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Optimization of ultrasonic-assisted extraction for herbicidal activity of chicory root extracts

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ABSTRACT

The present study evaluated the herbicidal potential of extracts from chicory (*Cichorium intybus* L.) roots on the germination of *Echinochloa crusgalli* L. Beauv and *Amaranthus retroflexus* L. Eight ultrasonic assisted-extraction (UAE) conditions were optimized, using an orthogonal matrix design. The extract concentrations that would yield the largest allelopathic effects on the plant species were estimated by a modeling analysis. Our results showed that an alcohol solvent extract of chicory root had significant herbicidal activity which depended on the extract concentrations and the target species. The half-inhibitory concentrations of crude extract of chicory root ranged from 0.5 $g\,l^{-1}$ to 40.5 $g\,l^{-1}$. At a frequency of 40 kHz, the optimum UAE conditions to produce an extract for use as herbicide against *E. crusgalli* L. included an ethanol content (Ec) of 50% (v/v), a solvent-to-solid ratio (SR) of 16:1, an ultrasound temperature (UT) of 35 °C, an impregnation time (Imt) of 24 h with two rounds of impregnation (Imr), a sonication period (St) of 120 min with two rounds of sonication (Sr) and an ultrasound input power (P) of 200 W. The optimum conditions to produce an extract for use against *A. retroflexus* L. included an Ec of 100% (v/v), a SR of 16:1, an UT of 20 °C, an Imt of 48 h with two Imr, a St of 30 min with one Sr and a P of 400 W. The extract had the largest inhibitory effects on the germination index and root growth of both *E. crusgalli* L. and *A. retroflexus* L. at concentrations ranging from 30.8 to 33.7 $g\,l^{-1}$. At a concentration of 4.2 $g\,l^{-1}$, the extract significantly enhanced the shoot growth of *A. retroflexus* L. Overall, chicory root extract has potential for use as a main ingredient in natural herbicides or for development as a novel plant-derived herbicide.

1. Introduction

Increasing public concern about environmental contamination has triggered a search for plant-derived resources and natural extracts for industrial agrochemical, cosmetic and pharmaceutical production. Many compounds derived from plants, such as chicory, have natural antimicrobial, antioxidant, pesticidal and herbicidal effects without causing harm to the environment (Pavela, 2010; Pavela et al., 2010; Santos et al., 2010; Tandon et al., 2010; Veloz et al., 2010).

Chicory (*Cichorium intybus* L.) has historically been grown worldwide, and this plant has many uses. For example, it was used in the folk medicine of ancient Greece and Rome (Perin, 1964), and it has more recently been used as a leafy vegetable (Mulabagal et al., 2009), bioactive forage (Kidane et al., 2009) and a traditional coffee surrogate (Baert and Bockstaele, 1992). It is also an important source of inulin, either because of its high root yield potential or because of its high root sugar content (Amaducci and Pritoni, 1998; Baert, 1997; Degidio et al., 1998; Meijer and Mathijssen, 1992, 1996). In recent years, a large number of medicinal functions of chicory have been researched. Chicory extracts have proven effective against Ehrlich ascites carcinoma (a kind of cancer) in mice (Hazra et al., 2002). The extracts have also been shown to decrease lipid activities (Shang et al., 2008), have hepatoprotective effects (Ammara et al., 2009) and contain some antioxidant components (Lavelli, 2008). Chicory has been demonstrated to contain potential anti-microbial (Wang et al., 2008b) and antifungal agents (Mares et al., 2005). Additionally, chicory has been made into anti-inflammatory medicine (Cavin et al., 2005). Importantly, chicory extracts are generally regarded as safe by the Food and Drug Administration (FDA) (Schmidt et al., 2007). Chicory is also recognized as a source of dietary fibers, such as inulin and fructo-oligosaccharides, which have health-promoting properties (Bais and Ravishankar, 2001).

Ultrasonic-assisted extraction (UAE) has been widely used to isolate bioactive substances from different parts of plants (Adje et al., 2010; Hromadkova et al., 1999; Liu et al., 2007). Previous studies have revealed that ultrasound could disrupt tissues and

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Table 1
Assignment of controlled factors and levels of experimental design using mixed orthogonal matrix L_{16} ($4^3 \times 2^6$) to optimize the UAE factors for largest yield of chicory root extract.

Factors[a]	Ec (%, v/v)	SR (v/w)	UT (°C)	Imt (h)	Imr (repetitions)	St (min)	P (W)	Sr (repetitions)	Vacancy
Level I	0	8	20	24	1	30	200	1	
Level II	50	16	35	48	2	120	400	2	
Level III	75	24	50						
Level IV	100	32	65						

[a] Columns Ec, SR, UT, Imt, Imr, St, P and Sr stand for ethanol content, solvent-to-solid ratio, ultrasound temperature, impregnation time, impregnation repetitions, sonication time, ultrasound input power, sonication repetitions, respectively, and last column vacancy is to account for statistical error.

cell walls, which enhanced the mass transfer of the solvents into the materials and the soluble constituents into the solvents (Zhang et al., 2009). Ultrasound is a highly efficient tool for the fast extraction of active compounds (Chen et al., 2009). The efficiency of the extraction process is influenced by many conditions, including the power, frequency, temperature and time of sonication (Lu, 2004). However, the reported methods have a wide range of optimized conditions; for example, studies have used the following frequency and power combinations: 25 kHz, 150 W (Cuoco et al., 2009), 35 kHz, 320 W (Ozcan et al., 2009) and 20 kHz, 450 W (Roldan et al., 2008); temperatures of 40–90 °C (Jalbani et al., 2006), 30 °C (Dong et al., 2010) and 25 °C (Ozcan et al., 2009; Roldan et al., 2008); and extraction times of 5 min (Ozcan et al., 2009), 5–20 min (Jalbani et al., 2006), 30–120 min (Liu et al., 2007) and 3 h (Durling et al., 2007). In light of these observations, and knowing that the conditions for extraction have both positive and negative interactions with each other, it is necessary to optimize conditions for extraction (Lee et al., 2010; Li et al., 2010). In this setting, an orthogonal design is the method of choice.

