Some Tropical Leaves as Feasible Sources of Dietary Protein[1]

Franklin W. Martin, Lehel Telek, and Ruth Ruberté[2]

ABSTRACT

Thirty-four species of tropical plants found in Puerto Rico were considered for possible use as high protein green leaf vegetables and as sources of leaf protein concentrate. They were tested for protein and carotenoid contents, and for toxins, including alkaloids, hydrocyanic glucosides, and oxalic acid. Six species (*Manihot esculenta, Sauropus androgynus, Cnidoscolus chayamansa, Canavalia ensiformis, Lablab niger,* and *Vigna unguiculata*) that combine high protein yields with suitable agronomic characteristics were selected for further trials and were described. Green leaves are concluded to be crops of high potential for Puerto Rico and the protein-hungry tropics.

INTRODUCTION

The worldwide effort to improve diets through protein-rich crops need not be dominated by a single species. Cereals and legumes may well be the most important sources of food, but other plants contribute some protein, and all are desirable in a balanced and appetizing diet. Perhaps the most neglected class of foodstuffs, yet one with much promise as a source of protein, is edible leaves (10). Probably no other single class is so widespread, and few foods are so easy to prepare.

As traditional foods comparable to spinach, green leaves are already widely used throughout the tropics, especially in West Africa and Southeast Asia. The leaves of many tropical plants grown for other purposes are also edible, and can be considered byproducts of other crops or ornamentals. Martin and Ruberté (5) have compiled an extensive list of species and have described the most important.

In general, large quantities of green leaves are not welcome in the diet. Although rich in vitamins, minerals, and protein, leaves are usually poor sources of carbohydrates and fats. In addition, they may contribute large quantities of fiber, which may or may not be needed, depending on other components of the diet. The green color of leaves is not usually appreciated in foods. For this reason, leaves are usually used alone as a side dish to the principal dish, and not mixed with other foods.

Protein of leaves can be used after it has been extracted (6) and concentrated. In the temperate zone, after considerable tests among

[1] Manuscript submitted to Editorial Board December 15, 1975.

[2] Plant Geneticist, Chemist, and Research Technician, Mayagüez Institute of Tropical Agriculture, Agricultural Research Service, Southern Region, USDA, Mayagüez, P.R.

possible sources, alfalfa (*Medicago sativa* L.) has been selected as the most promising species, and leaf protein concentrate (LPC) is a commercial reality in several forms (3). Alfalfa is seldom successful in the tropics.

The ideal plant for LPC should be a vigorous species, perennial if possible, that can be cut repeatedly within a short time. Some trees could qualify. Furthermore, the plant should be easily handled as a farm crop, and be relatively free of toxic materials, especially after LPC production. The initial concentration of protein in the leaf should be high, and it should be possible to extract a large part of the protein with present technology.

Requirements for vegetable and forage plants are less exact. The most important requisites are that the edible portions can be produced easily, in sufficient quantity, and free from toxic substances.

In studies of the food values of tropical green leaves, or in attempts to find LPC sources in the tropics, many species have already been tested for protein content. Some of the highest concentrations reported in the literature are given in table 1. In some cases total N content was used to estimate protein by multiplying by a factor of 6, and in other cases, by 6.25. No attempt is made here to adjust such differences. Only species with 25 percent protein or more on a dry weight basis are included.

A review of edible leaves of the tropics (5) prompted this study, for the authors became aware of the high protein sources already discovered in the tropics. High protein leaf sources for use either as vegetables or for LPC in Puerto Rico or other parts of the tropics were screened through a study of the literature and the authors' tests. Species selected fulfilled at least some of the requirements of an ideal species.

MATERIALS AND METHODS

The species used for these tests are given in table 2 with notes on plant habit and judgments on suitability as vegetables, forages, or LPC. Leaves of all species were harvested at the appropriate stages for use as fresh vegetables, and small quantities were eaten in most cases. The leaves were dehydrated in a forced draft oven at 56° C overnight and stored in tight plastic bags for a few weeks until analyzed. Samples were milled and passed through a 40 mesh screen. Those samples to be used for carotene determinations were stored in a nitrogen atmosphere until used.

N was measured by the AOAC technique (4). Protein was estimated by multiplying the N content measured by 6, a factor used in England (7) instead of the customary 6.25.

