TECHNICAL ASSISTANCE

IDENTIFICATION OF THE MAJOR GROUPS OF ACTIVE INGREDIENT CONTAINED IN THE LEAVES AND STEMS OF RAMIE.

This work was made in the laboratories of the Research Department of INDOTEC, in response to a request for assistance made by **Mr. Victor Ivan Valdez.**

The contents of the study has been elaborated and submitted according to the technicians of the proposal presented by INDOTEC to Mr Ramirez Váldez.

Introduction

Ramie occupies a prominent place within the plants that produce vegetable fibers, due to good performance and quality of it fiber.

Originally from the Chinese mainland, it has been very successful in getting acclimated in the Dom. Rep even existing in companies engaged to the operation of this plant in an industrial level.

For several months in the Department of Research and Development of INDOTEC this plant has been studied, from a dietary point of view. This new research covers a photochemical and pharmacodynamic study which aims to identify the presence or not of any substances that possess pharmacological properties, which might exert some kind of biological activity on the body. This study will provide guidance on the medicinal use of this plant.

In general, the description of medicinal plants is reserved for those species of plants that contain chemical compounds known as active ingredients, which are used in therapeutic medicine.

The active ingredients extracted from plants are commonly used in modern medicine. Digitoxin, Papaverine, Reserpine, Ergotamine, Cocaine, Vincamine, Pilocarpine, Caffeine and many others are examples of the importance of some plants due to its tested pharmacological activity.

The study presents a philosophical study of the leaves and stems of Boehmeria Nivea (ramie) whose results will mark the pattern to follow in the use of this species.

The plant material was subjected to rigorous tests to determine it purity and quality. The selection of working materials was made according to established rules.

A preliminary study made to the plant was used as a guideline to design a subsequent chemical analytical work.

Prior to the observation of the extract by solvents and application of heat, the sample was subjected to the process of maceration using water at room temperature, water and temperature artificially generated and a hydro-alcoholic solution to 50%. On the decanted liquids chemical tests were conducted to identify active ingredients.

An investigation was made to the extract obtained by solvent extraction with different polarities solvents to determine the presence of groups of natural compounds (active ingredients) following the methodology according to the characteristics of each group of active ingredient.

The results indicate that the plant material collected has purity and quality that meets the standards set by the pharmacopoeias. The presence of the active ingredients was detected such as taninos sterols and triterpenes, unsaturated sterols and cardiac.

Finally from the data obtained in this work we would prepare the conclusions and recommendations.

DESCRIPTION OF WORK MATERIAL

Reception

The material was received in sample reception of INDOTEC. It arrived in a black polythene bag with holes on the sides, to allow a slight aeration of the product.

It was moved to the research labs of INDOTEC for subsequent classification, selection and storage testing.

Description

The plant material was formed by a number of 36 branches composed of leaves and stems of ramie with a full vegetative growth.

The selection and storage was made according to the specifications for this kind of vegetable required for the French pharmacopoeia (11).

Selection and classification

The material was removed manually from foreign substances, impurities or any other substance that may cause confusion in the subsequent analysis (II).

PURITY DETERMINATION OF PLANT MATERIAL

a) **The purity determination** is the assessment made on plant material that will be used for the preparation of a medicine. This material must meet specific conditions that are determined according to standardized rules in pharmacopoeias, using specific techniques implemented earlier.

The leaves and stems of ramie were evaluated according to specific rules provided by the pharmacopoeia (11), to existing norms (10) and the techniques that are specified for that case.

Also a quantitative determination of impurities was made to the impurities from the area of cultivation (torn product attacked by insects and rodents, sick leaves, etc.). Quantitative determinations of foreign bodies (fragments of plants, mineral substances, etc.). See Table IV of the annex.

b) Quality Determination

This included the following assessments

Water content. To set the water content we follow a line under the methods prescribed in the pharmacopoeia, used by Ciulei (11) and the method contained in the A.O.A.C, 1978 amended by us.

Total ash and insoluble ash

The determination of total ash and insoluble ash in hydrochloric acid 10% was conducted in accordance with the specifications in the French pharmacopoeia used by Ciulei (11) and the method used by Albornoz (1). Table IV.

IDENTIFICATION OF THE ACTIVE CONTENT IN THE LEAVES AND STEMS OF D.F.L RAMI

Preliminary study of the plant product (leaves and stems).

Histochemistry Exam

This test was performed with the purpose of identify active ingredients contained in analyzed plant tissue. The exam was conducted with sections of tissue and extracts, through implementation of specific reactions to each case. See table V of the annex.

Qualitative Chemical Exam

To establish the chemical composition of the sample, we have followed the general outline used by Ciulei (13) and Dominguez (4).

