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# An HPLC-UV and HPLC-ESI-MS based method for identification of anti-inflammatory triterpenoids from the extracts of *Ugni molinae*

[Método CLAE-UV y CLAE-IES-MS para la identificación de triterpenoides anti-inflamatorios de los extractos de *Ugni molinae*]

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**Abstract**
The aim of this study was to establish an analytical method to detect the presence of the responsible triterpenoids of the anti-inflammatory activity of the leaves of *Ugni molinae* (murtilla). Successive leaves extracts of EtOAc (EAE) and ethanol (TEE) were prepared, obtaining for the first time from TEE a triterpenoid-rich sub-fraction (TF). The topical anti-inflammatory activity of TF was assessed (43.3% at 1 mg/ear) by means of the TPA-induced mouse ear oedema model, which was compared to EAE (83.1 ± 3.2%) and TEE (78.3 ± 11.8%) activities, both previously evaluated by us. These extracts were characterized in their triterpenoids by HPLC-UV and HPLC-ESI-MS. We demonstrated that TF has triterpenoids responsible in part of the anti-inflammatory activity, among them, madecassic and maslinic acids. These two compounds have been reported for the first time for this species. $ED_{50}$ for madecassic and alphitolic acids are also here reported.

**Keywords:** *U. molinae*, HPLC-ESI-MS, madecassic acid, alphitolic acid, maslinic acid, anti-inflammatory

**Resumen**
El objetivo de este trabajo fue establecer un método analítico para determinar la presencia de los triterpenoides responsables de la actividad anti-inflamatoria de las hojas de *Ugni molinae* (murtilla). Fueron preparados los extractos seriados de EtOAc (EAE) y etanólico (TEE) desde sus hojas, obteniendo desde el TEE por primera vez una sub-fracción rica en triterpenoides (TF). Se demostró la actividad anti-inflamatoria tópica del TF por el modelo de edema de oreja de ratón inducida por TPA (43,3% a 1 mg/oreja), la cual fue comparada con las de los EAE (83,1 ± 3,2%) y TEE (78,3 ± 11,8%) determinadas en nuestros estudios previos. Dichos extractos fueron caracterizados en sus triterpenoides por CLAE-UV y CLAE-IES-MS. Demostramos que el TF contiene triterpenoides responsables en parte de la actividad anti-inflamatoria, entre ellos, los ácidos madecásico y maslínico, reportados por primera vez para esta especie. Se informan además las $DE_{50}$ para los ácidos madecásico y alfitólico.

**Palabras Clave**: *U. molinae*, HPLC-ESI-MS, ácido madecásico, ácido alfitólico, ácido maslínico, antiinflamatorio

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## INTRODUCTION

The Chilean Myrtaceae *Ugni molinae* Turcz. is a small shrub found along the southern downhill Andean slopes (35º to 42º SL). It is commonly known as murtilla or murta and its edible berries are very appreciated by their sweet and aromatic taste. The leaves in either infusion or decoction are used by Chilean traditional medicine as an anti-inflammatory and analgesic agent to relieve kidney stones, urinary tract and back pains (Montenegro, 2000). This ethnopharmacological background was substantiated by pharmacological studies where the anti-inflammatory and analgesic effects of this species have been associated with pentacyclic triterpenoids in the leaves, such as betulinic, alphitolic, ursolic, oleanolic, corosolic and asiatic acids (Aguirre *et al*., 2006; Delporte *et al*., 2007). *U. molinae* leaves have also been reported as a source of antioxidant polyphenols (Rubilar *et al*., 2005), which adds to the pharmacological activities of the extracts. As the leaves of this species present potential as a medicinal plant, it is necessary to develop fast and reliable methods for the characterization of the active constituents in the crude drug, including sound analytical means.

HPLC-UV-ESI-MS is a technique that has important advantages over other chromatographic methods. It does not require extensive purification of mixture constituents, uses low volume of solvents, and is quick. Moreover, being an open system, it allows the analyses of different samples and different analytes simultaneously, which translates into savings in time, materials and reagents. ESI-MS provides the masses of analytes through their pseudomolecular ions and their identification through fragmented ions. The technique has been used to characterize the constituents of several Chilean crude drugs, including Boldo Folium (Simirgiotis and Schmeda-Hirschmann, 2010) and Mapuche medicinal plants infusions (Simirgiotis *et al*., 2012).

