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Antioxidant activity of *Blechnum chilense* (Kaulf.) Mett., *Curcuma domestica* Valeton and *Tagetes verticillata* Lag. & Rodriguez

[Actividad antioxidante de *Blechnum chilense* (Kaulf.) Mett., *Curcuma domestica* Valeton y *Tagetes verticillata* Lag. & Rodríguez]

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Abstract
Because of the increasing interest in improving human health worldwide, phytochemical antioxidants from medicinal and food plants are of great interest. The search for new sources of antioxidants is important for the best use of biodiversity. The objective of this work was to evaluate the antioxidant activity and the total phenolic compounds with DPPH and Folin-Ciocalteu assays for extracts and fractions of *Blechnum chilense*, *Curcuma domestica* and *Tagetes verticillata*. *B. chilense* water-methanolic and EtOAc fractions, follows of *C. domestica* EtOAc extract showed an important quantity of total phenolic compounds. Compared with *Aristotelia chilensis* MeOH extract, *T. verticillata* extract showed good activity, follows by EtOAc fraction from *B. chilense* and by EtOAc extract from *C. domestica*, with very similar results with *n*-hexane fraction from *B. chilense* and petroleum ether extract from *C. domestica*. All of these results were greater than α-tocopherol DPPH scavenging activity. The results suggest that all plants studied could be are new sources of antioxidants and the work are following with the identification of these compounds.

Keywords: Antioxidant activity, total phenolic compounds, DPPH, Folin-Ciocalteu; *Blechnum chilense*, *Curcuma domestica*; *Tagetes verticillata*.

Resumen
Debido al creciente interés mundial en el mejoramiento de la salud humana los antioxidantes provenientes de plantas medicinales y alimenticias se han convertido en compuestos de gran interés. La búsqueda de nuevas fuentes de antioxidantes es importante para el mejor uso de la biodiversidad. El objetivo de este trabajo fue evaluar la actividad antioxidante y el contenido de fenoles totales usando el método de Folin-Ciocalteu y la actividad inhibitoria del radical DPPH de fracciones y extractos de *Blechnum chilense* (Kaulf.) Mett, *Curcuma domestica* Valeton y *Tagetes verticillata* Lag. & Rodr. Las fracciones acuosa-metanólica y EtOAc de *B. chilense*, seguida del extracto EtOAc de *C. domestica*, mostraron una cantidad importante de compuestos fenólicos. La prueba con DPPH mostró que la actividad secuestrante más importante, comparada con la del extracto metanólico de *Aristotelia chilensis* (Molina) Stuntz, fue la del extracto de *T. verticillata*, seguido de la fracción EtOAc de *B. chilense* y el extracto EtOAc de *C. domestica*, con resultados similares a la fracción hexánica de *B. chilense* y el extracto obtenido con éter de petróleo de *C. domestica*, superando todas la actividad secuestrante de DPPH del α-tocoferol. Los resultados sugieren que todas las plantas estudiadas podrían ser nuevas fuentes de antioxidantes y se está trabajando para la identificación de los compuestos responsables de la actividad.

Palabras Clave: Actividad antioxidante, compuestos fenólicos totales, DPPH, Folin-Ciocalteu; *Blechnum chilense*, *Curcuma domestica*; *Tagetes verticillata*.

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INTRODUCTION

Antioxidants delay oxidative processes, often by inhibiting polymerization chains initiated by free radicals and subsequent oxidation reactions (Halliwell and Aruoma, 1991). Both natural and synthetic substances with this activity have been used for food preservation and for protection against neurodegenerative and cardiovascular diseases and cancer (Prior *et al*., 2005). Even at low concentrations compared with oxidizable substances such as DNA, protein, lipid, or carbohydrate, these compounds delay or prevent oxidative damage due to the presence of reactive oxygen species (ROS) (Halliwell and Aruoma, 1991). In the presence of low concentrations of antioxidants, the breaking of radical chain reactions is considered to be the predominant mechanism (Pokorny *et al*., 1988). As a result of this activity antioxidants possess a wide spectrum of biochemical properties, such as anti-mutagenic activity and the ability to modify gene expression (Marinova *et al*., 2005).