The extraction was done using the Soxhlet, working with solvents of increasing polarity, to achieve a complete exhaustion of plant material (petroleum ether → ethanol alcohol 80% → water). Fig. # 1

To identify the extracted compounds, the three extracts were analyzed separately, following the methodology in accordance with the physical and chemical characteristics of each group of active ingredient. See table in Annex VII.

IDENTIFICACION

The identification was made using:

a) Thin layer chromatography.
b) Color reactions produced by the action of a chromogen agent on the sample.

ANNEX - TABLE V

Photochemical tests conducted on a sample of ramie, received in the research labs of INDOTEC, sent by the Department of Technical Services.

Compounds Groups	Reagents Used	Heat		Presence
		Leaves	Trunk	
Volatile oils, fat, latex, resin.	Sudan III Solution	Red	Red	Sudan III Solution
Tannins	FeCl3 10 % Solution	Blue	Blue	FeCl3 10 % Solution
Anthraquinone	NH OH 5% and NaoH 5% Solutions	Red	Red	NH OH 5% and NaoH 5% Solutions
Flaconas	Sodium hydroxide 5% solution	Yellow	Yellow	Sodium hydroxide 5% solution
Mucilages	Hematoxylin	Red	Red	Hematoxylin
Lignin	Steimetz Reagent	Yellow		Steimetz Reagent

TABLE VI

Identification of active principles in leaves and stems of ramie, subjected to maceration process.

Chemical Groups	Water Maceration + Roon temperature		Water Maceration, afte wetting alcohol 80%		Maceration in alcohol 80%	
	Leaves	Trunks	Leaves	Leaves	Trunks	Tallos
Volatile oils	–	–	–	–	–	–
Fat	–	–	–	–	(±)	(±)
Alkaloids	–	–	(±)	–	(±)	–
Phenolic Compounds	–	–	(±)	–	(±)	–
2 dioxi sugars	–	–	–	–	–	–
	–	–				
Sterols	–	–	–	–	–	–
Flavonoids			–	–	–	–
Unsaturated Lactosas			–	–	–	–
Leucaanthocyanins					–	–
Heterixidos Antra-quinones	–	–	–	–	–	–
Saponins					(±)	(±)
Tannins			–	–	–	–
Triterpenes	–	–	–	–	–	–
Cardiac Glocer						

(-)=Indicates absence
(±)= Trace
+ = Slight presence
++= Presence
+++= Indicates abundant presence

TABLE VIII

Qualitative chemical analysis conducted in leaves and stems of the Bohemeria Nivea - Ramie.

Chemical Groups	Ether extract		Alcoholic extract		Water extract	
	Leaves	Trunks	Leaves	Leaves	Trunks	Leaves
Volatile oils	–	–			–	–
Fat	+	+	–	–		
Alkaloids	+	+	+	+	(+-)	(+-)
Phenolic Compounds					–	–
2 dioxi sugars			+	+		
Sterols			+	+		
Flavonoids	–	–	–	–		
Unsaturated Lactosas			+	(+-)		
Leucaanthocyanins			–	–		
Heterixidos Antra-quinones	–	–	–	–	–	–
Saponins			+	+	+	+
Tannins			+	+		
Triterpenes			+	(+-)		
Cardiac Glocer						

Indica:

(-)= Ausencia
(+-)= Trazas
+ = Ligera Presencia
++= Presencia
+++= Presencia abundancia

TECHNICAL ANALYSIS OF RAMIE'S SAMPLES

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Summary

We evaluated three (3) samples of Ramie, Boehmeria nivea (L) Grand, collected given ten days for twenty days to determine the optimum cut point, which was achieved on the assessment of the biochemical components of each sample.

The Ramie was established in an experimental plot, located in Santo Domingo, Dominican Republic, Owned by Ramirez and partners company.

Chemical-analytical determinations were made of crude protein (N. X 6.25), crude fiber, and fat (ether extract, ash, potassium and calcium). Carbohydrates (nitrogen-free extract) were determined by the difference between 100 and the amount of the protein, ether extract. In addition, we determined the percentage distribution.

The analysis of the results obtained in this investigation reveals that the sample for the second collection (17-11-86) represents the optimum cut point, because the leaves of this sample have high values of the constituents and high levels of the most essential amino acids.

INTRODUCTION

Ramie (Boehmeria nivea (L) Grand,) is a perennial textile plant, originally of java and Malaysian Islands. Has been extended to several countries in the world, including Dominican Republic, where it entered in 1882.

