IDECEFYN
Molecular Medicinal Chemistry
vol 21 January-April 2010, 113-116 http://www.idecefyn.com.ar ISSN 1666-888X

Detection of boldine via HPLC in *Peumus boldus* Molina propagated by *in vitro* culture

Daniela Ríos, Daniel Sandoval, Ángela Pineda[2], Cristian Gómez[1]

[1]Laboratorio de Biotecnología Vegetal, Facultad de Ingeniería y Tecnología, Universidad San Sebastián. [2]Laboratorio de Especialidades Farmacéuticas, Facultad de Ciencias de la Salud, Universidad San Sebastián. General Cruz 1577 Concepción, Chile.

ABSTRACT

Boldo (*Peumus boldus* Molina) is an endemic species from Chile. Its aroma is due to its essence that the active ingredients are mainly apomorphine alkaloids, among which boldine is predominant. Boldine, has presented antimicrobial activity, hepatoprotective, cholagogue, antispasmodic, sedative and antioxidant properties. The present study focuses on the evaluation of boldine content in plant extracts from *in vitro*-cultured nodal segments in relation to its mother plant and a sample of wild *Peumus boldus*, trough high performance liquid chromatography (HPLC). The sample analyses show the presence of boldine not only in samples of boldo from wild plants and mother plants but also in *in vitro*-propagated plant material. In conclusion the methodology developed allowed to detect boldine *in vitro*-propagated *Peumus boldus* Molina.

Keywords: boldine, HPLC.

Corresponding author: Corresponding Author: Cristian Gómez, e-mail: cgomez@uss.cl

Received: February 22, 2010. Accepted: March 10, 2010

Molecular Medicinal Chemistry
IDECEFYN
vol 21 January-April 2010, 113-116 http://www.idecefyn.com.ar ISSN 1666-888X

Introduction

Boldo (*Peumus boldus* Molina) is an endemic species from Chile belonging to the family Monimiaceae (Durán, 2005). Its geographical distribution is mainly in the central provinces, between Curicó and the Bio Bio region. Its aroma is due to its essence (1.5 to 2.4 mL per 100 g of dry leaves). The active ingredients are mainly apomorphine alkaloids (1-3% p/p), among which boldine is predominant (Vogel *et al*., 2008)

Boldine ($C_{19}H_{21}NO_4$; molecular mass 327.4 g/mol in anhydrous form), or [(S)-2.9-dihydroxy-1, 10-dimethoxyaporphine], has presented antimicrobial activity in *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* (Schrickel *et al*., 2001). It has hepatoprotective, cholagogue, antispasmodic, sedative, antioxidant properties, as well as regulating liver function (Muñoz *et al*., 2001).

In vitro culture is no only used for propagation purposes, but also for the production of secondary metabolites, given that this technique can help overcome natural and economic barriers (Namdeo, 2007).

The exploitation of the boldo leaf in Chile requires a management plan which must include justification for the extraction of raw material, sample characteristics and quantity in kilograms of dry leaf to be extracted (Ábalos, 2001). The present study focuses on the evaluation of boldine content in plant extracts from *in vitro*-cultured nodal segments in relation to its mother plant and a sample of wild *Peumus boldus*.

Experimental

Plant material

Wild *Peumus boldus* plant leaves (Ps), mother plant leaves (Pm) and seedlings from *in vitro*-cultivated mother plants Piv) were cut at the petiole and kept for 28 days in a Murashige & Skoog (1962) medium with a 25% reduction of macronutrients, supplemented with sucrose 30 g/L and agar 8 g/L. The material was dried on absorbent paper during 30 days in darkness at room temperature. The dried tissue was then ground in a mortar with liquid nitrogen.

Extraction

For every 50 g of dry powder obtained, 250 mL of 99.9% methanol was added for maceration at 50°C for 1 hour and then filtered. The product was concentrated at 50°C for two hours in a thermostabilized bath. 400 mL of acidified water was added to the extract with 2 to 3 drops of 1% acetic acid. It was then filtered and washed three times with 150 mL of ether, after which the aqueous extract was basified to pH 9.5 with 25% ammonium hydroxide (NH_4OH). It was subsequently washed three times with 200 mL of dichloromethane (CH_2Cl_2). The apolar extracts were dried under an extraction hood for 8 hours. Finally, 50 mL of 99.9% methanol was added for every 50 mg of residue and the extract was stored at 4°C in an amber bottle for posterior analysis.

