African Journal of Microbiology Research Vol. 5(16), pp. 2353-2358, 18 August, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.174
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Optimization of ultrasound-assisted extraction conditions using orthogonal matrix design to enhance the antimicrobial activity of extracts from *Cichorium intybus* root

Quanzhen Wang[1]*, Haitao Liu[1], Jinhong Du[1], Jian Cui[2], Guo Chen[1] and Yuyan Liu[1]

[1]Department of Grassland Science, College of Animal Science and Technology, Northwest Agriculture and Forestry University, 712100 Yangling, Shanxi Province, P. R. China.
[2]Department of Plant Science, College of Life Science, Northwest Agriculture and Forestry University, 712100, Yangling, Shanxi Province, P. R. China.

Accepted 30 July, 2011

Plant-derived compounds used as alternatives to chemical preservatives have been extensively researched for use as natural medical ingredients or food preservatives. Conditions for the ultrasound-assisted extraction (UAE) of chicory (*Cichorium intybus*) root (including type of solvent, impregnation time, number of sonication steps and ultrasonic power) were optimized to determine the best extract antibacterial activity by using orthogonal matrix design [L_{16} (4^5)]. The combination of 70% ethanol v/v, a 36 h impregnation time, three sonication rounds and 300 W ultrasonic power input provided the best antimicrobial activity results. Our results demonstrate that solvent composition has the largest effect on antimicrobial activity. Several extracts demonstrated antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis* and *Salmonella typhi,* and all extracts exhibited weak activity against *Bacillus subtilis*. To our knowledge, these results represent the first example of ultrasound-assisted chicory extracts aimed at increasing its potential for use in food industry.

Key words: *Cichorium intybus*, antibacterial, ultrasound-assistant extract, optimal conditions, orthogonal matrix design.

INTRODUCTION

There are a variety of useful compounds from higher plants that have not yet been identified. Medical and food industries tend to use natural antimicrobials, such as plant-derived compounds, as an alternative to chemical preservatives (Bachir and Mohamed, 2010). These preferences have led to the search for plant products that have natural antimicrobial and antioxidant effects. Such products have been extensively researched and used as both natural medical ingredients and food additive agents (Issa-Zacharia et al., 2010; Kumaravel et al., 2010; Liu et al., 2010; Mini et al., 2010; Somchit et al., 2010).

*Corresponding author. E-mail: wangquanzhen191@163.com. Tel: 0086-29-87091953 or 0086-13759942845. Fax: 0086-29-87092164.

Chicory (*Cichorium intybus*) is a perennial plant of the Asteraceae family, which is a native of to the Mediterranean region and widely grown in Europe, Western Asia, Egypt and North America. In traditional Indian medicine, chicory has been used to treat fever, diarrhea, spleen and liver enlargement, jaundice, gout and rheumatism (Mulabagal et al., 2009). In Belgium, France and the United States, chicory root has been used as a coffee additive for its bitter taste, which is caused by sesquiterpene lactones (Peters and Amerongen, 1996; Poli et al., 2002).

A number of studies report that chicory extracts possess antimicrobial activities. It was reported that water, ethanol and ethyl acetate extracts from chicory have antibacterial properties, and that root extracts have more intensive antibacterial activity than extracts from whole plants (Petrovic et al., 2004). Moreover, it was found that the sesquiterpene lactones extracted from

Table 1. Assignment of levels and condition factors using orthogonal matrix L_{16} (4^5).

Levels	Factors			
	A (Solvent)	B [Impregnation time (h)]	C (Sonication repetitions)	D [Ultrasonic input power (W)]
I	petroleum ether	12	1	200
ii	ethyl ether	24	2	300
iii	ethyl acetate	36	3	400
iv	70% ethanol v/v	48	4	500

chicory root can inhibit the growth of zoophilic and anthropophilic dermatophytes (Mares et al., 2005). Many gastrointestinal foodborne infections in humans, particularly those in developing countries, are often caused by enterobacteria (Ajayi and Akintola, 2010). Increasing concern over pathogenic and spoilage microorganisms in food is due to the growing prevalence of food borne disease outbreaks (Rahman and Kang, 2009). Considering the questionable safety of synthetic food preservatives, consumers are demanding more natural and fresh foods with fewer synthetic additives. However, to increase safety and maintain a long shelf life, food manufacturers are compelled to use natural or mild preservation techniques. Therefore, alternative sources of safe and effective natural preservatives must be explored (Abbasi et al., 2010; Negi et al., 2008; Hussain et al., 2009).

