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REGULAR ARTICLE

Extraction of mangiferin from *Mangifera indica* L. leaves using microwave-assisted technique

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Abstract

Mangiferin, a bioactive metabolite having potent antioxidant and pharmacological properties has been obtained in this work by microwave assisted extraction (MAE) technique, from *Mangifera indica* leaves. A Box–Behnken design was used to evaluate the effect of three parameters: microwave power, the time of extraction, and the ratio of solvent to raw material (mL/g) over the mangiferin yield. Irradiation time was the most important parameter on the recovery of mangiferin. The results indicated that the optimal conditions should be as follows: power of microwave 900 W, extraction time of 5 min and a solvent volume / vegetal material relation of 10. With these parameters the maximum mangiferin yield was 63.22%. The MAE process was compared with the conventional extraction in stirred tank. No significant statistical differences were found (p= 0.5639) between both extraction methods of mangiferin. The presence of the metabolite was confirmed using high performance liquid chromatography. The method was validated with the guidelines set on the International Conference on Harmonization (ICH), for the validation of analytical procedures.

Key words: Mangiferin, *Mangifera indica*, Microwave assisted extraction (MAE), HPLC

Introduction

Mango, (*Mangifera indica* L.), belongs to the family of Anacardiaceae, and is frequently found in tropical and sub-tropical regions. It is one of the best popular comestible fruits in the world. The chemical composition of this plant has been studied widely in the past and was reported that the extracts contain different chemical family such as triterpenes, phytosterols, flavonoids and polyphenols (Núñez et al., 2002; Núñez et al., 2002a; Núñez and Rastrelli, 2010; Núñez et al., 2007).

Mangiferin is a significant bioactive constituent of mango containing xanthone-C-glycoside, which has many pharmacological properties and is very important as phytochemical. It has antioxidant, immunomodulatory, antidiabetic, antitumour properties and is convenient in the treatment of asthma and inflammation (Jatoi et al., 2007). Some reports show the development of method for determination of mangiferin using a thin layer chromatography (TLC) and a high performance thin layer chromatographic (HPTLC) (Nedialkov et al., 1998). However, Liquid Chromatography (LC) method has not been reported before for the quantitative determination of mangiferin in extracts obtained from *Mangifera indica* L. leaves. Only a few reports on LC simultaneous determination of several components including mangiferin in Chinese traditional pharmaceutical preparations (Ronghua et al., 2004) and recently report measuring the amount of mangiferin permissible into the eye (Hou et al., 2010).

Extraction is considering the first basic stage in medicinal plant research due to the preparation of extracts from raw materials is the starting point for the separation and purification of chemical constituents (Romanik et al., 2007). Generally, the conventional methods for obtaining of natural products are mainly maceration, heat reflux and Soxhlet extraction. These processes are of easy operation; however, in most cases, they are arduous and highly time consuming with small yields and is

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possible to a severe risk of thermal degradation for most of the phytoconstituents. The traditional techniques of solvent extraction of plant materials are typically based on the correct choice of solvents of extractions and the use of methods (heat or agitation) with the objective to increase the solubility of the chosen metabolite and increase the mass transfer. The isolation of different constituents present in raw material is limited by the extraction stage (Mandal, 2007). In recent years, the use of microwave for obtain of constituents from plant material has shown great interest. The practice of microwaves in analytical sciences is not new; the first reported was in 1986 for the extraction of organic compound (Dean, 2010). Actually, MAE has attracted growing interest due to permit a rapid extraction of solutes containing into solid matrices, with extraction efficiency similar or superior to that of the conventional techniques (Camel, 2000). Heating occurs in a targeted and selective way in MAE with practically no heat being lost to the environment, and the apparatus can significantly diminished the extraction time (Huie, 2002). Different are the factors that affecting MAE, such as solvent type and volume, microwave power, extraction time, temperature and matrix characteristics (Mandal, 2007; Chan et al., 2011).

The feasibility of obtain mangiferin via MAE was reported by (Venkatesh et al., 2010). Longer time and moderate power were used, ethanol was employed by extraction solvent. In previous study MAE of mangiferin isolation from *Curcuma amada* was studied evaluating two independent parameters, microwave power and extraction (irradiation) time (Padmapriya et al., 2012). More recently, the influence of some variable such as microwave power, ethanol concentration, extraction (irradiation) time and pre-leaching time has been studied (Kullu et al., 2013).

Considering that other factor the importance in the extraction stage such as the type of the solvent and the relation vegetal material/solvent volume have been not evaluated, in the present paper, a more rigorous studied has been applied to know the influence of these independent variable on mangiferin extraction. The existence of metabolite in the final extract from *Mangifera indica* leaves was confirmed using high performance liquid chromatography (HPLC), employed mangiferin as standard.

