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Research Article

Study of Physicochemical Parameters of Different Cultivars of *Mangifera indica* L. leaves for their Use as a Source of Mangiferin.

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ABSTRACT
The leaves of *M. indica* are used as a source of mangiferin for the pharmaceutical industry. The present study deals with the phytochemical and physicochemical evaluation of different varieties in order to identify the most suitable one. The parameters included in this comparative study are extractable matter, mangiferin content, water content and total ash. ANOVA revealed a significant difference between the physicochemical parameters of the different cultivars. The Hierarchical Ascendant Classification divided the 29 cultivars into 4 clusters. Eventhough the Filipino and Biscochuelo cultivars showed a higher content of Mangiferin, the cluster of cultivars including the cultivar Corazón is the most suitable one taking the results of the extractable matter into account. In addition, the seasonal variation was investigated for the latter cluster, which revealed that optimal collection period includes the flowering and green fruit stages. Furthermore, based on the investigation of the influence of the drying and storage conditions, plant material for industrial isolation of mangiferin, can be dried using a sun dryer and stored in glass or polypropylene containers for one year.

Keywords: *Mangifera indica* L., leaves, cultivars, mangiferin

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruits worldwide in terms of production and consumer acceptance[1]. Extensive plant breeding has generated hundreds of cultivars, the fruits of which show a pronounced diversity in size, shape, color, flavor, seed size, and composition[2].

M. indica contains various constituents such as carbohydrates, tannins, proteins, saponins, mucilage, terpenoids, flavonoids and glycosides. Mangiferin (MgF), a xanthone glycoside is a main constituent of *M. indica* present in the leaves, fruits, stem bark and root. MgF has a wide panel of pharmacological activities such as antipyretic, antioxidant, antitumour, immunomodulatory and neuroprotective[3-7]. It is traditionally known to be useful for the treatment of a wide range of diseases like throat infection, burns, scalds, and is applied in antidiabetic, antioxidant, antimicrobial, antiviral and antibacterial treatments[8-14].

A careful survey of literature revealed that little work has been done on the phytochemical comparison, and more specifically the mangiferin content, of the leaves of *M. indica* from different cultivars grown in Cuba.

The aim of this work is a preliminary phytochemical characterization of *M. indica* leaves in order to use them as a source for obtaining MgF as a raw material for the pharmaceutical industry.

MATERIALS AND METHODS

Plant material

The cultivars studied were: 'Lancetilla','Delicia', 'Señora', 'Estero del Pinar', 'Keitt', 'Santa Cruz', 'Bombay tardío', 'Tommy Atkins', 'Minin', 'Mameyzón', 'San Felipe', 'Pedro', 'Biscochelo', 'Chino Amarillo', 'Chino Rojo', 'Filipino', 'Chino Esperón', 'Corazón', 'La Paz', 'San Diego', 'Julie', 'Super Haden', 'Delicioso', 'Dom', 'Ren', 'Reina de México', 'Haden', 'Springfield', 'Mario', 'Macho', 'Florida' and 'Smith'. Plant material was donated by the genebank of the Tropical Fruit Research in Artemisa, Cuba and the samples were authenticated by Dr. Victor Fuentes. Also leaves at different phenological stages, including. vegetative, flowering, green and ripe fruit were collected from July to October, 2012 and 2013. Voucher specimens (ROIG 4776) were deposited at the herbarium Medicinal Plant Experimental Station,"Juan Tomás Roig" San Antonio de

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los Baños, Mayabeque, Cuba. Leaves were washed in running water and dried using a sun dryer. They were homogenized to a fine powder (2-3mm) and stored in air tight packets.

Reagents

Mangiferin standard was donated by the Center of Pharmaceutical Chemistry and certified by the State Center for Drug Control (CECMED), Cuba. The HPLC purity of the reference material was 93.8%. The reagents used were of analytical grade (MERCK, Germany).

Physicochemical parameters

Physicochemical studies including total ash, loss on drying at 105 °C and extractive values (water soluble and ethanol soluble) were carried out according to the WHO guidelines[15].

Mangiferin content

Procedure

Mangiferin was quantified by HPLC. The separation was performed using a Knauer Smartline HPLC system (Knauer, Germany) equipped with a Smarline 1000 pump, a Smartline 2500UV detector, a Smarline 3900 Thermoautosampler, a Manager 5050 degasser and ClarityChrom software. A Luna C-18 reverse-phase analytical column (150 mm x 4,6 mm i.d., 5µm particle size), Phenomenex, USA was used. The following gradient was applied with mobile phase A being acetic acid 0.2% and acetonitrile (85:15) and B acetonitrile: 18 min, 100% A; 20 min, 20 % A; 22 min, 100% A. The flow rate was 1 mL min-1. Absorbance was monitored at 254 nm.