The *beta*-carotene of the leaves was extracted from 0.3 g samples of milled leaves with 8 ml petroleum ether. The mixture was agitated vigorously, allowed to stand 10 minutes, and then reagitated and filtered; this was repeated three times. The three extracts were combined and 2 ml 12% KOH in methanol was added to saponify the esters. These preparations were stored 20–24 hours in the dark. Water and petroleum ether were then added, and the mixture was shaken and allowed to separate. The aqueous phase was discarded and the ether phase was rewashed with water. After separation, an equal volume of 95% methanol was added to the ether phase, shaken well, and allowed to separate. The petroleum ether phase containing chiefly *beta*-carotene was ad-

TABLE 1.—*Previous reports of high protein content in leaves of tropical plants (1, 2, 8)*

Species	Common name	Percent protein	Reference
Amaranthus gangeticus	Amaranth	57.8	2
Amaranthus gangeticus	Amaranth	27.4	8
Amaranthus spinosus	Amaranth	30.6	8
Aspilia latifolia	Bush marigold	26.6	1
Atriplex rosea	Rose atriplex	57.3	2
Canavalia ensiformis	Horse bean	27.3	1
Carica papaya	Papaya	33.5	8
Cassia sophora	Sophora	25.2	1
Centrosema pubescens	Butterfly pea	27.2	1
Champeneia griffithii	Chemperai	25.0	8
Claoxylon longifolium	Salang	42.8	8
Clitoria ternatea	Butterfly pea	30.1	1
Colocasia esculenta	Taro	28.8	1
Coriandrum sativum	Coriander	60.8	2
Crotalaria juncea	Sun hemp	31.5	1
Desmodium uncinatum	Spanish clover	27.6	1
Dolichos lablab	Hyacinth bean	29.0	1
Glycine javanica	Forage soy	28.5	1
Gnetum gnemon	Belinjo	32.2	8
Hibiscus esculentus	Okra	27.6	1
Ipomoea batatas	Sweet potato	26.7	1
Leucaena glauca	Wild tamarind	31.5	1
Manihot esculenta	Cassava	26.8	1
Manihot esculenta	Cassava	41.7	8
Melia excelsa	Setang	27.4	8
Moringa oleifera	Horseradish tree	37.9	8
Phaseolus lathyroides	Phasemy bean	25.4	1
Physalis angulata	Ground cherry	32.0	1
Physalis peruvianum	Peruvian cherry	31.2	1
Pterococcus corniculatus	Bloodwood	46.0	8
Sauropus androgynus	Asin-asin	46.0	8
Sesbania grandiflora	Sesban	25.3	2
Sesbania grandiflora	Sesban	36.8	8
Stizolobium niveum	Velvet bean	26.8	1
Trigonella foenumgraceum	Fenugreek	32.6	2
Vigna sinensis	Cowpea	28.0	1

Table 2.—*Species in Puerto Rico observed as possible high protein leafy vegetables or LPC sources*

Scientific name	Common name	Annual or perennial	Growth habit	Judgment of suitability as:		
				Vegetable	Forage	Protein source
Adansonia digitata	Baobab	Perennial	Tree	Yes	No	No
Antidesma bunius	Bignay	Perennial	Tree	Yes	No	No
Basella rubra	Ceylon spinach	Perennial	Vine	Yes	No	No
Bauhinia purpurea	Poor man's orchid	Perennial	Tree	Yes	No	No
Cajanus cajan	Pigeon pea	Perennial	Shrub	Yes	Yes	Yes
Canavalia ensiformis	Horse bean	Annual	Vigorous vine	Yes	No	Yes
Canavalia gladiata	Sword bean	Annual	Vigorous vine	Yes	No	Yes
Carica papaya	Papaya	Perennial	Herbaceous tree	Yes	No	No
Cnidoscolus chayamansa	Chaya	Perennial	Tree	Yes	No	Yes
Coffea arabica	Coffee	Perennial	Small tree	Yes	No	No
Erythrina berteroana	Dwarf coral tree	Perennial	Tree	Yes	No	No
Erythrina variegata	Coral tree	Perennial	Tree	No	No	Yes
Ficus elastica	Indian rubber	Perennial	Tree	Yes	No	Yes
Hibiscus cannabinus	Kenaf	Annual	Semi-woody herb	Yes	No	Yes
Hibiscus sinensis	Hibiscus	Perennial	Shrub	Yes	Yes	Yes
Ipomoea batatas	Sweet potato	Perennial	Herb	Yes	Yes	Yes
Ipomoea pes-caprae	Beach morning glory	Perennial	Herb	Yes	Yes	Yes
Lablab niger	Hyacinth bean	Perennial	Vine	Yes	Yes	No
Mangifera indica	Mango	Perennial	Tree	Yes	No	Yes
Manihot esculenta	Cassava	Perennial	Shrub	Yes	No	Yes
Momordica charantia	Bitter gourd	Annual	Vine	Yes	No	No
Morinda citrifolia	Indian mulberry	Perennial	Tree	Yes	No	Yes
Moringa oleifera	Horseradish tree	Perennial	Small tree	Yes	No	Yes
Morus alba	Mulberry	Perennial	Tree	Yes	No	No
Musa paradisiaca	Banana	Perennial	Herbaceous tree	Yes	No	No
Nothopanax scutellarium	Cup panax	Perennial	Shrub	Yes	No	Yes
Peperomia pellucida	Pepper tea	Annual	Herb	Yes	No	No
Portulaca oleracea	Portulaca	Annual	Herb	Yes	Yes	Yes
Sauropus androgynus	Sauropus	Perennial	Shrub	Yes	No	Yes
Sechium edule	Chayote	Perennial	Vine	Yes	No	No
Tamarindus indica	Tamarind	Perennial	Herb	Yes	No	Yes
Urena lobata	Cadillo	Annual	Herb	Yes	No	Yes
Vigna unguiculata	Cowpea	Annual	Vine	Yes	Yes	Yes
Xanthosoma brasiliense	Tahitian taro	Perennial	Herb	Yes	No	Yes

