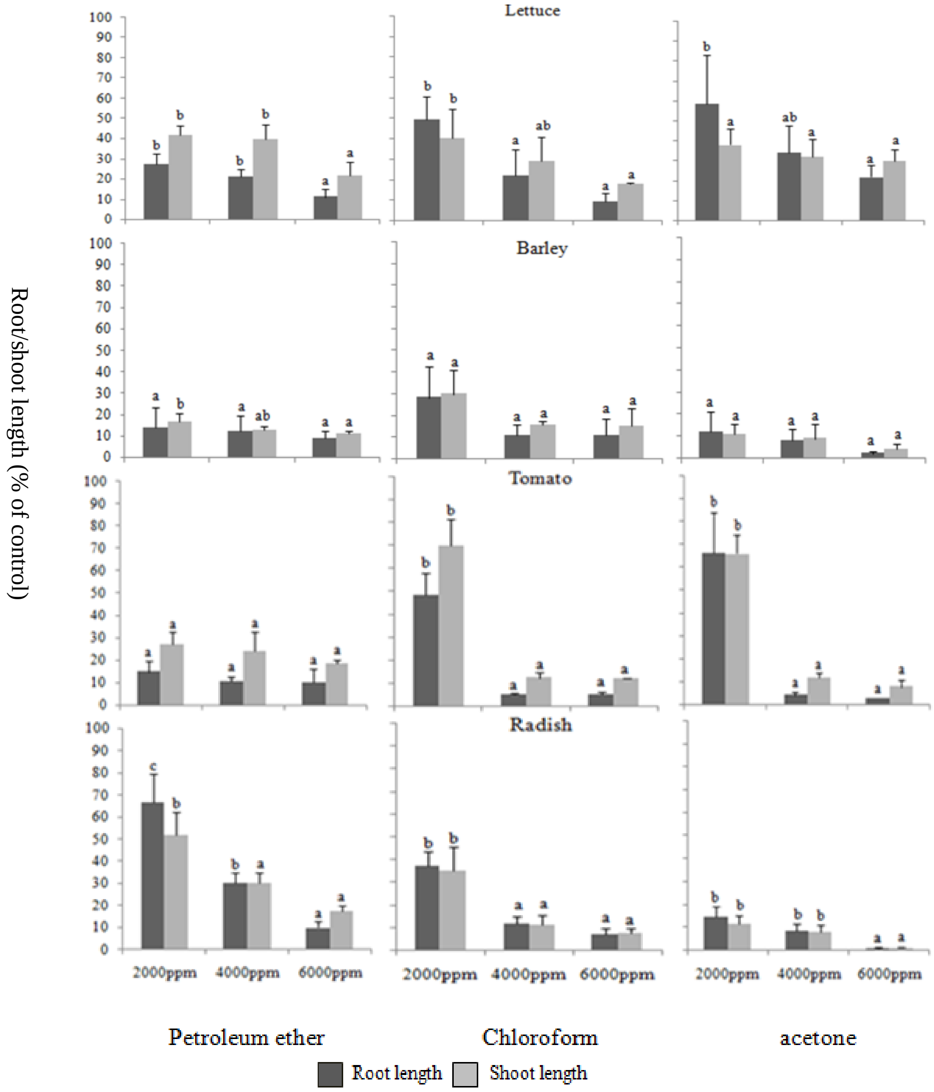

Figure 3. Root and shoot length (% control) of target species, 7 days after germination, in the presence of three concentrations (2000 ppm , 4000 ppm and 6000 ppm) of three organic extracts of *Tetraclinis articulata* leaves. The bars on each column show standard error. Value (N = 3 ± S.D.). Different letters on columns indicate significant differences among concentrations at $P < 0.05$ (Duncan test).