The stems of these plants are slender, 8.0 - 16 mm at the base, they can reach a height of 2.0 - 2.5 m, a size that is acquire in 45 or 60 days in suitable conditions for development. (13).

Has been shown to be an excellent forage for pigs when the plant is young and tender. These animals prefer it to the green corn and ripe bananas (16). For its industrial value, the ramie could be called the plant of a thousand uses.

This study's main purpose is to establish the groundwork to find the optimal cut point of Ramie, especially when this plant should be harvested with the intention to use it as forage.

El punto óptimo de corte, de acuerdo al objetivo planeado, corresponderá al período de desarrollo fisiológico de la planta, en el cual las determinaciones químicas de los nutrientes hechos a muestras recolectadas en ese tiempo exacto, arrojen, al compararlo con las otras muestras cosechadas a diferentes edades, los mejores resultados, es decir la mejor calidad nutritiva.

The optimal cut point, according to the planned target, corresponds to the period of physiological development of the plant in which chemical determinations of the nutrients made to collected samples at that exact time, yield, when compared with other samples harvested in different ages, the best results, that is the best nutritional quality.

Although the Ramie could be considered, as was indicated earlier, the plant of a thousand uses, has found little or no information of the nutritional quality of ramie, how their biochemical constituents vary with age and when is the most appropriate time for harvest, when its final use is as forage.

1. MATERIALS AND METHODS

On November 6, 17 and 27 of 1986, dates set by Ramirez Valdez and Associates, C. by A., Ramie (Boehmeria nivea)samples were collected from an experimental farm, located on the street Abelardo Rodriguez Urdaneta not. 55 Santo Domingo, DR, In order to determine the optimal cut point, through the determination of biochemical constituents in the samples.

The samples were harvested by hand (scissors) placed in polythene bags and transported to the laboratories of Research and Development Department of INDOTEC. Immediately the average weight and distribution (the percentage of usable and waste portion) was determined.

The usable portion (stem and leaf) was separated into two batches, the first batch was dried at 50 ° C for 24 hours, crushed in a Tecatoc's plastic mill mark , sifted in a sieve mesh 60, packaged in a plastic jars and stored in dried, until the time of analysis. In the second batch, the same day of harvest, samples were taken from separate stems and leaves to determine caroteo, vitamin C and humidity.

In the lot in dry state (leaves and stems) the following constituents were evaluated: fat, fiber, proteins (N X 6.25%) ash, calcium, potassium and amino acids.

The evaluation of moisture, fat, fiber, protein and ash, used methods of AOAC, 1980 (1) in the quantification of vitamin C was used the method of Madden, Paul (7), for the Carotene was used the Worker technique, N.A (19), and for calcium and potassium was used atomic absorption technique. In the quantification of essential and non-essential amino acids, quantitative assessment was made by gas chromatography (6) tryptophan was determined by the method of calorimeter Spiers and Chambers (17).

All the chemical tests conducted in this study were tested doubled in weight.

Vitamin C (ascorbic acid) and carotene (provitamin A), which are exposed in the tables of results were calculated on the basis of moisture, while the other constituents were calculated on the basis of hundred grams of dry samples. Carbohydrates also called nitrogen-free extract, were obtained by the following formula: carbohydrates% = 100 - (Σ % ash + fat, crude fiber + crude protein).

2. RESULTS AND DISUCION

The results of the physical and chemical made in three (3) Ramie samples are listed in Annex 1- 4.

The percentages of stems and leaves (Annex 1) for the first (6-11-86) and second (17-1-86) collection did not show significant variation, while the 2nd to 3rd showed that the percentage of leaves was increased by 17%. The portion of the waste in the first and second sample was not detectable, in the third was 0.5%.

According to standards set by pharmacopoeias the percentage of impurities from a plant material suitable for use in optimal conditions should not exceed 2%.

The chemical composition of the stem and leaves are outlined in Annex 2. In all the samples under study, the stem presents high levels of crude fiber and carbohydrates. Crude protein, carbohydrates and fats in the stem decreased gradually while the fibers increased in a period of 20 days (6-11 to 27-11). It is emphasized that the fiber content in the stem roughly doubled in 11 days (6-11 to 17-11). In pastures and forage lignifications happened in the cell walls of the stems, and thus the amount of fiber they contain increases, as it progresses in its vegetative growth and are approaching maturity (8)

Variations in the content of fiber in the leaves present a different profile than shown in the stem. It is appreciated that the maximum fiber content (13%) in the leaves was in the first sampling (6-11-86) and lower (9%) for the last sampling (27-11-86).

