Report on Guidance in Phytochemistry

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The author stayed in Paraguay as the dispatched expert of phytochemistry for two months from May 10, 1985. Various activity tests were conducted in consecutive order of extraction, on the extracts of 91 species of herbs collected during the stay, which were composed of 71 species bought at the 4th market in Asuncion City, 16 species purchased from a herb dealer (Yamawaki Company), and 4 wild species collected in the neighbourhood of Asuncion University campus. (as in a separate table)

1986. 1. 29

別 表

Material Extracted	上段 温浸(h) 下段 室温(r) Assay		Aldose Reducase (IC_{50})	Angiotensin-converting enzyme	β-Glucuronidase	Xanthine Oxidase	Urease	KB Cell	L5178Y Cell	Inflammation	Histamine	Diabetes
			10μ/ml 50% 以上	100μ/ml 50% 以上	IC_{50}<5μ/ml No.31~ 10μ/ml→70%以上+、90%以上++)	IC_{50} <10μ/ml	IC_{50}<50μ/ml	ED_{50}<50μ/ml	ED_{50}<50μ/ml (<100μ/ml±)	20 mg/rat *→+ **→++	100μ/ml >50%+ >70%++	
1. AMBAŶ	Ceropia adenopus Mart	hot (h) room (r)										
2. SARÁ MOROTÍ	Citharexylum myrianthum Cham	h r	++(2.2×10^{-6}) +(5.0×10^{-6})					+(54μ)	(N.T) +(37μ)			
3. PIPER Sp. No.2 (Hojas lunceoladas)									+			
4. PIPER Sp. No.1 (Hojas grandes anchas)		r								+?		
5. ÑANGAPIRŶ	Eugenia uniflora L.	h				+(6.6μ)						
6. CEDRÓN-CAPIÍ	Andropogon altratus	h r		+(50%)				±	+(34μ)			
7. TŶPŶCHA-KURATŨ	Scoparia dulcis L	h r	+(IC_{50} 4.7×10^{-6})		+(5.4)			± +(52μ)	+	++		
8. TAPECUÉ	Acanthospermum australe O. K.	h	++(IC_{50} 2.3×10^{-6})		+(4.0)			+(46μ)		++		
9. KOKÚ	Allophylus edulis Radrk.	h			+(5.0)			±				++
10. COLITA	Cordia salioifolia Cham.	h r		+(50%)	+(5.0)	±(9.5μ)		±	±(65μ)			
11. ŶVA HAI	Eugenia myrcianthes Niedenzu	h			+(5.0)	+(2.5μ)	+(28μ)					
12. PARAPARAI MÍ	Phyllanthus niruri L.	h r	++(IC_{50} 1.1×10^{-6}) ++(IC_{50} 1×10^{-6})	+(32%)		±		±(44μ)	(N.T) +(21μ)	(+)18.7%(3h) +	( )	
13. MIL HOMBRE	Aristlochia triangularis Chem. et schleht	h r		( )	( )	( )	( )	( )	(N.T) +(18μ)		++ ++	
14. MARCELA	Achyrochine satureioides (Lam)DC.	h r	+(3.1×10^{-6}) ++(2.4×10^{-6})		++(10μ-86.3%)	++(50μ-94%) ±(17μ)		+(50μ-66.8%) ±(72μ)	(N.T) +(34μ)		++ +	
15. COLA DE CABALLO	Equisetum giganteum L.	h r				±(50μ-59.8%)		+(53μ)	(N.T)		( )	
16. ROMERO	Rosmariuns officinalis L.	h r			±(filt)	+(filt)	++ ++(10.5μ)	+(50μ)	(N.T) +(50μ)		++	
17. BURRITO	Minthostachys mollis Kunth								(N.T)	(++)31.1%(1h)	( )	
18. YAGUARUNDI	Piper fulvencens C.D.C. =PIPER Sp. No.1	h			±				(N.T) ±(55μ)	h r	( )	
19. ALHUCEMA	Lavandula latifolia Vill.	h			±				(N.T)	23.2 +18.9%(4h)	( )	
20. ESPARTILLO GUAZU	Elinorus latiflorus	h							(N.T)	N.T	( )	
21. EUCALIPTO	Eucalyputus globulus	h r	++(2.2×10^{-6}) +(3.0×10^{-6})		+			+(50μ 78.2%) +(50μ 78.2%)	(N.T)	N.T	( )	

Material Extracted \ Assay		Aldose Reductase	Angiotensin-converting enzyme	β-Glucuronidase	Xanthine Oxidase	Urease	KB Cell	L5178Y Cell	Inflammation	Histamine	Diabetes
22. CANGOROSA	Maytenus ilicifolia Maxt					±					
23. SIETE, SANGRIA(S)	Cuphea racemosa (L.f.) Spreng										
24. CEDRON PARAGUAY	Lippia triphylla Kuntze										
25. SIEMPRE VIVA	Gomphrena parennis L.			+(10μ−70%)							
26. KAA HEE	Stevia rebaudiana Bert.	$+\sim++(2.0\times10^{-6})$		±							
27. AROMITA	Acacia farnesiana (L) Willd				+(50μ−77.3%)						
28. CHIRCA MELOSA	Baccharis articulata Pers	$+(3.1\times10^{-6})$		+(10μ−76.9%)						++	
29. CULANTRILLO	Adiantum cuneatum L.	$+(4.5\times10^{-6})$							N.T		
30. PIPER Sp. №3											
31. CAMBARÁ	Moguinia polymorpha Cab.	$+(3.0\times10^{-6})$		+				N.T	N.T		
32. PARATODO (PIRÉ)	Tabebuia caraiba Mart.								N.T		
33. ARACHICHU	Solanum nigrum L.								N.T		
34. ALTAMISA-(ITÉ)	Ambrosia artemisifolia L.						+(50μ−81%)		N.T		
35. CABELLO DE ANGEL	Cusenta xanthochortos Fugelm								N.T		
36. CAATAI	Polygonum acre H.B.K.								N.T		
37. CAARE {aerea (RAIZ)	Chenopodium ambrosioides L.						?(50μ−69%)		N.T		
38. SAUCO	Sambucus australis Chem. et Sch.								N.T		
39. MOLLE-I		+							N.T		
40. SALVIA		+							N.T		
41. CARAGUATÁ RUÂ	Eryngium floribundum Cham								N.T		
42. PINDO (RAPO)	Arecastrum romanzoffianum Bece.						±(50μ−57%)		N.T		
43. ŶSŶ	Protium heptaphyllum (Anth) Mart.	±							N.T		
44. GUAYACAN (CORTEZA)	Caesalpinia melanocarpa Griseb.	+	+(41%)						N.T		

Material Extracted \ Assay		Aldose Reductase	Angiotensin-converting enzyme	β-Glucuronidase	Xanthine Oxidase	Urease	KB Cell	L5178Y Cell	Inflammation	Histamine	Diabetes
45. PERDUDILLA NEGRA											
46. MALVA BLANCA	Sida cordifolia L.						?(50μ-67%)		N.T		
47. GUAYABA	Psidium guajava L.	+							N.T		
48. TAPERŶVA-HÚ	Cassia occidentalis L.		+(34%)						N.T		
49. PENICILINA											
50. VERBENA-Í	Verbena bonariensis L.	±		+					N.T		
51. TAMANDÁ CUNÁ	Catasetum barbatum Lindle								+(1hr 30.2%, 2hr 20.0%)	++(50%) (diphen. 7.2×10^{-8})	
52. AGUAPÉ PURUÁ	Eichhornia crassipes (Mart) Solms								N.T		
53. ŶVŶRÁ PŶTÁ (AEREA)	Peltophorum dubium (Spreng) Tamb.		+(52%)	+		+			N.T		
54. ALCANFOR DEL CAMPO		+							N.T		
55. CALAGUALA	Polypodium phyllitidis L.								N.T		
56. ŶVOPÉ	Gleditsia amorphoides Taub.						++(50μ-89%)		+		
		finished	finihed	finished		finished	finished	not tested	N.T not tested	finished	not tested

From the tests, the results of the study are herewith reported on Aldose Reductase inhibitory activity, anti-histamin activity as well as carrageenan edema inhibitory effect (topical application only).

I. Aldose Reductase (AR) inhibitory activity

In high blood sugar state as in diabetes, even due to slight rise in AR activity in cell, the reduction from aldose to alditol becomes intensified, thus encouraging the intracellular accumulation of the product in the lens. As a result, celluar acatastatia arises and progresses to cataracta. Hence, the substance that inhibitis AR activity can be expected to exhibit a therapeutic or preventive effect against cataract, one of complications of diabetes.

In this year, as the results of the examination on AR inhibitory activity of 29 kinds of Paraguayan herb extracts, a distinct activity was observed in the following 9 species. (The inhibition ratio exceeding 50% at the concentration of 10 μg/mℓ.) (Table 1)

Table 1. Species with AR inhibitory activity noticed

Exp. No.	Plant material	Original plant	*Evaluation (IC_{50} μg)
2	Sará morti	Citharexylum myrianthum Cham.	+ + (2.2)
7	Týpýchá-kuratũ	Scoparia dulcis L.	+ (4.9)
8	Tapecué	Acanthosperumum australe O.K.	+ + (2.3)
12	Para-paraí mi	Phyllanthus niruri L.	+ + (1.1)
14	Marcela	Achyrocline satureioides (Lam.) DC	+ (3.1)
21	Eucalipto	Eucalyptus sp.	+ + (2.2)
26	Caá heé	Stevia rebaudiana Bert.	+ + (2.0)
28	Chirca melosa	Baccharis articulata Pers.	+ (3.1)
29	Culantrillo	Adiantum cuneatum Langsd. et Fish	+ (4.5)

* In this column, + + symbol indicates the case where $IC_{50} < 2.5$ μg.

The extraction was as a rule conducted with 70% hot EtOH extraction, but some were extracted at room temperature because of the beginning circumstances in the laboratory of Asuncion Univesity. The materials on which the activity was observed in extracts at room temperature are 4 species, i.e. Exp. No. 2 (+ IC_{50} 5.0 μg/mℓ), No. 12 (+ + IC_{50} 1.0 μg/mℓ), No. 14 (+ + IC_{50} 2.4 μg/mℓ) and No. 21 (+ IC_{50} 3.0 μg/mℓ).

Among these specimens on which the activity was noticed, the fractionation and isolation for active constituent was conducted on Tapecú. As the result, 5,7,4'-trihydroxy 3,6-dimethoxy flavone was obtained, on which strong inhibitory activity was observed (IC_{50} 1×10^{-7} M/mℓ). The activity is stronger than that of quercitrin which has been admitted to be of strong AR inhibitory activity, and further, it is equivalent to or stronger than the activity of axillarin and LARI 1 which have been recently reported as flavonoids with stronger activity. The detail of the finding was published at the Pharmaceutical Society of Japan (the 10th annual meeting, held in Chiba, April 1986), and contributed. (A copy of the article attached.) Though Tapecué has been used in Paraguay for the treatment for blood stagnation, rheumatism, arthritis, blooding, etc., no information has been received yet that it is efficatious against diabetes and its complicaiton, cataract. At this time, as the AR inhibitory effect of this plant was unexpectedly observed, we expect the possibility of its use, for the treatment of cataract, the complication of diabetes. When we think of the fact that almost all of many currently used medicines were originally found in natural products, and that different activity from empirical pharmaceutical efficacy was noticed and previously unexpected medicine was made, the finding of AR inhibitory active constitent in Tapecué is significant, and so great expectation is entertained for the study of herbs which have been handed down by Guarani-Indio since old days.

II. Anti-inflammatory activity

1. Carraggenan edema inhibitory effect by topical application

Many herbs exhibit efficacy when their raw juice or extract is externally applied on a swelling, etc. Therefore, extracts of specimen herb were applied on carraggenan-induced edema to examine the anti-inflammatory effect. As the result, the effect was verified in the following 5 species out of 29 species (Exp. No. 1 ~ 30). (Table 2)

Table 2. Species with anti-flammatory effect

Exp. No.	Plant material	Original plant	*Evaluation (inhibition rate)
7	Tŷpŷchá-kuratũ	(Refer to Table 1.)	++ (46.8%)
8	Tapecué	(")	++ (61.7%)
12	Para-paraí mi	(")	+ (18.7%)
17	Burrito	Minthostachys mollis Kunth	++ (31.1%)
19	Alhucema	Lavandula latifolia Vill.	+ (18.9%)

* At 20 mg/rat, $*P < 0.05$ is indicated with +, while $** P < 0.01$ with ++.

Among those with observed activity, since Tapecué has been used as external application on swellings, the research for the ingredient of activity existed mainly in the dissolvable fraction in n-hexane was conducted. Isolation refinement was attempted with column-chromatograpy, etc., but only fatty acid and ursolic acid were isolated, because of insufficient quantity of material.

2. Inhibitory effect on histamin-induced ileum contraction

As for the way to examine the anti-inflammatory effect in the initial stage of acute inflammation, the inhibitory effect on histamine-induced contraction of the ileum isolated from guinea pigs was examined. The effect was noticed on 4 species out of 29 species. (Table 3)

Table 3. Species observed with contraction inhibitory effect

Exp. No.	Plant material	Original plant	*Evaluation
13	Mil hombre	Aristolochia trianglaris Cham. et Sch.	+ +
14	Marcela	Achyrocline satureioides (Lam.) DC.	+ +
16	Romero	Rosmarinus officinalis L.	+ +
28	Chirca melosa	Baccharis articulata Pers.	+ +

* Those of an inhibition raito exceeding 50% at 100 μg/mℓ are indicated with a symbol +, while those exceeding 70% with a symbol + +. (At this time, those with symbol + were omitted.)

Among those in which the activity was observed, the effective constituents of Mil hombre was investigated, because the preventive effect of Mil hombre against the bite by a venomous serpent or a scorpion has been handed down in South America, and its decoction is said effective when applied to external wound or swelling. As the result, compounds of 1-4were obtained from the effective fraction by various kinds of chromatography. It has been found that these are galbacin, 4'-hydroxy-3'-methoxy-3', 4'-desmethylenodioxygalbacin, cubebin and 3', 4'-dimethoxy-3', 4'-desmethylenedioxy cubebin, all of which have been reported to be isolated from this plant.

As the result of the investigation on the inhibitory effect of these compounds, the effect was observed in 2 (IC_{50} 1.1×10^{-5}) and 3 (IC_{50} 1.3×10^{-5}M). (In this connection, the value of Diphenhydramine, antihistamine medicine, is IC_{50} 6.0×10^{-8}.)

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Chemical and Pharmaceutical Studies on Medicinal Plants in Paraguay. I. Isolation and Identification of Lens Aldose Reductase Inhibitor from "Tapecué," *Acanthospermum australe* O.K.[1]

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The EtOH extract of "Tapecué," *Acanthospermum australe*, was found to have a potent inhibitory activity towards rat lens aldose reductase (AR). From the active fraction of the extract, 5,7,4'-trihydroxy-3,6-dimethoxyflavone was isolated. It was found to have higher activity ($IC_{50} = 1 \times 10^{-7}$ M) than quercitrin, which is a known inhibitor of AR ($IC_{50} = 1.8 \times 10^{-6}$ M in our bioassay).

Keywords——*Acanthospermum australe*; Compositae; 5,7,4'-trihydroxy-3,6-dimethoxyflavone; aldose reductase inhibitor; rat lens

There is a traditional system of medicine, "Medico de Yuyo," employing medicinal plants in Paraguay. In screening tests for biological activities of these plants "Tapecué," *Acanthospermum australe* (Compositae), showed weak inhibitory effects on β-glucuronidase activity and on the growth of KB cells and high inhibitory activity towards rat lens aldose reductase (AR). This paper deals with the isolation and identification of chemical constituents in "Tapecué," and identification of the active component inhibiting rat lens AR, which plays a significant role in the reduction of aldose to alditol under abnormal conditions such as diabetes.

"Tapecué" is an important crude drug which has traditionally been used for the treatment of blood stagnation, rheumatism and arthritis by internal administration, and of swelling and bleeding by external application in "Medico de Yuyo." Various diterpenes,[2] acanthospermal A, tridecapenta-3,5,7,9,11-yne-1-ene, thymol, isothymol, *etc.* have been isolated from this plant[3] but no studies in relation to the biological activity have been reported. Chemical and pharmacological studies of another plant of the same genus, *Acanthospermum glabratum*[4] have revealed no AR inhibitory activity.

EtOH : H_2O (7 : 3) extract (A) was suspended in water and extracted with *n*-hexane, $CHCl_3$ and *n*-BuOH successively to afford *n*-hexane extract (B), $CHCl_3$ extract (C), *n*-BuOH extract (E) and residue (F) (Fig. 1.)

The extract E (Table I), which was most active, was applied to a column of polyamide, and elution with MeOH : H_2O (3 : 2) followed by MeOH and $CHCl_3$ gave four fractions (fr. 1—4) (Fig. 1).

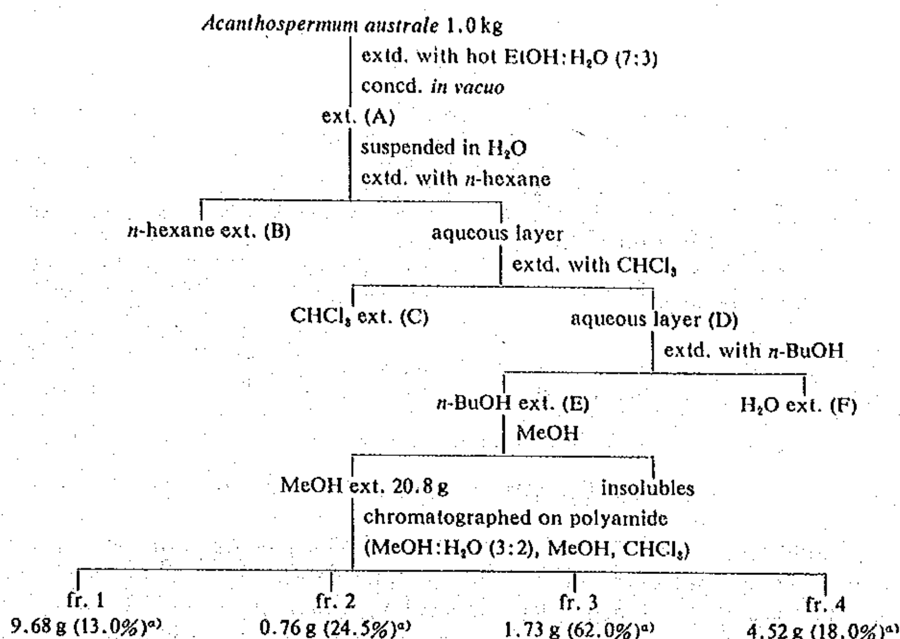

Fig. 1. Fractionation of Biologically Active Constituents of *Acanthospermum australe*

a) Values in parentheses indicate the inhibitory activities towards crude rat lens aldose reductase at the concentration of 1 μg/ml.

TABLE I. Inhibition of Crude Rat Lens Aldose Reductase by Extracts from *Acanthospermum australe* and Compounds 1—6

Extract	IC_{50} (μg)	Yield (%)	Compound	IC_{50} (μM)
A	2.3	100	1	0.1
B	20.0	13	2	—
C	4.0	14	3	3.2
D	2.6	—	4	9.2
E	1.5	29	5	4.8
F	13.0	43	6	—
			Quercitrin[a]	1.8

a) Quercitrin was assayed previously, and was tested again as a reference in this study.

Three crystalline compounds **1**, **2** and **3** were obtained from fr. 3, which exhibited higher activity than other fractions, by gel-filtration and silica gel column chromatography. Compounds **4**, **5** and **6** were obtained from fr.2 and fr. 4.