Preparation of standard

To construct the calibration curve, a stock solution of 100 ppm of reference compound MgF was prepared in a 50% dioxane solution. A series of dilutions were subsequently carried out to obtain 1/2, 1/4, 1/8, 1/16 and 1/32, of the original concentration for the preparation of standard solutions.

Sample Preparation

About 1 g of dried leaves were accurately weighed and processed by continuous extraction for 4 hours in a Soxhlet apparatus, using dioxane as a solvent. The extract was transferred to a 500 mL volumetric flask and made up to volume with a mixture of dioxane: water (1:1) v/v. A 5,0 mL aliquot was transferred to a 50 mL volumetric flask and made up to volume with the same mixture.

All the samples were analyzed in triplicate.

Statistical analysis

Table 1. Physicochemical parameters and MgF content of 30 cultivars of *M indica* leaves in the green fruit stage.

Cultivars	Loss on drying (%)	Ash (%)	Water soluble extractive value (%)	Ethanol soluble extractive value (70%)	Mangiferin (mg/100g)
Haden	6.3^{h}	10.0^{efg}	20.1^{ijk}	18.3^{l}	2.20^{ef}
Julie	5.5^{i}	8.3^{k}	21.7^{de}	26.0^{d}	3.39^{cd}
Bombay tardío	6.2^{h}	10.8^{d}	20.0^{jk}	18.5^{l}	2.09^{hij}
Keitt	7.2^{fg}	8.8^{j}	20.0^{ijk}	20.5^{i}	2.41^{gh}
Springfield	6.2^{h}	9.8^{gh}	20.1^{ijk}	18.9^{ef}	2.20^{ef}
Señora	5.6^{j}	10.8^{d}	17.6^{pq}	23.1^{h}	1.25^{lm}
Smith	4.7^{j}	10.1^{e}	20.3^{hi}	16.3^{o}	3.79^{bc}
Estero del Pinar	6.4^{h}	11.1^{c}	16.9^{r}	21.8^{i}	3.47^{cd}
Super Haden	4.4^{j}	9.7^{h}	20.6^{gh}	18.6^{kl}	3.66^{c}
Chino Rojo	5.3^{i}	11.2^{c}	18.9^{n}	17.5^{n}	3.10^{de}
Chino Esperón	6.8^{g}	11.6^{c}	18.2^{o}	19.1^{j}	2.46^{fgh}
Mario	4.8^{j}	11.1^{c}	16.2^{s}	20.4^{i}	1.47^{lm}
Corazón	7.4^{f}	10.2^{e}	17.2^{pq}	17.9^{m}	4.17^{b}
Lancetilla	6.3^{h}	7.4^{n}	19.5^{lm}	27.5^{d}	2.28^{hi}
La Paz	6.3^{h}	7.5^{n}	22.3^{c}	24.0^{g}	3.44^{cd}
Delicia	6.2^{h}	10.0^{ef}	19.2^{mn}	23.0^{h}	1.67^{klm}
Filipino	9.9^{e}	9.3^{i}	21.1^{f}	23.3^{h}	4.95^{a}
San Felipe	11.7^{c}	9.7^{fgh}	22.3^{c}	29.3^{c}	2.78^{efg}
San Diego	9.9^{e}	8.1^{l}	23.4^{b}	30.5^{b}	2.72^{efg}
Chino amarillo	9.8^{e}	10.0^{efg}	21.6^{e}	24.1^{g}	3.63^{c}
Tomy Atkins	11.3^{cb}	9.9^{efgh}	20.9^{fg}	25.6^{e}	1.24^{m}
Santa Cruz	11.4^{cb}	10.0^{ef}	18.2^{o}	23.1^{h}	2.83^{efg}
Minin	10.8^{d}	9.3^{i}	19.8^{kl}	24.8^{f}	2.80^{ef}
Reina de México	9.7^{e}	13.1^{b}	17.6^{pq}	22.0^{i}	2.90^{e}
Pedro	10.0^{e}	14.6^{a}	17.8^{p}	23.1^{h}	1.97^{ijk}
Bizcochuelo	10.6^{d}	7.9^{lm}	22.0^{c}	30.5^{b}	5.00^{m}
Mameyzon	11.2^{c}	7.8^{m}	23.7^{db}	27.6^{d}	2.13^{hi}
Dom	11.4^{cb}	10.0^{e}	19.4^{m}	24.1^{g}	1.67^{jkl}
Ren	13.2^{a}	8.3^{k}	25.3^{a}	32.5^{a}	1.90^{ijk}
Delicioso	11.6^{cb}	9.9^{efg}	20.4^{hi}	25.8^{de}	2.77^{efg}

Values with similar letters in a column do not differ significantly ($P<0.05$).