Several methods are employed to measure antioxidant activity. Among these are the ORAC method (Cao *et al*., 1993; Ou *et al*., 2001), the FRAP method (Benzie and Strain, 1996; Chavez *et al*., 2011) and the DPPH assay (Jiménez-Escrig *et al*., 2001; Thounaujam *et al*., 2010; García Rodríguez *et al*., 2011; Chavez *et al*., 2011). We have chosen the DPPH assay because the results have been shown to be proportional to the total phenolic composition of plant extracts (Jiménez-Escrig *et al*., 2001), the method is accurate, repeatable, rapid, no special expensive equipment is required and the reagents are relatively inexpensive. The DPPH assay is an indirect method based on the ability of the 2,2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors including phenols (Roginsky and Lissi, 2005). It should be noted, however, that the units of antioxidant activity obtained with different antioxidant assays are generally parallel but are not identical as the methods are based on somewhat different radicals and procedures (Jiménez-Escrig *et al*., 2001).

Although not the only important antioxidant compounds in plants, phenolic substances comprise a large percentage of these substances in many plants that have been examined. Further, it has been suggested that phenolic compounds are the substances with the greatest antioxidant activity from natural sources (Rice-Evans, 2000). Phenolic compounds can undergo redox reactions with ROS and inhibit oxidant activity in a concentration dependent manner. Further, in many cases, the antioxidant activity of plant extracts is proportional to their total phenol content (Rice-Evans *et al*., 1997), suggesting a causative relationship between these properties (Veglioglu *et al*., 1998).

Phenolic compounds constitute a large group of secondary metabolites (more than 8000 compounds) that are widely distributed in a large number of plant species. Although isolation and characterization of the total complement of flavonoids from any plant is challenging and requires sophisticated instrumentation such as HPLC, LC-MS, and NMR spectrometry, the Folin-Ciocalteu method is a relatively straightforward procedure that is useful for determining the total phenolic content of an extract.

As a part of our continuing studies of commonly used medicinal plants of Colombia and Chile, a number of species and types of bioactivity have been examined. Previous studies of the phytochemistry of three exceptionally active species, *Blechnum chilense* (Kaulf.) Mett., *Curcuma domestica* Valeton and *Tagetes verticillata* Lag. & Rodr., and relevant literature about these and related plants suggest that they are especially rich in bioactive compounds.

Because of its diversity of climatic and geomorphological features, Chile has a variety of habitats inhabited by about 190 fern taxa, both native and endemic (Gunkel, 1983; Marticorena and Rodríguez, 1995). Thirteen species of the fern genus *Blechnum* are widely distributed in the country, from Coquimbo in the north to Patagonia in the south. These plants are used for a variety of purposes and are well-known in Chilean medicinal folklore. Both *Blechnum hastatum* Kaulf. and *B. chilense* have been employed as emmenagogues and abortive plants (Looser and Rodriguez, 2004). Plants of *B. occidentale* L. have been used to treat pulmonary and urinary diseases (Toursarkissian, 1980).

The antioxidant properties of six ferns used in Chinese traditional medicine, known as "Guisubu", have been determined (Chang *et al*., 2007). Aqueous extracts of the ferns *Davallia mariesii* T. Moore ex Baker and *Davallia solida* (G. Forst.) Sw. exhibited high levels of polyphenols and strong scavenging ability against DPPH radicals (Chang *et al*., 2007). Flavonoids isolated with used microwave-assisted techniques (Lijun, 2006) and ethyl acetate, butanol and aqueous fractions from *Blechnum orientale* L. (Lai *et al*., 2010) had strong radical scavenging activity. A mixture of α-, γ- and δ-tocopherols has previously been isolated from *B. chilense* (Strzałka *et al*., 2009).