Compound **1**, yellow needles, exhibited a positive reduction test for flavonoids. Infrared (IR) and ultraviolet (UV) spectra of **1** showed the characteristic absorption patterns of flavonoids. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**, peaks due to four aromatic protons appeared as A_2B_2 type signals attributable to B ring protons. Another aromatic proton signal at 6.6 ppm assigned to the C-8 proton and a 6H singlet at 3.8 ppm attributed to two methoxyl groups were observed. The presence of three hydroxyl groups at C-5, C-7 and C-4′ in **1** was determined by analysis of the UV spectrum.[5] From the above results, **1** was concluded to be 5,7,4′-trihydroxy-3,6-dimethoxyflavone[6] and this identification

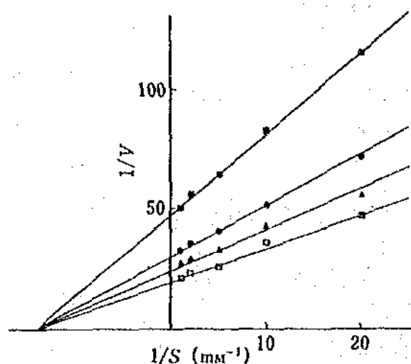

Fig. 2. Lineweaver-Burk Plots of Lens AR Activity

Enzyme activity was measured at each substrate concentration in the presence and absence of inhibitors. Key: (□) control, (●) in the presence of 10^{-7} M **1**, (▲) 5×10^{-8} M **1** and (■) 10^{-6} M quercitrin. The substrate is glyceraldehyde (S) and the velocity units (V) are changes in OD_{340}/200 s.

was confirmed by comparison of the physical and spectral data with those of an authentic sample.

Compounds **2**, **3**, **4** and **5** were identified as trifolin, hyperin, rutin and quercetin, respectively, by comparison of the physical and spectral data with those of authentic samples.

Compound **6**, a pale yellow powder, exhibited a negative reduction test for flavonoids and a positive color reaction to $FeCl_3$ and was concluded to be caffeic acid from the physical and spectral data.

Inhibitory Effect on Crude Rat Lens AR

Compound **1**, which has not previously been tested for inhibitory activity towards AR, exhibited the highest activity ($IC_{50}=1.0\times10^{-7}$ M) among compounds **1**—**6** and was about 18 times more potent than quercitrin ($IC_{50}=1.8\times10^{-6}$ M) (Table I).

According to Okuda *et al.*,[7] axillarin and LARI 1 are the most potent inhibitors of aldose reductase known so far ($IC_{50}=5.2\times10^{-8}$ and 4.2×10^{-8} M), respectively, being at least 6 times more potent than quercitrin (3.1×10^{-7} M). Some flavonoids showed varying activities depending on the solvent used,[8] and different values of IC_{50} of quercitrin were found by Varma *et al.*[9] and Okuda *et al.*,[7] and in this work, so the comparative potency of compounds should be estimated under the same conditions. As judged from the relative potencies (IC_{50}) of compound **1**, axillarin and quercitrin, **1** might be as potent as or more potent than axillarin.

We concluded that compound **1** is mainly responsible for the rat lens AR inhibitory activity of this plant.

Kinetics of Inhibition by Compound 1

Kinetic studies were conducted with **1** in order to determine the type of inhibition and the inhibition constant (K_i). The Lineweaver-Burk plots are shown in Fig. 2. Compound **1** was found to be a non-competitive inhibitor at the concentrations of 1.0×10^{-7} and 5.0×10^{-8} M, as was seen in the cases of quercitrin[7] and axillarin,[7] but it did not show the same type of inhibition at the concentration of 5.0×10^{-7} M. Okuda *et al.*[7] reported that many uncompetitive inhibitors display non-competitive inhibition at low concentrations and switch to uncompetitive inhibition at higher concentrations. In our experiment, **1** showed a similar action. The K_i value of **1** for lens AR was 2.05×10^{-7} M.

The inhibitory effect of **1** on lens AR was also checked in the presence of a large amount of bovine serum albumin (BSA). Compound **1** showed almost the same degree of inhibition in the presence and absence of BSA, suggesting that **1** inhibits the activity of lens AR even in the presence of other proteins.

Experimental

The melting point is uncorrected. IR and UV spectra were obtained with Hitachi 260-10 and Hitachi 220S spectrometers. ^{1}H-NMR spectra were taken with a Hitachi R-24B (60 MHz) spectrometer with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Mass spectra (MS) were obtained on a JEOL-JMS-D 200 instrument. Paper partition chromatography (PPC) was performed on Toyo filter paper No 51B employing the descending technique with AcOH : H_2O (15 : 85) and *tert*-BuOH: AcOH : H_2O (3 : 1 : 1) (TBA) as developing solvents, and the spots were detected under a UV lamp. Thin layer chromatography (TLC) was performed on Kieselgel 60F_{254} plates (Merck); spots were detected under a UV lamp and by heating after spraying 10% H_2SO_4.

Plant Materials——"Tapecué" was purchased from local dealers in Asunción., Paraguay and identified as *Acanthospermum australe* O.K. (aerial part) by Dr. H. Koyama, Faculty of Science, Kyoto University.

Bioassay——Crude AR was obtained from the supernatant fraction of the homogenate of rat lens according to the method of Kador and Sharpless.[10] One unit was defined as the amount catalyzing the oxidation of 1 μmol of reduced nicotinamide adenine dinucleotide phosphate per minute. Samples (1.4—2.0 units) were stored frozen until needed. The inhibitory effects of extract A—F and the isolated compounds on AR were assayed by the method previously reported.[8] Samples were dissolved in dimethylsulfoxide, which was found to have no effect on the enzyme activity at below 0.1% concentration.

Extraction and Fractionation——Dried powder (1 kg) of "Tapecué" was extracted with hot EtOH : H_2O (7 : 3) (1 h × 3). The EtOH : H_2O (7 : 3) solution was concentrated *in vacuo* to give the extract A (118 g). Extract A (100 g) was suspended in H_2O (600 ml) and extracted with *n*-hexane (500 ml × 3), $CHCl_3$ (800 ml × 3) and *n*-BuOH (670 ml × 3) successively to yield the biologically active extract E (29 g). The MeOH solubles (20.8 g) of E (21 g) was chromatographed on polyamide (Waco C-200, 280 g, 5 × 50 cm). Elution with MeOH : H_2O (3 : 2), MeOH and $CHCl_3$ gave fr. 1 (9.68 g), fr. 2 (0.76 g), fr. 3 (1.73 g) and fr. 4 (4.52 g). The most biologically active fr. 3 was subjected to gel-filtration (Toyopearl HW-40F) and silica gel column chromatography to give compounds 1 (13 mg), 2 (2 mg) and 3 (41 mg). From fr. 2, compounds 4 (21 mg) and 5 (7 mg) were obtained by column chromatography (silica gel and Sephadex LH 20). Compound 6 (110 mg) was obtained from fr. 4.

Compound 1 (5,7,4'-Trihydroxy-3,6-dimethoxyflavone)——Yellow needles, mp 199—200 °C ($CHCl_3$/MeOH). PPC *Rf* 0.34 (15% AcOH), 0.86 (TBA). Mg + HCl: orange; Zn + HCl; red-violet. MS *m/z*: 330 (M^+), 315. UV λ_{max}^{EtOH} nm (log ε): 341 (4.22), 270 (4.14). IR ν_{max}^{KBr} cm^{-1}: 3450, 1660, 1610.

Compounds 2—6——2, mp 236 °C (MeOH), 3, mp 233—234 °C (EtOH), 4, mp 192—195 °C (MeOH/H_2O), 5 mp > 300 °C, and 6, mp 220—222 °C (MeOH/H_2O), were identical with authentic trifolin, hyperin rutin, quercetin and caffeic acid, respectively.

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References and Notes

1) This work was presented in part at the 106th Annual Meeting of the pharmaceutical Society of Japan, Chiba, April 1986.
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Report on Direction in Phytochemistry

Assistant Professor, Dr. Munehisa Arisawa, Technical Expert, Phar. Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University
Period of stay: 2 months from May 10 to July 9, 1985

1. Straightening of a laboratory in the organization in the dispatched country, bringing-in of research equipments, setting-up and adjustment of the equipments

The above work was conducted with Suzuki Expert (dispatching one month) and Shimizu Expert (dispatching two months). In regard with the list of the equipments and materials, which were brought in, set up, and adjusted, the attached ① (P. 103～109) will be referred.

2. Purchase of research materials in the dispatched organization and preliminary survey of handed down drugs

After acquiring preliminary information on super market, pharmacy, etc. in Asuncion City, the preliminary survey on herbs in the 4th market in Asuncion City, the purchase of research materials, the collection of herbs in suberbs, photograph taking, etc. were conducted with the aforesaid two experts. See the attached ②. (P 110～111)

3. Preparation of herb extracts and guidance thereof in the dispatched organization

As hot-extraction equipment did not arrive yet, extracts were obtained by extraction at room temperature. That is, the exudate was obtained by exudation of materia, with 75% EtOH at room temperature. Then EtOH was distilled out from the exudate under reduced pressure with a rotary evaporator, and thus the extract was obtained. During the stay, 20 species of herbs extracts were prepared with Shimizu Expert, while the counterpart personnel was guided, as in the attached ③. (P 112)

4. Guidance to counterpart (Lucia Franco) during her training in the place to which she belonged in Japan (from July 26 to November 3, 1985)

(1) Isolation of chemical ingredients and elucidation of the structure

A training was conducted to isolate rutin from crude drug Sophoral Flos and to elucidate the structure. In this course, the below listed technical guidance was carried out.

1) Extracting process by a reflux condenser

2) Filtration process (including proper filtration to meet the necessity, how to fold filterpaper with creases, etc.)

3) Distillation under reduced pressure

4) Color reaction (flavone reaction, ferric chloride, etc.)

5) Use of paper partition chromatograph (P.P.C.)

6) Use of thin layer chromatograph (TLC)

7) Hydrolysis process

8) Acetylation process

9) Measurement of melting point

10) Ultraviolet (UV) absorption spector measurement

11) Infrared (IR) absorption spector measurement

12) Application of UV spector to elucidation of structure

13) Application of IR spector to elucidation of structure

14) Application of nuclear magnetic resonance (NMR) to elucidation of structure

(2) Inhibitory assay against angiotensin conversion enzyme (ACE)

After education and guidance were conducted on the participation of ACE in blood pressure adjusting mechanism, this assay method was practised, and following technical guidance was conducted.

1) How to use a pH meter

2) Preparation of buffer solution

3) Preparation of ACE original solution

4) How to handle a fluorescence photometer

5) Measurement of fluorescence luminosity

6) Procedure of ACE inhibitory assay

7) ACE inhibitory assay using standard inhibitor (captopril)

5. Assay for biological activity

(1) Inhibitory assay against ACE

The assay was conducted on herb extracts by the assay method in attached ④ (P 115～117), and the results were obtained as in attached ⑥. (P 100～102)

(2) Assay for cyto toxicity

The proliferation inhibition tests to KB cell obtained from rhinopharynx cancer and to L5178Y cell obtained from mouse leukomia were conducted by the assay method as in the attached ⑤ (P 118～119), whereby the results as in the attached ⑥ (P 100～102) were obtained.

JAPAN INTERNATIONAL COOPERATION AGENCY

Attached ①

P. O. Box 216, Mitsui Bldg., Shinjuku-ku, Tokyo, Japan.

Nos.	Description of	Quantity	Unit Price	Amount
1	COPY MACHINE (CANON NP-155)	1 set		676,000
	*CASSETTE (B4, B5, A4)			
	*COPY PAPER (B4/2box)(B5/2box)			
	(A4/2box)(A5/2box)			
	*STAND (WITH TONNER, 8pcs)			
2	CAMERA (ERUMO 2600AF)	1 set		126,000
	*MICROPHONE (EC-205)(1pc)			
	*LENS HOOD CASE (1pc)			
3	PROJECTOR (100V ERUMO SC-30)	1 set		154,000
4	FILM (KODACK KMA-594)	50 pcs	@¥ 1,700	85,000
5	FILM (KODACK ELA-594)	50 pcs	@¥ 2,000	100,000
6	FILM (KODACK KMA-580)	5 pcs	@¥ 4,450	22,250
7	SLIDE PRCJECTOR (220V AS3000A)	1 set		142,000
8	TYPEWRITER (OLIVETTI P-35)	1 set		115,000
	with CARBON RIBBON/2pcs			
	with TRANSE /1pc			
	with LIFT OFF TAPE/1pc			
9	PERSONAL COMPUTER (NEC PC-9801 F2)	1 set		358,000
10	COLOR DISPLAY (PC-KD 551K)	1 set		89,000
11	SYRIAL PRINTER (NM-9400S)	1 set		279,000
12	FLOPPY DISIC (PC-9836-4)	1 box		13,500
13	PRINTER PAPER (T-15131P)	1 box		6,000
14	RIBBON (NM-9004-001)	4 pcs	@¥ 2,000	8,000
15	AVR TRANSFORMER (1 kw)	1 pcs		150,000
16	ELECTRONIC DISPENSING BALANCE (PE-11)	1 pc		325,000
17	ELECTRONIC DISPENSING BALANCE (11712 MP-8)	1 pc		590,000
18	REFRIGERATOR (SR-521BF)	2 pcs	@¥150,000	300,000
19	AUTOMATIC WATER DISTILLATION	1 pc		784,800
	APPARATUS "AQUARIUS" GSR-27			
20	CENTRIFUGE (H-103NR)	1 pc		576,000
21	ROTARY EVAPORATOR (RE-51-A4)	2 pcs	@¥216,800	433,600
22	HANDY ASPIRATOR (JS-27K)	2 pcs	@¥ 66,800	133,600
23	WATER BATH (WH-12)	1 pc		66,000
24	HOTTING BATH (B-UP)	1 pc		20,000
25	LABORATORY JACK (30 × 30cm)	1 pc		29,500
26	MAGNETIC STIRRER (D-2S)	1 pc		43,000
27	MANTLE HEATER (AFS-50)	1 pc		54,000

Nos.	Description of Goods	Quantity	Unit Price	Amount
28	PH METER (F8DP)	1 pc		330,000
29	BATH, CONSTANT TEMPERATURE (ET-80)	1 pc		420,000
30	MILLS, WIREY (1029-B)	1 pc		220,000
31	GAS BURNER LPG	1 pc		19,500
32	TEST TUBE MIXER (TME-21)	1 pc		25,000
33	UV DETECTOR (CL-15)	1 pc		69,000
34	UV DETECTOR (UV -15)	1 pc		115,000
35	FORCED CONVECTION OVEN (FC-42T)	1 pc		356,000
36	Measuring Cylinder 200ml	2 pc	@¥ 1,160	2,320
37	-do- 100ml	2 pc	960	1,920
38	Measuring Pipette 10ml	10 pc	340	3,400
39	-do- 5ml	10 pc	270	2,700
40	-do- 1ml	10 pc	200	2,000
41	Triangle Flask 1,000ml	5 pc	880	4,400
42	-do- 300ml	10 pc	350	3,500
43	-do- 50ml	10 pc	270	2,700
44	Beeker 300ml	10 pc	260	2,600
45	-do- 100ml	10 pc	200	2,000
46	Washing Machine for Pipette	1 pc		13,000
47	Glass Flask 60φ	3 pc	350	1,050
48	-do- 105φ	3 pc	660	1,980
49	-do- 180φ	3 pc	1,500	4,500
50	Filter Paper № 2, 12.5/100 sheets	3 box	440	1,320
51	"SUNPU" Set M-type	1 pc		2,000
52	"SUNPU" № 1 Liquid 50ml	1 pc		600
53	"SUNPU" B-board/30 sheets	10 pc	250	2,500
54	"SUNPU" Sheet/100 sheets	3 pc	680	2,040
55	Glass Board for Electrophoresis Spencer 2m/m	3 pc	12,000	36,000
56	Spencer 1m/m	1 pc		12,000
57	Coam 2m/m, 13-kentai	2 pc	5,000	10,000
58	Centrifuge Tube 15×105N	100 pc	29	2,900
59	Test Tube Stand 16.5×50 pcs	4 pc	1,500	6,000
60	Silicone Tube 2.5mmφ	5 m	160	800
61	-do- 2.0mm	5 m	120	600
62	Milk Syringe (Ceramic)	20 pcs	300	6,000
63	Measuring Cylinder 1000ml	2 pcs	4,800	9,600
64	-do- 500ml	2 pcs	2,240	4,480
65	Filter Paper № 2 22.5φ	3 pcs	1,350	4,050
66	-do- 360φ	3 pcs	2,700	8,100
67	Spartel 150m/m	2 pcs	130	260

Nos.	Description of Goods	Quantity	Unit Price	Amount
68	-do- 240m/m	2 pcs	290	580
69	Micro. Spartel	2 〃	160	320
70	Stenless Forceps 125	2 〃	130	260
71	Silicon Teat 5ml	10 〃	180	1,800
72	Vvnil Bag 0.03×120×170/100 sheet	3 〃	900	2,700
73	"KIMU WAIPU" S-200	1 〃		11,000
74	Aluminume Foil 30cm×5m	3 〃	3,000	9,000
75	Glass Tube	10 〃	240	2,400
76	Rubber Tube 12mm×17mm	5 〃	3,800	19,000
77	Wrapping paper for Medicien/500 sheets	5 〃	590	2,950
78	Gauze 30cm×10m	5 〃	680	3,400
79	Glass Stirring Rod	10 〃	130	1,300
80	Plastic Bukets 15L	5 〃	1,100	5,500
81	Cleaning Plastic Bottle 500ml	3 〃	170	510
82	Loupe 20X	5 〃	4,500	22,500
83	KJELDAHI Type Flaks 100ml	5 pc	2,600	13,000
84	-do- 200ml	5 〃	2,650	13,250
85	-do- 300ml	5 〃	2,850	14,250
86	-do- 500ml	5 〃	3,300	16,500
87	-do- 1L	3 〃	3,650	10,950
88	-do- 2L	3 〃	5,250	15,750
89	-do- 3L	3 〃	6,650	19,950
90	Measuring Cylinder 100ml	2 〃	1,350	2,700
91	-do- 200ml	1 〃		1,680
92	-do- 500ml	1 〃		3,040
93	-do- 1L	1 〃		6,400
94	Flask 5ml	3 〃	1,360	4,080
95	-do- 10ml	3 〃	1,360	4,080
96	Measuring Pipette Tip 0.5ml	2 〃	510	1,020
97	-do- 1ml	2 〃	295	590
98	-do- 2ml	2 〃	295	590
99	-do- 5ml	2 〃	375	750
100	-do- 10ml	2 〃	485	970
101	Silicon Pipette № 3	10 〃	100	1,000
102	-do- № 5	10 〃	150	1,500
103	Silicon Pipetter № 10	1 〃		3,000
104	-do- № 25	1 〃		3,000
105	Plastic Bottle Washer 500ml	3 〃	170	510
106	TRAP Ball 29/42	1 〃		8,125
107	-do- 29/42 × 15/25	1 〃		7,850

Nos.	Description of Goods	Quantity	Unit Price	Amount
108	Liquid Dividing Funnel Cone 500ml	2 pc	5,650	11,300
109	-do- 1 L	2 〃	9,650	19,300
110	-do- 2 L	2 〃	12,500	25,000
111	-do- 3 L	2 〃	18,300	36,600
112	KOMAGOME Pipette 2ml	5 〃	100	500
113	-do- 3ml	3 〃	150	450
114	Glass Cutter	1 〃		4,200
115	Red Liquid Thermometer 0-100℃	3 〃	250	750
116	Holder for Tefron Meter	2 〃	2,600	5,200
117	Loupe 20X	1 〃		4,500
118	Ring 120	2 〃	1,150	2,300
119	-do- 85	2 〃	600	1,200
120	Funnell 100 × 9 × 100	3 〃	830	2,490
121	-do- 75 × 8 × 75	3 〃	500	1,500
122	-do- 50 × 8 × 65	3 〃	420	1,260
123	-do- 180 φ	3 〃	1,580	4,740
124	-do- 300 φ	2 〃	10,400	20,800
125	Glass Stick 8 m/m × 1,200 m/m	5 〃	180	900
126	Glass Tube 8 φ × 1,200 m/m	15 〃	100	1,500
127	-do- 10 φ × 1,200 m/n	5 〃	135	675
128	ELECTRONIC DISPENSING BALANCE PE-11TYPE	1 set		280,000
129	REFRIGERATOR WITH TRANSFORMER SR5218F(A)	1 〃		140,000
130	CENTRIFUGE H-Y03NR	1 〃		547,000
	*HOLDER, 15ml 3,500 R.P.M. (32pcs)			
	*-ditto- 50ml 4,000 R.P.M. (4pcs)			
	*1.5ml SETTLING TUBE (50pcs)			
	*50ml SETTLING TUBE (8pcs)			
	*BALANCER (1pc)			
131	MAGNETIC STARER	1 〃		61,000
132	TEST TUBE MIXER TM-100 WITH TRANS	1 〃		24,000
133	SLAB GEL ELECTROPHOREST APPARATUS SPG-1500W	1 〃		86,000
134	POWER SUPPLIES FOR ELECTROPHORESIS *ELEPOS PS-1510	1 〃		123,000
135	MICRO CYLINGE	1 pc		6,300
136	PH METER F-80P	1 set		313,000
137	HYDROGEN PEROXIDE (500g)	1 pc		300
138	ACRYLAMIDE MONOMER (500g)	2 pcs		7,300
139	N, N′-METHYLENEBISACRYLAMIDE (SP25g)	2 〃		5,500
140	N, N, N′, N′ TETRAMETHYLETHYLENDIAMINE(100g)	1 pc		4,300