For lettuce, roots were slightly more sensitive than shoots, with an average inhibition of 88.89%, 93.4% and 87.92% for roots and 88.17%, 82.03% and 69.85% for shoots at 6000 ppm of petroleum ether, chloroform and acetone extracts, respectively (Fig 3). At this concentration, the petroleum ether, chloroform and acetone extracts inhibited the root elongation of tomato by 90.07%, 94.89% and 97.39%, respectively and the shoot length by 91.47%, 88% and 93.36% (Fig 3). For barley, the acetone extract was most toxic at 6000 ppm and the root and shoot length was reduced by 97.82% and 95.99%, respectively (Fig 3).

It is believed that the phytotoxicity of extracts against germination and seedling growth of target species was generally attributed to the allelopathic potential of some terpenes (23) and organic acids such as hexadecanoic acid (major component in the chloroform extract) which have antibacterial and antifungal properties (2). The present study revealed the presence of allelochemical compounds in *T. articulata* leaves like terpenoids and phenolic compounds, which are inhibitory to the germination and seedling growth of various plants. The participation of phenolic compounds in the metabolic process inhibits the normal growth. The inhibition of growth of wheat radicles varies with the different concentrations of total phenolic compounds in different parts of sorghum (4). The release of phenolic compounds adversely affects the germination and growth of plants through their interference in energy metabolism, cell division, mineral uptake and biosynthetic processes (20). Furthermore, this study revealed the presence of hexadecanoic acid, α-pinene, camphor, thymol, limonene, borneol, bornyl acetate, delta-3-carene, P-cymene and linalool in *T. articulate* leaves. These compounds have been reported to have herbicidal activities (23).

BIOMASS ACTIVITY IN SOIL

Incorporation of *T. articulata* leaves powders in soil

The leaf residue was most toxic to all target species especially at 100 g/kg (Fig 4). Leaves residues at 100 g/kg caused total inhibition of lettuce and radish seedling growth. In barley, the leaf residue at 100 g/kg caused inhibition of 97.51% and 79.06 % in root and shoot length, respectively, and roots appeared more sensitive than shoots. Furthermore, 90.92 % and 93.05% inhibition occurred at 100 g/kg in root and shoot growth, of tomato respectively (Fig 4).

Irrigation with leaves leachate and aqueous extract

Soil irrigation with leachate of *T. articulata* leaves (at IC_{50}) caused inhibition of 85.83%, 30%, 35.33% and 38.08% in root length in lettuce, barley, radish and tomato, respectively and 85.66%, 40%, 64.32% and 47.78% in shoot length (Fig 4). Leachate at the minimum inhibitory concentration (MIC) caused total inhibition of seedling growth in lettuce and tomato. However, in radish and barley, the reduction in root length was 87.93% and 33%, respectively and 92.46% and 43% in shoot length (Fig 4).

Irrigation with aqueous extracts inhibited the seedlings growth of target species. The aqueous extract at IC_{50} caused inhibition of 75.96%, 20%, 57.49% and 84.35% in root length of lettuce, barley, radish and tomato, respectively, and 44.72%, 10%, 85.57% and 92.19% in shoot length (Fig 4). Radish and tomato roots were more sensitive than other species, their mean inhibition was 91.51% and 91.03%, respectively, while inhibition was 24% and 85.57% in barley and lettuce, respectively (Fig 4).

