ACTIVE METABOLITES AND BIOLOGICAL ACTIVITIES FROM *Malesherbia auristipulata*

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Summary

The antimicrobial activity of the resinous exudate from the aerial part of *Malesherbia auristipulata* showed an active mixture against Gram (+) bacteria: *S. aureus*, *B. subtilis* and *M. flavus* and less active against Gram (-) bacteria: *S. aeruginosa, S. aviatum* and *E. coli*. GC analysis revealed a mixture of polyunsatured fatty acids, being γ-linolenic acid the most abundant and the major compound responsible for this activity. Topic antiinflammatory and *per os* analgesic properties of *Malesherbia auristipulata* were also investigated and the major compounds of the dichloromethane and resinous bioactive extracts were isolated. An assessment of the antioxidant activity was performed for the different extracts from this species. Results of the evaluation of the topic antiinflammatory activities induced by arachidonic acid and phorbol-12-myristate-13-acetate for the extracts showed that this species has active constituents that might lower either cyclooxygenase or lipoxygenase activities, enzymes that allow the synthesis of pro-inflammatory endogenous substances such as prostaglandin E_2 or leukotrienes, respectively. Dichloromethane extract was the only that exhibited antioxidant activity. Our results corroborate the antiinflammatory and analgesic effects of this species. From the bio-active dichloromethane and resinous extracts, β-sitosterol, kaempferol and a mixture of highly saturated fatty acids were obtained; palmitic acid was the most abundant in the mixture. These compounds could partly explain the antiinflammatory and analgesic activities of this species.

Keywords: *Malesherbia auristipulata;* Biological activities; Chemical constituents.

Introduction

Malesherbia auristipulata Ricardi (Malesherbiaceae), vernacular for "ají de zorra", is a pre-Andean native species that grows in the stony gulches of Arica, first Region of Chile in the Azapa valley, 1850-2100 osl [1]. In Chile, 18 native species have been reported between the regions I Tarapacá and Region IV Coquimbo [2]. Species of *Malesherbia* genus have been used in respiratory affections [3] which suggest potential anti-inflammatory, analgesic and antimicrobial effects; however, scarcely information is available about their efficacy and there are no previous reports on its chemical composition. In this study, we assessed the antimicrobial, analgesic, anti-inflammatory and antioxidant action of the aerial part of this species.

Methods

Animals: 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.

General experimental procedures: Column chromatography (CC) was run using silica gel 60G (Merck 7734). TLC was performed on silica gel GF254 (Merck 5554); spots were detected under UV light, or after spraying Liebermann-Burchard reagent and then heating for about 5 min at 120º or using $AlCl_3$ (5%) or p-anisaldehyde-H_2SO_4 reagents. IR recordings were made in KBr cell; melting point was determined on a Kofler hot stage microscope and is uncorrected. UV spectra were recorded with a UNICAM UV-3 spectrometer. GC was performed in a HP 5890 with FID detector, capillary column fused silica BPX7 50 m, 0.25 µm film. Temperature was programmed from 160 to 230 ºC (2 ºC/min) and hydrogen was used as carrier gas. Fatty acid methyl esters were prepared according to the standard method [4].

Plant material: The aerial part of *Malesherbia auristipulata* Ricardi was collected in Arica Azapa valley near Cuesta del Aguila, Chile, 18º 30´ south, and was identified by Professor Eliana Belmonte. A voucher specimen is kept at Herbario de Escuela de Química y Farmacia (SQF 22215), University of Chile.

Extraction and isolation: The dried aerial part (1.72 Kg) of *M. auristipulata* was extracted by immersion in dichloromethane thus obtaining a resinous exudate extract (ERE, 27 g); then the plant was air dried and the vegetal material was ground and sequentially extracted with n-hexane, dichloromethane, and methanol at room temperature, yielding 2.3, 8.8 and 75.5 g of hexane (EHE), dichloromethane (EDCE) and methanol (EME) extracts respectively after the removal of the solvents in vacuo. These extracts were used to carry out all the pharmacological assays. ERE (21.20 g)