For the content of crude protein in the leaves shows that there is no significant variation between the first and second harvest. However, this component (%) decreases in the sample of (27-11-68). The decline in the protein content with age has been noticed by other researchers (Annex 5). It is important to note that the chemical composition of forage and pasture, and so on is a function of the chemical composition of soil and the climatic conditions where the plant growth (Annex 7).

In the leaves, soluble carbohydrates (sugars and starches) also experienced a decline with the age of Ramie, period (20 days, 6-11-86, 27-11-86), between the first and last harvest in which the size of the plant nearly tripled.

On the other hand, the total mineral (ash) in the leaves, contrary to what happened in the stem, experienced increases. Note that the total ash (leaves + stems) of Ramie increased during the time of harvest.
Different results have been found in studies of the defect of age on the chemical composition of forage (Annex 7). Note that the time interval between collections of samples presented in Annex 7 is greater than that chosen by us. We notice that the potassium content in the leaves did not show significant changes. In the stem this material was reduced (1.7 units) in a given period of time elapsed between the first and second harvest. Calcium, in both the stem and leaves only shows significant changes (%) from the second sample.

The values of the content of calcium obtained in this investigation could be considered high when compared with those presented in Annex 5 in which the goal is the age of some grass on the protein and mineral composition; it also presents values that are considered suitable for this element, as well as other constituents.

For the ascorbic acid and pro-vitamin A in the leaves, first increase and then decrease from the second sample. The carotene in the stem exhibits a similar behavior, to the point that in the third sample was not detectable. In contrast, the vitamin C in the stem decreased gradually during the time of harvest.

Regarding the essential and non-essential amino acids present in protein, are shown in Annexes 3 and 4, the results obtained in the aminograms made to Ramie. In Annex 3 can be seen the levels recommended by FAO in comparison with those of eggs and Ramie. The aminograms protein of Ramie shows that the sample of the second harvest (17-11-86) offers the best protein quality with high tenor of much of the essential amino acids. This highlights the apparent difference in the Ramie of metonymy and isoleucine.

The literature reports that the quality of a protein depends on one hand of the number of different essential amino acids entering its composition. Finally, the quality of each protein. (11).

Similarly literature reports, that there is an ideal structure of amino acids that are considered most suitable for nutrition in general. In some foods such as milk and egg protein has a structure similar to this ideal one and these are the most valuable food for nutrition, especially in the growth stage.

3. CONCLUSIONS AND RECOMMENDATIONS

In relation to the experimental data previously detailed and summarizing the information, we concluded:

-In a period of 10 days from November 6 to November 17 of 1986, in the leave of Ramie the nutrient content, crude protein, amino acids, ascorbic acid, carotene, ash, increased, while in the same period, from November 17 to November 27, 1986 except for the ash, the amount of those nutrients decreased. The ideal time to harvest a product is the optimal physiological development, where typically all the necessary chemical and physical factors are present.

- From the three harvested samples and chemically analyzed the sample leave collected in 17-11-89 had the best nutritional quality, with high tenor of the most essential amino acids and higher content of crude protein, vitamin C and carotenes.

- The optimal cut point for Ramie studied under the weather and ecological conditions existing at the time of harvest is the age that the plant had when the second sampling was taking place, Nov. 17, 1986.

It is estimated convenient to make further tests for determining the optimum cut point in Ramie using samples of different ages, harvested at different times of the year and essentially, cultivated, even on an experimental basis, in the land where the commercial planting would be installed, because of the effects of age, climate and ecological conditions on the nutritious composition of the forage.

In conducting the new tests is recommended to evaluate the next composition, chemical-analytical determination of crude fiber, crude protein, ash, fat, carbohydrates and dry matter, because first, the quantification of amino acids is costly and time-consuming, second, with the proximate analysis obtained information would shorten the number of samples that must be subject to amino acids quantitative assessment.

Annex 1

Percentage distribution in the samples of Ramie

Sampling Date	Average Weight in Grams	Stem Weight (Gr.)%	Leave	Waste
November 6, 1986	1150	60.86	39.14	-
November 17, 1986	1450	62.06	37.93	-
November 27, 1986	3753	44.64	54.88	0.5

Annex 2

Nutritional Characteristics in Samples of Ramie

Components	Sampling Date, Year 1986					
	November, 6		November, 7		November, 27	
	Leaves	Trunk	Leaves	Trunk	Leaves	Trunk
Humidity %	86.47	96.36	87.37	92.97	86.50	92%
Protein%	25.42	14.60	26.35	10.50	22.80	7.90%
Ash%	18.50	15.17	22.37	12.13	27.31	9.23%
Vitamin C.mg/100g %	8.83	11.04	14.0	8.36	7.20	8.50
Fat(Ether extract) %	4.40	2.36	3.70	2.30	4.00	1.44
Crude fiber%	12.91	16.02	9.64	30.20	9.1	39.53
Carbohydrates%	38.8	51.85	37.9	44.87	36.79	41.90
Potassium%	1.53	3.92	1.12	2.26	1.36	2.54
Calcium%	5.31	3.42	5.92	3.07	8.51	1.78
Carotenes%	2.56	4.25	8.66	5.51	2.15	

Not Disposable

All results except vitamin C and Carotene are expressed in dry basis.