Detection of boldine by means of HPLC

(Stévigny *et al*., 2004).

Stationary phase: Supelco Discovery® RP Amide C16 HPLC column (15 cm x 4.6 mm, 5 µm).

Mobile phase: Solvent A: acetonitrile 99.9% purity (Merck), Solvent B: ammonium acetate 10 mM, adjusted to pH 3 with acetic acid-acetonitrile solution 90:10 v/v, in gradient elution, as shown in Table 1.

Sample solutions: MeOH extract from wild plants (Ps), MeOH extract from mother plants (Pm) and MeOH extract from *in vitro*-cultivated seedlings (Piv).

Standard solution: Fluka Analytical®, B 3916-1G (Sigma Aldrich) boldine, in filtered 99.9% methanol (0.2µm) achieving a concentration of 1mg mL^{-1}

Sample treatment: prior to analysis via HPLC, the extract was filtered through a 0.2µm Millipore membrane.

Chromatographic conditions: flow: 0.7 mL/min; temperature: 22±2ºC; injection volume: 20 µL; UV detection: 302 nm; gradient conditions are shown in Table 1; integration parameter: area.

Results and discussion

The detection of the alkaloid boldine present in the MeOH extracts samples, was evaluated in relation to the peak and the retention time (t_R) of the standard solution during a total running time of 25 minutes.

The sample analyses show the presence of boldine not only in samples of *Peumus boldus* from wild plants and mother plants but also in *in vitro*-propagated plant material. These results confirm statements by Sepúlveda *et al*.(2003) in relation to the production of alkaloids as a response to both biotic and abiotic stress, given that the *in vitro* condition represents abiotic stress.

Molecular Medicinal Chemistry
IDECEFYN
vol 21 January-April 2010, 113-116 http://www.idecefyn.com.ar ISSN 1666-888X

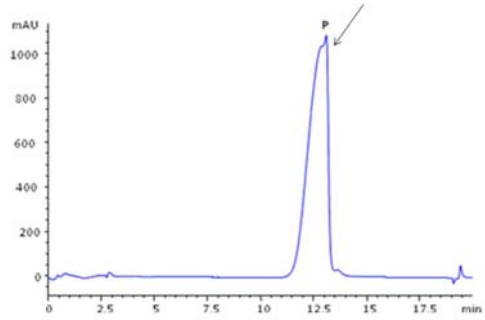

Figure 1. Chromatogram of standard solution of boldine (P) t_R: 12.097 minutes (see arrow).

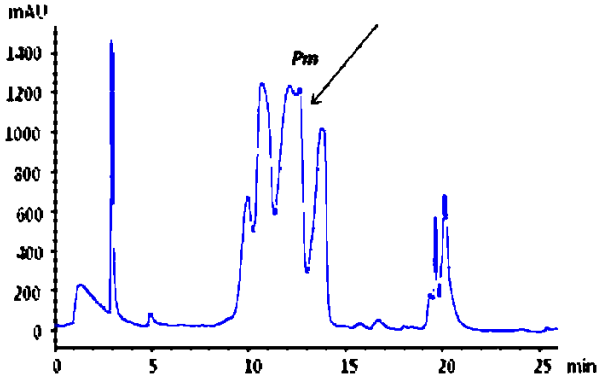

Figure 2. Chromatogram of the sample corresponding to *Peumus boldus* leaf extract from mother plants (Pm), t_R: 12.487 minutes (see arrow).

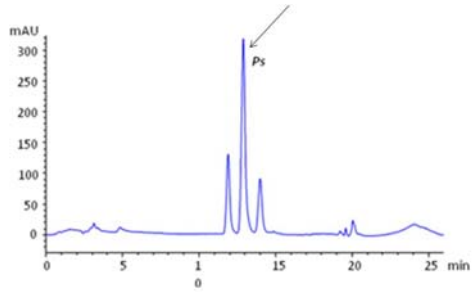

Figure 3. Chromatogram of the sample corresponding to *Peumus boldus* leaf extract from wild plants (Ps), tR:12.889 minutes (see arrow).

