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EFFECT OF EXTRACTION SOLVENTS ON THE CONTENT OF BIOACTIVE COMPOUNDS FROM *Larrea tridentata* LEAVES

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KEYWORDS

Larrea tridentata; nordihydroguaiaretic acid; kaempferol; quercetin; antioxidant capacity

ABSTRACT

Three organic solvents (methanol, ethanol, and acetone) at four different concentrations (90, 70, 50, and 30% v/v) were used for extraction of hormone-like phenolic compounds (phytoestrogens) from *Larrea tridentata* leaves, namely, nordihydroguaiaretic acid (NDGA), kaempferol and quercetin. Besides the phytoestrogens extraction the antioxidant potential and the content of total phenols in the produced extracts were also determined. Phytoestrogens recovery varied considerably according to the solvent and concentration used for extraction. The highest NDGA, quercetin and kaempferol contents (46.96 ± 3.39 , 10.46 ± 1.01 and 87.00 ± 6.43 mg/g DW plant, respectively) were recovered using 90% (v/v) methanol. All the produced extracts showed antioxidant capacity, but those obtained using 70% and 90% (v/v) methanol had significantly higher ($p < 0.05$) FRAP (ferric reducing antioxidant power) values (2.55 ± 0.09 and 2.73 ± 0.11 mM FE(II)/g DW plant, respectively) than the remaining ones. These findings allow concluding that 90% (v/v) methanol can be successfully used for the bioactive compounds (phytoestrogens) recovery from *Larrea tridentata* leaves.

INTRODUCTION

Larrea tridentata (Zygophyllaceae), commonly known as creosote bush, is a plant that grows in semidesert areas of Southwestern United States and Northern Mexico. This plant was traditionally used for centuries by North American Indians as a medicine for several illnesses including infections, kidney problems, gallstones, rheumatism and arthritis, diabetes and to

treat tumors (Ross, 2005). *Larrea tridentata* is an outstanding source of natural compounds with approximately 50% of the leaves (dry weight) being extractable matter. Among several bioactive compounds present in this plant, nordihydroguaiaretic acid (NDGA), kaempferol and quercetin can be found at considerable high concentrations (Brinker, 1993; Hyder et al., 2002).

The purpose of this study was to evaluate the effect of different organic solvents on the extraction of phytoestrogens, in particular, NDGA, kaempferol and quercetin, from *Larrea tridentata* leaves. The antioxidant potential of the produced extracts was also determined and are discussed.

METHODS

Air-dried leaves of *Larrea tridentata* were ground to fine powder and stored in dark bottles at room temperature for further use. Extractions were performed by mixing 1 g of plant material with 20 mL of organic solvent (methanol, ethanol or acetone, in a concentration of 90, 70, 50, or 30% v/v) or distilled water. The mixtures were heated during 30 min in a water-bath at 70 °C when using methanol, ethanol, or water, and at 60 °C when using acetone, due to its lower boiling point. After this time, the produced extracts were filtered through qualitative filter paper and stored at -20 °C until further analysis. NDGA, kaempferol and quercetin concentrations were quantified by high performance liquid chromatography. The free radical activity of *Larrea tridentata* extracts was determined by measuring the ability of the extracts to scavenge the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the ferric reducing/antioxidant power of the extracts was performed according to the method described by Benzie et al. (1996).

RESULTS & CONCLUSIONS

NDGA, kaempferol and quercetin extraction from *Larrea tridentata* leaves varied considerably according to the used solvent (Table 1), probably due to



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the polarity of each solvent and the solubility of the compounds in them (Wang and Weller, 2006). Low concentration levels of all the three phytoestrogens were observed on the aqueous extracts, which can be explained by their low solubility in water (Martins et al., 2010). The highest NDGA, kaempferol and quercetin contents (46.96 ± 3.39 , 87.00 ± 6.43 and 10.46 ± 1.01 mg/g dry wt plant, respectively) were recovered using 90% (v/v) methanol as extraction solvent.

Table 1. Bioactive compounds extraction from *Larrea tridentata* leaves using different organic solvents and antioxidant activity of the extracts.

Solvent (% v/v)	NDGA (mg/g)	Kaempferol (mg/g)	Quercetin (mg/g)	FRAP (mM FE(II)/g)
H ₂ O	2.12 ± 0.25^b	8.00 ± 0.94^c	2.28 ± 0.17^f	0.77 ± 0.02^g
Methanol				
90	46.96 ± 3.39^a	87.00 ± 6.43^a	10.46 ± 1.01^a	2.73 ± 0.11^a
70	33.57 ± 0.88^b	65.78 ± 3.00^b	8.68 ± 0.38^b	2.55 ± 0.09^a
50	22.53 ± 0.66^c	42.37 ± 3.85^c	5.91 ± 0.47^c	1.92 ± 0.18^d
30	13.31 ± 1.58^d	30.26 ± 3.66^d	5.00 ± 0.38^{de}	1.43 ± 0.02^f
Ethanol				
90	7.69 ± 0.15^f	48.96 ± 2.17^{cd}	5.54 ± 0.21^{cd}	1.52 ± 0.12^f
70	7.74 ± 0.10^f	49.82 ± 0.93^c	5.96 ± 0.50^c	1.90 ± 0.08^d
50	7.18 ± 0.24^f	47.52 ± 2.27^{cd}	5.25 ± 0.25^d	2.13 ± 0.06^{bc}
30	5.25 ± 0.17^g	38.29 ± 1.14^{cd}	4.99 ± 0.29^{de}	1.74 ± 0.06^e
Acetone				
90	10.82 ± 1.80^{de}	50.93 ± 1.74^{cd}	5.71 ± 0.12^c	1.89 ± 0.22^d
70	8.78 ± 0.11^{ef}	47.98 ± 1.28^{cd}	5.54 ± 0.14^{cd}	2.16 ± 0.05^b
50	6.71 ± 0.10^f	39.04 ± 1.28^{cd}	5.00 ± 0.41^d	1.81 ± 0.05^{de}
30	6.20 ± 0.28^{fg}	37.97 ± 2.19^d	4.95 ± 0.32^e	1.96 ± 0.06^{cd}

Different letters mean values statistically different at 95% confidence level.

All the produced extracts showed antioxidant potential with similar results for DPPH radical scavenging activity (data not shown). Nevertheless, different behavior was observed for FRAP results (Table 1) where extracts obtained using 70% and 90% (v/v) methanol had significantly higher ($p < 0.05$) values (2.55 ± 0.09 and 2.73 ± 0.11 mM FE(II)/g dry wt plant, respectively) than the remaining ones.

In brief, extraction with 90% (v/v) methanol can be considered as an efficient way to recover phytoestrogens (NDGA, kaempferol and quercetin) from *Larrea tridentata* leaves. The extract obtained under this

condition is also a valuable source of natural products with antioxidant capacity, and might find a number of industrial applications, particularly in the medicinal field.

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