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Antioxidant Capacity of *Prosthechea karwinskii* (Orchidaceae) Extracts Obtained by Sonication

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Abstract: *Prosthechea karwinskii* is a valuable ornamental orchid in Oaxaca, Mexico. In the Mixteca region, this plant has uses in traditional medicine as well as in religious ceremonies. The presence of phenolic compounds with antioxidant potential in this orchid has been reported, although not determined. Considering the advantages of the use of sonication in secondary metabolite extraction from medicinal plants, hydroethanolic and hydroethanolic extracts were obtained from each part of this orchid (pseudobulb, leaf and flower) using sonication pretreatment in order to determine antioxidant capacity. The results indicated that both hydroethanolic (IC₅₀: 3.52 ± 0.078 mg / ml) and hydromethanolic (IC₅₀: 8.88 ± 1.826 mg / ml) leaf extracts had a higher antioxidant capacity than pseudobulbs and flower extracts. Leaf extracts also exhibited inhibition in the breast cancer cell line (MCF-7). The results demonstrated the antioxidant capacity of this orchid and its potential for application in assessments related to oxidative stress diseases.

Key words: antioxidant activity index, cytotoxicity, extraction methods, medicinal plants.

INTRODUCTION

Plant extracts have been widely used in the food and pharmaceutical industries and their quality often depends on the extraction method used to prepare the compounds of interest in terms of antioxidant,

antifungal and flavoring properties. In the pharmaceutical industry the solvent extraction of bioactive compounds from plant material is a routine procedure. Recent technological advances and growing medical interest in plant-derived pharmaceuticals, however, have resulted in an increasing importance being placed on more efficient extraction methods that use alternative energy^{1, 2}. Sonication is one of the alternative methods for obtaining secondary metabolites; it favors performance and reduces extraction time. Sonication applies sound energy to produce cavitation bubbles that implode as a result of the shearing force produced in the solvent by the passage of an ultrasonic wave; causing the rupture of the cells, increasing solvent transfer into the sample matrix (or mass transfer) and increasing the contact surface area between the solvent and the secondary metabolites^{3, 4}. Phenolic compounds, one of the most important groups of compounds in plants, have attracted great interest for their ability to stabilize or deactivate free radicals before they attack cells. These compounds are thus able to protect organisms against damage caused by oxidative stress from free radicals⁵. *Prosthechea karwinskii* is an ornamental orchid with showy and fragrant flowers. In the Mixteca region of Oaxaca this plant is also used in traditional medicine for the treatment of hyperglycemia (pseudobulb and leaves), coughs (pseudobulb and flowers), and burns (pseudobulb) and to minimize the risk of a miscarriage (flowers)⁶. A previous study of this orchid identified several phenolic compounds⁷ associated with important antioxidant activity, which have not yet been identified in the present species.

In this work the methanolic and ethanolic extracts of pseudobulbs, leaves and flowers from *P. karwinskii* underwent sonication pretreatment in order to evaluate their antioxidant and cytotoxic activity.

METHODS

Plant material and extracts: The plant material was obtained from *Prosthechea karwinskii* specimens previously used as ornaments for Easter celebrations in Zaachila, Oaxaca, with the permission of the organizers of the festivities. A specimen was then entered in the Instituto Politécnico Nacional's Herbarium OAX. The plant material was separated into pseudobulbs, leaves and flowers; each portion was dried, pulverized and stored at room temperature (RT) until use. The hydroethanolic extract (HE) was prepared by placing 10 g of each part of the orchid in 400 ml of an ethanol-deionized water solution (1: 1) for 7 days at RT with constant stirring. The sample was then filtered and evaporated to RT. The hydromethanolic extract (HM) was obtained by placing the sample under reflux for 60 minutes with a methanol-water mixture (70:30). To obtain the extracts with sonication pretreatment prior to the extraction procedure noted above, each sample was sonicated (70W) for 20 minutes. The obtained extracts were evaporated mechanically for 24 hours at RT.

Determination of antioxidant activity index: The antioxidant activity of the extracts was determined by the method of DPPH in a concentration of 0.1 mM according to a modification of the method of Brand *et al.*⁸, the concentration needed to inhibit 50% of free radicals (IC50) was determined, the antioxidant activity index (AAI) was determined as the ratio of the final concentration⁹ of DPPH and the IC50.