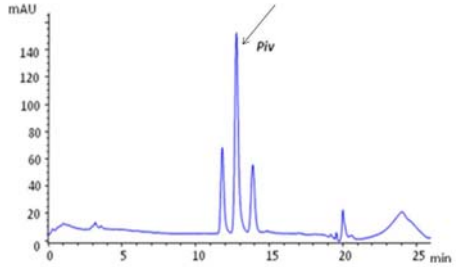

Figure 4 Chromatogram of the sample corresponding to *Peumus boldus* leaf extract from *in vitro*-cultivated seedlings (Piv), t_R: 12.717 minutes (see arrow).

In addition, qualitative changes are observed in the profiles of both Piv and Ps samples in relation to Pm. Further research should be carried out in the evaluation of boldine content in plant material of different origins, especially of *in vitro*-propagated origin.

Table 1. Gradient elution used in high efficiency liquid chromatography in samples of *Peumus boldus* Molina.

Time (min)	Solvent A (v/v)	Solvent B (v/v)
0	100	0.00
5	90.50	9.50
10	90.25	9.75
15	90.00	10.00
20	50.00	50.00
25	0.00	100

Conclusion

The methodology developed allowed to detect boldine *in vitro*-propagated *Peumus boldus* Molina.

Acknowledgements

The authors would like to thank the Subcomité Regional de Innovación (Regional Subcommittee for Innovation) INNOVA BIOBÍO CORFO for co-funding through the Universidad San Sebastián's seed capital sponsoring department (Unidad Patrocinadora Capital Semilla).

Note: Part of this study was presented at the 'II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

References

-Ábalos M., (2001). *Productos forestales no madereros en América Latina. Proyecto información y análisis para el manejo forestal sostenible. Integrando esfuerzos nacionales e internacionales en 13 países tropicales en América Latina*. FAO. 199p.

-Durán P. (2005). *Evaluación de la producción y productividad en biomasa aérea de Boldo (Peumus boldus Mol.) en un bosque esclerófilo de la comuna de María Pinto, provincia de Melipilla, región Metropolitana*. Memoria de título profesional de Ingeniero Forestal. Facultad de

Molecular Medicinal Chemistry

IDECEFYN

vol 21 January-April 2010, 113-116 http://www.idecefyn.com.ar ISSN 1666-888X

Ciencias Forestales. Universidad de Chile. Santiago, Chile.

-Muñoz, O., Montes M. y Wilkomirsky T., (2001). *Plantas medicinales de uso en Chile: química y farmacología. Universidad de Chile*. Editorial Universitaria. Santiago, Chile. 330p.

-Murashige, T. y Skoog F. (1962). A revised medium for rapid growth and bio assay with tobacco tissue culture. *Physiologia Plantarum* **15**: 473-497.

-Namdeo, A. G. (2007). Plant cell elicitation for production of secondary metabolites: A Review. *Pharmacognosy Reviews* **1**: 69-79.

-Sepúlveda G., Porta H. y Rocha M. (2003). La participación de los metabolitos secundarios en la defensa de las plantas. *Revista mexicana de fitopatología* **21**: 355-363.

-Schrickel, S. y Bittner, M. (2001). *La salud en nuestras manos: plantas medicinales en Chile, Riqueza Natural y Científica*. Editora y Gráfica Lamas. Concepción, Chile.

-Stévigny, C., Wautier, M., Habib, J.L., Chiap, P. y Hubert, P. (2004). Development and validation of a high performance liquid chromatographic method for quantitative determination of aporphine alkaloids from different samples of *Cassytha filiformis*. *Planta Med.* **70**: 764-770.

-Vogel, H., Razmilic, I., San Martín, J., Doll, U. y González, B. (2008). *Plantas medicinales chilenas, experiencias de domesticación y cultivo de Boldo, Matico, Bailahuén, Canelo, Peumo y Maqui*. Editorial Universidad de Talca. 2ª edición. Talca, Chile. 194p.