Orthogonal matrix design has been used to evaluate the interaction of different production parameters and how they affect product recovery. Furthermore, this method has been used to optimize multiple production parameters to establish optimum conditions (Hedayat et al., 1999). At present, ultrasound-assisted extraction (UAE) is considered a desirable method for organic compound extraction from different matrices (Khan et al., 2010). This method can shorten extraction time because the increased pressure favors penetration and transport while the temperature decreased may improve solubility and diffusivity (Pena et al., 2006).

In order to optimize conditions to achieve the best antibacterial activity for chicory extract use as a natural food preservative, the solvent, impregnation time, sonication repetitions and ultrasonic input power were evaluated by orthogonal matrix design. The L_{16} (4^5) experiment was adopted and sixteen different extraction groups were analyzed. The antibacterial activities of extracts derived using different extraction protocols were investigated.

MATERIALS AND METHODS

Plant materials

Five-year-old Puna chicory roots were freshly harvested in September 2009 in an experimental field at the Grassland Science Department at Northwest Agriculture and Forestry University (Shaanxi Province, China). The plant was identified by associate professor Quanzhen Wang (Northwest Agriculture and Forestry University, China). A voucher specimen was deposited in the Herbarium of the Laboratory of Grassland Science at the Faculty of Animal Science and Technology (Northwest Agriculture and Forestry University, China).

Preparation of plant extracts

Four levels for each of four different extraction conditions resulting in sixteen extraction method combinations (Table 1) were studied by an L_{16} (4^5) orthogonal matrix design (Hedayat et al., 1999). For each sample, 100 g dried ground powder (40 meshes) was soaked in 800 ml of various solvents for different times at room temperature until extraction was exhausted. The extraction temperatures were 30°C (ethyl ether), 50°C (petroleum ether and ethyl acetate) and 65°C (70% ethanol v/v), respectively. An ultrasonic apparatus (KQ-500DE, Kunshan Ultrasound Instrument Co., Ltd., China) was used for accelerated extraction. A beaker was partially submerged in an isothermal water bath to maintain the extraction temperature for 30 min. After extraction, the resulting mixture was passed through filter paper (Whatman No. 1.) by vacuum. The filtrate was concentrated on a rotary evaporator (SHB-III, Zhengzhou Science and Industrial Foreign Trade Co., Ltd., China) at 45°C and then stored at 4°C for further use. All samples were redissolved in dimethylsulfoxide (DMSO) at a concentration of 10 mg/mL and stored at 4°C.

Bacteria

The antibacterial activity of chicory extracts was determined using the following food-related bacteria obtained from the Department of Life Science, Northwest Agriculture and Forestry University: *E. coli*, *Staphylococcus aureus*, *Bacillus thuringiensis*, *Bacillus subtilis*, *Salmonella typh*. Cultures of each strain were maintained on beef cream-peptone culture media at 4°C (Monadi et al., 2010; Moussa and Hessan, 2010).

Antibacterial assays of disc diffusion

Antibacterial activity tests were conducted based on the disc diffusion method (Yan et al., 2009). A suspension of bacteria (2×10^8 CFU/ml) was spread on solid media plates. Sterile paper discs (6 mm diameter) were individually impregnated with solvent at 10 mg/ml. Discs with the solvent used for dissolution were used as negative controls and the standard reference antibiotic streptomycin (10 µg/disc) was used as a positive control for the bacteria in question. The plates were incubated at 37°C for 24 h after which the inhibition zone was measured in millimeters. Each assay in this experiment was performed in triplicate.