The statistical analysis was performed using Stargraphic software version 5.1. Results were considered statistically significant at P<0.05. The means and standard deviations for the physicochemical parameters were calculated. Hierarchical Ascendant Classification (HAC) was used to divide the *M. indica* cultivars in different clusters based on their extractable matter and MgF content.

Drying study

Four drying methods were used: oven drying at 40°C ± 1°C, under shade, sun and in a sun drier. The plant material of 5 cultivars (Macho, Haden, Springfield, Flipino and Florida) was dried until constant weight. Dried leaves were stored in an airtight amber bottle at temperature of 4° ± 1 °C, until the analyses were performed.

Storage study

The storage study was conducted at 25°C ± 2°C/ 70 % RH ± 5 %, using 4 kinds of containers: polypropylene, polyethylene, glass and paper. The dried leaves of Super Haden cultivar were stored for one year and were checked for residual humidity, MgF content and organoleptic characteristics at time points 0, 3, 6and 12months.

RESULTS AND DISCUSION

Comparison of different cultivars

The results for mangiferin content and the physicochemical parameters of 29 cultivars grown in Cuba are shown in table 1. The total ash ranged from 7.4±0.15 to 14.6±0.21 and the water from 4.4±0.12- 13.2±0.15 percent. The contents of water soluble and ethanol soluble fractions ranged from 16.2±0.12 to 23.7±0.35 g/100g, and 16.3±0.20 to 32.5±0.15 g/100g respectively and the mangiferin content ranged from 1.24±0.01 to 5.00±0.05 g/100g of dry leaves. The quality of the plant material is determined by its mangiferin content, while the total ash and water content are parameters that are indicative for the purity. In addition, the values of the determination of the extractable matter in ethanol 70% (v/v) and water allow us to predict the amount of other material extracted simultaneously with MgF, as these solvents are used as the first step in the industrial production of MgF as active pharmaceutical ingredient in our plant production site. Statistically significant differences between all parameters for the different cultivars were observed (Table 1.) A significant positive correlation among the majority of the physicochemical parameters evaluated was observed except for the total ash. All the characters, were negatively correlated with total ash. Substances soluble in water and in ethanol 70% show correlation between them (0.6404) and with the total ash (-0.6908 and -0.5014 respectively) and water content (0.4281 and 0.6965 respectively), all these correlations with statistical significance for a confidence level of 95%. The mangiferin content showed no statistically significant correlation with any of the parameters analyzed. The biosynthesis and the accumulation of MgF varies depending on the variety, but is independent from the effect of the physicochemical parameters analyzed in this study. The Hierarchical Ascendant Analysis of the results led to four clusters with variable characteristics (Table 2). Cluster 1 was formed by cultivars Haden, Bombay tardio, Keitt, Springfield, Señora, Chino esperón, Mario, Lancetilla, Delicia, Delicioso, Tommy Atkins, Minin, Pedro, Eldon; cluster 2:

Table 2. Characteristics of *M. indica* L. cultivars evaluated, organized into clusters by Hierarchical Ascendant Analysis.

Clusters	Water soluble extractive value (%)	Ethanol soluble extractive value (%)	Mangiferin content (mg/100g)
Cluster 1	19.2	22.3	1.97
Cluster 2	18.5	19.6	3.45
Cluster 3	21.7	25.8	3.90
Cluster 4	23.7	29.9	2.38

Table 3. Drying study of 5 cultivars using 4 drying conditions

Cultivars/ Parameters	Sun	Shade	Oven 40^0C	Sun dryer
Macho				
Mangiferin (%)	3.91[a]	3.86[a]	3.89[a]	3.69[a]
R. Humidity(%)	8.3[c]	9.8[d]	4.8[a]	6.9[b]
Haden				
Mangiferin (%)	1.68[a]	1.82[a]	1.51[a]	1.49[a]
R. Humidity(%)	8.9[c]	9.8[d]	7.8[b]	6.4[a]
Springfied				
Mangiferin (%)	1.95[a]	2.27[a]	1.62[a]	1.90[a]
R. Humidity(%)	10.8[c]	11.8[d]	6.6[a]	7.6[b]
Filipino				
Mangiferin (%)	3.36[a]	3.08[a]	2.70[a]	3.30[a]
R. Humidity(%)	8.2[c]	10.2[d]	6.6[a]	7.0[b]
Florida				
Mangiferin (%)	1.49[a]	1.48[a]	1.50[a]	1.57[a]
R. Humidity(%)	9.3[c]	10.0[d]	5.9[a]	7.8[b]

Values with similar letters in a column do not differ significantly (P<0.05).

