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Germination inhibitory activity of selected plants from Central South of Chile

[Actividad inhibitoria de la germinación de plantas seleccionadas del Centro-Sur de Chile]

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Abstract: Methanol extract obtained from aerial parts of 24 selected plants from Central Valley and Pre-andean foothill from Ñuble Province of Chile were tested for its inhibitory germination activity against *Trifolium repens* and *Raphanus sativus*. Many extracts (13/24 = 54%) showed inhibition of *T. repens* germination with IG% > 50%, but none on *R. sativus*.

Keywords: Native plants, Germination inhibition, phytotoxicity

Resumen: Extractos metanólicos obtenidos de partes aéreas de 24 plantas seleccionadas del valle central y preandino de la Provincia de Ñuble-Chile, fueron ensayados para determinar su capacidad inhibitoria sobre la germinación de semillas de *Trifolium repens* y *Raphanus sativus*. Los extractos (13/24 = 54%) muestran actividad inhibitoria de la germinación de semillas de *T. repens* con un IG%>50%, pero no tienen actividad significativa sobre *R. sativus*.

Palabras clave: Plantas nativas, Inhibición de la germinación, fitotoxicidad

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INTRODUCTION

From many plant extracts, semi purified and purified secondary metabolites have been studied for weeds control properties by many researches on over the world. Secondary metabolites from plants play a key defensive role against insect and act as plant growth inhibitory. There is an important factor in plant-plant interaction and is useful to study the field crop pests' behavior. Several compounds derived from plant are toxic to weeds; and some of them are highly active (Isman, 2006; Zhou *et al.*, 2013).

One of the main problems in agriculture is the competition of weeds against crops. The weeds compete for water, light and nutrients and are characterized by high productivity and longevity of their seeds and rapid growth. Therefore, control of weed is a key factor to consider in agricultural production systems. Seed germination and seedling establishment are two closely related processes with enormous importance in many aspects of plant biology, from plant ecology to crop productivity (Bewley & Black, 1983; Palacios *et al.*, 2010; Zhang *et al.*, 2012). Germination incorporates those events that start with the uptake of water by the quiescent dry seed and finish with the elongation of the embryonic axis (Bewley & Black, 1994; Holdsworth *et al.*, 2008). This process can be inhibited by a number of factors such as dormant embryos, thick seed costs and presence of inhibitors. Inhibitors are chemical compounds that inhibit development of embryos and metabolism including abscisic acid, phenolic compounds, isoprenoid and nonisoprenoids among others. If the seed does not germinate within a certain length, the embryo dies (Einhellig, 1995; Harborne, 1999; Kozubek, 1999; Mucciarelli *et al.*, 2001; Weitbrech *et al.*, 2011).

A lot of work has been recently focused on plant-derived materials as an alternative to herbicides (Kuk *et al.*, 2001). Allelopathy has emerged as a potential tool and as an alternative to synthetic herbicides for weeds managements.

We have previously demonstrated that diverse secondary metabolites have a different mechanism of action and different molecular targets when interact with the germination, seedling growth, and photosynthetic electron transport chain (Céspedes *et al.*, 1998, Céspedes *et al.*, 1999a, Céspedes *et al.*, 1999b, Céspedes *et al.*, 2000, Céspedes *et al.*, 2001a, Céspedes *et al.*, 2001b). In this report, we examine germination inhibitory properties of 24 selected plants from Central Valley and Pre-andean foothill from Ñuble Province, Chile against *Trifolium repens* and *Raphanus sativus* as model of weeds. This investigation is part of a program for the search of plant species as weed controllers.

MATERIAL AND METHODS

Plant material

Plants were collected on the hills of Ñuble Province-Chile, November 2012 to march 2013. Voucher specimens have been deposited in the Herbarium of the School of Agricultural Science, Universidad de Concepción, and were authenticated by Prof. Victor Finot. Plants were selected according to their availability, accessibility and especially to the lack of scientific information about their activity and/or chemical pattern.

Weed Seeds

Common *Trifolium repens* and wild radish *Raphanus sativus* species were used for phytotoxicity testing. These, were purchased from seeds section Homecenter Sodimac, Chillán, Chile, with a high % germination certified.