Nos.	Description of Goods	Quantity	Unit Price	Amoumt
141	VITAMIN B2 (1g)	1 pc		700
142	2-MERCAPTOETHANOL (25g)	1 〃		2,000
143	BROMOPHENOL BLUE (25g)	1 〃		3,000
144	POLYVINYL PYRROLIDONE K-30 (25g)	1 〃		550
145	COOMASSIE BRILLIANT BLUE R-250 (25g)	1 〃		4,000
146	UREA (500g)	1 〃		330
147	CHACOAL ACTIVATED POWDER (500g)	1 〃		1,330
148	RIVERSAL COLOR FILM	50 pcs	@¥ 2,000	100,000
149	ACETONE (500mℓ)	6 〃	@¥ 550	3,300
150	METHYL ALCOHOL (500mℓ)	18 〃	@¥ 400	7,200
151	HYDROCHLORIC ACID (500mℓ)	5 〃	@¥ 470	2,330
152	ACETIC ACID (500mℓ)	5 〃	@¥ 800	14,000
153	SCAT-20x-N (2KGS)	1 pc		3,300
154	SODIUM DODECYLSAL FATE (500g)	1 〃		7,000
155	DOTITE TMBZ (5g)	1 〃		22,000
156	TRIS(HYDROXYMRTHYL)AMINOMETHANE(500g)	2 pcs	@¥ 5,300	10,500
157	GLYCINE (AMINOACETIC ACID) (500g)	5 〃	@¥ 2,300	11,500
158	GLYCERIN (500mℓ)	2 〃	@¥ 1,250	2,500
159	SODIUM ACETATE CRYST (500g)	1 pc		330
160	AMMONIUM PERSULFATE (100g)	1 〃		300
161	STAINLESS MICRO SPARTEL 210	5 pcs	@¥ 160	300
162	-ditto- 180	5 〃	@¥ 160	300
163	STAINLESS SPOON' 165	3 〃	@¥ 60	180
164	-ditto- 210	3 〃	@¥ 175	325
165	STAINLESS TWEEZERS 150	3 〃	@¥ 150	430
166	-ditto- 130	3 〃	@¥ 180	340
167	VACUUM GUM TUBE 6 × 18	10 m	@¥ 1,060	10,300
168	FLASK 300mℓ	10 pcs	@¥ 400	4,000
169	-ditto- 500mℓ	10 〃	@¥ 580	5,300
170	-ditto- 1 L	5 〃	@¥ 1,080	5,400
171	-ditto- 2 L	3 〃	@¥ 2,200	6,500
172	-ditto- 3 L	2 〃	@¥ 2,900	5,300
173	STIRRING PICKUP ROD TEFLON	1 pc		2,650
174	REAGENT BOTTLE 250mℓ	5 pcs	@¥ 1,500	7,500
175	-DITTO- 500mℓ	5 〃	1,900	9,500
176	-DITTO- 1 L	3 〃	3,400	10,200
177	TEFLON STIRRING BARS 5 × 15	1 pc		330
178	-DITTO- 7 × 20	1 〃		320
179	-DITTO- 8 × 30	1 〃		350
180	FLASIC STAND 105φ	3 pcs	1,150	3,450

Nos.	Description of Goods	Quantity	Unit Price	Amount
181	-DITTO- 120φ	3 pcs	@¥ 1,150	3,450
182	JOINT CLAMP 15	5 〃	270	1,350
183	-DITTO- 29	3 〃	680	2,040
184	DIVIDE TUBE	2 〃	9,500	19,000
185	DIVIDE ADAPTER	2 〃	9,100	18,200
186	INDUCE ADAPTER	2 〃	9,100	18,200
187	DIVID TUBE	1 pc		25,000
188	CONDENSERS	2 pcs	15,000	30,000
189	BALL JOINL A	2 〃	3,900	7,800
190	-DITTO- B	2 〃	3,900	7,800
191	JOINT, SEPARATING	2 〃	3,900	7,800
192	OUALITATIVE FILTER PAPER 150φ	3 boxes	540	1,620
193	-DITTO- 300φ	3 〃	1,600	4,800
194	FILTER PAPER	1 box		5,400
195	PH TEST PAPER	1 〃		740
196	DEVELOPMENT TANK PAPER CHROMATOGRAPH	4 pcs	19,000	76,000
197	DYEING BAT	3 〃	1,000	3,000
198	TURN COLOR REACTION BOARD 2 × 6	2 〃	700	1,400
199	THREE-LEGGED STAND (M)	5 〃	2,500	12,500
200	-DITTO- (L)	3 〃	3,350	10,050
201	-DITTO- (LL)	3 〃	5,400	16,200
202	STAINLESS CAGE FOR TEST TUBE	3 〃	3,300	9,900
	(200 × 200 × 200)			
203	STAINLESS CAGE FOR TEST TUBE	2 〃	9,900	19,800
	(300 × 250 × 300)			
204	GLASS SPRAYER 30mℓ	2 〃	2,600	5,200
205	POLYETHYLENE BOTTLE 2L	5 〃	280	1,400
206	-DITTO- 3L	5 〃	420	2,100
207	-DITTO- 5L	5 〃	600	3,000
208	-DITTO- 10L	5 〃	1,120	5,600
209	MANTLE HEATER 3L	2 〃	27,000	54,000
210	POLYETHYLENE SIPHON	5 〃	250	1,250
211	PLASTIC BUCKET 10L	3 〃	850	2,550
212	-DITTO- 15L	1 pc		1,100
213	PLASTIC TUB 11L	3 pcs	950	2,850
214	BUSKET SHALLOW TYPE	2 〃	550	1,100
215	BUSKET DEEP TYPE	2 〃	700	1,400
216	SCAR ANGLE TYPE BOTTLE 50mℓ	10 〃	100	1,000
217	-DITTO- 100mℓ	10 〃	110	1,100
218	-DITTO- 250mℓ	10 〃	130	1,300

Nos.	Description of Goods	Quantity	Unit Price	Amount
219	RASP	1 pc		2,200
220	SECTIONAL STAND A TYPE	1 〃		33,500
221	SLYDUX	2 pcs	@¥ 36,000	72,000
222	PIPETTE MAN P-5,000	1 pc		45,000
223	-DITTO- P-1,000	1 〃		36,000
224	-DITTO- P- 200	1 〃		36,000
225	MICRO DISPENSER	1 〃		37,500
226	PIPETTE MAN CHIP C- 20	1 〃		11,250
227	-DITTO- C- 200	1 〃		11,250
228	-DITTO- C-6,000	1 〃		20,000
229	GAUZE 30cm × 10m	3 pcs	@¥ 680	2,040
230	CAPILLARY TUBE FOR DISPENSER	1 pc		3,500
231	ALUMINIUM FOIL 30cm × 25cm	3 pcs	1,800	5,400
232	COTTON 500g	3 〃	1,400	4,200
233	WIPE S-200	1 pc		11,000
234	STAINLESS WASHING CAGE	2 pcs	3,500	7,000
235	STAINLESS BLUSH № 4	5 〃	120	600
236	-DITTO- № 10	3 〃	140	420
237	MEDICINE WRAP PAPER	1 pc		500
238	CONE TYPE SETTLING TUBE WITH STOPPER	20 pcs	880	17,600
239	GUM TUBE 12 × 17m/m	2 roll	3,400	6,800
240	TEST TUBE WITH STOPPER	50 pcs	510	25,500
241	STAINLESS TEST TUBE STAND 15 × 50	2 〃	1,500	3,000
242	-DITTO- 165 × 50	2 〃	1,500	3,000
243	PARA FILM	1 pc		3,200
244	TEST TUBE WITH STOPPER 20φ × 125	50 pcs	210	10,500
245	CHEMT TUBE 7 × 10	10 m	450	4,500
246	-DITTO- 8 × 11	10 〃	540	5,400
247	SODIUM CHLORIDE (500g)	1 pc	700	700
248	POTASSIUM CHLORIDE (500g)	1 〃	670	670
249	SODIUM BICARBONATE	1 〃	1,310	1,340
250	SODIUM PHOSPHATE, DIBASIC, CRYST	1 〃	700	700
251	POTASSIUM DIHYDROGEN PHOSPHATE	1 〃	970	970
252	HYDROCHLORIC ACID SOLUTION (500g)	1 〃	570	570
253	SUL PHURIC ACID (500g)	1 〃	440	1,440
254	ENZYM	1 unit	43,880	43,830
255	DIMETHYL SULFOXIDE (500g)	1 pc	1,590	1,580
256	SODIUM HYDROXIDE, SOLID (500g)	1 〃	540	540
257	POTASSIUM HYDROXIDE SOLID (500g)	1 〃	610	640
258	CALCIUM CHLORIDE (500g)	1 〃	990	990

Attached ②

21 de mayo de 1985.

PLANTAS MEDICINALES DEL PARAGUAY

1. Santa Lucía blanca (Refrescante) Conhelia nudiflora.
2. Cepa caballo (Refrescante. Se machaca y toma en el agua)
3. Perdudilla (Refrescante. En agua fría) Gonphrena decumbens.
4. Zarzaparrilla (Refrescante. En agua fría) Smilax orficinalis.
5. Cola de caballo (Para los rinones. En agua caliente) Xanthium spinosum.
   Equisetum giganteum.
6. Aguapé puruá (Para la inflamacion del estomago. En agua caliente)
7. Cocú (Refrescante. En agua fria) Allophyllus edulis.
8. Typycha curatú (Para indigestiones) Scoparia dulcis.
9. Nangapyry (Para adelgazar) Eugenia uniflora.
10. Capii cati (Refrescante)
11. Mbocaya-i rapo (diurético)
12. Eatatilla (Refrescante)
13. Raíz de perejil* (Abortivo)
14. Cedrón Paraguay (Para calmar los nervios) Lippiacitriodora
15. Llanten (Raíz) (Remedio para todo. Caliente)
16. Menta-í* (Para calmar los nervios)
17. Taropé (Refrescante - oll part) Dorstenia brasiliensis.
18. Tupasy camby (Refrescante)
19. Poleo-í (Remedio caliente en te o mate - abortivo)
20. Toronjil* (Para el corazón)
21. Nuaty pytá (Para
22. Urusu caty (Para echar lombrices - en decocción)
23. Verbena-í (Para dolor de garganta - en decocción)
24. Cerdon capii (Para calmar los nervios - té)
25. Raiz de hinojo (Para dolor de estómago. En agua caliente)
26. Toyuya (Abortivo. Se toma en tereré o mate)
27. Ysypo mil hombre (Abortivo y refrescante. En el mate) (Aumenta virilidad) Aristolochia trianguar.
28. Malva rapo pire (Abortivo. Decocción o té)
29. Cana brava (Abortivo. Decoccion o te)
30. Yagua Rova (Abortivo. Se toma té o decocción)
31. Ruibarbo (Abortivo. Se toma te o decoccion)
32. Charmáa caa (Para el estómago, para despertar el apetito)
33. Achicoria (Purgante. En té o decoccion)
34. Pindo rapo (Abortivo. En té o decoccion)
35. Para todo pire (Uso desconocido) Tecoma aregentes.
36. Usuru mee (Para catarro. En mate o té)
37. Guayacan corteza (Dolor de barriga. En té o decocción)

38. Ybyrá pytá piré (Para lavar heridas o problemas de la piel. Se cocina y lava)
39. Yateí caa (Remedio caliente. En té o decocción)
40. Yaguareté caa (Para el estomago. En té o decocción) (Como depurativo)
41. Calaguala (Abortivo. En té o decocción o en mate)
42. Penicilina (Para limpiar heridas. Se hierve y lavar)
43. Doradilla (Abortivo. Té o decoccion)
44. Flor de mamón macho (En forma de jarabe para la tos de los ninos especialme- nte)
45. Cangorosa (Abortivo y para úlcera. Té o decocción)
46. Malva blanca (Para eliminar catarro. Té o decocción)
47. Yuruveva Té o decocción)
48. Ambay (Para la tos, catarro. Té o decocción)
49. Yerba de lucero (Para el estómago. Té o decocción)
50. Pata de buey (Para los riñones. En tereré o mate)
51. Yaguá rundy (Para la tos. Te o decocción)
52. Sauco (Para inflamación del estómago. Friccionar)
53. Alcanfor del campo En mate)
54. Yateí caá (Remedio refrescante. En tereré)
55. Barba de maíz (Avatí zogüé) (Para los rinones. En té o mate)
56. Savá morotí corteza (Para diabetes. Se machaca, sa hierve y se toma como agua)
57. Macho acá raíz (Para el corazon. Se machaca y se toma con agua fría)
58. Para para-i (Para los rinones, rompe piedras. Con agua fria o caliente)
59. Siete sangría (Para la presión. Con agua fría o en mate)
60. Ysypo pere (Para el cáncer. Se machaca. En te o decocción)
61. Tapecue (Problems de estomago. Para lavar heridas se hierve)
62. Ybahai (Para diabetes. Se hierve y se toma 2-3 veces al día o como té)
63. Siempre viva (Para el corazón y calmar los nervios. Se hierve y se toma en mate)
64. Calabacita (Para diabetes, se hierve y toma como agua o en tereré)
65. Caaré (Antihelmítico. Se hierve y se toma en ayunas)
66. Chirca melosa (Diabetes. Se hierve y se toma como agua o té)
67. Kino kino (Para dolores reumáticos y para golpes. Se machaca y se hierve)
68. Capí-una (Para el riñon. En té o decocción)
69. Agrial (Para dolor de garganta. Se hace gárgara con agua fría)
70. Curupaymí (Para el reuma. Se toma en mate o tereré) Prophyllum lanieolatum (F)
71. Yerba mata (Para el corazón. Se toma con agua fría)

Attached ③

GRUPO I

1. Ambay
2. Sarandy Morotí
3. Piper sp No. 2 (Hojas lanceoladas)
4. Piper sp No. 1 (Hojas grandes anchas) (Yaguarundy)
5. Nangapiry (Eugenia uniflora)
6. Cedron-capii (Cymbopogon citratus)
7. Typycha-Kuratu (Scoparia dulsis)
8. Tape-cué (Acantos perum)
9. Koku (Allophillus edulis)
10. Colita (Cordia salicifolia)
11. Yvahai (Eugenia myrcianthes)
12. Paraparaí mí
13. Mil hombre
14. Marcela
15. Cola de caballo
16. Romero
17. Burrito
18. Jaguarundí
19. Alhucema
20. Espartillo guazú

Attached ④

Assay of Inhibitory Activity of Angiotensin Converting Enzyme (ACE)

1. Reagents

Hippuryl-L-histidyl-L-leucine (HHL)
Angiotensin converting enzyme (ACE)
DMSO
2% O-Phthalaldehyde methanol solution, freshly made (OPA)
0.3N HCl
3N HCl
Buffer A
Buffer B
Buffer C
MeOH

2. Buffer

Buffer A (pH 8.30)
(1) ---------- KH_2PO_4 0.34 g / 50 ml H_2O
(2) ---------- K_3PO_4 0.53 g / 50 ml H_2O

Buffer B (pH 8.30)
(1) ------ KH_2PO_4 2.04 g, NaCl 3.5 g / 100 ml H_2O
(2) ------ K_3PO_4 3.18 g, NaCl 3.5 g / 100 ml H_2O

Buffer C (pH 8.30)
(1) ------ KH_2PO_4 6.8 g, NaCl 8.77 g / 500 ml H_2O
(2) ------ K_3PO_4 10.61 g, NaCl 8.77 g / 500 ml H_2O

All buffer solutions are prepared by mixing of (1) and (2) concurrently, adjusted to pH 8.30

3. Preparation of ACE solution

1) homogenized in Buffer A at 4°C
2) centrifuge at 40000 G
3) stock a supernatant below 0°C

4. Assay method

HHL 0.1 ml
Sample 0.01 ml + ACE 0.14 ml — incubation 37°C, 30 min.

+ NaOH — + OPA 0.1 ml — after 10 min. + HCl 0.2 ml

determination of fluorescence

1) prepared HHL (5 mM/1000 ml) 0.1 ml / tube
2) dissolve 0.01 ml of sample solution, add the ACE solution (30-40 μg protein / 0.14 ml) and start the enzyme reaction by incubation at 37°C for 30 min.
3) after the incubation add 0.3N NaOH to stop the reaction
4) add 0.1 ml of 2% OPA
5) after 10min., add 0.2 ml of 3N HCl and determined fluorescence (excitation wavelength at 340 nm and emission fluorescence wavelength at 455nm)
6) calculate the inhibition %

$$\text{Inhibition \%} = \frac{(A-B) - (C-D)}{A - B} \times 100$$

A; control — C; sample
B; blank of control — D; blank of sample

Attached ⑤

Cytotoxicity test against KB cell (*in vitro*)

1. Materials
2. Cell culture
3. Passage
4. Cell stock
5. Assay method

1. Materials

1. Preparation of reagents

PSS ---------------- NaCl 8.5 g / Dist.H_2O 1000 ml

PBS ---------------- NaCl 8.0 g
Na_2HPO_4· 12 H_2O 2.9 g
KH_2PO_4 0.2 g
KCl 0.2 g
/ Dist.H_2O 1000ml

0.025% CV----------- crystal violet 9 H_2O 35mg
/ Dist.H_2O 100ml

0.02% EDTA --------- EDTA 44 mg / PBS 200 ml

10% $NaHCO_3$ --------- $NaHCO_3$ 10 g / Dist.H_2O 90ml

Eagles' MEM medium ----- Dried E. MEM 9.4 g
/ Dist.H_2O 1000 ml

Glutamine solution ----- Glutamine 2.92 g / E. MEM m. 100ml

Hanks solution ---------- Dried Hanks s. 9.8 g / Dist.H_2O 1000 ml

0.25% Trypsin solution ----- Trypsin 0.5 g / PBS 200 ml
(is stirred over night at room temp.)