Statistical analysis

All experimental results are expressed the mean of four repeats.

Table 2. The L_{16} (4^5) matrix associated with analytical results.

Group	Factors and levels				Zone of inhibition in mm[a]				
	A	B	C	D	*Staphylococcus aureus*[b]	*Bacillus subtilis*[b]	*Bacillus thuringiensis*[b]	*Salmonella typhi*[c]	*Escherichia coli*[c]
1	1	1	4	3	10.2	7.2	9.7	8.6	9.7
2	2	1	1	1	11.6	6.5	9.4	8.2	8.1
3	3	1	3	4	8.5	8.5	13.1	12.3	11.1
4	4	1	2	2	12.2	8.9	10.8	7.5	9.5
5	1	2	3	2	12.1	7.1	11.8	10.2	9.2
6	2	2	2	4	11.7	8.1	8.9	10.1	11.5
7	3	2	4	1	9.2	9.6	9.6	11.6	12.0
8	4	2	1	3	9.8	8.2	12.7	8.6	11.7
9	1	3	1	4	11.0	7.9	8.6	10.4	7.6
10	2	3	4	2	9.4	9.8	10.7	14.2	11.2
11	3	3	2	3	8.6	7.5	10.2	10.9	10.9
12	4	3	3	1	11.1	6.7	10.3	12.2	13.6
13	1	4	2	1	8.3	8.7	10.2	7.4	10.2
14	2	4	3	3	8.4	7.1	9.7	9.7	6.9
15	3	4	1	2	9.1	7.5	9.4	11.4	8.1
16	4	4	4	4	11.5	9.1	11.5	7.1	10.1

[a] Values are means (mm) of four separate treatments. [b]Gram positive bacteria. [c]Gram negative bacteria.

Analysis of variance was performed by ANOVA. SAS software (version 8.2, USA) was used for statistical analysis. Results were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The antibacterial activities of chicory extracts (10 mg $disc^{-1}$) against the various microorganisms were assessed by measuring inhibition zone diameters *in vitro*. To evaluate the antibacterial activity of chicory extractions, four levels and four different extraction parameters were studied using an L_{16} (4^5) orthogonal matrix design. In total, sixteen different treatment groups with various parameters were established. The selected factors and levels are displayed in Table 1. Four different extraction parameters (factors) were statistically analyzed and are shown in Table 2 (Hedayat et al., 1999), and the ranges of the averages of the levels are shown in Table 3. The impact of the variables on the inhibition zone of *S. aureus* are shown as follows in decreasing order (ranges): solvent (A) > ultrasonic input power (D) > impregnation time (B) > sonication repetition number (C) (Table 3). The optimal extraction parameters were defined as the following: treatment with 70% ethanol v/v (A_4), 300 W ultrasonic input power (D_2), 12 h impregnation time (B_1) and two rounds of sonication (C_2). The impact of variables on the inhibition zone against *Bacillus subtilis* was measured in the following order: C > D > A > B. The optimal combination of parameters for this bacterium was to use four sonication rounds (C_4), 300 W ultrasonic input power (D_2), ethyl ether (A_2) and a 36 h impregnation time (B_3). The impact of variables on the inhibition zone of *B. thuringiensis* was in the following order: A > C > B > D. The optimal combination was to use ethyl acetate (A_3), three sonication rounds (C_3), a 12 h impregnation time (B_1) and 500 W ultrasonic input power (D_4). The impact of variables on the inhibition zone of *Salmonella typh* was in the following order: B > A > C > D. The optimal parameter combination was to use a 36 h impregnation time (B_3), ethyl ether (A_2), four sonication rounds (C_4), and 300 W ultrasonic input power (D_2). The impact of variables on the inhibition zone of against *E. coli* was in the following order: B > A > C > D. The optimal parameter combination was found using a 36 h impregnation time (B_3), 70% ethanol v/v (A_4), three sonication rounds (C_3), and 200 W ultrasonic input power (D_1). According to the results for antibacterial activities against all five types of bacteria, we found that extraction using 70% ethanol v/v, a 24 h impregnation time, three sonication rounds and 300 W ultrasonic input power is the optimal synthetic combination.

