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FLAVONOIDS OF *NOTHOFAGUS* SPECIES OF CHILE

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ABSTRACT.– The flavonoids isolated from the leaves and twigs of 9 species of the genus *Nothofagus* are, orobol 7-0 rhamnoglucoside, quercetin 3-0 rhamnoglucoside, quercetin 3-0 galactoside, 7-4' dihydroxyflavone 7-0 rhamnoglucoside, quercitrin, kaempferol, naringenin and dihydrokaempferol. The paired affinity between the species are calculated and phylogenetic relationship is discussed. Anticancer and antimicrobial activities of ethanol extracts of the leaves and twigs of *Nothofagus* species is reported.**

INTRODUCTION

Nothofagus species (Fam. *Fagaceae)* are well known for their comercial valuable timber. Nine species and one hybride *Nothofagus leonii (N. glauca* X *N. alesandrii)* grow in Chile. Flavonoids, as secondary metabolic products of plants due to their universal frequence in many plants have been used as valuable phyletic markers for classification of plants on evolutionary principles[2].

Interest in flavonoid constituents have also been due to their biological activities[3]. Due to these re reasons the phenolic constituents of the leaves and twigs of *Nothofagus* species growing in Chile were examined.

The present communication reports the flavonoid constituents of the leaves and twigs of *Nothofagus alesandrii* Espinosa, *Nothofagus alpina* (Poepp. et Endl.) Oerst. *Nothofagus antarctica* (Forst.) Oerst. *Nothofagus betuloides* (Mirb.) Blume, *Nothofagus dombeyi* (Mirb.) Blume, *Nothofagus glauca* (Phil) Krasser, *Nothofagus nitida* (Phil). Reiche, *Nothofagus obliqua* (Mirb.) Blume, and *Nothofagus pumilio* (Poepp. et Endl.) Krasser. The paired affinity[4] based on phenolic constituents between the species was calculated and the phylogenetic relationship is discussed. The extract of the leaves and twigs have been screened for anticancer and antimicrobial activities.

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** Antitumor testing is carried out through the auspicies of the Cancer Chemoteraphy National Service Centre (CCNSC), National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014, using established protocols (I). Antimicrobial testing has been carried out in the Department of Microbiology, University of Concepción, Chile.

RESULTS AND DISCUSSION

Table I records plants under investigation whith their botanical names, the month, year and the locality of collection. In table II the spectroscopic data of the isolated flavonoids are recorded. The flavonoids isolated and characterised from *Nothofagus* species of Chile are presented in table III. Orobol 7-0 rhamnoglucoside, quercitrin and quercetin 3-0 rhamnoglucoside were isolated from all the 9 species of *Nothofagus*. Kaempferol was isolated from *N. glauca*, *N. betuloides*, *N. dombeyi* and *N. nitida*. Dihydrokaempferol was isolated from *N. betuloides*, *N. dombeyi* and *N. nitida*. Naringenin was isolated only from *N. obliqua* and *N. dombeyi*. Quercetin 3-0 galactoside was isolated from *N. obliqua*, *N. antarctica* and *N. glauca*. 7-4'-dihydroxyflavone 7-0 rhamnoglucoside was isolated from *N. obliqua*, *N. alpina*, *N. antarctica* and *N. alesandrii*. The uncharacterised flavonoid compounds, the compound A, the compound K and the compound 0 were also isolated from these species. The compound A, a flavone derivative was found to occur in *N. obliqua*, *N. alpina*, *N. alesandrii*, *N. dombeyi*, *N. betuloides*, *N. nitida* and *N. pumilio*. The compound K, an aurone derivative was found to occur in *N. Obliqua*, *N. alpina*, *N. antarctica* and *N. dombeyi*. The compound 0, a chalcone derivative was found to be present in *N. betuloides*, *N. dombeyi* and *N. nitida*.

TABLE I. EXTRACTS OF NOTHOFAGUS SPECIES OF CHILE

N°	PLANT	PLACE OF COLLECTION.	DATE OF COLLECTION.
1	Nothofagus alesandrii Espinosa	Maule, Chanco.	October 1971.
2	Nothofagus alpina (Poepp. et Ends.) Oerst.	Malleco, Nahuelbuta.	January 1970.
3	Nothofagus antarctica (Forst.) Oerst.	Magallanes, Río Seco.	February, 1971
4	Nothofagus betuloides (Mirb.) Blume	Magallanes, Chorrillo de la Piedra.	February, 1971.
5	Nothofagus dombeyi (Mirb.) Blume	Maule, Chanco.	October, 1971.
6	Nothofagus glauca (Phil.) Krasser	Maule, Chanco.	October, 1971
7	Nothofagus nitida (Phil.) Reiche	Valdivia, Parque Nacional.	December, 1971.
8	Nothofagus obliqua (Mirb.) Blume	Ñuble, Quirihue.	December, 1971.
9	Nothofagus pumilio (Poepp. et Endl.) Krasser.	Magallanes, Chorrillo de la piedra.	February, 1971.