Complete MEM ----------- Glutamine solution 1.0 ml
10% $NaHCO_3$ 1.1 ml
FBS(fetal bovine serum)* 10.0 ml
E. MEM m.** ad. 100.0 ml
(aseptic manipulation in a clean bench)

* treated at 56°C for 30 min. and stocked at -25°C

Complete Hanks ---------- 10% $NaHCO_3$ 0.35 ml

FBS* 2.0 ml

Hanks s.*** ad. 100.0 ml

(aseptic manipulation in a clean bench)

* treated at 56°C for 30 min. and stocked at -25°C

2. Autoclaved sterilization

   0.02% EDTA

   10% $NaHCO_3$

   Eagles' MEM medium**

   Membrane filter set

   Culture bottles

   Test tube

   Silicone rubber stoppers

   Silicone rubber bulbs

   Bottles with screw cap

   Pastrur pipets

   Dispenser pipets

   Chips for dispenser pipet

3. Sterilization by membrane filter

   Glutamine solution*

   Hanks solution***

   0.25% Trypsin solution

4. Dry heat sterilization

   Pipets

2. Cell culture

1) storage cell was dissolved in warm water (37-42°C)
2) wash with 20 ml of complete Hanks solution in centrifuge tube by vibration
3) centrifugate at 1200 rpm for 5 min.
4) pipette off the supernatant in a clean bench
5) inoculate in a culture bottle with complete MEM and incubate at 37°C

3. Passage

1) reverse the bottle cell cultured and decant the medium
2) add 5 ml of 0.02% EDTA and wash a cell sheet for 3 min
3) decant the EDTA solution and add 5 ml of 0.25% trypsin
4) after 1 min. hit the bottom of the bottle and scrap the cell sheet by pipetting and then vibrator .
5) the cell suspension was washed with 20 ml of complete Hanks solution
6) centrifugate at 1200 rpm for 5 min
7) pipette off the supernatant in clean bench
8) inoculate in a culture bottle with complete MEM and incubate at 37°C

4. Cell stock

1) the cultured cells are counted by blood cell counting plate under microscopy
2) dilute to 1-3 X 10^6 cells/ml with complete MEM containing 10% DMSO and 20% FBS*
3) after freezed at -20°C for 30 min., stock at -80°C in deep freezer

* treated at 56°C for 30 min. and stocked at -25°C

5. Assay method

I) cell-count and dilute to 2-3 X 10^4 cells/ml with complete MEM

2) prepare the cell suspension 3.9 ml (0.8-1.2 X 10^5 cells)/tube

3) add 0.1 ml of sample, dissolve in H_2O, EtOH or DMF, and Vibrate

4) incubate at 37°C for 72 hr. (tube angle at 10°)

5) after 72 hr. incubation, add 1 ml of 0.025% CV and incubate at 37°C for 15 min. (tube angle at 10°)

6) decant excess dye and add 10 ml of PPS

7) rotate the tube gently to wash cells

8) decant the PSS perfectly, and add 3 ml of 50% EtOH and then vibrate

9) determine OD (optical density) at 570 nm

IO) calculate the inhibition %

$$\text{Inhibition \%} = \frac{B - S}{B} \times 100$$

B; OD_{570} blank

S; OD_{570} sample

Cytotoxicity test against L5178Y cell (*in vitro*)

1. Materials
2. Cell culture
3. Passage
4. Cell stock
5. Assay method

1. Materials

1. Preparation of medium

Fischer's medium -------- Dried F. medium 10.5 g
Kanamycin sulfate 0.12 g
/ Dist. H_2O 1000 ml

Fischer's medium containing 10% Horse serum (HS) ----------
Fischer's medium 900 ml
Horse serum* 100 ml
(aseptic manipulation in a clean bench)

* treated at 56°C for 30 min. and stocked at -25°C

2. Autoclaved sterilization

Membrane filter set
Bottles with screw cap
Test tubes with screw cap
Pastrur pipets
Chips for dispenser pipet
Dispenser pipets
Centrifuge tubes with screw cap

3. Sterilization by membrane filter

Fischer's medium

4. Dry heat sterilization

Pipets

2. Cell curture

1) storage cells are dissolved in warm water (37 - 42°C)
2) wash with 20 ml of Fischer's medium in centrifuge tube by vibration.
3) centrifugate at 1200 rpm for 5 min.
4) Pipette off the supernatant in a clean bench
5) inoculate in a centrifuge tube with Fischer's medium containing 10% HS and incubate at 37°C

3. Passage

discribed as cell culture 3), 4) and 5)

4. Cell stock

1) The cultured cells are counted by coulter counter
2) dilute to 1-3 X 10^5 cells/ml with Fischer's medium containing 20% HS and I0% DMSO
3) after freezed at -20°C for 30 min, stock at -80°C in deep freezer.

5. Assay method

1) cells are counted by coulter counter and dilute to 5 X 10^4 cells/ml with Fischer's medium containing I0% HS
2) prepare the cell suspension 2.9 ml/tube
3) add 0.1 ml of sample solution (H_2O, EtOH, DMF or DMSO, 3%)
4) incubate at 37°C for 48 hr.
5) after incubate, the cells are counted by coulter counter
6) caluculate the inhibition %

$$\text{Inhibition \%} = \frac{A - C}{A - B} \times 100$$

(A : final cell count of blank, B : initial cell count of blank, C : final cell count of sample)

Report on Guidance in Phytochemistry

Assistant Professor Dr. Toshimitsu Hayashi, Expert,
Faculty of Pharmaceutical Sciences,
Toyama Medical and Pharmaceutical University
Period of stay: 2 months from Sept. 10 to Nov. 9, 1985, and 6 months from Apr. 10 to Oct. 14, 1986

In FY 1985:

1. Services during the stay at the Faculty of Chemistry, Asuncion University, in the Republic of Paraguay (dispatched period: Sept. 10 ~ Nov. 9)

   1-1 Bringing, checking, setting, and adjusting of equipment and materials in Phytochemistry Division

   Following items were brought into Phytochemistry laboratory and checked:

   Equipment and materials enabling hot extraction of herbs

   Equipment and materials enabling the distillation of organic solvents
   Ion exchange resin and filters for the manufacture of distilled water

   The equipment and materials brought in May were re-checked, and newly brought equipment and materials were set and adjusted.

   1-2 Examination of the conditions for preparation of herb extracts and technical guidance to counterpart

   Extracting conditions:

   solvent: 70% EtOH (mixture of 7 parts of distilled EtOH from the article on the market and 3 parts of distilled water)

   specimen: 100 ~ 500 g. (dried specimen)

   time: 1 hour, 3 times

   subject specimens: Cangorosa, Siete sangría, Cedron Paraguáy, Siempre viva, Burrito, Marcela, Alhucema, Yaguarundy, Eucalipto, Parapara-i, Colita, Typychá Kuratũ, Tapecúe, Caáhée, Romero, Espartillo guazú, *Colade Caballo*, *Aromita*, *Chirca*, *Culantrillo*, *Piper sp.* No. 3, Yvahái

Extracts were prepared from the above specimens with counterparts (Esteban Ferro, Lucia Franco, and Cristina Theoduloz), with alloted task for each. The extracts were sent by mial to Japan for examination of biological activities.

1-3 Collection of herbs for extracts, and taking of photographs and 8 mm movie films (together with Expert Yoshizaki and counterpart of Botanical Division)

1st collection: Collectoin at Paraguari (October 3)

mil hombre, Araticu-í, Yvahái, Cardo santo, Piper, Marcela

2nd collection: Collection at Chaco (October 27 ~ November 4)

Kaatai, Altamisa, Kaaré, Cabello de ángel, Mandiyú-ra, Aguapé-purua, Guayacan, Salvia, Verbena-i, Quebracho blanco, Paratodo, Caarurupé, Llanten de tierva, taperyvá-hú, Aguapé, Cepa caballo, Pata de buey-í, Yvoty caarú

1-4 Hearing survey on herbs at the 4th market in Asuncion City

On October 1, together with Export Yoshizaki and Isabel Basauldo, 154 species of herbs were purchased, put in order and preserved. In regard with the results of survey, Material 1 will be referred.

1-5 Practice of seminor in Phytochemistry Division

2. Examination of biological activities of extracts from paraguayan herbs

On extracts from Paraguayan herbs delivered from Asuncion University, the inhibitory effects were examined, against Xanthin oxydase (relates gout), β-Glucuronidase (relates to hepatogenous jaundice) and Urease (relates to urinary tract calculus). The testing methods and the test results were indicated in Material 2 and 3.

3. Fractioning of extracts from Paraguayan herbs, and isolation and purification of active substances

3-1 Isolation and purification of Ureas inhibitory substances in romero

Concerning the extract from Romero, the fraction, isolation and purification of constituents are being carried out.

3-2 Isolation and purification of Xanthin oxydase inhibitory substances in Ñangapyry, and determination of structure

Making the Xanthin oxydase inhibitory activity an index, isolation and purification of active substances were conducted on the extract from Ñangapyry, and 2 kinds of flavonoid were isolated. Structure of these substances were examined by various spectral data, and thus those were identified as myricetin and myricitrin, respectively. Among them, Xanthin oxydase inhibitory effect was noted in myricetin.

3-3 Isolation and purification of β-Glucuronidase inhibitory substances in Typychá kuratü

On the extract of Typychá kuratü which exhibited strong β-Glucuronidase inhibitory activity, the active substance is being isolated and purified.

4. Recording of principal procedures for examination of biological activity in 8 mm movie films

In order to use as the teaching aids on technique transfer to cunterpart in Phytochemistry Division in FY 1986, all methods of bioassay which are being conducted in laboratory of pharmacognosy, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, were taken in movies.

In FY 1986:

1. Services during the stay at the Faculty of Chemistry, Asuncion University (dispatched period: April 15 ~ October 14)

1-1 Questionnairing to the users of Paraguayan herbs

For the purpose to make clear the status of use of herbs which were noticed in the survey at Asuncion 4th market in the first year, questionnaire survey was conducted. 1,000 questionnair sheets were printed and distributed, and answers of 701 persons were recovered. The results of survey such as the number of users, the part of herb to be used, ways of taking, and purpose of use, were arranged for each species respectively in Material 4. By the way, the breakdown of answers is as in Material 5 and 6.

1-2 Receiving of equipment and materials solely furnished to the pharmacology Division and those brought in FY 1986

1-3 Guidance of enzyme inhibition experiment to counterparts in the Phytochemistry Division

In order to enable to detect inhibitory substances against Xanthin oxydase and β-Glucuronidase, 8 mm movies on each enzyme inhibition experiment were exhibited, and made counterpart understand further at a seminar. Substantial technical guidance was conducted to Lucia Franco and Cristina Theoduloz.

1-4 Execution of a seminar in the Phytochemistry Division

Seminars were held four times, introducing literatures in concern with study on medicinal plants in the field of chemistry of natural products.

1-5 Straightening of laboratory of the Phytochemistry Division

Shelved cabinets for containing furnished materials were purchased, in which glaswares and reagents in the laboratory were straightened.

1-6 Collection of herbs for extracts at naturally growing places

1st collection: collected at San Lorenzo (September 26).
Salvia and Molle-í

2nd collection: collected at Paraguari (October 1).
Yvopé

1-7 Preparation of extracts from herbs

Extracts were prepared from Yvýra pyta, Molle-i, Cedron capií, Salvia, Chirca melosa, Typychá kuratũ, Romero, and Burrito, which were sent to Japan.

1-8 Taking of photographs of herbs

Photographs of herbs were taken, which were growing in San Lorenzo, Luque and in Asuncion University campus. Photos were also taken on the species purchased in Asuncion 4th market.

1-9 Tour outside the dispatched country

Tour to Argentine (June 7 ~ June 12)

Visited places and persons: Buenos Aires University, Proflessor J. Coussio
La Plata University, Professor M. Najera
La Plata Museum

Tour to Brazil (July 14 ~ July 19)

Visited places and persons: Oswald Cruss Laboratory, Dr. Jorge Bermudy
Rio de Janeiro Botanical Garden
Rio de Janeiro University, Professor Walter D. Mors
San Paulo botanical Garden, Dr. Marcos Buckeridge
San Paulo University, Dr. H. Yoshida and
Dr. M. Motidome

Besides above, visited and conversed with Mr. Tetsuo Nakasumi, a herb researcher and Mr. Goro Hashimoto, a botanist.

Tour to Bolivia (August 22 ~ August 26)

Visited places: Market at Santa Cruss
Market at La Paz
Clinic of natural remedy physician at La Paz (Dr. T. T. Valencia)

Besides above, conversed with instructors of the Faculty of Agriculture of Bolivia Univesity. (Dr. F. K. Saucedo, Dr. J. Magne, and Dr. R. C. Staffer).

PLANTAS MEDICINALES UTILIZADAS EN EL PARAGUAY - ADQUIRIDAS EN EL MERCADO 4 -

1. Ñangapiry: para adelgazar y colesterol.
2. Typych-a curatú: para afecciones del hígado.
3. Agrial: para gárgaras en afecciones de la garganta.
4. Eucalipto: para combatir la tos y para inhalaciones.
5. Hinojo: para el estómago.
6. Chicoria: para combatir la tos y laxante.
7. Taropé: abortivo.
8. Llanten: para combatir la inflamación.
9. Orégano: para afecciones del estómago.
10. Menta: para afecciones del estómago.
11. Tupasy camby: para frialdad - afecciones femeninas.
12. Cardo santo (raíz): para dolores menstruales.
13. Apio Paraguay: refrescante.
14. Tatú ruguay: para apendicitis.
15. Tapecue: para afecciones de la piel.
16. Pynó guazú (raíz): antiinflamatorio, para golpes.
17. Molle-í: para afecciones de la garganta.
18. Mil hombre: abortivo - diurético. Aumenta la virilidad.
19. Yaguá rová: para el reumatismo.
20. Pata de buey: para afecciones del hígado y riñones.
21. Cedrón capií: para calmar los nervious y para el corazón.
22. Cangorosa: para úlceras y anticancerígeno.
23. Uruzú heé: para bronquitis.
24. Ysypó peré: para curar el cáncer.
25. Albahaca: para el estómago - flatulencias.
26. Arachichú: para fuego de San Antonio.
27. Yerba de lucero: para diarreas y afecciones estomacales.
28. Ruda: para purificar la sangre.
29. Poleo-í: para afecciones del éstómago.
30. Malva de castilla: para palpitaciones del corazón.
31. Caarurupé: refrescante.
32. Tapecué: = I5.
33. Granada (fruto): para combatir diarreas.
34. Pacholí: para calmar los nervios.
35. Caraguatá rua: refrescante, corril.
36. Pata de buey-í: para afecciones del hígado y riñones.
37. Cocú: para afecciones del hígado.
38. Penicilina: para afecciones de la garganta.
39. Caá piky: refrescante.
40. Calaguala: para ácido úrico y el hígado.

41. Azafrán: para hepatitis.
42. Barba de choclo: para bajar la fiebre, refrescante.
43. Ysy: para bronquitis - cataplasma con sebo de buey.
44. Cabello de ángel: para el hígado.
45. Caraguatá (raíz): abortivo, corrial.
46. Toro ratí: para calmar la tos.
47. Caña brava: para el corazón.
48. Pyno-í: para purificar la sangre.
49. Mastuerzo: para el hígado y los riñones.
50. Tetŭ caá: para el corazón.
51. Teyuyá: abortivo.
52. Altamisa-í: abortivo.
53. Canchalagua-i: abortivo, para regular la menstruación.
54. Ajenjo: contraceptivo.
55. Rábano: para limpieza del estómago.
56. Ruibarbo: abortivo - corrial.
57. Perdudilla negra: para hepatitis.
58. Rosa mosqueta: laxante.
59. Guayacán: para orina con sangre, y dolores.
60. Zarza mora: corrial, diurético.
61. Penacho (flor): para purificar la sangre.
62. Llanten de agua: para el hígado y para adelgazar.
63. Caatai: para hemorroides.
64. Mbaracayá nambí: para afecciones del hígado.
65. Caaré (planta entera): antiparasitario.
66. Cerraja: para úlceras.
67. Mango (flor): para calmar la tos - bronquitis.
68. Cardo santo (semilla): para asma en ahogos.
69. Terciopelo (flor): para el corazón en palpitaciones.
70. Capií catí: refrescante - corrial.
71. Ambay: para calmar la tos.
72. Yua pecá: para hemorragias.
73. Yva hai: para diabetes.
74. Yvyrá pytá (corteza): para afecciones de la garganta.
75. Ynga (corteza): para el colesterol y diabetes.
76. Curupica-y (corteza): para el hígado.
77. Aguapé puruâ: para hepatitis, y estómago inflamado.
78. Cocu (especie diferente ?): para afecciones del hígado.
79. Caraguatá (fruto): antiinflamatorio.
80. Rosa china (flor): para hemorragias.

81. Tamanda cuna: para combatir la sífilis.
82. Mbuy-say yú: para los riñones.
83. Cola de ratón: para el hígado.
84. Malva rapó piré: para inflamación.
85. Almique (fruto): para dolor de oído.
86. Siete sangría: para el corazón.
87. Urupevó: para hemorragias.
88. Quebracho blanco (corteza): para bajar la fiebre.
89. Urucú (semilla): para jaquecas.
90. Curatú (semilla): para afecciones del estómago - flatulencias.
91. Yvyrá tai (hoja): para el reumatismo.
92. Curupay-mí: para el reumatismo.
93. Yva hai-poñy: para combatir la diabetes.
94. Caavó tyrey: para el hígado - hepatitis.
95. Malva de olor; para el corazón y dolores de cabeza.
96. Alfalfa: diurético y flatulencias.
97. Sandia (semilla): para bajar la fiebre, antigripal.
98. Girasol (semilla): sin datos.
99. Boldo (hoja): para el estómago.
100. Guavirá (fruto): para purificar la sangre.
101. Caá heé (hoja): para diabetes.
102. Naranja dulce (cáscara del fruto):
103. Caygua-í (semilla): emético.
104. Yacaré yrupé: para el hígado y los riñones.
105. Culantrillo: para el hígado y los riñones.
106. Yatei caá: para el estómago.
107. Yerba mate: para el corazón.
108. Suico: para el estómago.
109. Menta-í: para el estómago y para los nervios.
110. Batatilla: refrescante.
111. Vira-vira: para el hígado.
112. Cambará: para calmar la tos.
113. Sidra (hoja): para los nervios.
114. Salvia né: para los dolores menstruales.
115. Yaguarundí: para calmar la tos.
116. Pindó (raíz): abortivo.
117. Sauco: para dolores de estómago.
118. Malva blanca (flor): para bronquitis y catarros.
119. Mbocayá (plaútula): para el reumatismo y abortivo.
120. Uruzú catí: antiparasitario.
121. Yerba buena: para el estómago.

122. Curuguai: para los riñones y el hígado.
123. Para-para'í: para las piedras en los riñones.
124. Zarzaparrilla: diurético.
125. Laurel de España: para el estómago.
126. Cumandá yvyrai: para calmar la tos.
127. Perdudilla blanca: refrescante, corrial.
128. Marcela: para diarreas y para el estómago.
129. Borraja (flor): para calmar la tos.
130. Romero: para adelgazar.
131. Yaguareté caá: para el estómago.
132. Ytá poty: para hemorragias.
133. Mbocayá (hojas): diurético.
134. Catuaba: afrodisiaco.
135. Manzanilla: para el estómago.
136. Cola de caballo: para el hígado y los riñones.
137. Espartillo-í: abortivo.
138. Paratodo (corteza): para diarreas, úlceras.
139. Tilo (flor): para calmar los nervios, para el corazón.
140. Cebada Paraguay: refrescante para bajar la fiebre.
141. Cepa caballo: diurético.
142. Ceibo (corteza): para hemorroides.
143. Mbaracayá nambí (especie diferente ?):
144. Burrito: para el estómago.
145. Sará (corteza): para diabetes.
146. Santa Lucía morotí (raíz):
147. Charrúa caá: para combatir diarreas.
148. Verbena: para dolores de garganta.
149. Caaré (semilla): antiparasitario.
150. Anís: para el estómago y flatulencias.
151. Siempre viva: para el corazón.
152. Eneldo (semilla): para el estómago.
153. Doradilla: para el hígado.
154. Cedrón Paraguay: para palpitaciones del corazón y para calmar los nervios.