justed to 25 ml. The absorption maxima were observed and measured at the principal peak (449 nm) on a Beckman[3] recording spectrophotometer. The concentration of *beta*-carotene in solution was estimated from a curve derived from standard *beta*-carotene samples. From these data the carotene content was estimated as international units/g dried leaf.

Presence of HCN was detected by the Guinard reaction. Filter paper was soaked in 1% picric acid and dried, then dipped in 10% sodium carbonate and dried again. A sample of 0.25 g dried leaf was finely ground with about .25 ml water and placed in a test tube. A few drops of chloroform were added. A slip of the treated paper was placed in the tube above the liquid, and the tube was stoppered. After 1 hour changes from yellow to dark-brick red were noted and judged as negative or from slight to very strong.

Oxalic acid contents were determined by the method of Roughan and Black (9). Samples of 0.29 g dried milled leaves were extracted overnight with 5 ml of a solution of 100 ml anhydrous chloroform, 50 ml methanol, and 7.5 ml sulphuric acid. Then 5 ml water were added, the mixture was shaken mechanically 5 min and then allowed to separate for 2 hours. Fractions of 5 μl were spotted on silica gel thin layer plates containing hydroxylamine hydrochloride and ferric chloride containing various quantities of oxalic acid in the same sized spots after the plates were heated to 50° C for about 30 minutes. The amount of oxalic acid in samples was estimated by comparing the intensities of the test spots with those of the standards.

Presence or absence of alkaloids was determined by wetting 1 g sample of dried, milled leaves with 5 drops concentrated NH_4OH, and extracting in Soxhlet apparatus for 3 h with peroxide-free ethyl ether. The ether extract was re-extracted with 3, 2, and 1 ml of 10% H_2SO_4. One ml of H_2SO_4 extract was tested with Meyer reagent, and 1 ml of the same extract was tested with silicotungstic acid reagent. In the presence of alkaloid, white precipitates are formed with both reagents. The reaction was measured as negative, indefinite, or positive.

RESULTS

The results of tests for protein content (table 3) confirmed in general the data reported in the literature, and revealed some new species as especially rich sources of protein. Among the better species from the family Euphorbiaceae were *Cnidoscolus chayamansa, Manihot esculenta,* and *Sauropus androgynus* (table 3). In the family Leguminosae

[3] Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other equipment or materials not mentioned.

TABLE 3.—*Protein, carotene, oxalic acid, HCN, and other constituents of young dried leaves*