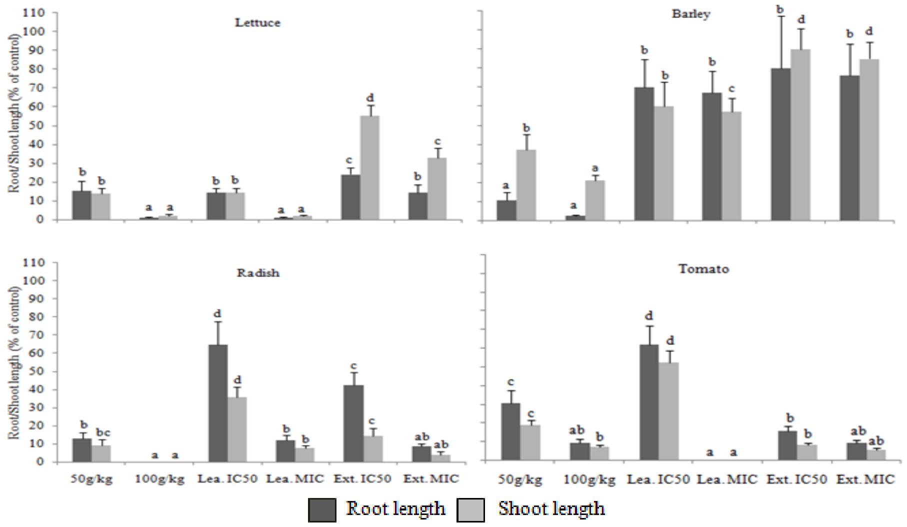

Figure 4. Effects of incorporation with soil of *T. articulata* leaves residue at 50g/kg and 100g/kg and irrigation with aqueous extract and leachate (at IC50 and MIC) of *T. articulata* leaves, on root and shoot length, expressed in % of control, of target species 30 days after germination. The bars on each column show standard error. Value ($N = 3 \pm$ S.D.). Different letters on columns indicate significant differences among concentrations at $P < 0.05$ (Duncan test).

The lab bioassay evaluates the allelopathic potentialities of plant species (7), however, pot cultures indicate the effects that could be reproduced under natural conditions and evaluate the biological activity of allelochemical compounds released from the plant residues. Residue incorporation in the soil or irrigation with leachate and aqueous extracts significantly inhibited the growth of target species. Effects of residues varied with recipient species and the inhibitory effects were attributed to their phytotoxicity (7). Plant residues and their decomposition products could be involved virtually in all biochemical processes in soil, which affects the plants directly or indirectly. After crop residue incorporation in soil, the elimination of phytotoxic activity of *T. articulata* residues over time is due to chemical decomposition or microbial degradation of organic compounds (14). In this study, irrigation with leaves aqueous extracts decreased the root and shoot length. The extract was phytotoxic to root length of lettuce, radish and tomato and caused inhibition of 85.57%, 91.51% and 91.03%, respectively.

The irrigation with aqueous extract of Wollemi pine proved a natural herbicide against ryegrass seeds (21). Likewise, irrigation with leachate (at MIC) decreased the root and shoot length. The leachate caused total inhibition of root and shoot length in lettuce and tomato and caused reduction of 90% in radish and 38% in barley in root and shoot length, respectively. The most common explanation for this variation in response would be the selectivity of allelochemicals for target species.

Chemical composition of extracts

The constituents of *T. articulata* leaves are listed in Table 3. The GC-MS analysis of petroleum extract resulted in detection of 59 components, representing 95.3% of the total yield. The extract was rich in oxygenated monoterpenes (21.18%), sesquiterpene