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Smith, Estero del Pinar, Super Haden, Chino rojo, Corazón, Santa Cruz, Reina de Mexico, cluster 3: Julie, La Paz, Filipino, Chino Amarillo, Bizcochuelo, cluster 4: Kent, San Felipe, San Diego, Mameyzon. Cluster 3 groups cultivars that combine a high content of MgF with the lowest content of extractable substances in water and ethanol 70%. Although a higher MgF content was found in Filipino and Biscochuelo cultivars, less compounds are extracted together with the metabolite of interest, both in water and in 70% Ethanol in the case of the Corazón cultivar. So we conclude that the group of cultivars that are part of the cluster number 2 would be the most suitable for use in obtaining MgF. Seasonal variation of Mangiferin content. The content of MgF in the leaves of different phenological states of seven cultivars were determined .From these results, i.e. for Super Haden cultivar during the stages of flowering, green fruit, ripe fruit and vegetative state was: 4.28 ± 0.86, 3.25 ± 0.03, 1.63 ± 0.11, 1.56 ± 0.12 respectively, it could be concluded that the highest content was found in the leaves collected during flowering and

green fruit. The highest content of mangiferin was found in the leaves collected during flowering , i.e. the phenological stage that leads to fruiting which could be related to the role of this metabolite in the plant as an important secondary metabolite to protect the plants against various forms of biotic and abiotic stress[16-18].

Drying study

In order to obtain a high quality of plant material to be used as a herbal drug it is important to determine which drying method is the best, i.e. the method leading in the shortest time to the starting material with the lowest amount of water. In our study all drying methods show differences in the residual moisture for the five cultivars studied while the MgF content remained unchanged for each cultivar studied. In general, the best results were obtained using artificial drying at 40 ^{0}C, except for Haden cultivar for which the residual moisture values were lower in the case of the sun dryer. For all methods this parameter remained below 12% w/w. (Table 3).

For improved energy efficiency in the production of medicinal plants technology, conventional hot air drying using fuel or electricity can be replaced by the use of solar dryers which has the advantage of energy savings with obtaining a dehydrated product quality and hygienic conditions where sanitation compared to the natural drying is higher.

Storage

In order to avoid possible damage and contamination by moulds, insects and other animal contamination, medicinal plant materials must be processed and packaged immediately after harvest. Proper long term storage of the drug should ensure product stability and conservation of physical, chemical, organoleptic and therapeutic properties. Therefore it is important to carefully analyze the influence of the type of packaging used and the conditions of storage.

Table 4 shows the results for the storage study during a year in four kinds of containers. The results showed no statistically significant differences in mangiferin content between the different types of containers or between the different time points while organoleptic characters changed, i.e. loss of color and odor, for packaging paper and nylon features after ninth months of storage. For samples stored in glass and polypropylene containers these features remained unchanged.

Table 4. Residual humidity, organoleptic characteristics and MgF content during the storage study

Time (months)	Residual Humidity(%)	Organoleptic characteristics		Mangiferin (%)
		Odor	Colour	
		Polypropylene		
0	8.51± 0.26	Green- yellow	Characteristic	2.75± 0.07
3	8.54± 0.05	Green- yellow	Characteristic	2.61± 0.20
6	8.66± 0.09	Green- yellow	Characteristic	2.70± 0.30
9	9.31± 0.09	Green- yellow	Characteristic	2.63± 0.06
12	9.73± 0.23	Pale green	Bit faded	2.57± 0.13
		Glass		
3	8.49± 0.29	Green- yellow	Characteristic	2.41± 0.07
6	8.73± 0.24	Green- yellow	Characteristic	2.76± 0.32
9	9.37± 0.10	Green- yellow	Characteristic	2.60± 0.23
12	9.85± 0.09	Pale green	Bit faded	2.54± 0.14
		Polyethylene		
3	8.00± 0.05	Green- yellow	Characteristic	2.77 ± 0.05
6	8.75± 0.15	Green- yellow	Characteristic	2.68± 0.21
9	8.91± 0.30	Green- yellow	Characteristic	2.30 ± 0.29
12	9.30± 0.12	Pale green	More faded	2.48 ± 0.28
		Paper		
3	7.8± 0.30	Green- yellow	Characteristic	2.63 ± 0.04
6	7.4± 0.25	Green- yellow	Characteristic	2.57 ± 0.14
9	9.3± 0.17	Green- yellow	Bit faded	2.56 ± 0.19
12	9.2± 0.35	Pale green	Very faded	2.30 ± 0.26

The residual water content is maintained in a range between 7.2 and 9.8. So the storage for one year in glass or polypropylene containers guarantees the retaining of all the physicochemical characteristics.

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