The genus *Curcuma*, family Zingiberaceae, has more than 80 species that are native to the Indo-Malayan region and are widely distributed in African and the Australian tropics (Sasikumar, 2005). Many of these species are used for medicinal and food purposes. The antioxidant properties of *Curcuma* species (Miquel *et al*., 2002), such as *C. longa* (Chen *et al*., 2008; Srinivas *et al*., 1992; Ramsewak *et al*., 2000; Kumar *et al*., 2006; Chan *et al*., 2008; Chen *et al*., 2008; Singh *et al*., 2010), *C. aromatica* Salisb. (Al-Reza *et al*., 2010), *C. zanthorrhiza* Roxb. (Masuda *et al*., 1992; Ruslay *et al*., 2007; Chan *et al*., 2008), C. *zedoaria* (Berg.) Rosc. (Mau *et al*., 2003), *C. aeruginosa* Roxb. *C. mangga* Valeton & Zijp (Chan *et al*., 2008), and *C. amada* Roxb. (Policegoudra *et al*., 2007), are well known and much of this activity is due to a single compound, curcumin (Ramsewak *et al*., 2000; Miquel *et al*., 2002; Ruslay *et al*., 2007; Ak and Gülçin, 2008).

Asteraceae is the largest family of vascular plants with more than 23,000 species (Jeffrey, 2007). The genus *Tagetes* belongs to this large family.The mostly American genus *Tagetes* (family Asteraceae) with more than 50 known species (Mc Vaugh, 1984) originated in Central and South America (Kaplan, 1958). Many of these are used medicinally. The antimicrobial activity of *T. lucida* has been examined (Cespedes *et al*., 2006).

Although antioxidant activity has not been determined for *Tagetes verticillata* of South America, high levels of antioxidant activity from other species including *T. patula* L. (Blum and Didyk, 2007), *T. mendocina* Phil. (Schmeda-Hirschmann *et al*., 2004), *T. minuta* L. (Ranilla *et al*., 2010), *T. maxima* Kuntze (Parejo *et al*., 2003; 2005), and *T. lucida* Cav. (Aquino *et al*., 2002) have been reported. The antioxidant carotenoid lutein occurs in flowers of *T. erecta* L. (Gao *et al*, 2009; Piccaglia *et al*., 1988; Wang *et al*, 2006) and *T. patula* (Piccaglia *et al*., 1988).

Despite the recognized importance of these three species, neither the total phenolic concentration nor their antioxidant properties have been adequately examined. This paper reports total phenolic compounds as determined using the Folin-Ciocalteu assay and DPPH radical inhibitory activity produced by fractions and extracts from *B. chilense, C. domestica* and *T. verticillata*.

MATERIAL AND METHODS

Chemical and solvents

All reagents used were either A.R. or chromatographic grade and were purchased from Merck, Chile. These included petroleum ether 35-60, methanol, *n*-hexane, ethyl acetate, Folin–Ciocalteu reagent, DPPH, and gallic acid.

Instruments

IR spectra were recorded on a Shimadzu FTIR-8400, and UV spectra on a Genesis 5 instrument.

Plant material

B. chilense was collected on Confluencia-Trehuaco Road Km. 5.4, Itata Riverside, Ñuble, Chile. This botanical specimen was identified by Prof. Dra. Patricia Arancibia A. Voucher specimens have been deposited in the Herbarium of the Departamento de Ciencias Básicas, Universidad del Bio-Bio, Chillán, Chile (Voucher number 2010/05).

T. verticillata was collected in a rural area near Guarné, Antioquia, Colombia. *C. domestica* was purchased from farmers of the Uraba Region, Antioquia, Colombia. Rhizomes were obtained by vegetative reproduction. These botanical specimens were identified by Prof. Dr. Ramiro Fonnegra, Departamento de Biología, Universidad de Antioquia, Medellín, Colombia. Voucher specimens have been deposited at the Herbarium of the Universidad de Antioquia, Colombia (Voucher number 174516 and 174515 respectively).