TABLE II. SPECTRAL PROPERTIES AND Rf VALUES OF THE FLAVONOIDS FROM NOTHOFAGUS SPECIES OF CHILE.

		max (MeOH) nm.													
N°	COMPOUND	MeOH		NaOMe		$AlCl_3$		$AlCl_3$/HCl		NaOAc		NaOAc/H_3BO_3		Rf x 100	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	Qg	361	257	410	272	440	275	405	264	380	277	380	265	47	43
2	R	359	257	408	272	435	275	402	271	393	271	387	262	45	53
3	Q	350	256	394	270	432	275	401	270	372	272	367	260	60	60
4	N	325	288	323	288	375	312	371	311		323		293	90	31
5	F	324	253 sh	358	294	327	255 sh	324	253 sh	334	260 sh	328	356 sh	90	43
6	Or	343 sh	262	335	290 sh	372	269	376	370	331 sh	263	332 sh	260	30	62
7	Dk	331 sh	293	325	246	382	316	378	280 sh	327	284 sh	336 sh	296	90	51
8	Ka	367	266	420	278	424	272	424	269	387	274	372	267	83	8
9	A	372	256	430	331		272		270	414	253	370	255	3	47
10	K	367	255	360	254	373	272	396 sh	271	392	287	390	277	8	10
11	O	354	263	343 sh	258	319	237	319	227	321	254 sh	299 sh	247 sh	34	7

Qg= Quercetin 3-O galactoside; R= Rutin; Q= Quercitrin; N= Naringenin; F= 4,7-Dihydroxyflavone; 7-O rhamnoglucoside; Or= Orobol 7-O-rhamnoglucoside; Dk= Dihydrokaempferol; Ka Kaempferol; A= Compound A; K=CCompound K; 0= Compound 0. TBA: Tert-butanol; Acetic acid; water (3:1:1); HoAc= Acetic acid 15%.

TABLE III. FLAVONOIDS OF NOTHOFAGUS SPECIES OF CHILE.

		Ka	Dk	Or	N	Q	R	Qg	F	A	K	O
1	Nothofagus ales andrii Espinosa	-	-	+	-	+	+	-	+	+	-	-
2	Nothofagus alpina (Poepp. et Endl) Oerst.	-	-	+	-	+	+	-	+	+	+	-
3	Nothofagus antarctica (Forst) Oerst.	-	-	+	-	+	+	+	+	-	+	-
4	Nothofagus betuloides (Mirb) Blume	+	+	+	-	+	+	-	-	+	-	+
5	Nothofagus dombeyi (Mirb) Blume	+	+	+	+	+	+	-	-	+	+	+
6	Nothofagus glauca (Phil) Krasser	+	-	+	-	+	+	+	-	-	-	-
7	Nothofagus nitida (Phil) Reiche	+	+	+	-	+	+	-	-	+	-	+
8	Nothofagus obliqua (Mirb) Blume	-	-	+	+	+	+	+	+	+	+	-
9	Nothofagus pumilio (Poepp. et Endl) Krasser	-	-	+	-	+	+	-	-	+	-	-

Ka= Kaempferol
Dk= Dihydrokaempferol.
Or= Orobol 7-O rhamnoglucoside.
N = Naringenin.
Q = Quercitrin
R = Rutin.
Qg= Quercetin 3-O galactoside.
F = 7,4' - dihydroxiflavone 7-O rhamnoglucoside.
A = Compound A.
K = Compound K.
O = Compound O.

The individual species of any genus have genetic differences which can be spected to produce chemical and morphological changes. The chemical aspect has been studied in *Nothofagus* species growing in Chile, by mean of the phenolic constituents present in the ethyl acetate and water soluble fractions of methanol extracts of the leaves and twigs of each specie. These fractions were resolved by two-dimensional paper chromatography. The individual phenolic constituents were located and compared by their characteristic fluorescence in ultraviolet light before and after exposure to ammonia. The comparative data thus obtained is recorded in table IV. Each distinct colour at any particular Rf value was regarded as a separate character[5] and the total number of characters for a specie is the total number of spots occuring for this specie. The paired affinity (P.A.) between the species was calculated by the method of Alston and Turner[4].

$$\text{P.A.} = \frac{\text{Spots common for A and B}}{\text{Spots in A + B}} \times 100$$

By this method the genus as a whole appears to be homogenous with respect to the phenolic constituents present in them. The maximum paired affinity between *N. glauca* and *N. betuloides* was found 71.5%, between *N. obliqua* and *N. alpina* 71%, between *N. nitida* and *N. betuloides* was 70%. A minor P.A. was found to exist in the following species. Between *N. dombeyi* and *N. antarctica.* The P.A. was found 48%, between *N. nitida* and *N. antarctica* and between *N. obliqua* and *N. betuloides* it was found to be 50%.