Variance analyses of the models of all bacteria but *B. thuringiensis* were found to be significant (Table 4. $Pr < 0.001$). Factors A, B and D were significantly effective for inhibiting *S. aureus* whereas factors C and D were significant against *Bacillus subtilis* (Table 4). Factors A, B and C consistently inhibited the Gram-negative bacteria *S. typhi* and *E. coli*; however, factor D (ultrasonic input power) was not significant (Table 4). Solvent type was found to be the most influential factor; increasing solvent polarity resulted in better antibacterial activities primarily because substances such as carbohydrates, proteins,

Table 3. Averages of levels with factors and ranges.

Factors	A	B	C	D	A	B	C	D
	Staphylococcus aureus				*Bacillus subtilis*			
Level i	10.4	10.63	10.38	10.05	7.73	7.78	7.53	7.88
Level ii	10.35	10.70	10.20	10.70	7.88	8.25	8.30	8.33
Level iii	8.85	10.03	10.03	9.25	8.28	7.98	7.35	7.50
Level iv	11.15	9.33	10.08	10.68	8.23	8.1	8.93	8.40
Range[a]	2.3	1.37	0.35	1.45	0.55	0.47	1.58	0.90
Order	1	3	4	2	3	4	1	2
	Bacillus thuringiensis				*Salmonella typhi*			
Level i	10.08	10.75	10.03	9.88	9.15	9.15	9.65	9.85
Level ii	9.68	10.75	10.03	10.67	10.55	10.13	8.98	10.83
Level iii	10.58	9.95	11.23	10.58	11.55	11.93	11.10	9.45
Level iv	11.33	10.2	10.38	10.53	8.85	8.90	10.38	9.98
Range[a]	1.65	0.8	1.2	0.79	2.7	3.03	2.12	1.38
Order	1	3	2	4	2	1	3	4
	Escherichia coli							
Level i	9.18	9.6	8.87	10.98				
Level ii	9.43	11.1	10.53	9.5				
Level iii	10.53	10.83	10.2	9.8				
Level iv	11.23	8.83	10.75	10.08				
Range[a]	2.05	2.27	1.88	0.58				
Order[b]	2	1	3	4				

[a] R means the average range for four average responses in each level for the inhibition zone against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Salmonella typhi*, *Escherichia coli*, respectively.
[b] The ordinal numeral for the range sequence of the eight factors in decreasing order.

Table 4. Variance analysis for the model and experimental factors.

Factor		*Staphylococcus aureus*[b]	*Bacillus subtilis*[b]	*Bacillus thuringiensis*[b]	*Salmonella typhi*[c]	*Escherichia coli*[c]
Model	F Value	3.85	3.72	1.87	4.93	4.33
	Pr > F	0.0003	0.0005	0.0608	<.0001	0.0001
A	F Value	7.62	1.16	3.39	6.63	5.01
	Pr > F	0.0003	0.3348	0.0248	0.0007	0.0040
B	F Value	3.49	0.88	1.14	8.11	6.19
	Pr > F	0.0221	0.4567	0.3415	0.0002	0.0011
C	F Value	0.22	9.74	2.15	3.58	3.89
	Pr > F	0.8807	<.0001	0.1059	0.0200	0.0140
D	F Value	4.08	3.09	0.81	1.39	2.23
	Pr > F	0.0113	0.0350	0.4933	0.2560	0.0956

[b]Gram positive bacteria. [c]Gram negative bacteria.