The aim of this study was to establish an analytical method to detect the presence of the responsible triterpenoids of the anti-inflammatory activity of the leaves of *Ugni molinae*.

## MATERIALS AND METHODS

### *Plant material*

The leaves of *Ugni molinae* Turcz. were collected at Provincia de Cauquenes, Chile (35º41'S; 71º40'W) on April 2010. A voucher herbarium specimen was deposited at the Escuela de Farmacia, Universidad de Chile (SQF-22462). The plant was identified by Dr. Carla Delporte following the monography of Reiche (1896).

### *Extraction and isolation*

Dry leaves (4.5 Kg) were powdered and consecutively extracted with hexane (HEX), $CH_2Cl_2$ (DCM), EtOAc (EAE) and EtOH (TEE) to obtain, after the concentration under reduced pressure, the corresponding dry extracts with a w/w yield of 1.4, 5.9, 3.7 and 22.2%, respectively. HEX and $CH_2Cl_2$ extracts were not included in this study since EAE and TEE have adequate physicochemical characteristics for pharmaceutical and cosmetic formulation, for instance solubility and color. A triterpenoid-rich sub-fraction (TF) was obtained from 5 g of TEE by means of a Soxhlet apparatus using EtOAc as a solvent (w/w yield: 10% of the starting material), hence concentrating the occurring triterpenoids in the TEE to compare the pharmacological activity of this proportion with the one of the EAE.

The TF (2.5 g) was partitioned by CC on silica gel 60, eluting with $CH_2Cl_2$-EtOAc and EtOAc-MeOH mixtures of increasing polarity. The $CH_2Cl_2$-EtOAc (30:70) dry fraction (22%) was constituted by asiatic acid and an unknown compound. This dry fraction (60 mg) was dissolved in 6 mL of methanol, filtered and the filtrate was concentrated to 2 mL under vacuum. The residual solution was applied to preparative TLC. The plates were developed with MeOH and two spots (Rf 0.6 y 0.3 of asiatic acid and unknown compound, respectively) were detected using anisaldehyde-sulphuric acid reagent. The band of the unknown compound was eluted in MeOH, filtered, taken to dryness yielding 10 mg of a white powder identified as madecassic acid by NMR spectroscopy and comparison with literature (Du *et al*., 2004).

### *HPLC analysis*

HPLC conditions were optimized in order to obtain the most descriptive qualitative analysis of the samples showing all the identified triterpenoids in *U. molinae* extracts. Considering the wavelength range used in previously reported methods for plant extracts with similar chemical nature (Rafamantanana *et al*., 2009), 201 nm was chosen as the detection wavelength which allowed us to obtain the optimum sensitivity for the secondary metabolites of our interest. A two-step gradient of solvent mixtures with a constant flow of 0.6 mL/min resulted to be the most suitable conditions to obtain a complete triterpenoids fingerprint. Most of

the peaks of the identified triterpenoids were observed with the best definition when using acetonitrile-water (60:40) during the first 40 minutes of run before changing immediately to acetonitrile-water (90:10) to observe the presence of the less polar compounds in the samples such as the structural isomers ursolic and oleanolic acids, which eluted as a single peak. Formic acid 0.1% in the water was used as ionization promoter. Blank of these mobile phases was previously run to demonstrate that the low formic acid concentration did not interfere with the analyte UV absorption.

HPLC-UV-ESI-MS fingerprints were acquired in a LC-MS system that consisted of an Agilent HPLC 1100 (Agilent Technologies Inc., CA, USA) hyphenated through a split to electrospray-ion trap Esquire 4000 mass spectrometer (Bruker Daltonik GmbH, Germany). ChemStation for LC 3D software (Agilent Technologies Inc., CA, USA) was used for HPLC control and EsquireControl 5.2 software (Bruker Daltonik GmbH, Germany) was used for mass spectrometer control.