was fractionated with flash CC in hexane 100%; hexane: CH_2Cl_2 (1:1); CH_2Cl_2 100%; CH_2Cl_2 : MeOH (1:1) and MeOH 100%. The hexane: CH_2Cl_2 (1:1) fraction was submitted to CC on silica gel 60 G, eluting with hexane: CH_2Cl_2 (1:1), 120 mg of compound **1** (C1) was isolated from 9-79 fractions of 100 ml. With increasing polarity, fractions eluted with CH_2Cl_2: EtOAc (8:2) to EtOAc: MeOH (7:3) in fractions 300 to 429 of 100 ml, yielded an impure compound **2** (C2). C2 purification with Sephadex LH-20 of hexane: CH_2Cl_2: MeOH (3:6.5:0.5) yielded chromatographic pure C2 (130 mg). Both compounds were identified by GC and submitted to the pharmacological assays. EDCE (6 g) was fractionated by CC, obtaining two fractions, one of them rich in steroidal compounds denominated FDC (eluted with CH_2Cl_2 100%; 310 mg) and another fraction, rich in flavonoids, was denominated FDK (eluted with CH2Cl2: EtOAc 1:1; 180 mg). Both were submitted to the pharmacological assays. A small amount, 5 mg, of a compound **3** (C3) was isolated from 260 mg FDC and then purified by CC of silica gel eluted with hexane: CH_2Cl_2 (8:2) in fractions 30 to 35 of 100 ml. An impure flavonoid fraction (FDK) of 130 mg was also obtained and from this fraction crystallization occurred in CH_2Cl_2 using MeOH drops and yielding 5 mg of compound **4** (C4). Identification of C3 and C4 were performed by direct comparison of the TLC, melting point and spectroscopic data IR for C3 and UV for C4 with authentic samples.

Antimicrobial assays: The antimicrobial activity of different samples were determined against *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (isolated from a patient), *Salmonella aviatum* (ATCC 2228*), Pseudomonas aeruginosa* (ATCC 14207), *Staphylococcus aureus* (ATCC 6538P), *Micrococcus flavus* (ATCC 10290), *Bacillus subtilis* (ATCC 6633) and *Candida albicans* (clinical isolate). They were dissolved in DMSO. Dilutions of 100 and 200 µg/ml were added to a fixed volume of Plate Count Agar (PCA). Then they were superficially inoculated with an overnight culture: bacteria were incubated at 37º C for 24 h and fungi at 28º C for 48 h. Results were recorded as growth or growth inhibition at each sample concentration [5,6]. The turbidimetric method [7] was used with serial dilutions of the extract in 4 ml of the Plate Count Broth or Tryptic Soy Broth. Both media were used to assay the minimum inhibitory concentration (MIC) of the essential oil against *S. aureus, B. subtilis* and *M. flavus*. Bioautographic agar overlay in TLC of the active samples (RE and C2) was carried out on silica gel 60G F_{254} glass plates and they were run with CH_2Cl_2: EtOAc 8:2 as solvent. The bioautograms were sprayed with an aqueous solution of thiazolyl blue (MTT). Positive antimicrobial reaction was observed as clear inhibition zones against a purple background [8,5,6].

Analgesic activity per os: For each dose of the sample under study, the analgesic activity was evaluated in groups of 8 mice and 16 control subjects, using an intraperitoneal injection of 0.5 ml of acetic acid 0.6 %. The analgesic effect was calculated by comparing the number of abdominal writhes of the treated and the control group; the latter received only the vehicle [9]. The number of abdominal

writhes of each mouse was counted for 30 min, beginning 5 min after acetic acid administration. The following equation was used to calculate the mean pain percentage : % P = [C sample /Control] x 100, where P stands for pain, C sample is the median writhes reached in sample treated animals and C control (41.6 ± 3.79) is the median writhes reached in control animals which received only the vehicle. The analgesic effect (An) was calculated according to the following equation: % An = 100 - % P. In analgesic assays, 1 h earlier than acetic acid administration, 600 mg/kg extract doses were orally administered by means of an intragastric catheter and suspended in saline arabic gum. The drug induced changes were statistically estimated using the Wilcoxon test for independent data [10]. The effects were significant for $p \leq 0.05$. The SEM ($SD/\sqrt{n}$) values were calculated for mean writhes.Sodium naproxen (SN), provided by Laboratorios Saval, Santiago-Chile, was used as a reference drug and was suspended in the same vehicle [11].