Method to Determine Inhibitory Activity against Xanthine Oxidase

I. Reagents

substrate --- xanthine $C_5H_4N_4O_2$ (0.15 mM, 22.8 mg/L)*
enzyme --- xanthine oxidase (0.04 unit /ml, in phosphate buffer)
HCl, Na_2HPO_4, KH_2PO_4

2. Buffer --- 1/15M phosphate buffer (pH 7.5)

3. Procedure

I) put 1.0 ml of test solution into the test tube

2) add 2.9 ml of buffer solution

3) add 0.1 ml of enzyme solution

4) after preincubation at 25 °C for 15 min, add 2.0 ml of substrate solution

5) after incubation at 25 °C for 30 min, add 1.0 ml of HCl

6) determine the absorbance of the assay mixture at 290 nm

* dissolved in distilled water at about 60 °C by stirring for 2 - 3 hrs; prepare just before use

	sample	blank 1	control	blank 2
test solution (ml)	1.0	1.0	-	-
dist H_2O(ml)	-	-	1.0	1.0
buffer (ml)	2.9	3.0	2.9	3.0
enzyme (ml)	0.1	-	0.1	-
preincubation at 25 ℃ for 15 min				
substrate (ml)	2.0	2.0	2.0	2.0
incubation at 25 ℃ for 30 min				
1N HCl (ml)	1.0	1.0	1.0	1.0
O.D. 290				

4. Calculation of the Inhibition %

$$\text{Inhibition \%} = \frac{(A-B)-(C-D)}{A-B} \times 100$$

A: optical density of control

B: optical density of blank 2

C: optical density of sample

D: optical density of blank 1

Method to Determine Inhibitory Activity against β-glucuronidase

1. Reagents

substrate --- p-nitrophenyl-β-D-glucuronide (0.1 M, 31.5 mg/ml)*
enzyme --- β-glucuronidase (from bovine liver, 15 units/g protein)**
AcOH, AcONa, HCl, tris(hydroxymethyl)aminomethane

2. Buffer --- 0.1M acetate buffer (pH 5.0)
0.01M tris-HCl buffer (pH 7.8)

3. Procedure

1) put 0.9 ml of test solution*** into the test tube
2) add 0.03 ml of substrate solution
3) add 0.1 ml of enzyme solution
4) after incubation at 37 ℃ for 30 min, add 0.25 ml of 0.2M Na_2CO_3
5) determine the absorbance of the assay mixture at 405 nm

* prepare just before use

** dissolve 20 mg of enzyme in 2 ml of 0.01 tris-HCl buffer (pH 7.8), centrifuge (10,000 rpm, 15 min) and use the supernatant as enzyme solution

*** test material is dissolved in 0.1M acetate buffer (pH 5.0)

	control	blank 1	sample	blank 2
acetate buffer (ml)	0.9	1.0	-	0.I3
test solution (ml)	-	-	0.9	0.9
substrate (ml)	0.03	0.03	0.03	-
enzyme (ml)	0.1	-	0.1	-
	↓ incubation at 37 C for 30 min			
Na_2CO_3(ml)	0.25	0.25	0.25	0.25
	↓ O.D. 405			

4. Calculation of Inhibition %

$E = A - B$

$S = C - (B + D)$

$$\text{Inhibition } \% = \frac{E - S}{E} \times 100$$

A: optical density of control

B: optical density of blank I

C: optical density of sample

D: optical density of blank 2

Method to Determine Inhibitory Activity against Urease

I. Reagents

Substrate --- Urea (3% urea in 0.1 M phosphate buffer, pH 6.7)

Enzyme --- Urease from Jack bean*

Indiacator --- Phenol red (0.1)**

2. Buffer --- 0.1 M Phosphate buffer (pH 7.7)

0.1 M Phosphate buffer (pH 6.7)

10 mM TEA (Triethanolamine) buffer (pH 7.0)

3. Procedure

I) put 0.25 ml of test solution into the test tube#

2) add 0.25 ml of enzyme solution

3) after incubation at 37 ℃ for 15 min, add 5.0 ml of substrate solution

4) measure the time of color change##

* Prepare the enzyme solution by dilution with TEA buffer in order to adjust the time of color change as 2 min in the case of blank

** Dissolve 0.1g of phenol red in 20 ml of ethanol and make up to IOO ml with distilled eater

\# Add 0.05 ml of 0.1% phenol red beforehand

\#\# The control solution is prepared with 5.5 ml of M phosphate buffer (pH 7.7) and 0.05 ml of o.1% phenol red. Measure the time when the test solution shows same color as solution

	sample	blank
test solution (ml)	0.25	-
enzyme (ml)	0.25	0.25
dist H_2O (ml)	-	0.25
	↓	incubation at 37 C for 15 min
phenol red (ml)	0.05	0.05
substrate (ml)	5.0	5.0
	↓	time

4. Calculation of the Inhibition %

$$\text{Inhibition \%} = \left(1 - \frac{\text{time of control}}{\text{time of sample}}\right) \times 100$$

Table 1. X.O.－Inhibitory Activities of Extracts from Medicinal Plants in Paraguay

Name of Medicinal Plants		Inhibition % at 50μg/mℓ	Name of Medicinal Plants		Inhibition % at 50μg/mℓ
AMBAY	〔r〕	42.2	COLA DE CABALLO	〔r〕	20.4
SARA MOROTI	〔h〕	30.1	ROMERO P/F	〔h〕	49.6/69.4
	〔r〕	7.8		〔r〕	52.2〔46〕
PIPER SP, № 2	〔r〕	6.1	BURRITO	〔h〕	28.3
PIPER SP, № 1	〔r〕	53.2〔46〕		〔r〕	14.1
ÑANGAPIRY	〔h〕	74.7〔6.6〕	YAGUARUNDI	〔h〕	39.4
CEDRON－CAPII	〔h〕	32.9		〔r〕	44.9
	〔r〕	16.5	ALHUCEMA	〔h〕	45.2
TYPYCHA－KURATU	〔h〕	16.7		〔r〕	53.7〔44〕
	〔r〕	37.2	ESPARTILLO GUAZÚ	〔h〕	34.9
TAPE－CUÉ	〔h〕	25.9		〔r〕	37.6
KOKU	〔h〕	21.9	EUCALIPTO	〔h〕	52.2
COLITA	〔h〕	68.0〔9.5〕		〔r〕	59.8〔35〕
	〔r〕	57.8〔36〕	CANGOROSA	〔h〕	52.6
YVAHAI	〔h〕	75.3〔2.5〕	SIETE SANGRÍA	〔h〕	49.5
PARAPARAI MÍ	〔h〕	72.4	CEDRÓN PARAGUAY	〔h〕	45.5
	〔r〕	79.4〔18〕	SIEMPRE VIVA	〔h〕	48.8
MIL HOMBRE	〔h〕	13.0	KAÁ HEÉ	〔h〕	42.4
	〔r〕	0.3	AROMITA	〔h〕	77.3
MARCELA	〔h〕	93.9	CHIRCA MELOSA	〔h〕	54.3
	〔r〕	81.9〔17〕	CULANTRILLO	〔h〕	37.0
COLA DE CABALLO	〔h〕	59.8	PIPER SP, № 3	〔h〕	19.2

〔 〕＝IC_{50}〔μg/mℓ〕
r＝room temperature　　P＝Precipitate
h＝hot temperature　　F＝Filtrate

Table 2. β-Glucuronidase Inhibitory Activities of Extracts from Medicinal Prants in Paraguay

Name of Medicinal Plants		Inhibition % at 10 $\mu g/ml$	Name of Medicinal Prants		Inhibition % at 10 $\mu g/ml$
AMBAY	(r)	44.0(13.5)	ROMERO(precip)	(h)	35.5
SARA MOROTÍ	(h)	41.0	(filtrate)	(h)	61.9
	(r)	36.5(20.0)		(r)	30.0
PIPER SP, № 2	(r)	24.0(23.0)	BURRITO	(h)	41.1
PIPER SP, № 1	(r)	31.0(30.0)		(r)	24.5(30.0)
ÑANGAPIRY	(h)	56.5(6.0)	YAGURARUND	(h)	29.1
CEDRON-CAPII	(h)	21.0(42.0)		(r)	27.5(34.0)
	(r)	17.5(32.0)	ALHUCEMA	(h)	63.9
TYPYCHÁ-KURATŨ	(h)	79.5(5.4)		(r)	24.0(15.0)
	(r)	34.5(15.0)	ESPARTILLO GUAZÚ	(h)	59.6
TAPE-CUÉ	(h)	63.0(4.0)		(r)	36.3(17.0)
KOKU	(h)	76.5(5.0)	EUCALIPTO	(h)	76.8
COLITA	(h)	62.5(10.1)		(r)	40.1(21.0)
	(r)	56.0(5.0)	CANGOROSA	(h)	36.5
YVAHÁI	(h)	54.0(5.0)	SIETE SANGRÍA	(h)	46.4
PARAPARAI MÍ	(h)	56.2	CEDRÓN PARAGUAY	(h)	56.5
	(r)	67.0(6.4)	SIEMPRE VIVA	(h)	70.3
MIL HOMBLE	(h)	18.6	KAÁ-HEÉ	(h)	64.0
	(r)	16.0(72.0)	AROMITA	(h)	56.5
MARCELA	(h)	83.6	CHIRCA MELOSA	(h)	76.9
	(r)	32.0(16.5)	CULANTRILLO	(h)	48.9
COLA DE CABALLO	(h)	52.4	PIPER SP, № 3	(h)	17.0
	(r)	14.5(30.0)			

( ) ; IC_{50} ($\mu g/ml$)
r ; room temperature
h ; hot temperature

Table 3. Inhibitory Activities of Extracts from Medicinal Plants against Urease in Paraguay

Material		Crude ext	n-Hexane ext	Insol	$CHCl_3$	H_2O ext	n-BuOH ext
AMBAY	r	33.2	—	—	—	—	—
SARA MOROTI	r	32.6	—	—	—	—	—
PIPER SP № 2	r	19.1	—	—	—	—	—
PIPER SP № 1	r	28.3	—	—	—	—	—
ÑANGAPIRY	r	47.4	31.5	39.7	23.0	—	70.4
CEDRON CAPÍI	r	11.0	—	—	—	—	—
	h	12.0	—	—	—	—	—
TYPYCHÁ-KURATŨ	r	29.2	—	—	—	—	—
	h	3.9	—	—	—	—	—
TAPE-CUÉ	r	9.2	—	—	—	—	—
KOKU	r	2.5	—	—	—	—	—
COLITA	r	35.2	—	—	—	—	—
	h	11.6	—	—	—	—	—
YVAHÁI	r	66.0	17.1	53.4	9.4	65.7	77.4
PARAPARAI-MÍ	h	32.1	—	—	—	—	—
MIL HOMBRE	h	6.9	—	—	—	—	—
MARCELA	r	32.9	—	—	—	—	—
	h	33.4	—	—	—	—	—
COLA DE CABALLO	r	33.2	—	—	—	—	—
	h	50.1	—	—	—	—	—
ROMERO	r	93.0	95.0	51.9	72.9	10.9	—
(precip)	h	95.0	—	—	—	—	—
(filtrate)	h	64.0	—	—	—	—	—
BURRITO	r	28.7	—	—	—	—	—
JAGUARUNDI	h	26.4	—	—	—	—	—
ALHUCEMA	h	18.7	—	—	—	—	—
ESPARTILLO GAUZU	h	8.8	—	—	—	—	—
EUCALIPTO	r	30.0	—	—	—	—	—
	h	40.8	—	—	—	—	—
CAÑGOROSA	h	62.1	—	—	—	—	—
SIETE SANGRIA	h	43.2	—	—	—	—	—
CEDRON PARAGUÁY	h	34.2	—	—	—	—	—
SIEMPRE VIVA	h	16.1	—	—	—	—	—
KAA HEÉ	h	34.8	—	—	—	—	—
AROMITA	h	36.8	—	—	—	—	—
CHIRCA MELOSA	h	37.9	—	—	—	—	—
CULANTRILLO	h	32.2	—	—	—	—	—
PIPER SP. № 3	h	31.0	—	—	—	—	—

Inhibitory Activity of Crude Extracts of Plants from PARAGUAY
(— Not Test, Inhibition % at 50 μg/ml)

RESULTADO DE LA ENCUESTA SOBRE PLANTAS MEDICINALES UTILIZADAS EN PARAGUAY

Nº	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMPLEO
1	AMBAY	354	Hoja	Tos – Catarro	Decocción – Te – Mate
2	SARÁ MOROTÍ	23	Hoja – Corteza	Diabetes	Te
5	ÑANGAPIRY	210	Hoja	Hipertenssión – Diurético Adelgazante	Tereré – Decocción – Mate
6	CEDRÓN CAPIÍ	209	Hoja	Estómago – Corazón Tranquilizante	Te – Decocción – Mate
7	TYPYCHÁ CURATÚ	135	Planta entera	Estómago – Digestivo	Te – Decocción – Tereré
			Hoja	Estómago – Digestivo	Te – Tereré – Decocción
8	TAPECUÉ	256	Hoja	Desinfectante – Cicatrizante	Lavaje – Te
			Planta entera	Desinfectante – Cicatrizante	Lavaje – Decocción – Te
9	COCÚ	432	Hoja	Refrescante – Hepatitis – Diurético	Tereré – Te – Con agua
11	YVAHAI	50	Hoja	Diabetes	Te – Decocción – Mate
12	PARA-PARA-Í	165	Planta entera	Diurético–Riñón–Cálculo vesic.	Tereré – Te – Mate
			Hoja	Riñón–Cálculo vesic–Diurético.	Tereré – Te – Mate
13	MIL HOMBRE	206	Tallo	Diurético – Abortivo	Decocción – Mate – Tereré
14	MARCELA	65	Flor y Hoja	Digestivo	Decocción – Mate
			Hoja	Digestivo	Decocción – Te
15	COLA DE CABALLO	146	Hoja	Refrescante – Diurético	Tereré – Te
			Hoja y Tallo	Refrescante	Tereré

Nº	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMOLEO
16	ROMERO	175	Hoja	Colesterol – Digestivo	Te
17	BURRITO	469	Hoja	Estómago – Digestivo	Te – Decocción – Mate
18	YAGUARUNDI	202	Hoja	Tos – Catarro	Te – Decocción
19	ALHUCEMA	24	Hoja	Estómago – Reumatismo	Te – Decocción
20	ESPARTILLO GUAZÚ	16	Planta entera	Dolor de muela	Decocción
21	EUCALIPTO	350	Hoja	Expectorante – Tos	Te – Inhalación – Decocción
22	CANGOROSA	329	Hoja	Cicatrizante–Abortivo–Cáncer	Te – Mate
			Hoja y Raíz	Cicatrizante	Decoccion – Mate
23	SIETE SANGRÍA	112	Planta entera	Hipertensión – Corazón	Mate – Te
			Hoja	Hipertensión	Te
24	CEDRÓN PARAGUAY	230	Hoja	Corazón – Sedante	Te – Mate
25	SIEMPRE VIVA	78	Flor	Corazón – Sedante	Mate – Te
26	CAÁ HEÉ	195	Hoja	Diabetes	Te – Mate – Decocción
27	AROMITA	116	Flor	Corazón	Decocción – Te
28	CHIRCA MELOSA	26	Hoja	Estómago	Decocción
29	CULANTRILLO	95	Hoja	Refrescante	Tereré – Decocción
31	CAMBARÁ	107	Hoja	Tos	Te – Decocción
32	PARATODO (PIRÉ)	24	Corteza	Herida – Sarna – Refrescante	Mate – Lavaje – Te
34	ALTAMISA ITÉ	98	Hoja	Dolor de cabeza	Directamente – Te – Decocción
35	CABELLO DE ANGEL	127	Cabello	Diurético – Infec. vías urinarias	Te – Mate

№	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMPLEO
36	CAATAI	96	Hoja	Sarna	Uso externo – Decocción
			Planta entera	Sarna – Desinfectante	Lavaje
37	CAARÉ	189	Hoja	Vermífugo	Decocción – Te
			Hoja	Vermífugo	Decocción – Te
38	SAUCO	91	Hoja	Hepatitis	Te
39	MOLLE–Í	67	Hoja	Garganta	Gárgaras – Te
			Hoja y Raíz	Garganta	Gárgaras – Te
40	SALVIA	303	Hoja	Antiespasmódico – Digestivo	Te – Mate – Decocción
41	CARAGUATA RUA	86	Hoja	Refrescante	Tereré
42	PINDÓ (RADÓ)	35	Raíz	Abortivo – Diurético	Te – Decocción
43	YSY	19	Gomorresina	Piel – Bronquitis	Friccionar
44	GUAYACÁN	56	Corteza	Diarrea	Decocción
45	PERDUDILLA NEGRA	51	Planta entera	Refrescante	Tereré – Mate
			Raíz	Refrescante	Tereré – Mate
46	MALVA BLANCA	331	Hoja y Flor	Tos – Catarro	Decocción – Te
			Hoja	Tos – Catarro	Decocción – Te
47	GUAYABA	326	Hoja	Garganta – Diarrea	Decocción – Gárgaras – Te
48	TAPERYVÁ–HÚ	94	Raíz	Vermífugo	Te – Decocción
49	PENICILINA	108	Hoja	Antiséptico	Lavaje
50	VERBENA–Í	277	Hoja	Depurativo – Digestivo Garganta	Te – Decocción

№	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMPLEO
51	TAMANDÁ - Í	3	Fruto	Asma - Bronquitis	Decocción
			Corteza	Asma - Expectorante	Decocción
52	AGUAPÉ PURUÁ	173	Hoja	Hepatitis - Refrescante	Tereré - Con agua
			Planta entera	Hepatitis - Refrescante	Tereré - Decocción - Con agua
53	YVYRA PYTA	23	Corteza	Garganta - Desinfectante	Te - Gárgaras
54	ALCANFOR DEL CAMPO	48	Hoja	Estómago - Catarro	Te - Decocción
55	CALAGUALA	135	Hoja	Abortivo - Amenorrea	Te - Decocción
56	YVOPE	85	Fruto	Anticaspa	Lavaje
			Semilla	Anticaspa	Lavaje
57	CARDO SANTO	99	Raíz	Depurativo - Digestivo	Decocción - Tereré
			Hoja	Dolor de vientre	Decocción - Te
58	PYNÓ GUAZÚ	50	Hoja	Refrescante	Tereré
			Planta entera	Depurativo	Te
59	TAYUYÁ	19	Raíz	Diurético	Tereré - Decocción
60	CAÑA BRAVA	88	Raíz	Diurético - Reumatismo	Tereré - Te - Decocción
				Refrescante	
61	TAROPÉ	103	Raíz	Refrescante	Tereré
			Planta entera	Refrescante	Tereré
62	YAGUÁ ROVA	71	Raíz	Abortivo - Reumatismo	Te - Decocción
63	MOLLE	68	Hoja	Garganta	Buche - Gárgaras - Decocción
			Raíz	Garganta	Gárgaras - Decocción
			Hoja y Tallo	Garganta	Gárgaras - Decocción

RESULTADO DE LA ENCUESTA SOBRE PLANTAS MEDICINALES UTILIZADAS EN PARAGUAY

Iguazú	12
Asunción	251
Lambaré	14
San Lorenzo	111
La Colmena	7
Areguá	6
Caacupé	6
Minas Cué	7
Isla Pucú	2
Itacurubí de la Cordillera	15
Itaguá	6
Fernando de la Mora	20
Paraguarí	5
San Bernardino	2
Ipacaraí	1
Luque	49
Capiatá	82
Villeta	4
Quiindy	4
Yaguarón	3
Itá	11
Tobatí	9
Ñemby	7
Carapeguá	4
San Juan Bautista (Misiones)	1
San José de los Arroyos	1
Caaguazú	1
Villa Hayes	1
Mcal. Estigarribia (Chaco)	1
Villa Elisa	2
Mariano Roque Alonso	2
Ypané	1
Mayor Martínez	1
Ayolas	7
Encarnación	11
Pirapó	5
Zeballos Cué	2
Lugar sin nombre	27
TOTAL	701

RESULTADO DE LA ENCUESTA SOBRE PLANTAS MEDICINALES UTILIZADAS EN PARAGUAY

Items of answers

Age	Male	Female
0 – 20	4	16
20 – 29	30	71
30 – 39	51	88
40 – 49	41	127
50 – 59	40	104
60 – 69	22	48
70 – 79	9	17
80 – 89	1	6
90 – 99	–	1
Uncertain	7	18
Total	205	496
Average of age	44.0	44.1

Report on Training at Pharmacology

Department of Pharmacology, Faculty of Medicine
Toyama Medical and Pharmaceutical University
Directed by: Professor Seuhiro Nakanishi
Associate Professor Ryuji Takeda
Assistant Professor Yasunori Momose
Assistant Professor Hiromi Yamazaki

In order that Dr. Ibarrola may establish the pharmacological investigation such as drug effect with various medicinal plants in Paraguay, we had designed his practices including lecture and experiments of pharmacology as follows. He had done these subjects at our department from May 13 to December 25 in 1985.

I. Biochemical pharmacology

1. Subcellular fractionation of animal tissue

   He studied how to prepare subcellular fractions of liver and brain from mice or rats.

2. Determination of enzyme activity which is localized in the subcellular fractions of brain and liver

   He measured the activity of alcohol dehydrogenase in the soluble fraction as well as aldehydrogenase (ALDH) in the soluble, mitochondrail and microsomal fractions. He also examined the effects of some drugs after in vivo treatment on these enzyme activities.

3. He learned how to determine kinetic parameters such as Km and Vmax values for hepatic ALDH isozymes in the subcelluler fractions.