A simple filtration was carried out on the samples using a 0.22 µm PTFE filter before LC-MS analysis. For HPLC separation of 20 µL of 3 mg/mL of TF and EAE, and 10 mg/mL of TEE in MeOH a Hibar Purospher Star RP-18 (250×4 mm, 5 µm) column (Merck GmbH, Germany) was used at room temperature using 0.1% aqueous formic acid (A) and 0.1 % formic acid in acetonitrile (B) as mobile phases with the following program: 0-40 min 60% B, 40-41 min 60-97% B and 41-60 min 97% B at flow rate of 0.6 mL/min and UV detection at 201 nm. The ionization process by electrospray was carried out at 3000 V using nitrogen as nebulizer gas at 325 ºC and as dry gas at 30 psi and 10 L/min. The ion trap was set in the ion control mode (ICC activated). The collision-induced dissociation was carried out by means of helium present in the trap. The ESI-MS/MS fragmentation was carried out by means of the following parameters: SmartFrag, 30-200%; fragmentation amplitude, 1.00 V; fragmentation time, 40 ms; isolation width MS/MS, 4 m/z; spectra average MS/MS, 5; precursor ion number AutoMS/MS, 5 signals; the AutoMS/MS threshold was previously set to each injection. Additionally, for the precursor ion selection the active exclusion mode was used (data dependent analysis) with the following parameters: exclusion, 2 spectra; exclusion time, 1 min. Mass spectra of each of the chromatographic peaks are available on request to the corresponding author.

Data were acquired in the negative polarity mode for interval of 20-1000 m/z. For the analysis of chromatograms and mass spectra the DataAnalysis 3.2 software (Bruker Daltonik GmbH, Germany) was used. To establish the retention time, authentic triterpenoids were injected.

$^{1}$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) spectra of all the isolated compounds were acquired on a Bruker UltraShield 400 spectrometer (Bruker Daltonik GmbH, Germany) with TMS as internal standard. The triterpenoids NMR spectra were recorded in DMSO-d6 for asiatic, corosolic, maslinic, alphitolic, betulinic, ursolic and oleanolic acids and in MeOD-d4 for madecassic acid. NMR spectra of the isolated triterpenes are available on request to the corresponding author.

Column chromatography (CC) was performed using silica gel 60 (0.063 - 0.200 mm) (Merck 7754) and thin layer chromatography (TLC) was carried out using silica gel G $F_{254}$ (Merck 5554). Spots were detected under UV light and spraying with anisaldehyde - sulphuric reagent. Madecassic, asiatic, maslinic, betulinic, ursolic and oleanolic acids standards were purchased from Sigma (MO, USA). Preparative TLC was carried out on 20 x 20 cm, 1-mm thick silica gel G 60 plates (Merck) using MeOH as developing solvent. Alphitolic acid and a mixture of corosolic and maslinic acids were isolated from TF as described by Aguirre *et al*. (2006).

***HPLC reproducibility***

Under the established conditions intra- and inter-day reproducibility was evaluated, injecting 20 µL of EAE, TEE and TF three times per day. Intra-day relative standard deviation (RSD) of the retention time and peak height of the extracts were less than 0.6% and 1.4%, respectively (n = 3). Inter-day RSD of the retention time and peak height were less than 1.5% and 2.0%, respectively (n = 9). These results indicate that the method shows good stability and reproducibility. Madecassic, asiatic, maslinic, betulinic, ursolic and oleanolic acids standards also showed adequate reproducibility in their respective retention times.

***Topical anti-inflammatory activity***

All animal experiments were performed according to the ethical guidelines suggested by the "International Norms for the Biomedical Investigation with Animals", elaborated by the Council of International Organizations (1990) and the bio-ethics norms of the

Commission of the Chilean Public Health Institute and Faculty of Chemical and Pharmaceutical Sciences.