alkaloids, tannins, glycosides and amines increase in abundance with increased solvent polarity. Impregnation time, sonication repetition number and ultrasonic input power were found to be subordinate factors. Most

bioactive compounds such as polyphenolics (e.g., tannins and flavonoids) exist in higher polarity solvents. Additional compounds found in the extracts may either enhance or attenuate the effect of phenolic compounds. The 70% ethanol and acetic ether extracts exhibited significant antibacterial activity against many of the bacteria in this study. This antibacterial activity may be ascribed to polyphenols, tannins and coumarins found in crude extracts (Kilani et al., 2008). Phenolic compounds can attack cell walls and membranes by affecting their permeability, enabling the release of intracellular constituents and interfering with membrane functionality (Bajpai et al., 2009). There were differences in chicory extract antibacterial activity against Gram-positive and Gram-negative bacteria. Gram-positive bacteria were found to be more susceptible to chicory extracts than Gram-negative bacteria, possibly because the hydrophilic cell walls of Gram-negative bacteria are composed of lipopolysaccharide (LPS), which inhibits the accumulation of phenolic compounds in a target cell membrane (Bezic et al., 2003).

Chicory contains several sesquiterpene lactones, particularly in the roots (Beek et al., 1990; Peters and Amerongen 1996; Poli et al., 2002). Poli et al. has reported the isolation of two guaianolides with different chemical structures, including 8-deoxylactucin and 11 β, 13-dihydrolactucin, from the root extracts of *C. intybus* var. 'Rosso di Chioggia' (Picman, 1986). Two pure isolated lactones possess similar biological activity to chicory extracts against several phytopathogens, which cause morphological anomalies that have been observed using scanning electron microscopy (Mares et al., 2005).

Conclusions

The antibacterial activity of chicory extracts was found to be affected by the type of solvent used for extraction. The optimal UAE conditions were obtained to produce the best chicory root extract antimicrobial activity. It was concluded that combining 70% ethanol v/v, a 36 h impregnation time, three sonication rounds and 300 W ultrasonic input power could produce the highest antibacterial activity in the extracts. Extracts from chicory roots can be used as a natural food preservative as well as an antibacterial agent. To our knowledge, these results represent the first example of a practical application for UAE aimed at increasing the potential use of chicory extracts in food industry. Chicory root antibacterial activities against additional bacterial strains should also be studied.

ACKNOWLEDGMENTS

This work was supported by The Ministry of Science and Technology of the Peoples Republic of China, International Cooperation Research Project (2008DFA31650) and Shaanxi Province International Cooperation Research Project (2008KW-29). We are grateful to Prof. Hu Tianming and postgraduate students Sun Luanzhi and Wu Chunhui.

REFERENCES

Abbasi FM, Ahmad H, Perveen F, Inamullah, Sajid M, Brar DS (2010). Assesment of genomic relationship between *Oryza sativa* and *Oryza australiensis*. Afr. J. Biotechnol., 9(12): 1312-1316.

Ajayi AO, Akintola TA (2010). Evaluation of antibacterial activity of some medicinal plants on common enteric food-borne pathogens. Afr. J. Microbiol. Res., 4: 314-316.

Bachir RG, Mohamed B (2010). Antimicrobial activity determination of the gum of *Pistacia atlantica* Desf. Oil. Afr. J. Microbiol. Res., 4: 2457-2460.

Bajpai VK, Al-Reza SM, Choi UK, Lee JH, Kang SC (2009). Chemical composition, antibacterial and antioxidant activities of leaf essential oil and extracts of Metasequioa glyptostroboides Miki ex Hu. Food Chemic. Toxicol., 47: 1876-1883.

Beek TV, Maas P, King B, Leclercq E, Voragen A, Groot AD (1990). Bitter sesquiterpene lactones from chicory roots. J. Agric. Food Chem., 38: 1035-1038.

Bezic N, Skocibusic M, Dunkic V, Radonic A (2003). Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. Phytother. Res., 17: 1037-1040.

Hedayat A, Sloane N, Stufken J (1999). Orthogonal arrays: theory and applications: Springer Verlag.