Topic anti-inflammatory activity: For each dose of the sample under study, the anti-inflammatory activity was evaluated in groups of 8 mice and 16 control ones. After 5 minutes, the treated animals received 2 mg of arachidonic acid (AA) or 5 µg of phorbol-12-myristate-13-acetate (TPA) dissolved in 20µl acetone. The control subjects only received the same dose of AA or TPA. The inflammatory agents, AA or TPA, and the samples were applied to the inner (10µl) and outer (10µl) surfaces of the right ear of mice. The left ear always receives the vehicle; acetone in this case. Mice were sacrificed by cervical dislocation and a 6 mm diameter section of the right and left ears were cut and weighed [12]. Dermal anti-inflammatory activity (T) was evaluated according to the following equation: %T= [Wc – Ws/Wc] x 100, where Wc and Ws are median values of the weights of the right and the left ear sections of the control and the treated animals respectively [13]. The reference drugs were provided by Laboratorio Chile S.A., Santiago-Chile.

Xanthine oxidase activity: Different extracts were evaluated in 50 µg/ml bidistilled water and when their inhibition value was >50 %, additional tests were performed for IC50 determination [14]. Inhibition of the xanthine oxidase (XO) activity was measured in relation to the amount of uric acid from xanthine. This quantity was spectrophotometrically measured at 290 nm. Extract evaluation required a mixture of 1.0 of extract solution, 2.9 ml of phosphate buffer (Na_2HPO_4/ KH_2PO_4; pH = 7.5) and 0.1 ml of enzyme solution at the concentration of 0.042 µ/ml. After preincubation of the mixture at 25º C for 15 min, the reaction was initiated by adding, in bidistilled water, 2.0 ml of 150 µmolar xanthine as substrate. This assay mixture was incubated at 25º C for 30 min. When 1.0 ml of 1N HCl was added, the reaction stopped and the absorbance was measured. The inhibition percentage of xanthine oxidase activity (% I) was calculated as :%I = (A-B)-(C-D)/(A-B) x 100 , where A is the XO activity without testing extract (total uric acid); B, the blank of A without XO; C, the enzyme activity with testing extract (residual uric acid); and D, the blank of C without the

enzyme. The IC50 determination of allopurinol, used as reference drug, was 0.035 μg/ml (0.267 μM). For XO activity, the extract-induced changes were statistically determined with the Wilcoxon test for independent data [10]. Effects were significant for $p < 0.05$. Both xanthine and xanthine oxidase (XO) from cow milk were purchased from Sigma Co. and the standard inhibitor allopurinol was provided from Laboratorios Saval, Santiago-Chile.

Results

Chemical constituents**:** ERE yielded C1 (0.57%) constituted by highly saturated fatty acids (78%), palmitic (27.68%), myristic (8.34%) and stearic acid (7.64%) among others (Table 1).

The fatty acids were detected by GC, palmitic acid being the most important (27.68%). Present Rf: 0.3 in silica gel with CH_2Cl_2: EtOAc (7:3), and brown colour with p-anisaldehyde-H_2SO_4 reagent. By the other hand, C2 (0.61%) is an oil formed by various polyunsaturated fatty acids, mainly γ-linolenic acid (58.04%) and a small amount of linoleic acid (0.7%) among others (Table 2).
C2 was a yellow oil; Rf : 0.5 in silica gel with CH_2Cl_2: EtOAc (8:2), and blue colour with p-anisaldehyde-H_2SO_4 reagent. Both ß-sitosterol (C3) and kaempferol (C4) were isolated from FDC and FDK respectively. Identification of ß-sitosterol was performed through direct comparison with authentic β-sitosterol of the data obtained by chromatography (TLC), melting point and spectroscopic (IR). Mp: 138-139 ºC. Rf: 0.6 in silica gel with n-hexane: EtOAc (7:3); and purple colour with Lieberman-Burchard reagent [15]. Identification of kaempferol was performed through direct comparison with authentic kaempferol of the data obtained by chromatography (TLC), melting point and spectroscopic (UV) [16]. Mp: 226-227. Rf: 0.5 in silica gel with CH_2Cl_2: EtOAc (7:3); and yellow colour with $AlCl_3$ (5%).
Antimicrobial activity: The aerial part of *Malesherbia auristipulata* showed antibacterial activity. Only ERE was active. This extract was active against Gram (+) bacteria: *S. aureus, B. subtilis* and *M. flavus* at 100 and 200 μg/ml. All extracts were inactive against the fungi *Candida albicans*. C2 was responsible for the antimicrobial activity of this extract against the Gram (+) bacteria: *S. aureus*, *B. subtilis* and *M. flavus* and less active against Gram (-) bacteria: *P. aeruginosa , S. aviatum* and *E. coli*, all detected through bioautographic assay. MIC for C2 were 120, 30 and 30 μg/ml for *S. aureus, B. subtilis* and *M. flavus* respectively. As reference, MIC for ampicillin for these strains were 5, 10 and 20 μg/ml, respectively. Analgesic and antiinflamatory activities:Table 3 shows the results for the pharmacological assays of the various extracts, FDC and FDK fractions, C1, and C2 together with the maximum effect of SN for *per os* analgesic activities, and antiinflammatory dermal maximum effect of nimesulide (NM) and indomethacin (IND) [11].