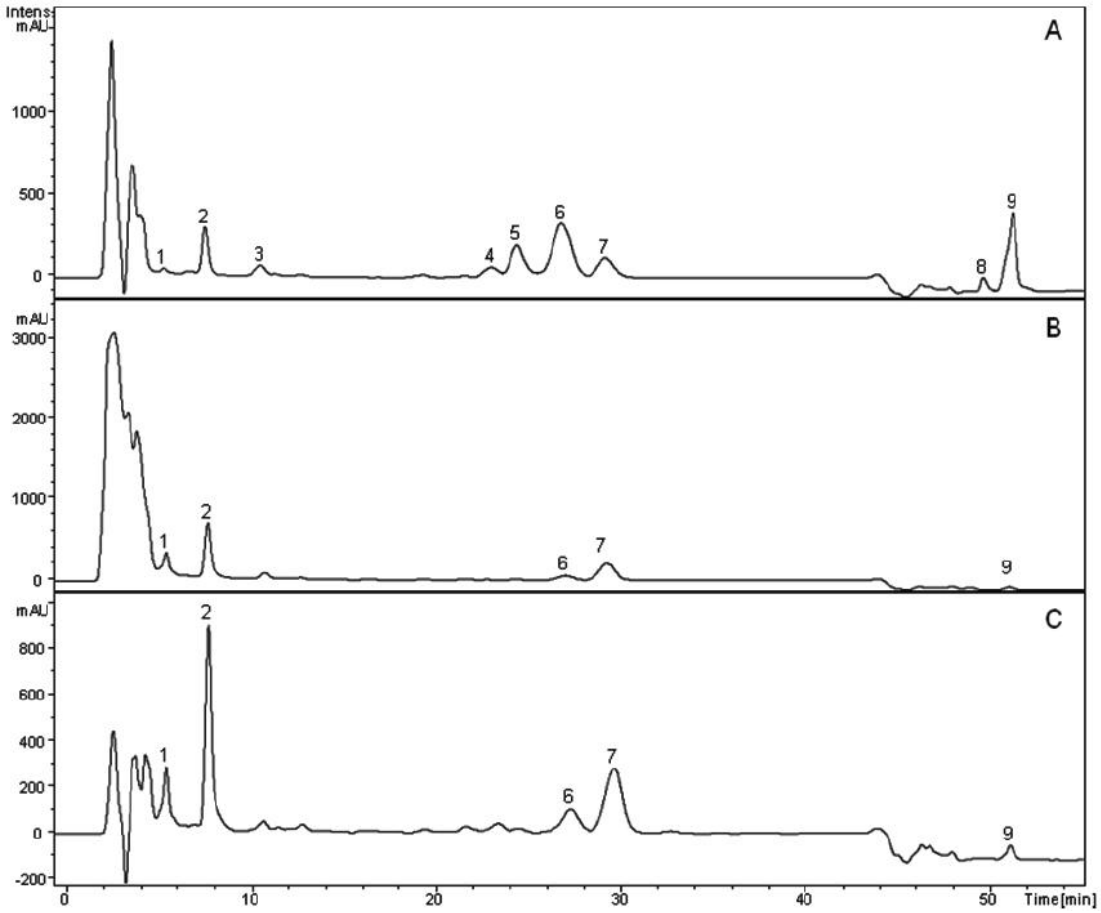



**Figure 1**
HPLC chromatograms of selected extracts and fractions of *Ugni molinae* leaves. Detection: UV, 201 nm. (A): EtOAc, 3 mg/mL; (B): TEE, 10 mg/mL; (C): TF, 3 mg/mL. Compounds. **1**: madecassic acid; **2**: asiatic acid; **3**: not identified; **4**: not identified; **5**: alphitolic acid; **6**: corosolic acid; **7**: maslinic acid; **8**: betulinic acid; **9**: ursolic and oleanolic acids

Adult male CF-1 mice (20-25 g), obtained from a stock maintained at the Chilean Public Health Institute, were used to assess the anti-inflammatory effect. All animals were housed in a climate- and light-controlled room with a 12 h light-dark cycle, fasted overnight before the day of the assays, with free access to water.

For each dose of the samples under study, the anti-inflammatory activity was evaluated in groups of 8 treated and 16 control mice. After 5 minutes of sample treatment, mice received 5 µg of phorbol 12-myristate 13-acetate (TPA) (Sigma; St. Louis, MO), as pro-inflammatory agent, dissolved in 20 µL of acetone (solvent does not interfere with the assay). Control subjects only received TPA at the same concentration. Both, the sample and the TPA, were applied to the inner (10 µL) and outer (10 µL) surfaces of the right ear. The left ear only received acetone. Mice were sacrificed by cervical dislocation after 4.5 h of TPA and a 6 mm diameter section of the right and left ears were cut and weighed, the weight difference between bot ear sections correspond to the oedema value (Lloret and Moreno, 1995). Dermal anti-inflammatory activity (TA) was evaluated according to the following equation: %TA= [Wc-Ws/Wc] x 100; where Wc and Ws are the different median values of the weights of the right and the left ear sections of the control and the treated animals respectively (Delporte *et al*., 2003).