Preparation of Extracts plants

The plant material was air-dried at room temperature, crushed and extracted with methanol in a Soxhlet apparatus. Yields of each viscous extract are expressed as percentage weight of air-dried plant material, and are shown in Table 1. The resulting methanol extract was concentrated at reduced pressure at 40 °C and 200 mb obtaining syrup extract. A portion of the methanol extract was dissolved with distilled water to a ratio 60/40 methanol/water. Then this solution was placed in a separator funnel and a liquid/liquid distribution with 150 ml of n-hexane was made 3 times. Then n-hexane phases were combined and concentrated under reduced pressure. An identical process was repeated with ethyl acetate, remaining a residual mixture methanol/water.

Germination bioassay

Thirty seeds of *T. repens* or *R. sativus* were placed in a 9-cm Petri dish lined with filter paper, treated with 2 ml of ethanolic solution of extract at 10 mg/ml and 4ml of distilled water after ethanol was evaporated. Controls received ethanol (2 ml), evaporated and 4 ml of distilled water. Each treatment was replicated three times. The dishes were placed in a growth chamber (25 ± 1 ºC, 70–75% relative humidity, with a photoperiod of 16:8 light–dark cycles). After 2 and

7 days for *T. repens* and *R. sativus*, respectively, germination was assessed and the inhibitory germination index (IG) was calculated as IG% = [1−(T/C)]×100, where T and C are the number of seeds germinated in the treatment and the control, respectively. 2,4-D was used as a positive control.

The extracts showed greater than 75% IG were partitioned with solvent increasing polarity, obtained three fractions: n-hexane, ethyl acetate and water. The germination inhibition index was calculated.

Table 1
Inhibitory germination inhibitory effects of extract from plants from Central Valley and Preandean foothill of Ñuble province, Chile

Plants species	Family	Yield[a] (%)	IG[b,c] (%)	
			T. repens	*R. sativus*
Baccaris linearis	Asteraceae	43.32	76.47	6.89
Baccaris sp.	Asteraceae	38.95	52.94	13.8
Bidens aurea	Asteraceae	14.66	29.41	6.89
Brassica rapa	Brassicaceae	42.83	76.47	10.34
Bartsia trixago	Orobanchaceae	21.48	47.11	20.68
Cirsium vulgare	Asteraceae	24.32	82.35	6.89
Chenopodium ambrosioides	Amaranthaceae	25.30	47.05	6.89
Colletia spinosissima	Rhamnaceae	32.20	82.35	10.34
Echium vulgare	Boraginaceae	47.49	23.52	17.24
Erodium moschatum	Geraniceae	23.97	35.49	6.89
Escallonia pulverulenta	Escalloniacae	28.28	52.94	3.44
Eschscholzia californica	Papaveraceae	77.55	5.84	3.44
Euphorbia maculat a	Euphorbiaceae	16.22	88.23	17.24
Euphorbia platyphyllos	Euphorbiaceae	53.82	35.29	17.24
Galega officinalis	Fabaceae	20.45	82.35	13.7
Malva nicaensis	Malvaceae	20.51	41.47	3.44
Margyricarpus pinnatus	Rosaceae	12.53	52.94	3.44
Podocarpus salignus	Podocarpaceae	13.22	29.41	-3.44
Portulaca oleracea	Portulacaceae	11.57	41.17	10.34
Raphanus sativus	Brassicaceae	73.03	58.82	13.79
Saponaria officinalis	Caryophyllaceae	18.34	88.23	3.44
Silybum marianum	Asteraceae	13.54	70.58	6.89
Sisymbrium officinale	Brassicaceae	28.28	52.94	10.34
Verbascum virgatum	Scrophulariaceae	13.54	17.64	3.44
2,4-Dichlorophenoxyacetic acid (2,4-D)		100µg/ml	100	100
		50 µg/ml	95	96

[a] Yield of extract per 100 g of plant material
[b] Data represent the mean on three replicates
[c] IG% = Inhibitory Germination Index

RESULTS AND DISCUSSION

The inhibitory effect on germination of each extract was evaluated in a bioassay against two species, *Trifolium repens* and *Raphanus sativus*, representing dicotyledonous plants. The results of screening are presented in Table 1. Many plants extract (13/24 = 54%) showed effects on *T. repens* with IG% > 50%, but none on *R. sativus*. Eight (8/24 = 33.3%) extracts possessed an IG between 70 and 88 % against *T. repens* (Table 1).

The extract with an IG% > 75% was fractionated with solvents of increasing polarity, as indicated in the methodology and evaluated their ability to inhibit the germination of T. repens and R. sativus. The results are showed in Table 2 and 3. These results confirm the low level of inhibitory activity on germination by all fractions obtained from selected plants against *R. sativus*.