Table 1: Fatty acid composition of compound C1 (0.57%), determinate as methyl esters percentage.

Saturated Compounds	Fatty Acids	% ± S.D.	Rt C1* C18:0	Rt St* C18:0
Caprilic	C8:0	0.33 ± 0.00	0.27	0.25
Capric	C10:0	0.66 ± 0.00	0.29	0.29
Lauric	C12:0	3.98 ± 0.00	0.34	0.34
Tridecanoic	C13:0	2.13 ± 0.00	0.39	0.39
Miristic	C14:0	8.34 ± 0.12	0.47	0.47
Pentadecanoic	C15:0 p	2.28 ± 0.00	0.63	0.60
Palmitic	C16:0	27.68 ± 0.10	0.69	0.69
Heptadecanoic	C17:0	4.09 ± 0.00	0.84	0.84
Stearic	C18:0	7.64 ± 0.01	1.00	1.00
Eicosanoic	C20:0	6.08 ± 0.00	1.38	1.38
Heneicosanoic	C21:0	1.77 ± 0.00	1.59	1.58
Docosanoic	C22:0	7.52 ± 0.00	1.80	1.80
Tetracosanoic	C24:0	3.98 ± 0.00	2.23	2.23
Total Saturated		76.48		
Monounsaturated				
Miristoleic	C14:1	3.78 ± 0.00	0.57	0.57
Palmitoleic	C16.1	2.01 ± 0.02	0.76	0.77
Octadecenoic	C18:1 tr- isomer	0.46 ± 0.01	1.03	1.04
Oleic	C18:1w9	3.20 ± 0.05	1.07	1.07
Eicosenoic	C20:1	0.66 ± 0.00	1.43	1.42
Docosenoic	C22:1	0.27 ± 0.02	1.88	1.88
Total Monounsaturated		10.38		
Polyunsaturated				
Linoleic	C18:2w6	2.76 ± 0.01	1.34	1.34
γLinolenic	C18:3w6	1.88 ± 0.01	1.27	1.26
αLinolenic	C18:3w3	0.70 ± 0.00	1.34	1.34
Total Polyunsat.		5.34		
Non Identified	-	7.80		

*The standard mixture of methyl esters used to identify the fatty acids using Relative retention times (Rt) to Compound C1 and stearic acid was Supelco TM 37 Component FAME Mix. Sigma Catalogue Nº 47885-U. P:Probably.

Table 2.- Fatty acid composition of compound C2 (0.61%) determinate as methyl esters percentage.

Saturated Compounds	Fatty Acids	% ± S.D.	Rt C2* C18:0	Rt St * C18:0
Miristic	C14:0	0.18 ± 0.01	0.47	0.47
Pentadecanoic	C15:0 p	0.25 ± 0.01	0.63	0.60
Palmitic	C16:0	0.80 ± 0.03	0.69	0.69
Heptadecanoic	C17:0	0.40 ± 0.15	0.84	0.84
Stearic	C18:0	0.50 ± 0.04	1.00	1.00
Eicosanoic	C20:0	0.56 ± 0.01	1.38	1.38
Total Saturated		2.69		
Monounsaturated				
Palmitoleic	C16.1	0.31 ± 0.02	0.76	0.77
Oleic	C18:1 w9	2.52 ± 0.07	1.07	1.07
Eicosenoic	C20:1	10.50 ± 0.03	1.43	1.42
Docosenoic	C22:1	0.27 ± 0.02	1.88	1.88
Total Monounsaturated		13.60		
Polyunsaturated				
Linoleic	C18:2w6	0.70 ± 0.09	1.19	1.19
γLinolenic	C18:3w6	58.04 ± 0.05	1.27	1.26
αLinolenic	C18:3w3	0.70 ± 0.00	1.34	1.34
Eicosatrienoic	C20:3w3	16.63 ± 0.05	1.70	1.70
Docosadienoic	C22:2	3.05 ± 0.03	1.93	1.93
Total Polyunsat.		79.12		
Non Identified	-	4.59		