Extraction of plant material

Dried leaves (646.5 g) of *B. chilense* were powered and extracted twice with methanol for a total of five days at room temperature. The combined extract was evaporated under reduced pressure to yield a greenish-gummy residue (crude extract) (198.2 g). The methanolic crude extract was then partitioned between *n*-hexane and EtOAc. From this procedure, an *n*-hexane fraction (BH), an EtOAc fraction (BE) and a residue of the aqueous-methanolic fraction (BWM) were obtained.

Based on preliminary assays of bioactivity, a petroleum ether extract of dried leaves of *T. verticillata* was prepared. Dried leaves (250 g) of *T. verticillata* were powered and extracted with petroleum ether at room temperature. The solvent was evaporated under reduced pressure to yield a *T. verticillata* petroleum ether extract (TPE) (8.2 g).

Based on preliminary assays of bioactivity, two extracts of dried rhizomes of *C. domestica* were prepared. Dried rhizomes (350 g) of *C. domestica* were powered and extracted with petroleum ether at room temperature. The extract was evaporated under reduced pressure to yield a *C. domestica* petroleum

ether extract (CPE) (5.2 g). A second batch of dried rhizomes (300 g) of *C. domestica* were powered and extracted with EtOAc at room temperature. The solvent was evaporated under reduced pressure to yield a *C. domestica* EtOAc extract (CE) (10.3 g).

Determination of total phenolic content

The total phenolic content of extracts was determined using the Folin–Ciocalteu (F-C) procedure. To evaluate the linearity of the F-C assay, calibration curves with gallic acid solution were prepared. These were made from 0.5 g of gallic acid plus 10 mL of ethanol, distilled water was added to make the total gallic acid solution to 100 mL. Aliquots of 0.2 mL extract at 8000 ppm or distilled water (control) were introduced into test tubes followed by 15.8 mL of distilled water, 1 mL of F-C reagent and 3 mL of sodium carbonate (20% w/v). Tubes were maintained in alternate cycles of agitation and settling during the test to keep a homogenous solution. Absorbance was read at 675 nm. Total phenolic contents were expressed as gallic acid equivalents (mg per 100 gram of extract). The gallic acid standard line has the equation $y = -0.00266 + 0.00163x$ ($R2 = 0.99232$), where y is absorbance at 675 nm and x is concentration of gallic acid in mg/L. All assays were conducted in triplicate (Bordeau and Scarpa, 1998; Yan *et al*. 2006) and the data obtained was analyzed with Origin 6.1 software.

Antioxidant activity:
(2,2-diphenyl-1-picrylhydrazyl (DPPH) essay

The free radical scavenging activity of the extracts and fractions was measured by the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of the extract. Samples of 4.9 mL of extracts and fractions at 8000 ppm. α-Tocopherol and methanolic extract from *Aristotelia chilensis* (Mol.) Stuntz as reference extract were added with 100 L de DPPH 5 mM (Cespedes et al., 2010). These solutions were allowed to settle for 30 minutes at 37° C. Subsequently, the absorbance was read at 517 nm using methanol as a blank. Absorbance decrease (AD) can be found from experimental data using: AD = DPPHA – DPPHSA + SA. DPPHA = DPPH absorbance; DPPHSA = sample with DPPH solution absorbance; SA = sample absorbance. The % inhibition = (AD of sample /AD of control)*100 (Masuda *et al*., 1999). This formula eliminates the effect of the extract absorbance. Later, the AD is compared with the AD from a known reference antioxidant extract and use of the following formula: (Sample AD/Reference antioxidant AD) x 100. We used *A. chilensis* as a standard because of its strong antioxidant properties (Miranda-Rottmann *at al*., 2002; Cespedes *et al*., 2008; Avello *et al*., 2009). Assays were carried out in triplicate.