Orthogonal array designs have been used to efficiently discover how different parameters interact and how they affect product recovery (Stenlund et al., 2009). Previous knowledge of the variables, past experiences and intuition are all very helpful in arranging the variables and levels of the experiment because orthogonal array designs only cover a predefined subset of possible interactions (Wang et al., 2008a). This type of optimization procedure requires the use of a strategically designed experiment that deliberately introduces changes to identify factors affecting the procedure in order to estimate the factor levels required to yield an optimum response. Orthogonal array design was applied to optimize the factors affecting herbicide extraction from soils (Diez et al., 2008).

The inappropriate use of agrochemicals may give rise to undesirable side effects. It may be necessary to develop new management systems based on ecological manipulations to reduce dependence on synthetic herbicides and insecticides (Kohli et al., 1998; Xuan et al., 2003). Plants are known to produce secondary metabolites that affect the germination and growth of other plants. This mechanism of interaction between plant species has been defined as allelopathy (Weidenhamer, 2005; Zhang et al., 2010). Allelopathy is a biological interaction between two plants that occurs through the production of chemical compounds (allelochemicals) released by leaching, volatilization, decomposition or root exudation. Hence, allelopathy, together with competition, is a promising, environmentally friendly tool for weed management (Kalinova, 2010) because the phytotoxins contribute to the ecological stability of a plant community (De et al., 2010). The exploitation of allelopathic plants for weed control has grown in importance (Xuan et al., 2003).

As previously mentioned, there are a number of studies that have focused on the biochemical activities of extracts from chicory (Ripoll et al., 2007), but information concerning the herbicidal activity of these extracts is rarely available. Therefore, the main objective of this study was to evaluate the herbicidal activity or allelopathic effects of extracts from chicory roots, with the goal of developing an effective plant-derived herbicide. The study determined the herbicidal activity of the extracts against *Echinochloa crusgalli* L. Beauv and *Amaranthus retroflexus* L., combining orthogonal optimization and model analysis. Because both *E. crusgalli* L. Beauv (Khanh et al., 2008) and *A. retroflexus* L. (Rezaie and Yarnia, 2009) are cosmopolitan weeds, their presence lowers crop yields as they compete for fertilizer, water and sunlight. These two weed species are often seen in crop fields, and they have very strong vitality (Ottis and Talbert, 2007). Therefore, the use of chicory root extract as a herbicide could serve as a control for these two plants.

2. Materials and methods

2.1. Reagents and materials

2.1.1. Preparation of plant materials

Five-year-old Puna chicory roots, freshly harvested in September 2009 after growing in an experimental field of the Grassland Science Department, Northwest A&F University, Shanxi Province, China, were washed several times with water, cut into pieces 3–5 mm in thickness and dried in an oven at 50 °C until a constant weight was obtained (Chen et al., 2009; Jiang and Liu, 2008). The dried material was then ground into a powder and passed through a 40-mesh sieve with a particle diameter of less than 0.35 mm (Diouf et al., 2009; Yu et al., 2009). The chicory root powder was then stored in sealed bags for future use.

2.1.2. Reagents

Ethanol was analytical grade from Xi'an Chemical Reagent Factory (PR China), distilled water was from Logistics Group of Northwest A & F University (PR China).

2.2. Experimental design

Controlled factors and levels of the experimental design were assigned using a mixed orthogonal matrix L_{16} ($4^3 \times 2^6$) (Table 1) (Hedayat et al., 1999). In the 16 treatments, 8 key factors, included ethanol content (Ec), the solvent-to-solid ratio (SR), the sonication temperature (UT), the impregnation time (Imt), the number of (either one or two) impregnations (Imr), the sonication time (St), the ultrasonic input power (P) and the number of (either one or two) sonication repetitions (Sr), that significantly influenced the extractive efficiency of UAE (aside from a fixed frequency of 40 kHz) were investigated. The first three factors (Ec, SR and UT) were investigated at four levels; the remaining 5 factors were investigated at two levels. For example, the treatment 11 (T_{11}) is at $(Ec)_3$, $(SR)_3$, $(UT)_2$, $(Imt)_2$, $(Imr)_2$, $(St)_1$, $(P)_2$ and $(Sr)_2$, which mean the Ec at 75% (volume ratio: v/v), the SR at 24:1 (v/w), the UT at 35 °C, the Imt with the ethanol solvent for 48 h, the Imr twice, the St at 30 min, the P of 400 W and the Sr twice. The last column, vacancy was left to account for the statistical error of the orthogonal method (Hedayat et al., 1999) (Table 2). When impregnation was performed twice, the filtrate was collected and the solid was extracted with the same volume of fresh solvent (Huang et al., 2009).