Species	Protein content	Carotene	Oxalic acid content	HCN reaction	Alkaloids
	%	*IU/100 g*			
Adansonia digitata	10.0	5200	None	Negative	None
Antidesma bunius	9.8				
Basella rubra	No sample	4300	Trace	Negative	None
Bauhinia purpurea	14.3	2300	None	Negative	None
Cajanus cajan	19.1	15000	None	Negative	None
Canavalia ensiformis	24.4	25000	Trace	Negative	None
Canavalia gladiata	22.1	9500	Trace	Negative	None
Cnidoscolus chayamansa	26.3	4200	None	Strong	None
Coffea arabica	18.1	1800		Negative	None
Erythrina berteroana	19.2	1800	Medium	Negative	Trace
Erythrina variegata	16.0	4200	Medium	Negative	Trace
Ficus elastica	4.8	1800	None	Slight	None
Hibiscus cannabinus	15.6	7800	Trace	Negative	None
Hibiscus sinensis	16.8	9500	Trace	Negative	None
Ipomoea batatas	20.1	2500	None	Negative	None
Ipomoea pes-caprae	13.6	6700		Negative	None
Lablab niger	27.1	5000	Trace	Negative	None
Mangifera indica	6.4	2500	None	Negative	None
Manihot esculenta	25.5	9500	Trace	Strong	None
Momordica charantia	19.5	5400	None	Slight	None
Morinda citrifolia	11.7	2800	Trace	Negative	None
Moringa oleifera	19.4	4400	Trace	Negative	None
Morus alba	22.1	14000	None	Negative	None
Musa paradisiaca	16.4	200	Medium	Slight	
Nothopanax scutellarium	25.1	8800	Trace	Negative	None
Peperomia pellucida	20.3	2500	Trace	Negative	None
Portulaca oleracea	26.6	20000	High	Negative	Uncertain
Sauropus androgynus	25.3	12000	None	Negative	None
Sechium edule	23.3	2000	Trace	Negative	None
Tamarindus indica	12.5	2700	Trace	Negative	None
Urena lobata	10.3	3500	Trace	Negative	None
Vigna unguiculata	29.8	9200	Trace	Negative	None
Xanthosoma brasiliense	21.3	5400	Medium	Negative	None

the richest sources were *Canavalia ensiformis*, *Lablab niger*, and *Vigna unguiculata*. A few unexpectedly high sources of protein were *Nothopanax scutellarium* (family Araliaceae), and *Portulaca oleracea* (Portulacaceae). All high protein species merit further examination.

Carotene content was high to very high in almost all species tested, as compared to other sources of this nutrient. Outstanding sources were *Cajanus cajan*, *Canavalia ensiformis*, *C. gladiata*, *Morus alba*, *Portulaca oleracea*, *Sauropus androgynus*, and *Vigna unguiculata*. Some of the best sources of protein were also best sources of carotene.

With respect to toxic compounds, results found scattered throughout the literature were confirmed for some species, but in others new toxicities were found. Hydrocyanic acid was found in appreciable quantity only in *Manihot esculenta* and the rather similar species *Cnidoscolus chayamansa*. Weak reactions in several other species suggest insignificant amounts of HCN (table 2). Alkaloids were found in the two *Erythrina* species, and perhaps in small quantity in *Portulaca*.

Oxalic acid was present at least in trace amounts in most of the leaves tested. *Portulaca* contained large amounts, and thus cannot be recommended as a leafy vegetable. The two *Erythrina* species, the tender leaves of banana, and leaves of *Xanthosoma brasiliense* contained moderate amounts.

Six species were selected that yield high percentages of protein and that appear to have the ability to produce large amounts of foliage in repeated cuttings. These species are adapted to Puerto Rico and should be good sources of leaf protein. The promising species are described below.

Manihot esculenta, or cassava, is a perennial, vigorous, much branched, large but soft-wooded shrub. Grown throughout the tropics for its principal product, edible starchy roots, it is also used as a source of leaves for use as greens. Its advantages are ready propagation from stem cuttings, rapid growth, high yield, and drought resistance. Unfortunately all parts of the plant contain hydrocyanic glucosides, destroyed by careful cooking.

Sauropus androgynus is a perennial, tall, spindly shrub that leans for support on other plants or climbs through the branches of small trees. Known as a source of edible leaves in India and Southeast Asia, *Sauropus* is frequently planted as a hedge. Its advantages are propagation from stem cuttings, which should not be dried out, vigor once established, and high yield. In trials of leaf production in Sarawak, *Sauropus* has outyielded other species to which it has been compared.

Cnidoscolus chayamansa, or chaya, is a perennial, large, vigorously growing, soft-wood shrub. Known as a source of edible leaves in Mexico and Central America, the leaves and stems have few to many stinging hairs that are eliminated by cooking. Its advantages are: Propagation from cuttings, that are easily rooted if taken from the soft wood, vigor, high yields of leaf, rapid recuperation after harvesting, disease resistance, and drought resistance of the cutting. Unfortunately the foliage contains hydrocyanic glucosides, which are destroyed by cooking.

Canavalia ensiformis, or horse bean, is an extremely vigorous bushy or somewhat twining annual herb which yields very large pods with large seeds. Young pods and leaves are sometimes eaten, but are somewhat toxic and should be treated cautiously. Although a species native to the New World, the horse bean has been extended throughout the tropics, where it is often used as a green bean. The advantage of horse bean as a leaf source is rapid and vigorous growth, but its annual nature and poisonous character are disadvantageous.