Statistical analysis

Between F-C method and DPPH assay results we did a Simple correlation analysis. The data information was analyzed with Origin 6.1 software.

RESULTS

Estimation of total phenolic content

Based on the results of the Folin-Ciocalteu assay, only the aqueous-methanolic and EtOAc fractions of *B. chilense* and to a lesser degree the EtOAc extract of *C. domestica* contain the highest quantities of total phenol compounds (Figure 1, Table 1).

Antioxidant activity:
(2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Based on the DPPH assay, the most important scavenging activity (compared with an extract of *Aristotelia chilensis*, was shown by a petroleum ether extract of *T. verticillata* (TPE), followed by an EtOAc fraction from *B. chilense* (BE). The EtOAc extract of *C. domestica* (CE), the *n*-hexane fraction from *B. chilense* (BH) and the petroleum ether extract from *C. domestica* (CPE) produced similar, but somewhat lower results. All of these results were greater than those observed for α-tocopherol DPPH scavenging activity (Figure 1, Table 1).

The correlation between total phenolic content and DPPH radical inhibitory activity show a no statistically significant correlation in evaluated extracts ($R^2 = 0.2073$; P value > 0.05).

DISCUSSION

A majority of plant-derived antioxidants can be divided into groups based on solubility of the compounds involved. Best-known among the lipophilic type are α-, γ- and δ-tocopherol. These are generally soluble in non-polar solvents such as petroleum ether, butanol, and to a lesser degree ethyl acetate.

Antioxidant activity of lipophilic extracts

The total tocopherol content of *B. chilense* consists of a mixture of α-, γ- and δ-tocopherols in amounts of 56.3 ± 1.6; 1.9 ± 0.4 and 1.1 ± 0.07 μg/g DW, respectively (Strzałka *et al*., 2009). The DPPH

scavenging activity of EtOAc and butanol extracts of this fern had a lower IC_{50} than α-tocopherol (Lai *et al*. 2010), although in the present study EtOAc and *n*-hexane fractions from *B. chilense* showed greater DPPH inhibitory activity than α-tocopherol. As these fractions showed greater DPPH inhibitory activity than pure α-tocopherol, tocopherol content in lipophylic extracts of *B. chilense* may be only partially responsible for antioxidant activity.

Curcuma species have pronounced antioxidant activity (Ramsewak *et al*., 2000; Miquel *et al*., 2002; Ruslay *et al*., 2007; Ak and Gülçin, 2008). The scavenging of DPPH radical activity with both ethyl acetate (CE) and petroleum ether (CPE) extracts (75.0% and 73.8%, respectively) of *C. longa* (syn. *C. domestica*) was greater that of α-tocopherol (Table 1). The DPPH scavenging activity of essential oil from *C.*