Data analysis: The results obtained in the determination of total flavonoids and AAI are expressed as the mean \pm standard deviation. Data were evaluated using the Minitab 16 software. Statistical significance was determined with an analysis of variance and a Tukey multiple range test was used to test for significant differences between the different extractions methods used. For all analyses the level of significance was $P < 0.05$.

Assay of cell growth inhibition: The extracts showing the highest antioxidant activity index were evaluated for cytotoxic activity on six human cancer cell lines: central nervous system glia (U251), prostate (PC-3),

leukemia (K562), colon (HCT-15), breast (MCF-7) and lung (SKLU). The cell lines were obtained from the National Cancer Institute (NCI) from the United States of America. The cytotoxicity of the extracts was determined in microcultures, measuring cell viability and growth with the Sulforhodamine B assay according to procedures validated by NCI.

RESULTS

The results in Table 1 indicate that the *Prosthechea karwinskii* extract with the highest antioxidant activity was the maceration with ultrasound leaf extract (HESleaves) with values of $IC_{50} = 3.52 \pm 0.078 \mu\text{g/ml}$ and $AAI = 11.2 \pm 0.251$. These values are higher than those reported for the ethanol-water leaf extract of *Bauhinia kalbreyeri*¹⁰ with AAI 2.1406 and methanolic leaf extract of the orchid *Dendrobium speciosum*¹¹ with $AAI 3.46 \times 10^{-5}$.

Table 1: 50% inhibitory concentration (IC_{50}), antioxidant activity index (AAI) of the hydromethanolic and hydroethanolic extracts of *Prosthechea karwinskii*

Extract	IC_{50} (mg/ml)	AAI
HESpseudobulb	11.81 ± 0.320	3.33 ± 0.090
HESleaves	3.52 ± 0.078	$11.2 \pm 0.251^*$
HESflowers	44.92 ± 3.740	0.79 ± 0.151
HMSpseudobulb	15.46 ± 1.421	2.55 ± 0.233
HMSleaves	8.88 ± 1.826	4.54 ± 0.838
HMSflowers	24.70 ± 1.381	1.59 ± 0.086
C ₆ H ₆ O ₆	2.68 ± 0.03	$14.71 \pm 0.165^*$

C₆H₆O₆: ascorbic acid, HE: hydroethanolic extract, HM: hydromethanolic extract, S: sonication. The values represent the mean \pm SD, superscript values show statistically significant difference as revealed by the Tukey test ($P < 0.05$).

The HESpseudobulb extract for *P. karwinskii* AAI was 3.33 ± 0.090 , higher in comparison to hydromethanolic and chloroform pseudobulb extracts reported for another Mexican orchid, *Prosthechea michuacana*¹² with AAI values of 0.0901 and 0.1268, respectively. For HMSleaves *P. karwinskii* extract, the value of 4.54 ± 0.838 AAI is higher compared with other plant extracts obtained in the same method as in leaves of *Juglans regia*¹³, AAI = 1.98×10^{-9} *Jasminum humile*,¹⁴ AAI=0.5598 and rhizomes of *Curculigo orchoides*,¹⁵ AAI=0.3720.

Table 2: Percent inhibition in cancer cell line

Extract	U251	PC-3	K562	HCT-15	MCF-7	SKLU-1
HESleaves	NE	2.6	NE	7.8	13.8	4.8
HMSleaves	NE	NE	NE	6.0	13.0	NA

Concentration, (50 $\mu\text{g/ml}$) vehicle DMSO. U251= central nervous system glia, PC-3= prostate, K562= leukemia, HCT-15= colon, MCF-7=breast, SKLU= lung, NE= no effect.

In Table 2 the percent inhibition results in cancer cell lines are shown. The leaf extracts with antioxidant activity that received sonication pretreatments were more effective in the inhibition of growth in breast cancer cells (MCF7). The antioxidant activity of a sample is considered as an indicator that favors the activation of redox type metabolism in cells where a highly oxidative environment¹⁶ is generated due to cellular inflammation, forming reactive oxygen species. These cause oxidative cell damage generating pro-

inflammatory mediators such as cytokines, chemokines and prostaglandins that are converted into angiogenesis initiators, a fundamental process in the malignant transformation of tumor growth and other diseases. Hence, it is understood that at low concentrations of reactive oxygen species, due to the antioxidant effect, the response mechanism against diseases such as cancer, diabetes, arthritis, atherosclerosis, ischemia, immune and endocrine system is enhanced^{16,17}.