Table 2
Inhibitory germination effects of fraction from plants from Central Valley and Preandean foothill of Ñuble province, Chile

	R. sativus					
	%IG[a,b] 24 h			%IG[a,b] 120		
plants	n-hexane	ethyl acetate	MeOH/water	n-hexane	ethyl acetate	MeOH/water
Baccaris linearis	**29.41**	**27.78**	**11.76**	**10.00**	**5.00**	**0.00**
Brassica rapa	**17.65**	**27.78**	**29.41**	**5.00**	**-5.26**	**10.00**
Cirsium vulgare	**-5.88**	**0.00**	**11.76**	**0.00**	**5.26**	**10.00**
Galega officinalis	**11.76**	**27.78**	**35.29**	**5.00**	**5.26**	**5.00**
Euphorbia maculata	**17.65**	**33.33**	**29.41**	**10.00**	**5.26**	**0.00**
Saponaria officinalis	**23.53**	**11.11**	**11.76**	**15.00**	**0.00**	**10.00**
Colletia spinosissima	**-3.53**	**4.44**	**3.53**	**0.00**	**-1.05**	**10.00**

[a] Data represent the mean on three replicates
[b] IG% = Inhibitory Germination Index

Table 3
Inhibitory germination effects of fraction from plants from Central Valley and Preandean foothill of Ñuble province, Chile

	T. rapens					
	%IG[a] 24 h			%IG[a] 120 h		
plants	n-hexane	Ethyl acetate	MeOH/water	n-hexane	ethyl acetate	MeOH/water
Baccaris linearis	**72.73**	**42.86**	**42.86**	**18.75**	**42.86**	**15.38**
Brassica rapa	**100.00**	**85.71**	**14.29**	**0.00**	**14.29**	**0.00**
Cirsium vulgare	**54.55**	**85.71**	**85.71**	**6.25**	**21.43**	**46.15**
Galega officinalis	**63.64**	**57.14**	**14.29**	**-12.50**	**35.71**	**23.08**
Euphorbia maculata	**9.09**	**57.14**	**14.29**	**18.75**	**0.00**	**0.00**
Saponaria officinalis	**72.73**	**85.71**	**-14.29**	**12.50**	**0.00**	**0.00**
Colletia spinosissima	**63.64**	**57.14**	**57.14**	**12.50**	**28.57**	**-15.38**

[a] Data represent the mean on three replicates
[b] IG% = Inhibitory Germination Index

Moreover, it was observed that the n-hexane fraction of *B. linearis* has an IG of 72% on *T. rapens*. However, ethyl acetate and MeOH/water fraction have an IG < 50% respectively, on the same species. The literature show phytochemical and biological investigations about 120 species of *Baccharis* genus. Those results are mainly the isolation of diterpenoids of clerodane and labdane type, phenolics as phenylpropanoids and flavonoids aglicone with flavone unit being most frequent (Gongaza-Verdi *et al.*, 2005)

Against *T. repens* Br. *rapa* possess high activity with IG of 100% and 85% showed by n-hexane and ethyl acetate fractions respectively. Metabolic characterization of different cultivars of *B. rapa* was previously done by NMR-based metabolomics analyses from which some phenylpropanoids, flavonoids and glucosinolates were identified. Among the identified compounds in Brassicaceae, glucosinolates has attracted a great deal of attention as they seem to be involved as phytoanticipins in the chemical defense of the plants (Abdel-Farid *et al.*, 2007, Abdel-Farid *et al.*, 2009, Abdel-Farid *et al.*, 2010).

Against T. repens the ethyl acetate and MeOH/water fractions from *C. vulgare* showed an IG of 85%. The n-hexane fraction from this plant do not showed any activity. From *C. vulgare* have been isolated brassicasterol, campesterol; stigmasterol; 4,6-tetradecadiene-8,10,12-triyn-1-ol; 1,11-trideca-diene-3,5,7,9-tetrayne; p-coumaric acid; caffeic acid; ferulic acid; p-hydroxybenzoic acid; protocathuic acid; vanillic acid; genkwanin-4'-O-glucoside; kaempferol-3-O-glucoside; quercetin-3-O-glucoside; quercetin-3-O-galacatoside (Jordon-Thaden & Louda, 2003).