*The standard mixture of methyl esters used to identify the fatty acids using Relative retention times (Rt) to C2 and stearic acid was Supelco TM 37 Component FAME Mix. Sigma Catalogue Nº 47885-U.

In per os assays, ERE and EDCE, at the doses of 600 mg/kg, exhibited the strongest analgesic activities (67.9 and 60.3 % respectively) similar to the reference drug (SN 70%). In relation to the results obtained in antiinflammatory studies, RE and DCE showed the strongest effect against AA (50.0 and 54.7 % respectively) and TPA (77.0 and 84.7 % respectively), all at the dose of 3 mg/ear, and were similar to the reference drugs (NM and IND 48.8 and 92.9 % respectively) and EME did not exhibit significant activity against AA (Table 3).

Table 3. Antiinflammatory, analgesic and topic antiinflammatory of different extracts, FDC, FDK, C1 and C2 from *M. auristipulata* and reference drugs

Sample	Dose	% An ± SEM	%TA ± SEM	%TP ± SEM
RE	600 A	67.9* ± 13.4		
RE	3.0 B		50.0* ± 8.8	77.0* ± 7.9
HE	600 A	12.8* ± 13.1		
HE	3.0 B		38.0* ± 12.6	65.3* ± 4.9
DCE	600 A	60.3* ± 14.4		
DCE	3.0 B		54.7* ± 6.1	84.7* ± 8.6
ME	600 A	59.0* ± 9.8		
ME	3.0 B		8.3 ± 7.9	85.2* ± 14.0
INF	402 A	49.3* ± 11.2		
INF	3.0 B		15.2 ± 11.3	6.0 ± 9.3
FDC	100 A	73.3* ± 9.1		
FDC	1.0 B		6.3 ± 10.5.	62.2* ± 4.5
FDK	100 A	47.4* ± 7.9		
FDK	1.0 B		1.0 ± 16.0	42.9* ± 10.5
C1	100 A	23.7* ± 8.0		
C1	1.0 B		0.0 ± 15.0	45.5* ± 8.4
C2	100 A	7.9 ± 3.5		
C2	1.0 B		0.0 ± 9.3	12.0* ± 6.5
SN	12.5 A	↑70.0* ± 4.0	n.t.	n.t.
NM	1.0 B	n.t.	↑48.8* ± 4.0	n.t.
IND	0.5 A	n.t.	28.0* ± 9	↑92.9 ± 3,2

Dose:A:mg/kg; B:mg/ear. n.t., non tested; An, analgesic effect; TA and TP, topical antiinflammatory effect induced by AA and TPA respectively; RE, resinous exudate; HE, hexane extract; DCE, dichloromethane extract; ME, methanol extract; INF, aqueous extract; FDC, fraction rich in β-sitosterol; FDK, fraction rich in kaempferol; C1, compound 1; C2, compound 2; SN, sodium naproxen (d-2-(6-methoxy-2-naphthyl) propionic acid); NM, nimesulide (4-nitro-2-phenoxymethanesulfonanilide); IND, indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid); ↑, maximum effect; *, $p \leq 0.05$; without asterisk, $p > 0.05$. Each group represents the median ± SEM of 8 mice pretreated with sample or reference drugs.

Among the other extracts under study, methanolic extract exhibited analgesic activity (59% at the dose of 600 mg/kg) but, as anti-inflammatory agent, it was active only against TPA (85,2% at the dose of 3 mg/ear); the infuse extract (EINF) only showed analgesic activity while EHE exhibited a mild analgesic action (12.8%) and a moderate anti-inflammatory effect against AA (38%) and TPA (65.3%) respectively.