Doses of the samples were selected according to previous work in the same type of biological assay (Aguirre *et al*., 2006), confirming the activity of EAE and TEE and establishing it for TF at a dose of 1

mg/ear. Madecassic and alphitolic acids were both evaluated at 0.1, 0.2, 0.7, 0.8 and 1.6 µmol/ear while 0.2, 0.4, 0.7 and 1.4 µmol/ear doses of the positive control indomethacin were also assayed.

### *Statistical analysis*

Statistical significance was evaluated using the Kruskall–Wallis test, followed by Dunn's multiple comparisons test. The criterion for statistical significance was set at $P \leq 0.05$. Data were expressed as median values ± SEM calculated from the weight of the oedema for treated and untreated animals considering control values as a 100% of inflammation.

## RESULTS AND DISCUSSION

### *Chemical characterization of EAE, TEE and TF*

The herein established fingerprints for the anti-inflammatory EAE, TEE successive extracts and TF, show the phytochemical profile of the bioactive triterpenoids found in *U. molinae*. Figure 1 shows the HPLC-UV chromatograms at 201 nm of EAE (A) TEE (B) and TF (C). Table 1 details the ESI-MS and MS/MS main fragments for each peak observed in the chromatograms, corresponding to the triterpenoids: Asiatic acid (7.5 min), alphitolic acid (24.3 min), corosolic acid (26.8 min), betulinic acid (49.6 min) and a mixture of ursolic and oleanolic acids (51.2 min). We could also observe the presence of two other triterpenoids not yet identified in this species: madecassic acid (5.2 min) and maslinic acid (29.1). The HPLC trace of TF, as a purified triterpenoid sub-fraction from TEE, shows an important enhancement of the triterpenoids area compared with its parent TEE (Table 2).

**Table 1**
**MS ionization and MS/MS fragmentation of the triterpene acids occurring in *Ugni molinae* leaves extracts**

| Peak | Rt (min) | m/z | | | | | | Identification |
|---|---|---|---|---|---|---|---|---|
| | | MS | | | MS/MS of $[M-H]^-$ | | | |
| 1 | 5.2 | 503.7 $[M-H]^-$ | 539.4 $[M+Cl]^-$ | | 501.1 | 389.2 | 437.2 | Madecassic acid |
| 2 | 7.5 | 487.4 $[M-H]^-$ | 523.3 $[M+Cl]^-$ | 975.6 $[2M-H]^-$ | 441.2 | 409.2 | | Asiatic acid |
| 3 | 10.5 | 487.6 $[M-H]^-$ | 523.4 $[M+Cl]^-$ | 339.2 | 441.2 | 469.1 | 423.6 | n.i. |
| 4 | 22.9 | 471.5 $[M-H]^-$ | 507.4 $[M+Cl]^-$ | 339.2 | 393.1 | 405.1 | | n.i. |
| 5 | 24.3 | 471.4 $[M-H]^-$ | 943.7 $[2M-H]^-$ | | 369.2 | 405.1 | | Alphitolic acid |
| 6 | 26.8 | 471.4 $[M-H]^-$ | 943.6 $[2M-H]^-$ | | 423.1 | 393.1 | 409.2 | Corosolic acid |
| 7 | 29.1 | 471.4 $[M-H]^-$ | | | 423.1 | 405.1 | 393.1 | Maslinic acid |
| 8 | 49.6 | 455.5 $[M-H]^-$ | | | 419.0 | 395.0 | 437.1 | Betulinic acid |
| 9 | 51.2 | 455.4 $[M-H]^-$ | | | 407.1 | | | Ursolic and oleanolic acids |