Against *T. repens*, *G. officinalis* showed low activity with an IG of 63 and 57% by n-hexane and ethyl acetate fraction, respectively. Three flavonol triglycosides kaempferol 3-[2Gal-(4-acetylrhamnosyl) robinobioside], kaempferol 3-(2Gal-(4-rhamnosylrobinobioside) and quercetin 3-(2G-rhamnosylrutinoside) have been isolated from a methanolic extract of *G. officinalis* aerial parts. Also have been identified from *G. officinalis* a rare norterpenoid glucoside: dearabinosyl pneumonan-thoside (Champavier *et al.*, 1999; Champavier *et al.*, 2000).

Against *T. repens*, only ethyl acetate fraction of E. maculata has an IG of 57%. The literature reported nine compounds isolated from the ethanol extracts from this plant, whose chemical structures were elucidated by spectroscopic and chemical methods: including quercetin, kaempferol, apigenin-7-O-glucoside, luteolin-7-O-glucoside, quercetin-3-O-arabinoside, ethyl gallate, ellagic acid, scopoletin and umbelliferone (Runhui & Lingyi, 2001).

Against *T. repens* the n-hexane and ethyl acetate fraction from *S. officinalis* have an IG of 72 and 85% respectively. From this plant a complex mixture of triterpenoid saponins has been isolated (Jia *et al.*, 1998; Koike *et al.*, 1999; Jia *et al.*, 1999).

Against *T. repens*, *C. spinosissima* showed an IG activity slightly higher than 50% for all fractions. Two quaternary benzyltetrahydroisoquinoline alkaloids have been isolated from aerial parts of *C. spinosissima*, (Sanchez & Comin, 1967). The neutral components from the stems of *C. spinosissima* include lupenone, sitosterol, lupeol, daucosterine (Pacheco *et al.*, 1973).

In a parallel manner the root growth inhibition was measured (Table 4). In general it can be observed that the different fractions of the selected plants do not show significant effect on root growth, even in some cases the root growth is unaffected. The obtained n-hexane fraction of Br. rapa, *E. maculata* and *S. officinalis* produce root growth inhibition with 71.56, 79.72, and 70.97% of inhibition, respectively.

Against *R. sativus* only the ethyl acetate fraction of *C. vulgare* showed an inhibitory activity of 69.24% inhibition on root growth. The MeOH / water fraction of *Br. rapa* produces an inhibition of 80.66% on the root growth of *R. sativus* seeds. In particular, it was observed that seeds treated with MeOH / water fraction of *C. spinosissima* are not able to germinate and develop leaves.

The decrease in root growth is one of the consequences of germination inhibition (Corbineau *et al.*, 1988; Staswick *et al.*, 1992). Inhibition of seed germination and root elongation increases with increasing concentrations of MeJA as growth inhibitors. One of the reasons for this inhibition may be related to inhibition of ethylene production by natural inhibitors which are suspected to be a required factor in seed formation and germination (Nojavan-Asghari & Ishizawa, 1998). Therefore, we assumed that this inhibition is related to endogenous release of ethylene and it has been reported that application of exogenous MeJA causes the inhibition of germination by reducing ethylene production (Nojavan-Asghari & Ishizava, 1998; Kepczynski *et al.*, 1999).

The results obtained in this investigation indicate the need to deep the investigation about this property and phytotoxic elucidating the metabolites responsible for phytotoxic activity and its possible use as field-level control weeds in agricultural production systems. We are doing experiments with monocotyledoneous species for dissect the selectivity of assayed species.

Table 4
Root growth inhibitory effect of fraction from plants from Central Valley and Preandean foothill of Ñuble province, Chile.

	RGI[a,b] %					
	n-hexane		ethyl acetate		MeOH/water	
plants	*T. repens*	*R.sativus*	*T. repens*	*R. sativus*	*T. repens*	*R. sativus*
Control	**0**	**0**	**0**	**0**	**0**	**0**
Baccaris linearis	**20.23**	**19.47**	**56.41**	**17.35**	**30.76**	**35.59**
Brassica rapa	**12.6**	**71.56**	**41.03**	**47.78**	**33.13**	**80.66**
Cirsium vulgare	**24.05**	**14.42**	**44.23**	**69.24**	**58.65**	**66.53**
Galega officinalis	**-0.76**	**9.67**	**60.19**	**33.47**	**67.61**	**43.59**
Euphorbia maculata	**-3.21**	**79.72**	**44.68**	**7.64**	**-16.83**	**31.78**
Saponaria officinalis	**28.85**	**70.97**	**33.59**	**31.76**	**-47.79**	**28.26**
Colletia spinosissima	**28.85**	**19.81**	**33.59**	**19.47**	**-47.79**	**-0.63**

[a] Means of three experiments.
[b] RGI= Root Growth index

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