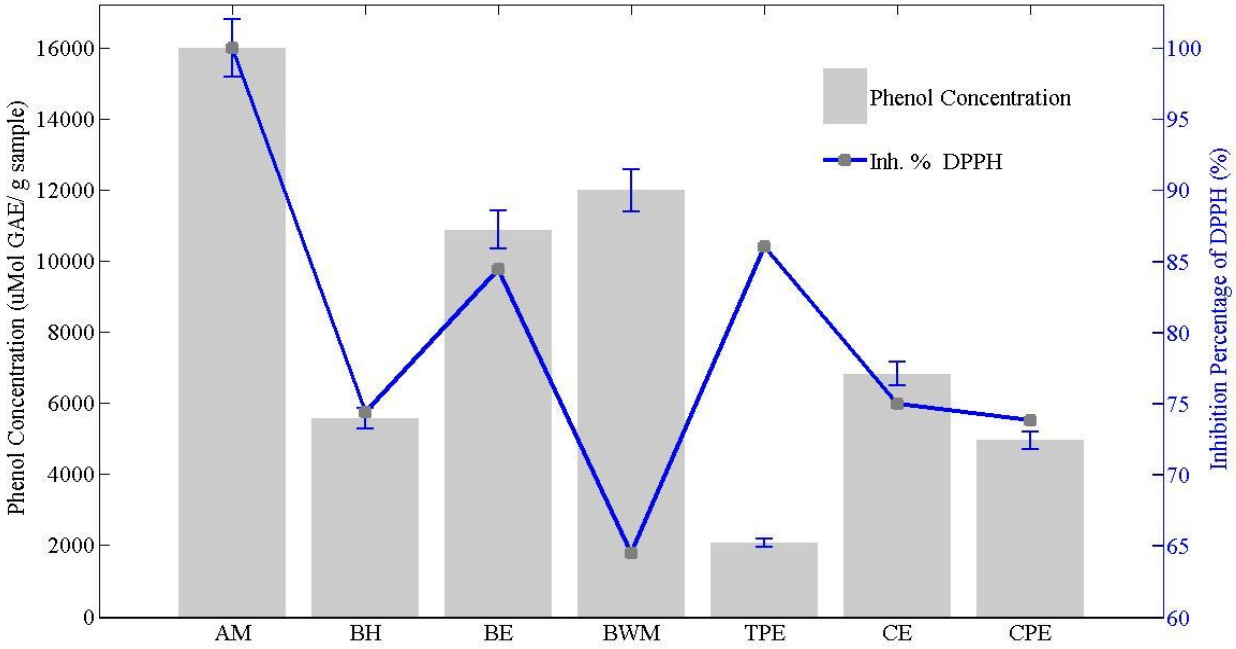

Figure 1. Total phenolic concentration and inhibitory percentage of every extract and fraction. (AM= *A. chilensis* methanolic fraction, BH= *B. chilense* hexane fraction , BE= *B. chilense* EtOAc fraction , BWM=. *B. chilense* aqueous-methanolic fraction , TPE= *T. verticillata* petroleum ether extract, CE= *C. domestica* EtOAc extract , CPE= *C. domestica* petroleum ether extract).

Table 1. Total phenolic content and inhibitory percentage of DPPH comparing to *Aristotelia chilensis* of every extract studied

Extract	Phenolic concentration* (μMol GAE/g sample)	Inhibition Percentage of DPPH (%) **
MeOH, fruits of *A. chilensis***	15,987.00 ± 799.35	100.00%
n-hexane *B. chilensis*	5,571.82 ± 278.89	74.43%
EtOAc *B. chilensis*	10,879.01 ± 543.95	84.47%
H_2O/MeOH (1:1) *B. chilensis*	11,988.72 ± 599.44	64.55%
Petrol ether *T. verticillata*	2,067.51 ± 103.38	86.02%
EtOAc *C. domestica*	6,829.63 ± 341.48	75.00%
Petrol ether *C. domestica*	4,951.55± 247.58	73.87%
α-Tocopherol	Not determined	68.16%

* GAE: Gallic acid equivalents.

** With respect to reference antioxidant extract (MeOH *A. chilensis*)

***μMol Cat-Eq/g sample

zedoaria at 20 mg/mL was comparable to that of α-tocopherol (Mau *et al*., 2003), but differences in the material tested make this value difficult to compare to those of the present study. However, based on a number of studies, the main antioxidant from *Curcuma* species is a lipophylic phenylpropanoid/polyketide-derived phenolic compound curcumin (Ramsewak *et al*., 2000; Miquel *et al*., 2002; Ruslay *et al*., 2007; Ak and Gülçin, 2008). In one species, *C. xanthorrhiza*, structurally similar compounds such as bisdemethoxycurcumin and demethoxycurcumin contribute to the activity (Ruslay *et al*., 2007).

Three of four fractions of a butanolic extract from *T. lucida* had higher DPPH inhibitory activity than α-tocopherol (Aquino *et al*. 2002), as did the *T. verticillata* petroleum ether extract in the present work.