TABLE IV. PHENOLIC CONSTITUENTS OF NOTHOFAGUS SPECIES ENDEMIC TO CHILE.

Spot	N ant		N ale		N alp		N bet		N dom		N gla		N nit		N obl		N pum		FLUORESCENCE			
	EA	H_2O	EA	H_2O	EA	H_2O	EA	H_2O	EA	H_2O	EA	H_2O	EA	H_2O	EA	H_2O	EA	H_2O	V	V (NH_3)	UV	UV (NH_3)
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	red	red	red	red
2			+																yellow	yellow	brown	brown
3	+		+		+		+		+		+		+		+		+		yellow	yellow	yellow	yellow
4	+		+		+		+		+		+		+		+		+		yellow	yellow	yellow	yellow
5	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+	brown	brown	brown	brown
6	+				+		+				+		+		+		+		yellow	yellow	yellow	yellow
7					+														yellow	yellow	yellow	yellow
8	+										+								yellow	yellow	yellow	yellow
9	+										+				+				yellow	yellow	yellow	yellow
10							+		+										yellow	yellow	brown	brown
11		+		+		+		+		+		+							yellow	yellow	brown	yellow
12		+		+				+		+		+		+		+		+	brown	brown	brown	green
13		+				+		+				+		+		+		+	yellow	yellow	brown	yellow
14							+				+								violet	violet	violet	violet
15																			yellow	yellow	yellow	yellow
16				+															---	---	violet	brown
17								+	+	+	+			+	+				---	---	brown	black
18				+						+		+							---	---	blue	white
19		+		+				+				+				+			---	---	blue	white
20				+												+			---	---	violet	brown
21			+									+							---	---	yellow	black
22		+		+		+		+		+	+			+		+			---	---	blue	green
23		+		+										+					---	---	blue	blue
24						+													---	---	blue	blue
25						+								+					---	---	blue	white
26						+				+						+		+	---	---	yellow	yellow
27						+										+		+	---	---	blue	blue
28		+						+		+									---	---	violet	brown
29		+																+	---	---	brown	brown
30		+																	---	---	brown	yellow
31								+		+				+					---	---	brown	brown
32							+	+											---	---	blue	yellow
33										+								+	---	---	blue	black
34										+									---	---	yellow	black
35										+									---	---	yellow	yellow
36										+						+			---	---	brown	brown
37				+						+		+		+				+	---	---	yellow	yellow
38				+		+		+		+				+		+		+	---	---	blue	white
39				+															---	---	white	white
40	+		+	+		+		+				+		+		+			---	---	brown	brown
41	+		+	+						+						+			---	---	brown	brown
42	+		+	+										+					---	---	blue	green
43	+			+				+						+					---	---	yellow	yellow
44	+			+				+						+					---	---	yellow	black
45	+																		---	---	yellow	yellow
46	+																		---	---	yellow	yellow
47						+		+		+		+							---	---	brown	yellow
48								+						+					---	---	yellow	yellow
49								+											---	---	yellow	yellow
50								+											---	---	yellow	yellow
51												+							---	---	red	red
52														+					---	---	blue	blue
53														+					---	---	brown	green

V= visible light
UV= ultraviolet light
EA= ethyl acetate
N. ant= N. antarctica
N. ale= N. alessandrii
N. alp= N. alpina
N. bet= N. betuloides
N. dom= N. dombeyi
N. gla= N. glauca
N. nit= N. nitida
N. obl= N. obliqua
N. pum= N. punilio