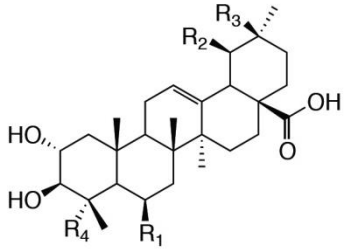
**Rt: retention time; n.i.: not identified**

**Table 2**
**Comparison of triterpenoid constituents in the ethanol serial extract (TEE) and triterpenoid-rich sub-fraction (TF) (relative peak areas)**

| Peak | Rt (min) | Area | |
|---|---|---|---|
| | | TEE 10 mg/mL | TF 3 mg/mL |
| 1 | 5.3 | 4305 | 4078 |
| 2 | 7.6 | 16541 | 21681 |
| 6 | 27.0 | 3046 | 5838 |
| 7 | 29.2 | 12595 | 17133 |
| 9 | 51.0 | 1282 | 1601 |

**Rt: retention time**

The presence of madecassic acid in EAE and TF detected by HPLC-UV-ESI-MS was confirmed by TLC analysis where a blue spot (anisaldehyde-sulphuric acid reagent), with Rf 0.22 (n-buOH-EtOAc-$NH_3$-$H_2O$ (6:4:0.5:1) was coincident with a standard sample of madecassic acid. An additional proof of occurrence of madecassic acid (Figure 2) was the isolation from TF by CC and subsequent preparative TLC. NMR data were in agreement with literature (Du *et al*., 2004). Maslinic acid (Figure 2) was also identified in the fingerprints of EAE, TEE and TF by the MS/MS fragmentation pattern (Li *et al*., 2009), its structure was confirmed by NMR spectral data (Tanaka *et al*., 2003).




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Madecassic acid | OH | $CH_3$ | H | $CH_2$-OH |
| Maslinic acid | H | H | $CH_3$ | $CH_3$ |

**Figure 2**
**Structure of madecassic acid (compound 1) and maslinic acid (compound 7)**

***Topical anti-inflammatory activity***

The anti-inflammatory activity against the TPA-induced mouse ear oedema of 1 mg/ear of TF was 43.3 ± 4.2 %. In our previous studies (Aguirre *et al*., 2006), the same dose of EAE and TEE showed an activity of 83.1 ± 3.2 and 78.3 ± 11.8%, respectively. The effect was statistically significant compared to the control group. The anti-inflammatory effect might be in part related to the occurrence of corosolic, asiatic, betulinic, ursolic and oleanolic acids, presenting an $ED_{50}$ value of 0.19 and 0.13, 0.66, 0.21 and 0.65 µmol/ear, respectively (Aguirre *et al*., 2006; Banno *et al*., 2004; Banno *et al*., 2005). It is also important to remark that the existing polyphenols in this species could contribute to the anti-inflammatory activity, since Rubilar *et al*. (2005) showed that *U. molinae* leaves are a rich source of antioxidant polyphenols: flavonoid glycosides of quercetin, myricetin and kaempferol, which are widely known for their anti-inflammatory and analgesic activity (Delporte *et al*., 2007; Parveen *et al*., 2007) which are mainly present in TEE, as was confirmed in our laboratory by the Folin-Ciocalteu method (data not shown). Both triterpenoids and polyphenolic compounds occur in TEE and contribute to the anti-inflammatory effect of this extract, which resulted almost twice more active than its triterpenoid-rich sub-fraction, TF (Micelia *et al*., 2005). The HPLC-UV-ESI-MS fingerprint of TF demonstrated that this triterpenoid-rich sub-fraction maintains and enhance the triterpenoid composition of TEE (Table 2). We could observe in EAE, TEE and TF chromatograms, qualitative differences in their

triterpenoid composition and also relative quantitative differences when comparing the areas under curve among TEE and TF (Table 2).

In this work we demonstrated that madecassic and alphitolic acids also show a dose-dependent topical anti-inflammatory activity with an $ED_{50}$ of 0.11 and 0.20 µmol, respectively, compared to an $ED_{50}$ of 0.38 µmol of indomethacin (Table 3). Both pentacyclic triterpene acids inhibited TPA-induced inflammation with higher potency than the reference drug. As for maslinic acid, Banno et al., (2005) demonstrated that this triterpene reduced oedema in the TPA model ($ED_{50}$ = 0.27 µmol), which was supported by Sosa et al. (2005) and later by Márquez *et al*. (2006) who suggested that this compound possesses potential biopharmaceutical use due to its suppressive effect on the production of nitric oxide, IL-6 and TNF-α.