Antioxidant properties of methanolic and aqueous methanolic extracts

Phenolic compounds comprise the largest group of antioxidant compounds soluble in solvents of medium-polarity such as methanol and aqueous-methanol mixtures. These include simple phenolic compounds, phenylpropanoids, stilbenes, and flavonoids of several structural types such as flavones, flavonols, anthocyanins, and proanthocyanidins. Solubility is determined by the number of unsubstituted phenolic hydroxyl and carboxyl groups as well as sugar substitutions, prenyl groups and other more non-polar substituents. High levels of hydroxycinnamic acid and quercetin derivatives (32.0 ± 2.0 and 10.0 ± 1.0 mg/g dw expressed as chlorogenic acid and quercetin aglycone, respectively) were found in phytochemical analyses of *T. minuta* extracts (Ranilla *et al*., 2010). Although flavonoids are frequently responsible for antioxidant activity, this finding suggests that other phenolic compounds are responsible for much of the high antioxidant activity observed in extracts of this species.

As noted above, the phenolic content of many plants is correlated with antioxidant properties of plant extracts usually measured as radical scavenging ability (Rice-Evans *et al*., 1997; Veglioglu *et al*., 1998). Alternatively, other studies observed low phenolic content and moderate to strong DPPH inhibitory activity (Kähkönen *et al*., 2001; Atoui *et al*., 2005; Meda *et al*., 2005; Echavarría *et al*., 2009; Moein and Moein, 2010). In a similar manner, we found no statistically significant correlation between total phenolic content and DPPH radical inhibitory activity ($R^2 = 0.2073$) in evaluated extracts.

The antioxidant activity of *C. amada* appears to be independent of total phenolic content (Policegoudra *et al*., 2007). In our study, the phenolic content of *Curcuma* extracts (6,829 and 4,951 mMol GAE/g sample, DPPH inhibitory percentages of 75.00 and 73.87%) was lower but the DPPH radical inhibitory activity higher (per amount of antioxidant in the plant) than that of the *B. chilense* EtOAc fraction (10.879 mMol GAE/g sample, DPPH inhibitory percentages of 84.47%). In an earlier study, methanolic extracts from *C. longa* were shown to exhibit variation in phenolic content and DPPH scavenging activity (Chen *et al*., 2008). These results suggest only a loose correlation between total phenolic content and DPPH inhibition activity from *C. longa* extracts.

Based on literature reports, total phenolic content and the complement of compounds present in *Tagetes* species appears to vary. Based on the Folin-Ciocalteu assay, an aqueous extract of *T. minuta* contained 67.0 ± 7.0 mg/g total phenolic compounds (Ranilla *et al*. 2010). The Southern Cone species *T. mendocina* has been demonstrated to possess 3% total phenolic compounds and correspondingly high DPPH inhibitory activity (Schmeda-Hirschmann *et al*., 2004). High levels of total phenolic compounds in an EtOAc fraction from *T. maxima* were found. In this instance, both non-polar and more polar fractions were reported to have high levels of DPPH inhibition activity (Parejo *et al*., 2003).

Many phenolic compounds have been reported from *Tagetes* species. Use of assay-guided isolation of antioxidant compounds in extracts of *T. mendocina* led to isolation and c - hydroxyacetophenone, protocatechuic acid, syringic acid, patuletin, quercetagetin 7-*O*-β-D-glucoside, patuletin 7-*O*-β-D-glucoside and axillarin 7-*O*- β-D-glucoside (Schmeda-Hirschmann *et al*., 2004). Thus, these results suggest that quercetin derivatives are the most important compounds responsible for antioxidant activity of several *Tagetes* species. Methanolic extracts of *T. maxima* contained a series of acylated quercetagetin glycosides: quercetagetin-7-*O*-(6-*O*-caffeoyl-β-D-glucopyranoside), quercetagetin-7-*O*-(6-*O*-*p*-coumaroyl-β-D-glucopyranoside), quercetagetin-7-*O*-(6-*O*- - - - -tri-*O*-methylquercetagetin-7-*O*-β-D-glucopyranoside (centaureidin-7-*O*-β-D-glucopyranoside), quercetagetin-7-