Discussion

It must be emphasized that there are no reports about the presence of saturated and highly polyunsaturated fatty acids, ß-sitosterol and kaempferol for *M. auristipulata.* There are several reports in the literature on the antimicrobial activity of γ-linolenic acid. Lee *et al*., (2002) [17] reported MICs for γ-linolenic acid against *Bacillus cereus* and *S. aureus* of 20 and 50 ppm respectively. Synergic effect of γ-linolenic acid and antibiotics such as amikacin and cephtazidime has also been reported [18]. On the other hand, C1 was inactive as antimicrobial agent.

C1 showed a significant anti-inflammatory activity against TPA (45.5%) at 1 mg/ear doses, being inactive against AA-induced inflammation, and it showed a weak analgesic activity at 100 mg/kg doses. Palmitic acid was the most important compound of C1, this fact could partly explain its in vivo pharmacological activity. Its anti-inflammatory activity has been reported for various species, such as *Plantago major* [19]. Its mechanism of action would occur through the lipid peroxidation reduction that could reduce the level of cyclooxygenase -2 - enzyme and the alpha tumoral necrosis factor, TNF-α [20]. The most important compound of C2 was γ-linolenic acid and it has been reported that it has anti-inflammatory activity [21,22,23]. However, C2 only had a mild anti-inflammatory activity against TPA. FDC and FDK obtained from bioactive DCE exhibited analgesic (73.3 and 47.4 respectively at doses of 100 mg/kg) and anti-inflammatory activities against TPA (62.2 and 42.9 % respectively at 1 mg/ear doses) and not against AA. The results obtained particularly for ERE and EDCE in all in vivo assays suggested to do further research on their chemical composition to isolate and to identify other new bioactive molecules with analgesic and/or anti-inflammatory effect. Kaempferol and β-sitosterol were the main isolated compounds from FDK and FDC respectively.

In previous studies, β-sitosterol has been reported as anti-inflammatory, analgesic and antipyretic steroidal compound after *per os* administration [24,25]. This steroid has also proved to be an effective topical anti-inflammatory agent mainly in acute TPA-induced inflammation; its effect on leukocyte migration to the inflamed site might be an important issue of its mechanism of action [26]. Therefore, this steroid is one of the responsible compounds of the effects exhibited for FDC and DCE against TPA-induced oedema and per os analgesic activity. The anti-inflammatory and analgesic activity of FDK could be partly due to kaempferol. This flavonoid shows a strong and extended anti-inflammatory effect. This in vitro activity was assessed by determining its inhibitory effect on chemical mediators released from cells, neutrophils, macrophages, and microglial cells [27,28]. In addition, kaempferol enhanced both random and f-Met-Leu-Phe-directed migration in in vitro murine neutrophils [29]. Therefore, this flavonoid is one of the responsible compounds of the effects showed for FDK and DCE against TPA-induced oedema and per os analgesic activity. In xanthine oxidase activity, only DCE was active against xanthine oxidase with 49% of inhibitory effect ($p < 0.05$). This effect could be partly due to kaempferol [30,31].

In this study we demonstrate that the antimicrobial activity of *Malesherbia auristipulata* is explained by the presence of a mixture of polyunsaturated fatty acids formed mainly by γ-linolenic acid. In addition, the antioxidant action of the dichloromethane extract would be caused by kaempferol.The *in vivo* antiinflammatory and analgesic effects confirm the medicinal uses as antiasmathic of some *Malesherbia* species, effect are partly responsible for kaempferol, β-sitosterol and a mixture of highly saturated fatty acids mainly constituted by palmitic acid.