**Table 3**
**Topical anti-inflammatory effects of alphitolic acid, madecassic acid and indomethacin (reference drug) in the TPA-induced mouse ear oedema model**

| Dose (µmol/mouse) | % Anti-inflammatory effect ± SEM | | |
|---|---|---|---|
| | Alphitolic acid | Madecassic acid | Indomethacin |
| 1.6 | 97.5 ± 2.1* | 84.1 ± 10.0* | n.a. |
| 1.4 | n.a. | n.a. | 92.9 ± 13.0* |
| 0.8 | 96.7 ± 1.1* | 70.5 ± 8.5* | n.a. |
| 0.7 | 95.4 ± 2.6* | 69.8 ± 6.5* | 71.7 ± 11.3* |
| 0.4 | n.a. | n.a. | 48.2 ± 17.1* |
| 0.2 | 41.9 ± 11.5* | 61.1 ± 6.2* | 19.8 ± 9.4* |
| 0.1 | 20.2 ± 5.4* | 55.8 ± 7.2* | n.a. |

**Each value represent the median ± SEM of the results obtained from eight animals treated with samples; * $p \leq 0.05$ shows that there is a significant difference between the treated group and the control group (in the latter we considered 100 % inflammation); n.a. not assayed**

It has been established that TPA induces inflammation via the activation of NF-κB signalling pathway (Ban *et al*., 2009). This is consistent with the *in vivo* anti-inflammatory effect of madecassic acid we observed and the iNOS, COX-2, TNF-α, IL-1β, and IL-6 inhibition via downregulation of NF-κB described by Won *et al*. (2010).

A number of triterpene acids with the ursane, oleanane, and lupane skeletons are known for their potent anti-inflammatory activity, which is comparable to that of synthetic non-steroidal anti-inflammatory drugs (Recio *et al*., 1995; Liu, 1995). Several reports on the topical anti-inflammatory activities of plant extracts have been ascribed to these compounds, and more specifically to the widespread ursolic acid (Banno *et al*., 2005; Ismaili *et al*., 2001).

Our results and literature data show that 2α-hydroxy pentacyclic triterpene acids (alphitolic, asiatic, corosolic, madecassic and maslinic acids) were more potent than indomethacin as anti-inflammatory agents. Inhibition of phorbol ester-induced changes was demonstrated several years ago for corosolic acid (Ahn *et al*., 1998) and other pentacyclic triterpene acids (Huguet *et al*., 2000). More recently, corosolic acid, among many other triterpene acids, was shown to be a strong inhibitor of TPA-induced inflammation in the mouse ear assay (Banno *et al*., 2004; Banno *et al*., 2005). Moreover, the results reported in the two latter papers suggest that the introduction of additional hydroxyl groups at C-2 and/or C-23 in the oleanane and ursane skeletons can lead to increased anti-inflammatory activity.

## CONCLUSIONS

In Chile several dermocosmetic formulations based on *U. molinae* extracts are commercially available. Moreover, due to the anti-inflammatory activity of the extracts of the leaves of this native species, these own an important potential in the pharmaceutical field. Hence, the herein established HPLC-UV fingerprints represent a valuable analytical contribution to develop quality control assessments. This chromatographic method could be used for authentication and identification of the plant material or its herbal products.

The anti-inflammatory activity of *Ugni molinae* leaves are due mainly to the presence of pentacyclic triterpene acids (betulinic, ursolic, oleanolic acids) including the 2α-hydroxy derivatives alphitolic, asiatic, corosolic, madecassic and maslinic

acids. The results allow us to demonstrate a dose-dependent contribution of alphitolic and madecassic acids in the anti-inflammatory activity. Madecassic and maslinic acids were identified for the first time in this species.

The better knowledge of bioactive compounds in *U. molinae* leaves sustain the traditional use of this species in Chile and its potential for pharmaceutical preparations from the crude drug.

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