4. SDS-polycrylamide gel (PAG) electrophoresis

   He studied polymorphism of cytochrome P-450 by SDS-PAG electrophoresis.

5. Preparation of isolated hapatocytes

   He learned the anatomy of the liver and practiced the selective isolation of hepatocytes from periveneous or perioportal region.

II. Physiological pharmacology

The following practices were done with various animals.

1. With dog

   (a) He practiced to inject intravenously or intraperitoneally and learned how to record changes in respiration and blood pressure. He also examined the pharmacological effects of typical compounds on respiration and blood pressure.

   (b) He studied to expose the ureter through a retroperitoneal approach and to insert a catheter. He learned to analyze diuretic effect caused by a hypertonic solution and other diuretic agents used clinically.

2. With guinea-pig

   He studied the drug action on the movement of isolated ileum.

3. With mouse

   He determined LD50 of strychnine nitrate after intraperitoneal injection according to up and down method.

4. With rabbit

   He examined the effects of some drugs on the respiration and blood pressure as well as on the movement of isolated jejunum.

5. With frog

   He prepared isolated frog heart by Yagi method and studied the effect of a few drugs on it.

Training at other laboratories

1. He visited the department of pharmacology of Shinshu University (Prof. Shigetoshi Chiba) and was shown the pharmacological experiments on cardiovascular system with dog for a week.

2. He visited Kawanishi Pharma Research Institute of Nippon Boehringer Ingelheim and was shown the experiments on general pharmacology (Head of pharmacology division, Dr. Hiroshi Kohei) and toxicology (Head of toxicology division, Dr. A. Kast).

Report on Training of C/P Isabel Basualdo in Japan

Herbal Garden, Faculty of Pharmaceutical Sciences
Toyama Medical and Pharmaceutical University
Period: from May 11 to August 2, 1986

This training aims at studying morphology of crude drugs, putting Paraguayan herbs in order, cultivating herbs, and straightening and maintaining a herb garden.

In the practice of the training, the cooperation by Suzuki, assistant, and technical personnel of the garden was devoted.

Paraguayan materials which were brought by Isabel was too much damaged to be used, so c/p received the training (Suzuki was in charge) on processes of morphological study with specimens in this garden. In regard with putting Paraguayan species in order, a part of those which were surveyed and collected in Paraguay was sticked to board to be placed in order, and botanical names as well as common names were classified on some of these species.

Regarding cultivation, the guidance on cuttage practice was conducted by technical personnel. Besides, c/p observed and studied procedures practised in this garden.

Concerning the straightening and maintenance of a herb garden, the constitution of our garden was so completely explained that it could become a good reference in maintaining a botanical garden in Asuncion University, and essential matters in practice were guided. In addition, following places were visited to grasp the functions. Herb Cultivation and Guidance Center of Toyama Prefecture, Medicinal Plants Garden of Kyoto Pharmaceutical University, the Kyoto Botanical Garden, Kyoto Herbal Garden, Pharmacognosy Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Sugadaira Herb Garden of Nagano Prefecture, Kitamimaki Experiment Land of Nagano Prefecture, etc., were visited, and the practice of distinguishing was conducted.

We convince, from above technical experiences, c/p has sufficiently acquired the outline of the technique required for the study of herbs. Owing to the cooperation of Suzuki, assistant, and technical personnel, discussion was lastly held relating botany in Asuncion University. Then the content of the discussion was straightened, and the sentence was made to determine the purport of further cooperation in study in the future, which was mutually understood.

REPORT OF THE TRAINING IN THE MEDICAL AND PHARMACEUTICAL UNIVERSITY OF TOYAMA

Instructer: Dr. Shoichi Suzuki

1) Use of electroforesis to detect morfological variation

We have analysed 4 species of trichosanthes (Flia. cucurbitaceae), Datura (Flia. Solanaceae) and Cassia (Flia. Leguminosae).
We have worked with leaf, root and seed (0,5 grs.), prepared the sample of prottein concentration by extracted them mixed in TRIS, and polyvinil pyrrolidine at PH 6,8.
The polyacrilamide gels was used in final concentration of 12,5 %.
The sample was loaded onto the gel (about 60 mc.) and the electroforesis was continued until the tracking was migrated nearly to the gel bottom.
We used 50 volt during 2 hours and then have changed to 170 volt. Finally the gel was stain in coomassie blue during 45 minutes after this, was destain in mixed of metanol, acetic acid and water.
The stainded protein bands was photographed before dry it.
The protein banding pattern were analysed with a computer, using a program called "Multi variant analysis".
We could not get a good result because took a long time before we can use correctly the technique.

2) Determination of the botanical origins of cruds drugs

The method is called S.U.M.P. (Suzuki's Universal Macro Printing).
We printed the leaves of Cassia and have observated it with a microscopy, then we took pictures, develoment and printed on paper. We could analyses the hair and the cell of the leaves.

3) Studies on propagation method for cultivation

For the cuttage test has used 43 species of the medicinal plants from the garden. The samples were cutted and putting into water during one day before to plant then.

Work outside

8 - 8 - 85 - Went to Togamura

8 - 23 - 85 - Went to Tateyama mountain to study the different between plants from cold and hot place.

8 - 30 - 85 - Visited the Pharmaceutical company in Toyama.

9 - 4 - 85 - Visited the Kyoto Botanical Garden.

9 - 5 - 85
9 - 6 - 85 - Attended to meeting of Medical and Pharmaceutical Society for Wakan Yoku in Kyoto.

9 - 20 - 85 - Visited the Medicinal plants research Center of Toyama Prefecture.

NOTE: This repot is already included in the first report of the Project.

Nélida Soria

LABORATORY REPORT. DEPARTMENT OF PHARMACOGNOSY . MEDICAL AND PHARMACEUTICAL UNIVERSITY. TOYAMA.

BY: DR. ESTEBAN A. FERRO BERTOLOTTO

FROM: JUNE,23,1986

TO : JULY,22,1986

DIRECTED BY: Associate Professor Dr. MINEO SHIMIZU

Along this month were performed the following activities:

- -Use of Hitachi 220 U.V. spectrophotometer.
- -Preparation of the enzyme Aldose Reductase(AR) from rat lenses for inhibition assays.
- -AR assay following the time course using water and DMSO.
- -AR assay using diferent solvents.
- -AR assay using plant extracts and pure compounds to measure the inhibition percent and calculate the IC_{50} of the active samples.
- -Checking of the AR enzyme activity.
- -Preparation of urease from Canavalia ensiformis DC (Jack bean).
- -Solvent fractionation of a crude extract of Marcela and further AR inhibition test of each fraction.
- -Bibliographic search of the genus Citharexylum(Sara moroti).

PREPARATION OF AR CRUDE ENZYME FROM RAT LENSES

Rats(Wistar strain) weighting 200-300 g. were put in a glass jar with ether.Then they were killed by broking their necks and the eye lenses were extracted using scissors . The lenses were put in a phosphate buffer solution 0.1 M,pH: 6.8 containig 1 mM of 2-mercaptoethanol and 1 mM of NADP. This solution was kept in a ice bath, using 0.1 ml of solution per lens, and was stored frozen at -25° C until the enzyme preparation. The lenses with the buffer solution were melted using an ice bath, and then tranfered to an tissue homogeneizer and stirred till complete lenses disruption and milky aspect of the mixture.This mixture were put into cool certrifuge tubes and centrifuged 15 min.at 4° C at 12000 r.p.m.(10000 g.). The supernatant contains the crude AR enzyme and was transfered to vials for further assays. The enzymatic

activity was tested using the assay conditions,phosphate buffer 0.1 M, pH 6.2,NADPH ,with and without the substrate($\pm$ glyceraldehyde)and measuring the absorbance decay at 340 nm. The enzyme was diluted using phosphate buffer 0.1M pH 6.8 to get an absorbance decay of 70% in 200 seconds. The crude enzyme was separated in aliquots of 1.0 , 1.3 ,1.5 and 1.8 ml. and stored at -78° C.

AR ASSAY

The AR assay was performed using a phospnate buffer solution 0.1 M,pH 6.2 containing NADPH 0.104 mM. A part of this solution (Substrate +) was used for disolving the substrate,$\pm$ - glyreraldehyde 10 mM. All the assays were performed following this scheme:

	CONTROL	BLANK-1	SAMPLE	BLANK-2
PO_4 buffer(ml)	0.97(+)	0.97(-)	0.97(+)	0.97(-)
Sample(ml)	-	-	0.01	0.01
Water or DMSO(ml)	0.01	0.01	-	-
AR crude enz.(ml)	0.02	0.02	0.02	0.02

The assay was performed at 25° C,keeping the buffer solution in a water bath and sirring every 5 min.The solutions were transfered using dispensors to 1 ml. UV cells,and sirring with glass rods after adding the reagents. The enzyme was added and mixed 40 seconds before starting the scan. Unless other conditions be reported the assays were plotted using a 220 Hitachi VIS/UV spectrophotometer at 340 nm,slit 2,response 4,time drive 60 mm/min. and scale 0.00-- 0.05. Two sets of cells were used and always in the same way and position.

AR TIME-COURSE USING WATER(CONTROL)

TIME (minutes)	ABSORBANCE	TIME(minutes)	ABSORBANCE
2	0.0009	20	0.182
6	0.058	26	0.222
10	0.094	30	0.244
12	0.140	36	0.269
14	0.132	40	0.281
16	0.150	44	0.290
18	0.157	50	0.297

This data were plotted giving a linear relationship till 10-14 minutes after adding the enzyme.

AR ASSAY USING DIFERENT SOLVENTS

In the usual conditions of the AR assay were tested diferent solvents during 300 seconds and noted the effect in the absorbance decay.

SOLVENT	ABS.DECAY AT 200''	ABS.DECAY AT 300''
Water	44 %	67%
DMSO	44.2%	67%
Ethanol	41%	61%
Propilenglycol	10.5%	16.3%
Methanol	43%	63.3%

The propylenglycol exibits a strong inhibitory effect on AR.The others solvents have a very close response among them.

AR ASSAY USING PLANT EXTRACTS AND PURE COMPOUNDS. MEASURING OF INHIBITION % AND IC_{50}.

Using the AR assay conditions noted previously were tested several crude extracts of medicinal plants of Paraguay. Also were checked isolated compounds,fractions and a reference(quercitrin). In each batch is noted the inhibition percent of each sample,the initial and final value of the quercitrin and the IC_{50} of the samples with strong inhibitory effect. The samples that exibited inhibition % more than 50 were repeated and the data showed is an average of this results. The inhibition percent was calculated using a control line,obtained plotting the variation of absorbance of DMSO along the assay. Blank control was performed till get a stable condition(about 40-70 minutes after disolving NADPH)and every 4 samples. This blank line shows a constant slope till 230-260 minutes after disolution of NADPH. So the useful time for assay is arround 3 hours.

For the calculation of inhibition % was used the formula:

$$\text{Inhibition \%} = \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} \cdot 100$$

or Inhibition % = $\frac{(CONTROL - BLANK\ 1) - (SAMPLE - BLANK\ 2)}{(CONTROL - BLANK\ 1)} \cdot 100$

The samples of crude extracts were tested using a concentration of 1×10^{-5} g/ml (10 micro g/ml). For the calculation of IC_{50} were tested several concentrations from 5×10^{-5} to 5×10^{-7} g/ml and plotted the inhibition % againt the log of the concentrations. The concentration that exibit 50% inhibition corresponds to the IC_{50}. This value is expressed in g/ml for crude extracts and fractions and using moles/l for pure compounds.

July,3.1986

AR Lot#3

SAMPLE	INHIBITION %
Quercitrin 1×10^{-6}g/ml	56.6
Quercitrin 5×10^{-6}g/ml	68.3
Quercitrin 1×10^{-5}g/ml	87.2
Propylenglycol(0.01ml)	68.8
Sará Morotí Bark(5×10^{-6}g/ml)	40.3
Kaá-Heé	23.8
PAR 2+6(5×10^{-6}g/ml)	34.7
Sará Morotí Leaves(5×10^{-6}g/ml)	61.7

IC_{50} of quercitrin: 4.6×10^{-7} g/ml

July,7,1986

AR Lot#3

SAMPLE	INHIBITION%
Quercitrin1×10^{-5} g/ml	76.3
Quercitrin 5×10^{-6} g/ml	72.3
Quercitrin 1×10^{-6} g/ml	47.2
Quercitrin 5×10^{-7} g/ml	26.7
Quercitrin 1×10^{-7} g/ml	16.5
Cambará	85.4
Araticuí	31.0
Altamisa-ité	36.3
Cabello de Angel	35.0
Caa-tai	20.1
Caa-rê(roots)	8.2

SAMPLE	INHIBITON%
Caa-rê(aerial parts)	9.2

IC_{50} quercitrin=$1x10^{-6}$ g/ml ----- 2.45×10^{-6} M

July,9,1986

AR Lot#3

SAMPLE(g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	50.4
Cambará $1x10^{-5}$	85.7
Cambará $5x10^{-6}$	56.2
Cambará $1x10^{-6}$	36.4
Cambará $5x10^{-7}$	16.5
Cambará $1x10^{-7}$	19.0
Sauco	28.2
Molle-í	55.1
Salvia	50.4
Caraguata-ruá	23.8
Pindó	16.4
Ysy(leaves)	48.6
Guayacán(bark)	79.9
Quercitrin $1x10^{-6}$	24.2

IC_{50} Cambara:3×10^{-6} g/ml

July,11,1986

AR Lot#3

SAMPLE	INHIBITION %
Quercitrin $1x10^{-6}$	47.4
Perdudilla Negra	15.0
Malva Blanca	30.6
Guayaba	54.4
Taperyva-hu	28.2
Penicilina	26.6
Verbena-í	49.2

SAMPLE	INHIBITION%
Aguape-puruá(roots)	14.6
Aguape-puruá(aerial parts)	21.1
Yvyra-pytá	11.2
Quercitrin $1x10^{-6}$	32.7

July,12,1986

AR Lot#3

SAMPLE	INHIBITION %
Quercitrin $1x10^{-6}$	70.7
Alcanfor(de hoja)	81.1
Calahula	29.9
Ybopé(Gleditsia sp.)	34.4
Páratodo(leaves)	44.7
Sará moroti bark(E.Ferro)	51.3
S.Moroti bark(E.Ferro)precipitate	53.7
S.Moroti bark(E.Ferro)n-BuOH	77.7
S.Moroti bark(E.Ferro)water	30.2
Quercitrin $1x10^{-6}$	67.0

July,15,1986

AR Lot#3

SAMPLE(g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	61.0
S.Morotíleaves E(Horie)$5x10^{-6}$	66.0
S.Moroti 1(Horie)	43.8
S.Moroti 2(Horie)	33.1
S.Moroti 3(Horie)	78.7
S.Moroti 4(Horie)	84.3
S.Moroti 1 (6) (Horie)	63.2
S.Moroti 3 (6) (Horie)	6.1
Molle-í $5x10^{-5}$	86.7
Molle-í $1x10^{-5}$	54.0
Molle-í $5x10^{-6}$	18.4
Molle-í $1x10^{-6}$	17.2
Molle-í $5x10^{-7}$	1.0

SAMPLE	INHIBITION %
Salvia $5x10^{-7}$	0.0
Salvia $1x10^{-6}$	6.3
Salvia $5x10^{-6}$	14.1
Salvia $1x10^{-5}$	39.4
Salvia $5x10^{-5}$	78.0
Ysy $5x10^{-7}$	0.0
Ysy $1x10^{-6}$	7.0
Ysy $5x10^{-6}$	14.9
Ysy $1x10^{-5}$	57.9
Ysy $5x10^{-5}$	94.1
Quercitrin $1x10^{-6}$	61.5

July,16,1986

AR Lot#3

SAMPLE	INHIBITION %
Quercitrin $1x10^{-6}$	71.3
Guayacán(bark)$5x10^{-7}$	10.3
Guayacán(bark)$1x10^{-6}$	17.4
Guayacán(bark)$5x10^{-6}$	53.1
Guayacán(bark)$1x10^{-5}$	85.5
Guayaba $5x10^{-7}$	7.4
Guayaba $1x10^{-6}$	0.0 (?)
Güayaba $5x10^{-6}$	22.5
Guayaba $1x10^{-5}$	65.3
Guayaba $5x10^{-5}$	81.8
Verbena-í $1x10^{-6}$	3.1
Verbena-í $5x10^{-6}$	27.0
Verbena-í $1x10^{-5}$	61.0
Verbena-í $5x10^{-5}$	92.0
Alcanfor(dehoja)$5x10^{-7}$	0.0
Alcanfor(de hoja)$1x10^{-6}$	17.1
Alcanfor(de hoja)$5x10^{-6}$	35.6
Alcanfor(de hoja)$1x10^{-5}$	67.3
Alcanfor(de hoja)$5x10^{-5}$	90.8
Quercitrin $1x10^{-6}$	45.2

IC_{50} Guayacán (bark)= 4.3 x 10^{-6} g/ml

IC_{50} Guayaba = 7.8 x 10^{-6} g/ml

IC_{50} Verbena-í = 8.0 x 10^{-6} g/ml

IC_{50} Alcanfor(de hoja) = 7.0 x 10^{-6} g/ml

July,17,1986

AR Lot#3

SAMPLE (g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	68.0
S.Morotí bark(E.Ferro) $1x10^{-6}$	1.5
S.Morotí bark(E.Ferro) $5x10^{-6}$	15.4
S.Morotí bark(E.Ferro) $1x10^{-5}$	35.0
S.Morotí bark(E.Ferro) $5x10^{-5}$	84.0
S.Morotí bark(EF)n-BuOH $1x10^{-6}$	2.6
S.Morotí bark(EF)n-BuOH $5x10^{-6}$	29.6
S.Morotí bark(EF)n-BuOH $1x10^{-5}$	71.2
S.Morotí bark(EF)n-BuOH $5x10^{-5}$	94.7
PAR 1(Horie)$5x10^{-7}$	82.6
PAR 2 (Horie)$5x10^{-7}$	34.9
PAR 3m(Horie)$5x10^{-7}$	44.2
PAR 3a(Horie)$5x10^{-7}$	0.18
PAR 4(Horie) $5x10^{-7}$	7.2
PAR 5(Horie) $5x10^{-7}$	0.0(?)
PAR 6(Horie) $5x10^{-7}$	29.3
Quercitrin $1x10^{-6}$	60.0

IC_{50} Sará Morotí Bark(E.Ferro) Crude extract=1.6 x 10^{-5} g/ml

IC_{50} Sará Morotí Bark(E.Ferro) n-BuOH = 7.0 x 10^{-6} g/ml

July,19,1986

AR Lot#3

SAMPLE(g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	70.0
Marcela A $1x10^{-5}$	83.0
Marcela B $1x10^{-5}$	31.8
Marcela C $1x10^{-5}$	58.9
Marcela E $1x10^{-5}$	85.8
Marcela F $1x10^{-5}$	66.5
S.Morotibark (EF)$1x10^{-5}$	40.9
S.Moroti bark(EF)$5x10^{-5}$	81.2
S.Moroti bark(EF)BuOH $1x10^{-5}$	70.7
S.Moroti bark(Horie)$1x10^{-5}$	69.0
S.Moroti bark(Horie)$5x10^{-5}$	96.5
Quercitrin $1x10^{-6}$	64.7

The strongest inhibitory activity of Marcela fractions was found in the n-BuOH fraction(E). This fraction will be processed for the isolation of the active compounds.
The comparison of two extrcts of Sara Moroti bark at hot conditions show different activities. Both samples were collected in the same season in different ,but close,places. TLC compairson should be done with both extracts.

CHECKING OF THE AR ENZYME ACTIVITY

Was calculated using the formumla:

$A = \varepsilon \cdot b \cdot c$

A: absorbance of the control at the middle stable control line. This value was calculated using several control lines of the same enzyme lot.(#3).The mean value was corrected to 60 seconds.

ε: $\varepsilon_{NADPH} = 6.22 \times 10^6\ cm^2 / mol$

b: path length = 1 cm

c: concentration of NADPH.

The A value in the assay conditions was: 0.05 ± 0.0014 using a scale value of 0.05. The c value in the formula gives the rate of comsuption of NADPH and the enzyme activity when the A value is corrected for 60 seconds. With these data the AR activity is : 1.4.