Table 3. Chemical composition of *T. articulata* leaves

No	RI	Compounds	Compounds content (%)		
			P E	C E	A E
1	1033	α-pinene	2.56	3.56	8.23
2	1055	α -Fenchene	0.63	0.12	0.21
3	1060	Ethyl isovalerate	-	0.27	-
4	1071	Camphene	-	0.42	-
5	1089	Isobutyl isobutyrate	-	0.38	-
6	1100	Undecane	-	0.77	-
7	1124	Sabinene	-	-	0.36
8	1126	Verbenene	-	0.74	0.45
9	1148	δ3-Carene	-	-	0.63
10	1180	α - Terpinene	-	-	0.59
11	1192	Limonene	-	0.31	0.93
12	1198	Methyl hexanoate	0.84	0.79	-
13	1200	Dodecane	0.83	0.47	0.8
14	1228	γ - terpinene	-	0.39	0.52
15	1234	(Z)-beta-Ocimene	-	2.31	-
16	1250	p - Cymene	-	1.1	1.39
17	1285	Octanal	-	0.79	-
18	1300	Tridecane	1.03	1.52	0.56
19	1314	(Z)-3-Hexenyl acetate	-	2.11	-
20	1400	Tetradecane	0.57	-	-
21	1420	m-Di-tert butylbenzene	10.84	5.56	0.46
22	1435	α-dimethylstyrene	-	-	1.03
23	1442	Camphene hydrate	2.18	0.52	-
24	1445	1-Tetradecene	0.67	0.75	-
25	1452	2-Furfural	-	0.49	1.1
26	1474	Campholenal	0.21	0.37	-
27	1487	α-Copaene	0.29	0.31	0.47
28	1500	Pentadecane	0.78	0.28	-
29	1517	Camphor	8.77	6.03	7.69
30	1524	β-Bourbonene	1.32	0.32	-
31	1529	α- Gurjunene	-	0.52	0.32
32	1537	Linalool	0.95	0.39	-
33	1551	Linalyl acetate	0.44	0.8	-
34	1566	Isocaryophyllene	-	0.32	-
35	1583	Bornyl acetate	6.82	4.31	5.43
36	1587	α-funebrene	0.38	0.34	-
37	1590	β-Elemene	-	-	0.33
38	1593	β- caryophyllene	1.33	0.83	1.36
39	1623	Isothujol	-	0.43	-
40	1628	α-Patchoulene	-	-	0.34
41	1644	Widdrene	3.36	2.52	0.99
42	1660	β -Cedrene	3.81	1.1	3.22
43	1677	β -Gurjunene	0.69	0.5	1.12
44	1690	Borneol	6.47	4.31	5.3
45	1695	Germacrene D	3.05	2.06	1.63
46	1700	Heptadecane	-	-	0.6
47	1710	δ-amorphene	0.58	-	0.42

48	1720	α - Muurolene	0.82	0.62	0.56
49	1726	γ -Amorphene	-	0.55	-
50	1728	Zingiberene	0.43	0.47	-
51	1733	α-Selinene	0.19	-	-
52	1740	γ -Cadinene	0.61	-	-
53	1756	δ - Cadinene	3.15	2.17	3.52
54	1762	Citronellol	0.73	0.79	0.35
55	1786	Cadina-1,4-diene	-	0.72	0.43
56	1792	Myrtenol	0.69	0.99	-
57	1804	Carveol	0.45	0.31	0.49
58	1808	1s,cis-calamenene	0.34	0.38	0.52
59	1826	trans-Calamenene	1.86	1.76	-
60	1834	Hexanoic acid	0.21	-	-
61	1852	p-Cymen-8-ol	0.31	-	-
62	1860	trans-Myrtanol	0.73	0.28	-
63	1910	α -Calacorene	0.21	0.72	0.27
64	1920	Tetradecanal	1.16	-	-
65	1933	Neophytadiene	-	1.45	0.46
66	1943	Isocaryophyllene oxide	-	0.69	-
67	1975	Caryophyllene oxide	2.03	1.48	1.24
68	1986	Humulene oxide	0.33	0.39	0.41
69	2025	Ledol	1.11	0.69	0.64
70	2065	α - Cedrol	0.71	-	-
71	2080	β-Elemol	0.3	-	1.49
72	2100	Heneicosane	0.55	0.45	0.34
73	2120	Spathulenol	0.29	-	-
74	2140	Widdrol	0.35	-	-
75	2156	γ -Eudesmol	-	-	0.65
76	2163	α-acorenol	0.2	0.31	0.32
77	2170	Thymol	0.94	0.29	0.43
78	2180	Farnesol	-	0.76	0.9
79	2190	β-acorenol	-	0.69	1.44
80	2195	p-Isopropylphenol	1.17	-	0.55
81	2209	Caryophyllene alcohol	9.57	7.07	0.39
82	2310	Octadecanal	1.09	-	1.21
83	2318	Epimanoyl oxide	-	-	1.11
84	2325	Cedr-8-en-13-ol	0.7	0.39	0.25
85	2380	Totarol	-	0.96	7.6
86	2495	Ferruginol	0.3	-	3.85
87	2510	Methyl linoleate	-	0.29	4.42
88	2530	1-Octadecanol	0.64	1.47	4.46
89	2603	Phytol	0.41	2.21	1.04
90	2822	Pentadecanoic acid	-	-	0.74
91	2890	Hexadecanoic acid	2.66	16	7.82
92	2900	Nonacosane	-	0.32	2.84
93	2920	Kaur-16-en-18-oic acid, acetate	0.66	-	0.55
		Total identified	**95.3**	**94.87**	**97.77**

PE: Petroleum ether extract; CE: Chloroform extract; AE: Acetone extract

Table 3a. Summary of Table 3 Data.