Issa-Zacharia A, Kamitani Y, Muhimbula H, Iwasaki K (2010). Antimicrobial effect of slightly acidic electrolyzed water for inactivation of *Salmonella* spp. and *Escherichia coli* on fresh strawberries (*Fragaria* L.). Afr. J. Microbiol. Res., 4: 2174-2180.

Khan MK, Abert-Vian M, Fabiano-Tixier AS, Dangles O, Chemat F (2010). Ultrasound- assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. Food Chem., 119: 851-858.

Kilani S, Sghaier MB, Limem I, Bouhlel I, Boubaker J, Bhouri W (2008). *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus*. Bioresource Technol., 99: 9004-9008.

Kumaravel K, Ravichandran S, Sritama bose S (2010). *In vitro* antimicrobial activity of shrimps haemolymph on clinical pathogens. Afr. J. Microbiol. Res., 4: 2592-2596.

Liu GQ, Lin QL, Guo-Pu Ren GP, Han WJ, Wang XL, Zhang HY (2010). Use of response surface methodology to optimize critical medium components for biomass and extracellular polysaccharide production by *Ganoderma sinense.* Afr. J. Microbiol. Res., 4: 2735-2741.

Mares D, Romagnoli C, Tosi B, Andreotti E, Chillemi G, Poli F (2005). Chicory extracts from *Cichorium intybus* L. as potential antifungals. Mycopathologia, 160: 85-92.

Mini NV, Ida B, Seema D, Shital D, Riva DS, Astrida R (2010). Antimicrobial activity of ten common herbs, commonly known as 'Dashapushpam' from Kerala, India. Afr. J. Microbiol. Res., 4: 2357-2362.

Monadi AR, Mirzaei H, Javadi A, Hosseinzade N, Amjadi Y (2010). Effect of some probiotics on *Salmonella typhi* during associated growth in milk. Afr. J. Microbiol. Res., 4: 2708-2711.

Moussa IM, Hessan AM (2010). Rapid detection of community acquired- methicillin resistance *Staphylococcus aureus* recovered from King Saudi Arabia. Afr. J. Microbiol. Res., 4: 2804-2810.

Mulabagal V, Wang H, Ngouajio M, Nair MG (2009). Characterization and quantification of health beneficial anthocyanins in leaf chicory (*Cichorium intybus*) varieties. European Food Res. Technol., 230: 47-53.

Negi PS, Jayaprakasha GK, Jena BS (2008). Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria. Lwt-Food Sci. Technol., 41: 1857-1861.

Pena A, Ruano F, Mingorance MD (2006). Ultrasound-assisted extraction of pesticides from olive branches: a multifactorial approach to method development. Analytic. Bioanalytic. Chem., 385: 918-925.

Peters AM, Amerongen AV (1996). Sesquiterpene lactones in chicory (*Cichorium intybus* L) - Distribution in chicons and effect of storage, Food Res. Int., 29: 439-444.
Petrovic J, Stanojkovic A, Comic L, Curcic S (2004). Antibacterial activity of *Cichorium intybus*. Fitoterapia, 75: 737-739.
Picman A (1986). Biological activities of sesquiterpene lactones. Biochem. Syst. Ecol., 14: 255-281.
Poli F, Sacchetti G, Tosi B, Fogagnolo M, Chillemi G, Lazzarin R (2002). Variation in the content of the main guaianolides and sugars in *Cichorium intybus* var. "Rosso di Chioggia" selections during cultivation. Food Chem., 76: 139-147.
Rahman A, Kang S (2009). *In vitro* control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. Food Chem., 116: 670-675.
Somchit MN, Abdul RR, Abdullah A, Zuraini A, Zakaria ZA, Sulaiman MR, Arifah AK, Mutalib AR (2010). *In vitro* antimicrobial activity of leaves of *Acalypha indica* Linn. (Euphorbiaceae). Afr. J. Microbiol. Res., 4: 2133-2136.
Yan RW, Yang Y, Zeng Y, Zou G (2009). Cytotoxicity and antibacterial activity of *Lindera strychnifolia* essential oils and extracts. J. Ethnopharmacol., 121: 451-455.