PREPARATION OF UREASE FROM CANAVALIA ENSIFORMIS SEEDS

200 g. of powder of C.ensiformis DC (seeds) and 3 spoons of Hyflo Super Cell were extracted 4-5 min. at 0-5° C(ice bath) with 1 l. of acetone 30%(aqueos) containing 8-10 ml of 2-mercaptoethanol and 1 mM of Na_2EDTA. The suspension was filtered in vacuo at 0-5° C during 15-25 min.(optimun 15 min) To the clear solution was added cold acetone dropwise, 170 ml in a cool room,and the mixture was kept in this conditions over nigth. After decanting the supernatant the precipitate was centrifuged 10 min. at 3000 r.p.m and 4° C. The precipitate was dissolved using 0.1M phosphate buffer pH 6.7 containing 0.1 M of triethanolamine(TEA),15 ml. This mixture was centrifuged 20 min. at 12000 r.p.m. and 4° C. The supernatant contains the urease and is stored at 5° C. The precipitate of the last process can be dissolved again using the PO_4-TEA buffer to get more enzyme. The activity of the enzyme was measured using assay conditions and the following formula:

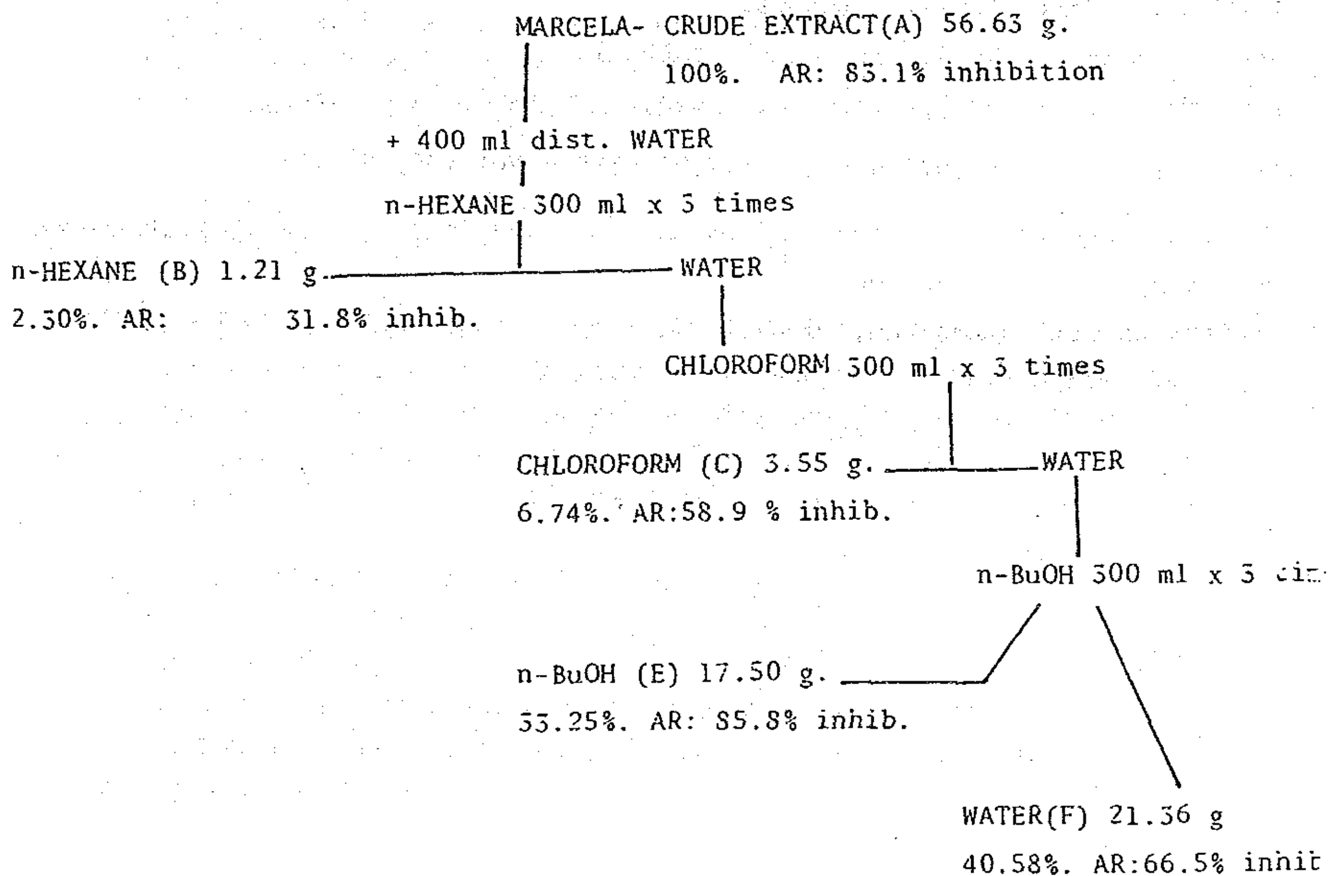
$$\text{Urease(Summer units/ml)} = \frac{50 . f . 0.4 . n}{V . t}$$

f: temperature factor
n: dilution % of the enzyme solution
V: volume of enzyme solution tested
t: time to change pH from 6.7 to 7.7

The urease is stable at 4° C. one month and 8 units/tube are used in each assay.The urease preparation was performed in the Hygienic Sciences Lab of this University.

SOLVENT FRACTIONATION OF A CRUDE EXTRACT OF MARCELA

52.63 g of an extract obtained from Marcela in room temperature conditions at the Faculty of Chemical Sciences(Paraguay) were suspended with 400 ml of water and sonicated during 20 min. The plant material was extracted using 70% aqueos Ethanol. The solvent fractionation was made at room temperature using n-Hexane, Chloroform and water saturated n-Buthanol(n-BuOH). Each fraction was washed with 50 ml. of water(100 ml of n-BuOH saturated water for the n-BuOH fraction)and these water washes were mixed with the water layer before the next solvent extraction. Each solvent was shaked 30 min(10 min x 3 times) before layers separation. The extraction scheme was as follow , and for each fraction is showed the yield and the AR inhibition % at $1x10^{-5}$ g/ml.



MARCELA FRACTION	SOLVENT	AMOUNT(g)	YIELD(%)	AR INHIBITION (%)
A(crude extract)	70%EtOH	56.63	100	83.1
B	n-Hexane	1.21	2.30	31.8
C	$CHCl_3$	3.55	6.74	58.9
E	n-BuOH	17.50	33.25	85.8
F	H_2O	21.36	40.58	66.5

REFERENCES ABOUT THE GENUS CITHAREXYLUM (SARA MOROTI)

The search was made using the following sources:
Konstitution und Vorkommen der Organischen Planzenstoffe. Walter Karrer
Birkhauser Verlag(1958). Bassel.
Annual Index of the Reports on Plant Chemistry.
Hirokawa Publishing Company Inc. Tokyo.(1958-1972)

Chemical Abstracts.1970 untill now(July,1986- Vol 105, N° 1)

Was found one reference about Citharexylon genus.Also was searched the genus Verbenoxylon as a synoname.
" Citharexylum solanaceum,iridoids of,taxonomy in relation to" 91:189758w
Z.Naturforsch,C Biosci 1979,34C(5-6) 316-29.

LABORATORY REPORT. DEPARTMENT OF PHARMACOGNOSY. TOYAMA MADICAL AND PHARMACEUTICAL UNIVERSITY. TOYAMA.

BY: DR. ESTEBAN A. FERRO BERTOLOTTO
FROM: JULY,23,1986
UNTIL: AUGUST,22,1986.

During this month were performend the following activities:
- -Preparation of the AR enzyme from rat lenses(lot#5) and checking of the enzyme activity.
- -Protein assay of AR preparations(lots #3,#4, and #5).
- -Calculation of AR specific activity(lot #3).
- -Fractionation of the n-BuOH fraction(E) of a crude extract obtained at room temperature from Marcela.
- -Use of a Droplet Counter Current Chromatograph(DCCC).
- -Assistance to the 6th Symposium on the Development and Application of Naturally Occurring Drug Materials(July,25-July,26) at Nagoya.

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PREPARATION OF AR CRUDE ENZYME FROM RAT LENSES

From 26 Wistar rats (6-7 weeks old)were obtained the lenses, and the enzyme preparation was performed using the procedure reported previously. After the usual activity check and dilution, the crude enzyme was aliquoted in 3 fractions of 1 ml. and 5 fractions of 1.3 ml. and stored at - 25° C. This batch was labeled as Lot#5.

PROTEIN ASSAY OF ARPREPARATIONS(LOTS #3,#4 and #5)

The protein concentration of each lot was measured using the Bio-Rad Protein Assay Kit(Bio-Rad Laboratories,Richmond,USA),based on the diferential absortion of Coomasie Brillant Blue G-250 dye when it's bound to proteins.

The assay was performed using as protein standard bovine serum globulin(BSG**at different concentrations. The standard solutions and samples were diluted using a pH 7.2 buffer solution containing 6.8 g of KH_2PO_4 and 8.76 g of NaCl in 1000 ml of dist. water. pH was adjusted using KOH solution. Fallowing the assay procedure 0.1 ml of satndards and samples were placed in test tubes. The samples(AR crude enzyme) were diluted 1:50 using the pH 7.2 buffer solution. 0.1 ml of buffer solution was used as blank. 5.0 ml of the diluted dye reagent(1:5) were added to each tube and incubated at room temperature 30 minutes after gentle mixing. The absortins at 595 nm were measured using a Hitachi 220 Spectrophotometer, and the OD_{595} were plotted against standard concentrations to get a satandard curve for read the unknown values.For each standard concentration and sample were made 3 tubes and the values plotted are the average.

SAMPLE	PROTEIN CONCENTATION (MICRO G)	OD_{595}
Standard 1	153.0	0.989
Standard 2	107.1	1.009
Standard 3	76.5	0.741
Standard 4	45.9	0.493
Standard 5	22.9	0.269
AR Lot#3 (1:50)	79.0*	0.759
AR Lot#4 (1:50)	68.5*	0.668
AR Lot#5 (1:50)	59.1*	0.586

* From standard curve.

This assay gave for the AR lots the fallowing protein concentrations :

AR LOT#3: 3950 micro g = 3.95 mg/ml
AR LOT#4: 3425 micro g = 3.42 mg/ml
AR LOT#5: 2955 micro g = 2.95 mg/ml

**BSG = 1.53 mg/ml(stored at -78° C) . Stock solution.

AR SPECIFIC ACTIVITY (Lot#3)

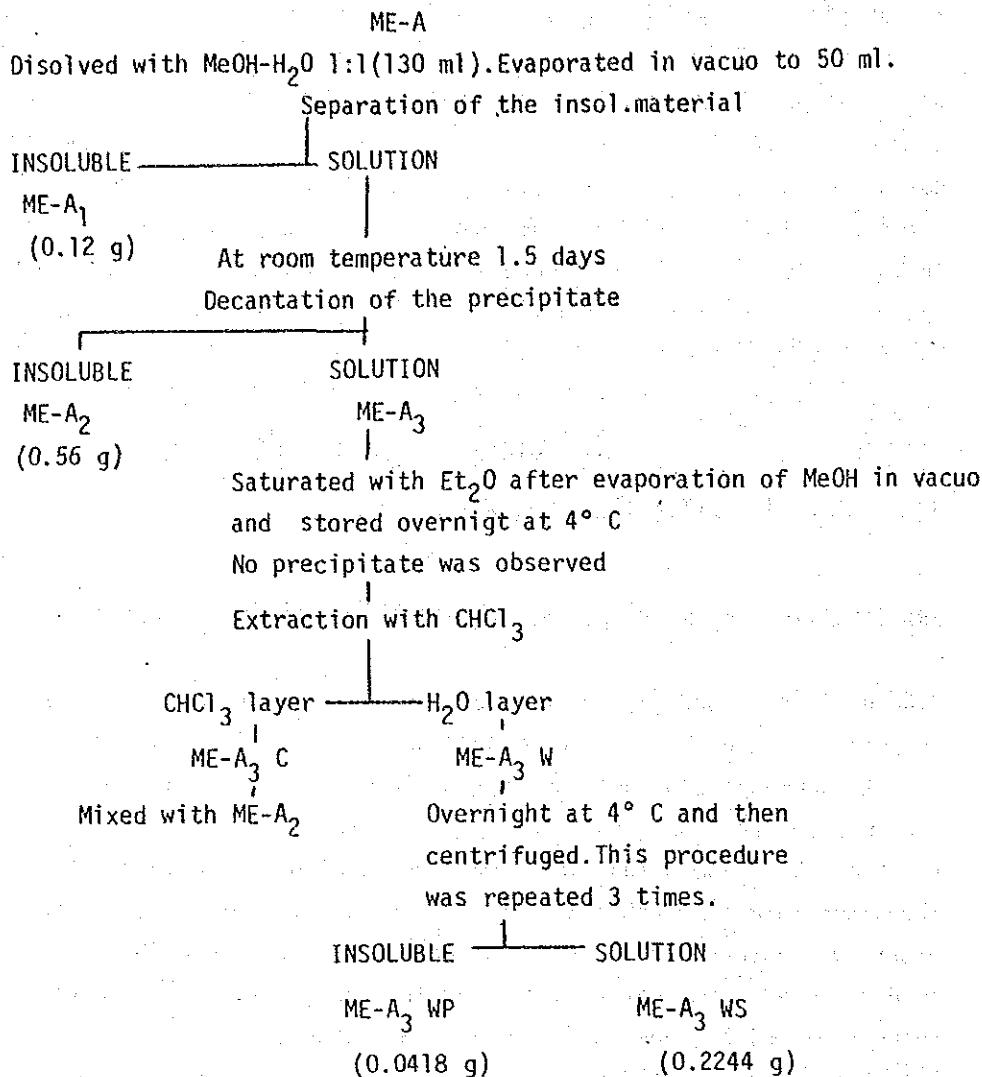
Using the previouly reported value for the AR(Lot#3) activity based on the rate of consuption of NADPH and the protein concentration,was calculated the specific AR activity.
For AR,1 Unit = 1×10^{-9} mol of NADPH consumed per minute.
In the AR assay conditions there is 1.45 Units (0.02 ml of AR crude enzyme), so there is 72.50 Units/ml.
For AR Lot#3, Protein = 3.95 mg/ml

$$\text{AR SPECIFIC ACTIVITY} = \frac{\text{AR ENZIMATIC ACTIVITY (UNITS/ML)}}{\text{AR PROTEIN CONCENTRATION (MG/ML)}}$$

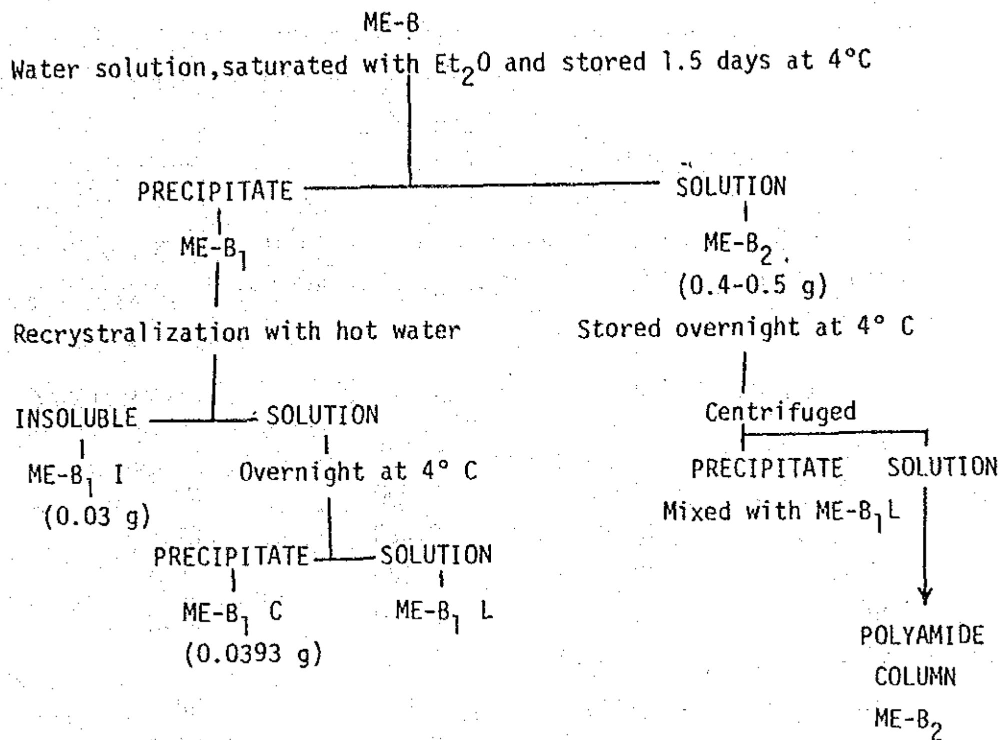
AR SPECIFIC ACTIVITY= 18.35 Units/mg

FRACTIONATION OF THE N-BUTHANOL (E) FRACTION OF MARCELA EXTRACT

2.06 g of the n-BuOH(E) fraction of Marcela extract,obtained at room temperature with 70% EtOH, were suspended in 30 ml of dist. water at room temperature and sonicated during 10 minutes. The insoluble material was separeted by centrifugation,disolved in MeOH,evoparated in vacuo at 40° C and dried. This fraction labeled ME-A weighs 1.43 g. The water soluble fraction was mixed with 120 ml of MeOH,but no precipitate was observed.After evaporation in vacuo of the MeOH,was added cold Acetone to the water extract and no change was noted.The water soluble material ,labeled ME-B,weight was 0.60 g.
The ME-A fraction was suspended in 30ml of boiling dist. water, and after 10 minutes was filtered off . The hot water soluble material was mixed with ME-B for further separations and the insoluble material remains as ME-A. ME-A and ME-B fractions were treated separatelly according with the fallowing schemes.The fractions showing similar TLC patterns were mixed.



The TLC patterns were made using Silica Gel and Cellulose pre-coatted plates. Silica plates were eluted with the following solvent mixtures EtOAc-MeOH 10:3 ; $CHCl_3$-MeOH-H_2O 35:65:40(lower layer) and $CHCl_3$-MeOH 2:1. The spots were observed under UV light,with and without NH_3 fumes and spraying 10% H_2SO_4 or AcOH-H_2SO_4-H_2O and heating at 100° C in both cases.



POLYAMIDE COLUMN ME-B_2

A clear water solution of ME-B_2 (about 0.5 g) was chromatographed in a column(2.5 cm Ø,35 cm long) filled with Polyamide C-200 (Wako Pure Chem.Ind) and packed with water.The column was eluted according with the fallowing scheme:

SOLVENT	AMOUNT (ML.)	FRACTIONS (ML.)
Water	500	--
MeOH-Water 10:90	600	1-2(300)
MeOH-Water 20:80	200	3 (200)
MeOH-Water 50:50	700	4-5(150) 6-7(50) 8-15(30)
MeOH-Water 70:30	600	16-24(30) 25-49(15)
MeOH	500	50-71(15) 72-78(50)
5% Na_2CO_3(water sol.)	300	80-92(15)

The fractions were checked using Ployamide TLC plates(Polyamide FM Plate,Wako Pure Chem.Ind) eluted with MeOH-Water mixtures(3:1, 5:1) and the spots were observed under UV light with and without NH_3 fumes and after spraying with $FeCl_3$ solution.Fractions with the same TLC pattern were mixed and labeled as ME-B_2 fractions . Some of them were tested with the AR inhibition test. The weight of the fractions and the AR inhibition % is showed in the fallowing scheme. Also some fractions from n BuOH(E) extract of Marcela were tested with that enzymatic assay.