O-β-D-glucopyranoside, as well as 6-hydro-xykaempferol-7-*O*-(6-*O*-caffeoyl-β-D-glucopyranosi-de) and patuletin-7-*O*-β-D-glucopyranoside (Parejo *et al*., 2005). The most powerful antioxidant active fractions were shown to contain quercetagenin 7-*O*-β–D-glucopyranoside, quercetagenin 3-methyl ether 7-*O*-β–D-glucopyranoside, 6-hydroxykaempferol-7-*O*- - - -dimethyl ether as the main components (Aquino *et al*., 2002).

In a later paper, Parejo *et al*. (2005), found that the EtOAc fraction from *T. maxima* had the highest levels of total phenolic content and DPPH inhibitory activity, but the *n*-hexane fraction showed a higher level of total phenolic content (123.03 ± 11.36 GAE/mg extract) than the same fraction in the previous paper (28.8 ± 0.7 GAE/mg extract) (Parejo *et al*., 2003). This finding suggests that the DPPH inhibitory activity of the EtOAc fraction from *T. verticillata* should also be investigated. The observation that some extracts of *T. verticillata* with a low level of total phenolic compounds exhibit potent DPPH radical inhibition, such the petroleum ether extract (2,067.5 μMGAE/g, 86 % inhibition of DPPH, (Table 1) is puzzling.

These correlations can be explained because other readily oxidizable compounds also may react with F-C reagent (Meda *et al*., 2005). Further, the susceptibility of compounds to oxidation in the F-C assay also depends on their chemical structure as well as other components of the sample. Thus, the radical scavenging activity of an extract cannot be predicted only on the basis of its total phenolic content because, in many cases, the antioxidant activity results from the presence of different compounds and their mixtures (Parejo *et al*., 2002; Atoui *et al*., 2005; Meda *et al*., 2005). This could be the case of the *B. chilense* aqueous-methanolic extract (high levels of total phenolic compounds and low radical DPPH inhibition levels) with a great possibility of the presence of different kinds of compounds in the extract due to solvent properties.

The relationship between antioxidant activity and total phenolic content of some plants is complex and, for that reason, is so difficult to describe it taking in account only the presence of phenolic compounds (Kähkönen *et al*., 2001). This can be explained by the antioxidant properties of individual compounds in a mixture. The composition of the mixture can vary considerably and even though the mixture possesses the same levels of total phenolic compounds it may not have the same level of antioxidant activity.

Different methods for measurement of the antioxidant activity are based on different reaction mechanisms and sometimes give different results.

Another explanation is that extracts are complex mixtures of compounds with different polarities and antioxidant and prooxidant properties, and those changes in activity may be caused by synergies and antagonisms between or among these compounds (Kähkönen *et al*., 2001; Parejo *et al*., 2002). We concludes that total phenolic content can not be used to predict the radical scavenging activity of an extract, a conclusion shared with Kähkönen *et al*. (1999) and Parejo *et al*. (2002).

CONCLUSIONS

The aqueous-methanolic and EtOAc fractions of *B. chilense*, the *C. domestica* EtOAc extract, and the *T. verticillata* petroleum ether extracts are promising candidates for future identification of individual compounds and subsequent determination of their antioxidant activity. The activity observed may be due to mixtures or individual compounds including the possibility of synergies or antagonisms.

The results obtained demonstrate examination of plants with the Folin-Ciocalteu and free radical DPPH inhibition assays may reveal new sources and interactions of antioxidant compounds. This potential could be useful in the food, pharmaceutical and cosmetology industries. It is strongly recommended that studies of the isolation and identification the compounds responsible for antioxidant activity from each of these plants be pursued.

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