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References

1. Ricardi M. Gayana 1965; 12**:** 3-10.
2. Marticorena C. Quezada M. Catálogo de la flora vascular de Chile. Gayana 1985; 42 (1-2)**:** 57-58.
3. Pastor S. Angeles E. Alvarez JL, Gutiérrez L. Jayos E. Briceño I. Rosales M. Gómez L. Sevilla R. Del Carpio C. Rivera CJ, Informe FAO. Perú: Informe nacional para la Conferencia Técnica de la FAO sobre recursos fitogenéticos. Leipzig 1996; 133.
4 Aenor C. Asociación española de normalización norma UNE 55037-73. Catálogo de Normas UNE, Madrid; 1991.
5. Erazo S. Negrete R. Zaldívar M. Backhouse N. Delporte C. Silva I. Belmonte E. López-Pérez JL, San Feliciano A. Planta Med 2002a*;* 68**:** 66-67.
6. Erazo S. Zaldívar M. Delporte C. Backhouse N. Tapia P. Belmonte E. Delle Monache F. Negrete R. Planta Med 2002b; 68**:** 361-363.
7. Balows A. Hausler Jr. WJ, Hermann KL, Isenberg HD, Manual of clinical microbiology, 5th edn. Shadomy H.J. (ed). Wahsington DC: American Society for Microbiology 1991; 1105-1112.
8. Rahalison L. Hamburger M. Hostettmann K. Monod M. Frenk E. Phytochem Anal 1991; 2: 199-203.
9. Davies NM, Roseeth AG, Appleeyaard CB, Mcknight W. Del Soldado P. Calignano A. Cirino G. Wallace JL, Aliment Pharmacol Ther 1997; 11**:** 69-79.
10. Hollander M. Wolfe DA, Nonparametric statistical methods. J. Wiley and Sons: New York, 1973; 68-70.
11. Delporte C. Backhouse N. Salinas P. San-Martín A. Bórquez J. Loyola A. Bioorg Med Chem 2003; 11**:** 1187-1190.
12. Lloret S. Moreno JJ, Biochem Pharmacol 1995; 50(3)**:** 347-353.
13. Delporte C. Backhouse N. Erazo S. Negrete R. Vidal P. Silva X. López-Pérez JL, San Feliciano A. Muñoz O. J Ethnopharmacol 2005; 99 (1): 119-124.
14. Noro T. Oda Y. Miyase T. Ueno A. Fukushima S. Chem Pharm Bull (Tokyo) 1983; 31: 3984-3987.
15. Nakanishi K. Infrared absorption spectroscopy practical. Holden-Day Inc: San Francisco and Nankodo Company Limited, Tokio, 1962; 233.
16. Mabry TJ, Markham KR, Thomas MB, The systematic Identification of Flavonoids. Springer-Verlag: New York, 1970; 58.
17. Lee JY, Kim YS, Shin DH, J Agric Food Chem 2002; 50**:** 2193-2199.
18. Giamarellos-Bourboulis EJ, Grecka P. Dionyssiou-Asteriou A. Giamarellos H. J Antimicrob Chemother 1995*;* 36**:** 327-334.

19. Ringbom T. Huss U. Stenholm A. Flock S. Skattebol L. Perera P. Bohlin L. J Nat Prod 2001; 64(6)**:** 745-749.
20. Nanji AA, Zakim D. Rahemtulla A. Daly T. Miao L. Zhao S. Khwaja S. Tahan SR, Dannenberg A.J. Hepatology 1997; 26(6):1538-1545.
21. Goffman FD, Galletti S. J Agric Food Chem 2001;49(4)**:** 2104.
22. Song C. Li X. Leonard BE, Horrobin DF, J Lipid Res 2003;44(10): 1984-1991.
23. Ziboh VA, Miller CC, Cho Y. Am J Clin Nutr 2000;71(1 Suppl)**:** 361 S6-S.
24. Handa SS, Chawla AS, Sharma AK, Fitoterapia 1992; 63**:** 3-21.
25. Santos ARS, Niero R. Cechinel Filho V. Yunes RA, Pizzolatti MG, Delle Monache F. Calixto JB, Planta Med 1995; 61: 329-332.
26. Gómez M. Saenz M. García M. Fernández M. Z Naturforsch [C] 1999*;* 54**:** 937-941
27. Della Loggia R. Ragazzi E. Tubazo A, Fascina G Vertua, Pharmacol Res Commun 1988*;* 20(5)**:** 91-94.
28. Goel RK, Pandey VB, Dwivedi SP, Rao YV, Indian J Exp Biol 1988; 26(2)**:** 121-124.
29. Kenny MT, Balistreri FJ, Torney HL, Immunopharmacol Immunotoxicol 1990;12**:** 527-541.
30. Iio M. Ono Y. Kai S. Fukumoto M. J Nutr Sci Vitaminol (Tokyo) 1986; 32**:** 635-642. 31.Chang WS, Lee YJ, Chiang HC, Anticancer Res 1993; 13**:** 2165-2170.

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