COLUMN FRACTION	SAMPLE	WEIGHT(G)	AR INHIBITION %
7-18 19-20	ME-B_2 1+2	0.0628	NP
21-30	ME-B_2 3	0.0580	NP
31-34	ME-B_2 4	0.0209	67.9
35-48	ME-B_2 5	0.0692	58.6
49-53	ME-B_2 6	0.0082	55.8
54-57	ME-B_2 7	0.0095	NP
58-66	ME-B_2 8	0.0159	NP
67-70	ME-B_2 9	0.0056	64.3
71-73	ME-$B_2$10	0.0108	NP
74-77	ME-$B_2$11	0.0163	67.8
78-79	ME-$B_2$12	0.0061	57.6
80-83	ME-$B_2$13	0.1215	83.5
84-92	ME-$B_2$14	0.0643	NP
-----	ME-A_3 WP	0.0418	76.8
-----	ME-A_3 WS	0.2244	85.2
-----	ME-B_1 C	0.0393	61.9

The AR inhibition test was performed using the usual procedure,and the samples were tested in DMSO solution of 10 micro g/ml. A standard sample of Quercitrin was tested twice at 1 micro g/ml giving a AR inhibition % of 67.8.- The AR enzyme belongs to the Lot #3.

** NP= not performed.

COMPARISON OF SARA MOROTI CRUDE EXTRACTS

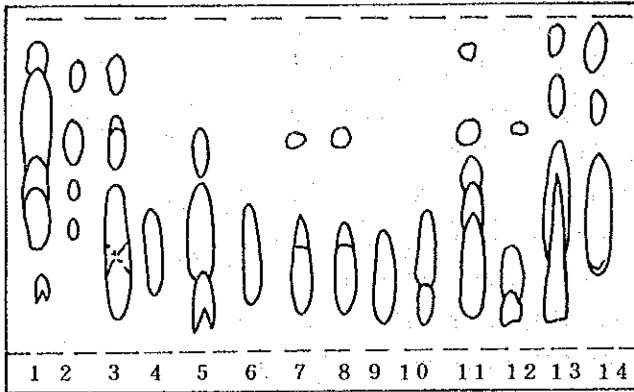
Two different crude extracts from bark of Sara Moroti,obtained both in hot extraction conditions were tested with the AR inhibition test as shown in the first report. Since the inhibition % value of the samples was different , TLC of both was performed using Silica Gel plates eluted with $CHCl_3$-MeOH 3:1 and observed under UV ligth and after spraying with AcOH-H_2SO_4-Water 80:10:10 and heating. Also TLC with cellulose plates eluted with n-BuOH-AcOH-Water 4:1:2 and observed under UV light(w. & wo. NH_3 fumes)and after spraying with $FeCl_3$ solution was made. Both extracts show the same main spots,but the first one collected at the National University Campus(SanLorenzo) exibit a bigger amount of chlorophil and low polarity compouds. The second sample collected at Capiataseems to be from an older plant. Compairson of these extracts with the samples purchased from the market should be done using chemical and biological methods.

USE OF D.C.C.C.

A short trainig was made using the droplet counter current chromatograph.The solvent system was $CHCl_3$:MeOH:Water 35:65:40. The lower layer was used as stationary phase and the upper layer was used as mobile phase. A sample containing a dye mixture of Guinean Green, Naphtol Yellow and Ponceau-SX was separated.(1.7 mg of sample in a mixture 1:1 of both layers).The equipment was set for working with 120 tubes, and 10 ml fractions were collected with a fraction collector working overnight. All the operations for the previous set up of the apparatus were performed.

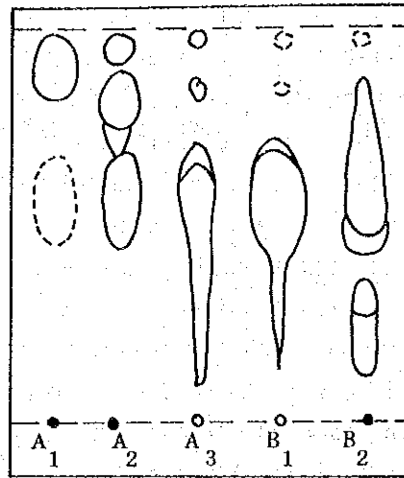
SUMMARY OF SOME TLC PLAFES

MARCELA(E)
POLYAMIDE COLUMN FRACTIONS(ME-B_2)

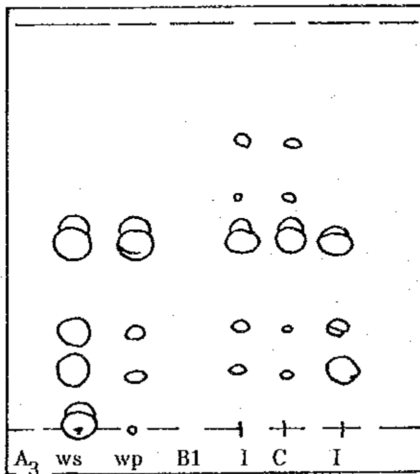


Polyamide FM. $MeOH-H_2O$ 5:1. UV light

MARCELA(E). WATER SOL/INSOL. FRACTIONS

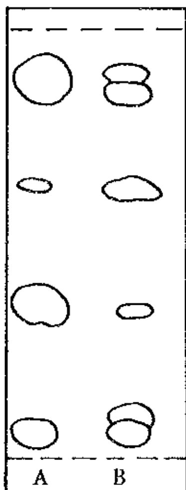


Cellulose. $CHCl_3-MeOH-H_2O$
65: 25:4. uv light



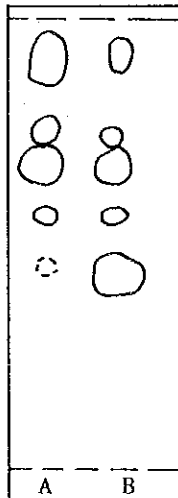
MARCELA(E)
Silica Gel
$CHCl_3-MeOH-H_2O$ 35:65:40(lower layer)
UV light

SARA MOROTI CRUDE EXTRACTS

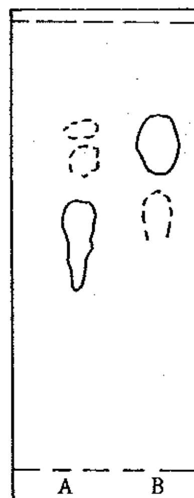


Silica Gel
$CHCl_3$-MeOH
3 : 1
UV light

(1)



(2)



Cellulose
n-BuOH-AcOH-H_2O
4 : 1 : 2
(1):UV light
(2): $FeCl_3$

A=Campus sample
B=Capiata sample

Final report about the training course in Toyama (Japan)

By : Lucia Franco

Place: Toyama Medical and Pharmaceutical University - Japan

Field: Phytochemistry

Chief of the department: Prof. Dr. Noakata Morita

Instructor: Assistant Prof. Dr. Munehisa Arisawa

Duration of the training course: From July 26th to October 3rd (1985)

Part one

- Extraction - purification and structural elucidation of active principles focussed on flavonoids
- Cromatography methods
- Determination of melting point
- Spectroscopic methods: Basic training on Ultraviolet Spectra (U.V.) Infrared Spectra (IR) Nuclear Magnetic Resonance (N.M.R.)
- Hydrolysis methods (Acidic Hidrolysis)
- Acetilation methods

Finally was presented a report concerning to this part of the training course.

Part two

Bioassays (in vitro)

Test 1: Inhibition of Angiotensin Converting Enzyme (ACE)

- Procedures for determining the protein concentration of the enzyme solution.
- Determination of the enzyme concentration required for the assay.
- Determination of the incubation time optimum.
- Determination of the inhibition of Angiotensin Converting enzyme by Captopril.

    Calculation of IC50

I have never tried this assay using a plant extract.

Test 2: Cytotoxicity against KB cells

- Methodology of cells culture: Medium used . Counting cells method.
- Assay of cytotoxicity against KB cells:

  Determination of inhibition percentage. ED50

  Material: a) Simaba multiflora

  b) Acanthospermum australe

Visits to other institutions

8 - Aug - 85

- Visit to Togamura (observation of growing plants in high places).

30 - Aug - 85

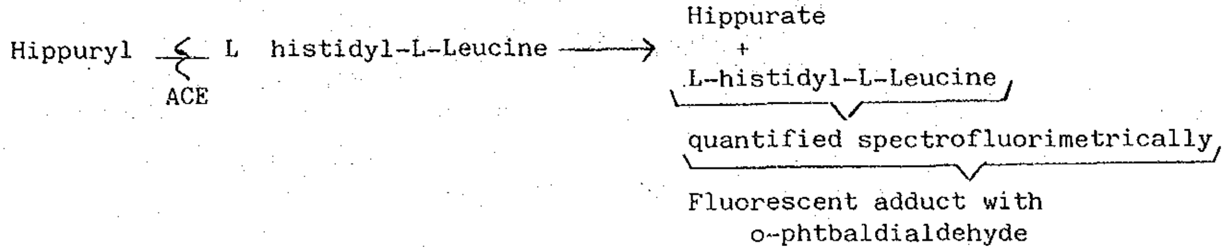
- Visit to Kokando Pharmaceutical Company.

9 - Sep - 85

- Symposium of Medical and Pharmaceutical Society (Wakun-Yaku) in Kyoto City.
- Visit to Kyoto Botanical Garden.

Angiotensin converting enzyme (ACE)

Assay is based on:

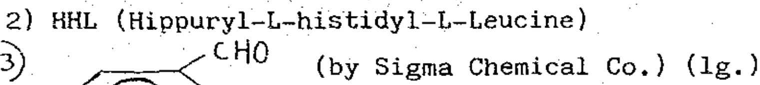


1) Angiotensin convertin enzyme

Rabbit lung acetone power

(by Sigma Chemical Co.) (lg.)

2) HHL (Hippuryl-L-histidyl-L-Leucine)



(by Sigma Chemical Co.) (lg.)

3) OPA (o-phtaldialdehyde) for biochemistry

(by Nakarai Chemical Co.)

4) 0,3 N NaOH (S.G.)

5) 3 N HCl (S.G.)

6) Buffer solution

Type A
1) KH_2PO_4 (S.G) 0,34 g/50 ml. H_2O
2) K_3PO_4 (S.G) 0,53 g/50 ml. H_2O

Instead of K_3PO_4, use $K_3PO_4 \cdot 3H_2O$: 0,665 g/50 ml. H_2O

Type B
1) KH_2PO_4 2,04 g, NaCl 3,50 g | 100 ml. H_2O

2) K_3PO_4 3,18 g
* ($K_3PO_4 \cdot 3H_2O$ 3,99 g)
NaCl 3,50 g / 100 ml. H_2O

<u>Type C</u> 1) KH_2PO_4 6,80 g / NaCl 8,77 g — 500 ml. H_2O

2) K_3PO_4 10,61 g

* ($K_3PO_4 \cdot 3\ H_2O$ 13,31 g)

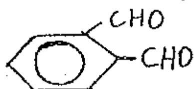
NaCl 8,77 g — 500 ml. H_2O

All type 1) + 2) ⟶ PH 83

Reagents

1) 3N HCl — C. HCl 128,75 ml/H_2O 500 ml.
   d= 1,18
2) 0,3 N NaOH — NaOH 6g/ H_2O 500 ml.

3) OPA 2,0 W/V %



20 mg / MeOH 1ml.
(on occasion)

4) Substrate (HHL) (Mw = 429,47)

Hippuryl-Histidyl-Leucine

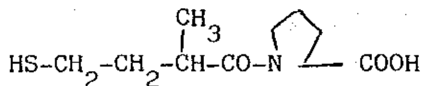
107,375 mg/ Buffer C type 50 ml.

* Dissolve in

5) Enzyme Powder 1 g/ Buffer A type 20 ml.

Homogenized and then ultracentrifugated

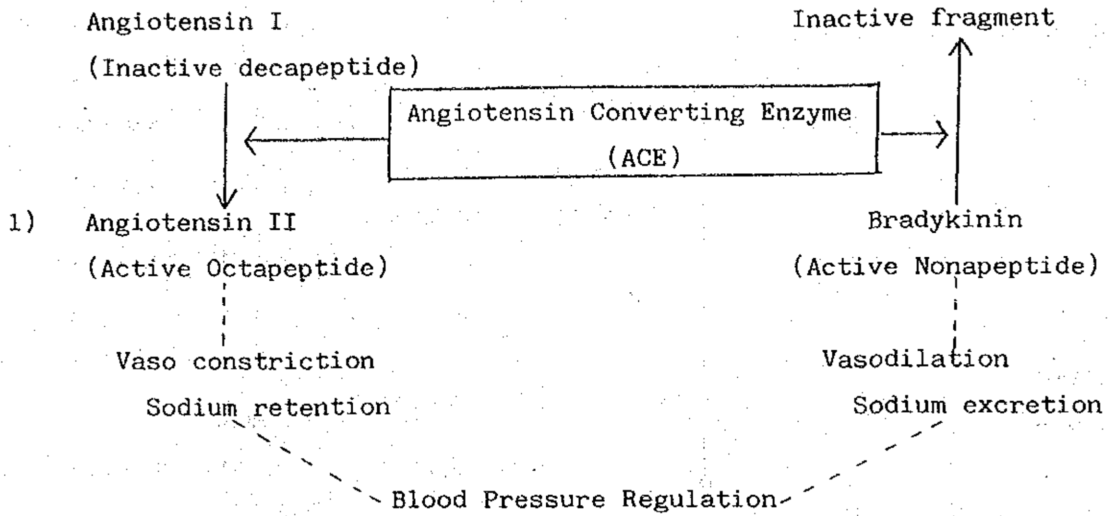
6) Captopril



MW 217

Reactions catalyzed by ACE

1)



2)

Protein assay

Standard Assay Procedure

Prepare several dilutions of protein standard (Standard: 1,32 mg/ml), and enzyme solution.

1. Place 0,1 ml of standards in test tubes and 0,1 ml of buffer type C in blank test tube.
2. Add. 5,0 ml diluted dye reagent (Ej: 20 ml + 80 ml H_2O)
3. Vortex
4. Incubation. After a period of 30 minutes ($37^{o}C$) measure OD 595.
5. Plot OD 595 vs concentration of standards. Read un Knowns from the standard curve.

Enzyme concentration assay

Enzyme concentration prepared: 1/5, 1/10, 1/15

Blank Buffer type B

Procedure:

	Enzyme		Blank	
Substrate (HHL)	0,1 ml		0,1 ml	
Enzyme	0,14 ml ↓	Incubation 37°C 30 min	---	(vortex)
NaOH 0,3 N	1,45 ml ↓		1,45 ml	(vortex)
? Enzyme	—		0,14	
2% OPA	0,1 ml ↓	10 min	0,1 ml (no wat. bath)	
HCl 3N	0,2 ml ↓ read		0,2 ml	

Plot Intens vs concentration of enzyme solution and choose the best concentration.

Incubation time

15, 30, 45, 60 minutes

Procedure:

	Enzyme		Blank
Substrate	0,1 ml		0,1 ml
Enzyme (1/15)	0,14 ml ↓	Incubation 37°C	---
NaOH 0,3 N	1,45 ml		1,45 ml
Enzyme	---		0,14 ml
2% OPA	o,1 ↓	10 min	0,1
HCl	0,2 ↓		0,2
	340 nm	455 nm	

Ploted Intens vs time and choosed the best incubation time

Inhibition of ACE by captopril

Captopril doses (with Buffer C) Ex:

$C_1 = 1,28.\ 10^{-7}$ mol/l

$C_2 = 6,4\ .\ 10^{-8}$ mol/l

$C_3 = 2,56.\ 10^{-8}$ mol/l

$C_4 = 1,28.\ 10^{-9}$ mol/l

Enzyme solution: 1/15

Procedure

	Enzyme (E)	blank (B)	Sample	Sample blank
Substrate	0,1	0,1	0,1	0,1
Sample (Captopril solution)	---	---	0,01	0,01
Buffer C	0,01	0,01	---	---
Enzyme	0,14	---	0,14	---
		↓ Incubation 30 min.		
NaOH 0,3 N	1,45	1,45	1,45	1,45
Enzyme	---	0,14	---	0,14
OPA 2%	0,1 ml	0,1 ml	0,1	0,1
		↓ 10 min.		
HCl 3 N	0,2	0,2	0,2	0,2

Calculation

$$\text{Inhibition (\%)} = \frac{E - S}{E} \times 100$$

E = with out inhibitor

S = sample with inhibitor (captopril)

IC 50 = $1,8.10^{-7}$ mol/l

Reference: A sensitive fluorimetric assay for Serum

Angiotensin. converting enzyme

Joan Friedland PH D et al

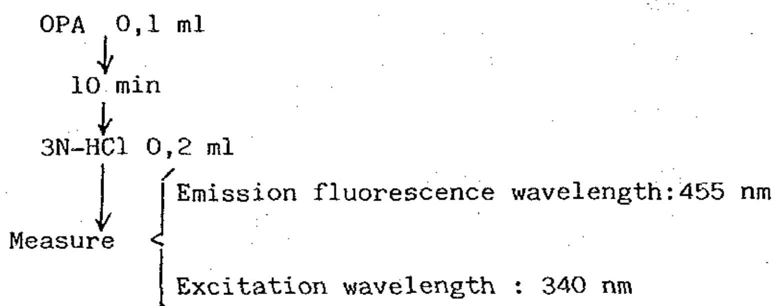
Am. J. Clin. Pathol, 66.416 (1976)

ACE - Assay method

Sample: 2,5 mg/1ml DMSO ⟶ 100 μg/ml

His-Leu Mw=252 # Assay method :

	A Control	B Control Blank	C Sample	D Sample blank
Substrate	0,1	0,1	0,1	0,1
Sample	---	---	0,01	0,01
DMSO	0,01	0,01	---	---
Enzyme	0,14	---	0,14	---
	37°C	30 min	incubation	
0,3 N NaOH	1,45	1,45	1,45	1,45
Enzyme	---	0,14	---	0,14



$$\text{Inhibition (\%)} = \frac{(A - B) - (C - D)}{A - B} \times 100$$

Note: If we get very high inhibition we can change the concentration of sample with another one; but if we get very small inhibition with this concentration, we musn't change the first concentration

Ref: Pharmacological Training at Department of Pharmacology, Faculty of Medicine, Toyama Medical and Parmaceutical University
(From May to December in 1986)

By: Dr. D. Ibarrola

A) Experiments of Biochemical Pharmacology

Hepatocytes from male rats of Wistar strain were isolated by separate perfusion with collagenase by the method of Nakanishi et al.. Kinetic studies on aldehyde dehydrogenase, alcohol dehydrogenase, glutamate dehydrogenase, cytochrome c oxidase, NADPH-cytochrome c reductase and glucose-6- phosphatase were carried out according to the method of Lineweaver and Burk, using mitochondrial, microsomal and cytosolic fractions of isolated hepatocytes.

B) Experiments of Physiological Pharmacology

I) Intact Preparation

Anaesthetized dogs were used. The femoral artery,femoral vein,and trachea were cannulated and ureter was catheterized. Mesurements of blood pressure,respiration activity,heart rate and urine volume,were carried out after administration of drugs.

II) Isolated Preparations

The frog heart (Yagi-Hurtung's method) and the intestine of rabbit and guinea-pig (Magunus's method) were used. In both methods the responses to the drugs were recorded mechanically. The drugs have been added to the organ bath.

Conclusion

I have considered that the training at the department of pharmacology is very efficient to success the purpose of the JAPANESE- PARAGUAYAN project. Because all these experiments mentioned above could be applied to the pharmacological studies of medicinal plants.

Concluding remarks

Since the chemical and pharmaceutical study on Paraguayan herbs was started in May 1985, Japanese technical experts of various fields visited the National Asuncion University, and conducted the collaboratory research jointly with Paraguayan personnel, overcoming the difficulties in language as well as in circumstances, and getting over the deficiency in research equipment and materials, as well as in water, electricity, etc. We are convinced of that the results during recent one and a half year are appeciable, since in botany the names of the original plants of herbs on the market were identified, in phytochemistry various activities were found by biological tests (experiment using enzyme) concerning the efficacy of herbs, and the fundamentals of pharmacology to be started for the first time in the university of locale were learned in the significant training for a year, and so forth.

In the remaining short period of the project, the studies in 3 divisions will be naturally proceeded, but a herbarium and a herbal garden for Paraguayan herbs must be in completed, and it is very important to bind the color photographs of Paraguayan herbs into a complete book so that it can be preserved with the results of studies.

We would like to express our sincere thanks to Japan International Cooperation Agency that kindly assisted us in this study by furnishing with equipment as well as materials, etc., and to the stuff members of the Faculty of Chemistry, National Asuncion University.

JICA