The isolation of a chalcone derivative (compound 0, spot No. 17 table IV) from *N. betuloides*, *N. dombeyi* and *N. nitida* the most primitive species of the *Nothofagus* genus as it is shown by their perennial character is important since chalcones represent the earlier stages of the biogenesis of flavonoids[8] and this supports the phylogenetic relationship that exists in the species of this genus. The hydroxyflavonoids such as quercetin, kaempferol and their derivatives are known to have antifungal activity[3]. These hydroxyflavonoids are found to occur frequently in *Nothofagus* species which supply valuable timber. Ethanol extracts of the leaves and twigs of *N. obliqua*, *N. alpina*, *N. antarctica*, *N. dombeyi* and *N. pumilio* were screened for anticancer activity against the cells derived from human epidermoid carcinoma of the nasopharinx (KB) *in vitro*, *in vivo* against the P-388 lymphocytic leukemia (PS) and L-1210 lymphoid leukemia (LE) in mice. Marginal cytotoxic activity was found in the extract of *N. obliqua* ED^{50} (2.6 x $10^{(1)}$), *N. antarctica* ED^{50} (2.1 x $10^{(1)}$), and *N. dombeyi* ED^{50} (2.3 x $10^{(1)}$). The extracts of *N. pumilio* and *N. alpina* were found inactive in these test systems used. The ethanol extract of *N. alpina* and *N. obliqua* were also evaluated for antimicrobial activity against *Sarcina lutea*, *Staphilococcus aureus* and *Escherichia coli*. Moderate antibacterial activity was found against all the three test systems in the extracts of *N. alpina*. The extract of *N. obliqua* was found active against *Staphilococcus aureus*.

EXPERIMENTAL

Plant material.– The leaves and twigs of each *Nothofagus* species were collected separately. The date, year and the locality of collection of each plant is given in table I. The collected plant material was dried in a drying oven.

Extraction and fractionation.– The powdered leaves and twigs in each case were extracted separately with MeOH in Soxhlet. The solvent from the MeOH extract was removed in a rotatory evaporator to give the methanolic extract. This extract was treated with water at 100° and the precipitated obtained was filtered. The aqueous filtrate was first extracted with $CHCl_3$ and then with EtOAc. The $CHCl_3$ extracts which gave negative test for flavonoids were discarded. The solvent from EtOAc extracts and water from the remaining aqueous layer was removed *in vacuo* to give ethyl acetate and water soluble fractions. The flavonoids were isolated by two dimensional chromatography in Whatman 3MM chromatographic paper and were characterised by the usual spectroscopic techniques and by co-chromatography with authentic samples.

Chromatography on polyamide.– The ethyl acetate and water soluble fractions were chromatographed on polyamide column by the method of Markham and Mabry[12]. The elution was monitored by T.L.C. (cellulose). The spots were detected under UV light before and after exposure to ammonia.

Chromatographic Examination.– Two-dimensional chromatograms were prepared using first t-butanol, acetic acid, water (3:1:1) (TBA) and then 15% acetic acid (15% HOAc). One-dimensional chromatograms were also prepared using these solvents and HCl, acetic acid, water (3:1:10) (Forestal). The chromatograms were examined under UV (254 and 365 nm) light before and after exposure to NH_3 vapours. The chromogenic sprays used were $AlCl_3$ 5% in methanol for phenolics and Patridge reagent modified for sugars.

Silica Gel (G.F. 254 E. Merck) plates were used. The solvent systems were: I-chloroform, ethyl acetate, formic acid (5:4:1); II-toluene, ethyl formic acid (5:4:1); III-chloroform, acetic acid (8:2); IV-ethyl acetate, ethanol (1:1). Polyamide plates were prepared by the standard procedure. The solvent used were: methanol, acetic acid, water (90:5:5) and chloroform, methanol, butane-2-one (1:1:1). Whatman 3MM paper was used for the preparative paper chromatography, and the solvent system was the upper layer of n-BuOH, AcOH, H_2O (4:1:5) (BAW).

Cellulose plates were prepared by the standard procedure. The solvent system used were ethyl acetate, acetic acid, water (3:1:3); n-butanol, acetic acid, water (5:1:4) or (6:1:3) and the spots were detected by the usual method.

Ultra violet spectra of the flavonoids were recorded with a Unicam SP 800 spectrophotometer.

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CONTENIDO ESTEROIDAL DE *YUCCA FILIFERA* (HORT. EX ENGELM.) AISLAMIENTO DE LAS FILIFERINAS (SAPONINAS ESTEROIDALES)***

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ABSTRACT.– From the leaves, stem, peduncle, flowers and seeds of *Y. filifera,* after acid hydrolysis, sarsasapogenin (1) was isolated as the only aglycone. The highest content of sarsasapogenin was found in the seeds, where it is linked to different sugars, constituing a mixture of glycosides. Hydrolysis of these mixed glycosides which we have called filiferines afforded sarsasapogenin in 8% yield.

Las plantas del género *Yucca* (Liliáceae) han sido muy estudiadas debido a que en sus diferentes especies es común encontrar productos de naturaleza esteroidal. Se han estudiado casi todas las especies de este género, entre ellas, las que son nativas de los Estados Unidos de Norteamérica[1-5] y las cuatro especies que crecen en Egipto[6].

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