CONCLUSIONS

Sonication promotes plant cell breakage, thus increasing the mass transfer between the solvent and the secondary metabolites and enhancing extracts biological activity. The effect of sonication was higher in the extracts obtained from *Prosthechea karwinskii* leaves as demonstrated in the results of AAI.

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REFERENCES

1. J. Londoño-Londoño, V. Rodrigues de Lima, O. Lara, A. Gil, T.B. Crecsynski Pasa, G.J. Arango, J.R. Ramírez Pineda. Clean recovery of antioxidant flavonoids from citrus peel: Optimizing an aqueous ultrasound-assisted extraction method. *Food Chem*, 2010, (119):81-7.
2. L.M. McCune, T. Johns. Antioxidant activity relates to plant part, life form and growing condition in some diabetes remedies. *J Ethnopharmacol*, 2007, (112):461-9.
3. L. Petigny, S. Périno-Issartier, J. Wajsman, F. Chemat. Batch and Continuous Ultrasound Assisted Extraction of Boldo Leaves (*Peumus boldus* Mol.) *Int. J Mol. Sci*, 2013, (14):5750-64
4. M.N. Soares Melecchi, V. Flores Péres, C. Dariva, C.A. Zini, F.C. Abad, M. Martínez, E.B. Caramao. Optimization of the sonication extraction method of *Hibiscus tiliaceus* L. Flowers. *Ultrason Sonochem*, 2006 (13):242-50
5. M. Biesaga. Influence of extraction methods on stability of flavonoids. *J Chrom A*, 2011, . (1218):2505-12.
6. G. Cruz García, R. Solano Gómez, L. Lagunez Rivera. Documentation of the medicinal knowledge of *Prosthechea karwinskii* in a Mixtec community in Mexico. *Rev Bras Farmacogn*, 2014, (24): 153-8.
7. O. Mijangos-Ricardez. *Optimización de métodos de extracción con energías auxiliares y caracterización de la fracción fenólica de Prosthechea karwinskii y Prosthechea varicosa*. Instituto Politécnico Nacional CIIDIR Oaxaca Pp, 2010, 54-60.
8. W. Brand-Williams, M.E. Cuvelier & C. Berset. Use of a free radical method to evaluate antioxidant activity. *LWT – Food and Sci Tech*, 1995, (28): 25-33.
9. R. Scherer, H.T. Godoy. Antioxidant Activity Index (AAI) by de 2, 2-diphenyl-1-picrylhydrazyl method. *Food Chem*, 2009, .(112) 654-8
10. E. Murillo, O. Lombo, M. Tique, J.J. Méndez. Potencial antioxidante de *Bauhinia kalbreyeri* Harms (FABACEAE). *Información Tecnológica*, 2007, 18(6):65-74
11. M. Moretti, L. Cossignani, F. Messina, L. Dominici, M. Villarini, M. Curini, M.C. Marcotullio.

- Antigenotoxic effect, composition and antioxidant activity of *Dendrobium speciosum*. *Food Chem.*, 2013, 140 (4):660-5
12. A.M. Neira-González. *Aislamiento e identificación de los compuestos con actividad antioxidante del extracto de cloroformo de la orquídea comestible Prosthechea michuacana*. Instituto Politécnico Nacional. México D.F.Pp, 2009, 45-119
 13. M. Carvalho, P.J. Ferreira, V.S. Mendes R. Silva, J.A. Pereira, C. Jerónimo, B.M. Silva. Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. *Food and Chem Toxicol*, 2010, (48):441-67
 14. P. Nain, A. Kumar, S. Sharma, J. Nain. In vitro evaluation of antimicrobial and antioxidant activities of methanolic extract of *Jasminum humile* Leaves. *Asian Pac J Trop Med.*, 2011, 804-7.
 15. A.R. Bafna, S.H. Mishra. In vitro antioxidant activity of methanol extract of rhizomes of *Curculigo orchoides* Gaertn. *Ars Pharm*, 2005, 2 (46): 125-38
 16. P. Rajendran, N. Nandakumar, T. Rengarajan, R. Palaniswami, E.N. Gnanadhas, U. Lakshminarasiah, J. Gopas, I. Nishigaki. *Clin Chim Acta*, 2014, (436):332-347
 17. C. Costa, J. Incio, R. Soares. Angiogenesis and chronic inflammation: cause or consequence? *Angiogenesis*, 2007, (10):149–66.

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