Lablab niger or *Dolichos lablab*, or hyacinth bean, is a highly variable species often grown throughout the tropics as a food source. Both bush and vine forms are common. Some of the latter are vigorous perennials that provide edible beans, as well as fodder. The hyacinth bean makes a vigorous cover that can overwhelm weeds. As a native of the Old World, it finds its greatest use in India. Appropriate varieties offer the advantages of vigor, high yields of leaf, and perennial nature.

Vigna unguiculata, or cowpea, is a variable species with both bush and climbing forms that is normally grown as an annual. Some varieties are remarkably vigorous and produce tremendous quantities of foliage that smother weeds. As a source of edible seeds, cowpeas are ancient cultivars in Africa and Asia, but are now distributed throughout the tropics. Some varieties might be suitable for once-over harvesting of foliage for LPC. The leaves are already widely consumed as a very nutritious leafy vegetable.

The following high protein leaf sources are considered to be of only secondary value for the reasons that follow: *Nothopanax scutellarium* is a slow-growing species. *Portulaca oleracea* is suitable only as a garden vegetable and contains excessive amounts of oxalic acid.

A few other species tested have been reported as poisonous. The mango produces an allergic reaction in some people. The bitter gourd leaves, even though used as a vegetable, contain a substance that reduces levels of sugar in the blood. Undoubtedly a few others could be found to be poisonous under special circumstances. The frequent occurrence of toxic materials in leaves illustrates the need for complete knowledge and thorough testing before new leaves or their products are used in the diet.

DISCUSSION

The results of this trial of easily obtained species now available in Puerto Rico suggest that suitable high protein sources can be easily found for extraction of leaf protein or for use as high protein vegetables. It appears appropriate now to proceed with production trials and to perfect extraction techniques for the major part of the protein. Efforts should be concentrated at first on *Manihot esculenta* and on *Cnidoscolus chayamansa*, believed to be the most promising species. Processing of leaves that can be grown in the tropics could open a new industry and supply some of the protein commonly lacking in tropical diets.

RESUMEN

Se estudió la posibilidad de usar como fuentes de proteína 34 especies de plantas tropicales ricas en proteínas que se encuentran en Puerto Rico. También se determinaron sus contenidos de carotenoides y la presencia de venenos como glucósidos de ácido prúsico, alcaloides y ácido oxálico. Seis de estas especies que combinan riqueza proteica y características agronómicas deseables se seleccionaron y se describen. Éstas se estudiaron más extensamente y se consideran de elevado potencial proteico no solo para Puerto Rico sino para el mundo tropical.

LITERATURE CITED

1. Byers, M., Extraction of protein from the leaves of some plants growing in Ghana, J. Sci. Food Agr. 12: 20-9, 1961.
2. Devi, V. A., Rao, N. A. N., and Vijayaraghavan, P. K., Isolation and composition of leaf protein from certain species of Indian flora, J. Sci. Food Agr. 16: 116-20, 1965.
3. Edwards, R. H., Miller, R. E., Fremery, D. de, Knockles, B. E., Bickoff, E. M., and Kohler, G. O., Pilot plant production of an edible white fraction leaf protein concentrate from alfalfa. J. Agri. Food Chem. 23: 620-26, 1975.
4. Horowitz, W., Methods of Analysis of the Association of Official Agricultural Chemists, Association of Official Agricultural Chemists, Washington, D. C., pp. 744-45, 1965.
5. Martin, F. W., and Ruberté, R., Edible Leaves of the Tropics, Antillean College Press, Mayagüez, P.R., 235 pp., 1975.
6. Pirie, N. W., Leaf Protein: Its Agronomy, Preparation, and Use, International Biological Program Handbook 20, Blackwell Scientific Publications, Oxford, 192 pp., 1971.
7. ——, Proteins, in: Modern Methods of Plant Analysis IV, p. 26, 1955.
8. Rosedale, J. L., and Melsien, J. N., Malay leaf and other vegetables and their analysis, General Series, Dpt. of Agr. Straits Settlements and Fed. Malay States, Kuala Lumpur, No. 31, 21 pp., 1940.
9. Roughan, P. G., and Black, C. Roger, Simple methods for routine screening and quantitative estimation of oxalic content of tropical grasses, J. Sci. Food Agr. 24: 803-11, 1973.
10. Terra, G. J. A., The significance of leaf vegetables, especially of cassava, in tropical nutrition, Trop. & Geog. Medium, 2: 97-108, 1964.