Grouped compounds	Compounds content (%)		
	PE	CE	AE
Oxygenated monoterpenes **I**	21.18	14.42	13.83
Sesquiterpene hydrocarbons **II**	19.37	14.84	14.47
Oxygenated sesquiterpenes **III**	15.59	11.78	7.73
Aromatic compounds **IV**	11.15	6.75	2.88
Other terpenes derivatives	7.92	5.11	5.98
Paraffins	6.81	5.87	6.17
Monoterpene hydrocarbons	3.19	7.85	11.92
Fatty acids	2.87	16	8.56
Phenolic compouds	2.41	1.25	12.43
Aldehydes	2.25	1.28	2.31
Fatty acid esters	0.84	3.84	4.42
Olefins	0.67	0.75	0
Alcohols	0.64	1.47	4.46
Oxygenated diterpenes	0.41	2.21	2.15
Diterpene hydrocarbons	0	1.45	0.46
Total	95.3	94.87	97.77

Kovats retention index (RI) relative to C8-C28 n-alkanes on the HP innowax capillary column
(PE: Petroleum ether extract; CE: Chloroform extract; AE: Acetone extract) (-) compound absent in the fraction

hydrocarbons (19.37%), oxygenated sesquiterpenes (15.59%) and aromatic compounds (11.15%). Major components were m-Di-tert butylbenzene (10.84%), caryophyllene alcohol (9.57%), camphor (8.77%) and bornyl acetate (6.82%). In chloroform extract, 68 compounds were identified representing 94.87% of the total yield, the extract was rich in fatty acids (16%) followed by sesquiterpene hydrocarbons (14.84%), oxygenated monoterpenes (14.42%) and oxygenated sesquiterpenes (11.78%), the predominant compounds were hexadecanoic acid (16%) followed by caryophyllene alcohol (7.07%) and camphor (6.03%). While, acetone extract contains sesquiterpene hydrocarbons (14.47%), oxygenated monoterpenes (13.83%), phenolic compounds (12.43%) and monoterpene hydrocarbons (11.92%), among the sixty identified components with the yield of 97.77%. The α-pinene (8.23%) was major one followed by Hexadecanoic acid (7.82 %), camphor (7.69%) and Totarol (7.6%) (Table 3).

The herbicidal effects of extracts resulted from the combined reactions of additive, synergetic or antagonistic effects of several compounds (25). The individual monoterpenes (α-pinene, limonene, camphor and thymol) strongly inhibits the seed germination and seedling growth of some agricultural crops and weeds (23). This study revealed the presence of > 7 compounds [hydrocarbonated monoterpenes (α-pinene, limonene, δ-3-carene and p-cymene) and oxygenated monoterpenes (Linalool, borneol and bornyl acetate)] in *T. articulata* leaves with herbicidal activity (25).

Both monoterpenoids and sesquiterpenoids are mostly involved in the herbicidal activity of extracts, although the exact mechanisms of extracts on germination and seedling growth inhibition remain unclear. Inhibitions may be caused by terpenes, which interfere with physiological and biochemical processes in target species.

CONCLUSIONS

T. articulata possess allelopathic activity and released the allelochemicals in field and laboratory experiments. It exerts strong inhibitory effects on seed germination and radicle elongation in test crops (lettuce, barley, radish and tomato), which may be attributed to one or more of the above-mentioned mechanisms. In depth physiological and biochemical investigations with chemical studies are needed to fully utilize its allelopathic potential to control weeds.

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