Annex 3

Amino acid content in samples (leaves) of Ramie gr/100 gr. Protein

	Sampling Dates				
Amino acids	6-11-86	17-11-86	27-11-86	FAO pattern	Egg
Lysine	3.5	6.1	2.6	4.2	6.5
Valine	2.6	4.6	3.9	4.2	7.3
Leucine	4.3	6.2	6.05	4.8	8.9
Isoleucine	0.79	2.1	1.5	4.2	6.7
Threonine	1.07	2.8	2.2	2.8	5.1
Methionine	0.19	0.54	0.49	2.2	3.2
Phenylalanine	2.8	3.2	1.51	2.8	6.8
Tryptophan	0.98	1.5	1.95	1.4	1.6
Tyrosine	-	-	-	2.8	4.6
Alanita	2.6	4.4	6.05	-	-
Aspartic acid	2.2	4.80	3.02	-	-
Glutamic Ac	3.9	6.6	6.7	-	-
Proline	3.1	5.5	4.7	-	-
(Reference)				13	13

Annex 4

Aminograms from samples of Ramie in gr/100 dry samples (leaves)

	Sampling Dates		
Aminoacid	6-11-86	17-11-86	27-11-86
Lysine	0.90	1.6	1.38
Valine	0.66	1.2	0.88
Leucine	1.1	1.6	1.4
Isoleucine	0.20	0.56	0.4
Threonine	0.27	0.73	0.49
Methionine	0.05	0.14	0.11
Phenylalanine	0.72	0.85	0.34
Tryptophan	0.23	0.41	0.35
Alanita	0.66	1.2	1.4
Glisina	0.57	1.2	0.88
Proline	0.79	1.5	1.1
Serina	0.52	0.66	0.44
Aspartic acid	0.56	1.3	0.69
Glutamic Ac	1.0	1.8	1.5

Annex 5

Effect of Age on the composition of some herbs

Species	Age(days)	Crude Protein(%)	Phosphorus(%)	Calcium(%)
Elephant	40	9.9	0.24	0.35
	60	7.9	0.18	0.28
	90	5.4	0.13	0.23
Guinea	40	9.0	0.27	0.88
	60	7.0	0.27	0.78
	90	5.6	0.16	0.64
Fish	40	9.2	0.25	0.39
	60	7.2	0.21	0.35
	90	4.8	0.15	0.29
Pangola	30	12.5	0.22	0.42
	45	9.6	0.22	0.36
	60	8.0	0.17	0.34
Star	30	10.6	-	-
	60	7.8	-	-
	70	7.5		

Suitable Levels

P : Greater than 0.17 %
Ca : Greater than 0.20 %
CP : Greater than 7.04 %

P = Phosphorus
Ca = Calcium
CP = Crude Protein

Source: Reference (2).

Annex 6

Ash content (percentage) of 10 Tropical Herbs Forage

Species	harvest interval	ASH %
Afican crad	days	
Venezuela elephant	30	10.8
Pangola	60	8.4
Signal	90	6.6
Buffel	120	6.9
Jaragua	150	6.9
Limpo	180	6.1
Congo	Mean	7.7
Mean		
Giant Pangola		

Source : Reference (4).

Annex 7

Variations in composition within species of forages. All values on dry basis

	Crude Protein%	Crude fiber%
Guinea:		
Season of Rain	13.3	34.1
During Drought	8.1	35.9
Tnder, lots of leaves	13.1	25.8
Solid, Stem	4.5	39.7
Napier of 6 weeks	9.7	29.2
of 8 weeks	7.4	34.3
of 10 weeks	6.8	33.6
of 12 weeks	4.4	38.4
of 14 weeks	4.4	36.6
of 15 weeks	4.5	40.0
Ladino clover:		
Tender, dehydrated	26.1	14.7
Principle dried flowers shade	22.5	19.5
Little dried flower	23.4	16.4
Tender, sun dried	19.6	23.0
Blue Grass (Poa Pratensis)		
Hay, tender cutting	16.9	26.9
Hay, Half Cutting bloom	10.6	31.7
Hay, Full cutting bloom	8.9	32.5

Source: Reference (3).