Materials and Methods

Plant material

The leaves of *Mangifera indica* L. were collected from plants grown in a fruit farm in San Antonio de los Baños, Mayabeque, Cuba and successively dried and milled to obtain particles of around 2-3 mm. The content of mangiferin and the water is 3.23% and 6.5%, respectively.

Chemicals and Reagents

Acetic acid and methanol were chromatography grade from Merck (Darmstadt, Germany) and used without further purification. The reference standard (RS) of mangiferin was a house standard with 94.8% of purity determined by NMR, HPLC and DSC. Water, HPLC grade, was obtained using a Millipore Milli Q plus purification system (Billerica, MA, USA).

Quantification of mangiferin in the extracts from *Mangifera indica* leaves by HPLC

The determination of mangiferin was achieved by high performance liquid chromatography (HPLC). An HPLC system (Shimadzu LC20 model), equipped with two solvent delivery pump (LC20AD), UV/VIS detector (SPD20A), autosampler (SIL20AC) and a column oven (CTO20A) was used for the quantification of mangiferin in the extracts. The chromatographic separation was made on a RP18 column (Superspher 100, 250 mm x 4.6 mm; 5 µm) using methanol and acid acetic 2.5% (28/72 v/v) as the mobile phase at 1 mL min^{-1} and room temperature. The injection volume was 20 µL and detection was achieved at 254 nm. The chromatogram were acquired and processed by LCMS Solution software (Shimadzu). The guidelines set on the International Conference on Harmonization (ICH) for the validation of analytical procedures was using to validate of analytical method [ICH, 1995]. With the objective to validate the method of analysis were used the parameters linearity, accuracy, range, precision, limit of detection (LOD), limit of quantitation (LOQ) and specificity.

Equipment for the microwave-assisted extraction (MAE)

Microwave-assisted extraction was realized using a microwave apparatus (*SANYO* EM-T109SS) in a closed vessel system, with operation frequency of 2 450 MHz. It was equipped with one 1000 mL container collocated in the space with mayor radiation, determined previously (Pérez, 2012). Around 10.0 g of leaves powder was put into extraction vessel and processed under different MAE situations. The ranges of the variables considered are showed in Table 1.

Table 1. Level of independent variable chosen for BBD.

Variables	(-1)	(-1)	Unit
Time	1.0	5.0	min
Power	540.0	900.0	W
Solvent volume /vegetal material rate	10.0	20.0	mL/g

Reflux extraction (RE)

Powdered *Mangifera indica* leaves (10.0 g) was refluxed with 200 mL of water (Rusakova et al., 1985). Subsequently filtration through the filter paper, the end extracts was analyzed by HPLC.

Experimental procedure

Box–Behnken design was used to optimize the extraction variable and evaluate some statistical parameters as main effects, interaction effects and quadratic effects over the extraction yields of mangiferin that is the response variable. Were taken as the factors the microwave power, extraction time, and raw ratio of solvent to raw material, which have strong effects on the yields. A total of 15-run experiment were realized. Three assays were carried out in the plane center. After extraction the liquor was separate from material vegetal waste and filter by reduce pressure. Finally was determined the mangiferin content by HPLC inside liquor. The yield of drying was calculated to take account the initial content of mangiferin present in the mango leaves and the content of mangiferin existent in the liquor at the end of the experiments.

For calculating the optimal point, a second-order polynomial model was fitted to correlate link between independent variables and response (mangiferin yield). Analysis of the experimental design results were carried out using a statistical program. The regression analysis (ANOVA) was effected, and the fitness of the polynomial equation was evaluated by the coefficient of determination R^2. Both model and regression coefficients were considered statically significant when the p-values were lower than 0.05.

Result and Discussion

HPLC method validation

Different parameters were determined such as linearity, accuracy, precision, specificity and selectivity. The calibration curve in the rage of 5 and 100 µg/mL was found to be linear with good correlation coefficient 0.998-0.999. The limit of detection and quantification estimated from the calibration curve were 0.1 and 0.62 µg/mL respectively. For repeatability six samples at three different concentrations were prepared and analyzed by the HPLC method to determine variation arising from method and expressed as % RSD. The relative standard deviation (RSD) values were found to be below 5%. The results show that the average recovery at each level between 98 and 102%. A study of peak purity of the chromatographic peak mangiferin was made by the comparison of the UV spectrum at different parts of the peak. No differences were observed in the UV spectra obtained for mangiferin peak, so we can conclude that no exist interferences in the determination of mangiferin in the extracts under the analysis conditions used (Figure 1).

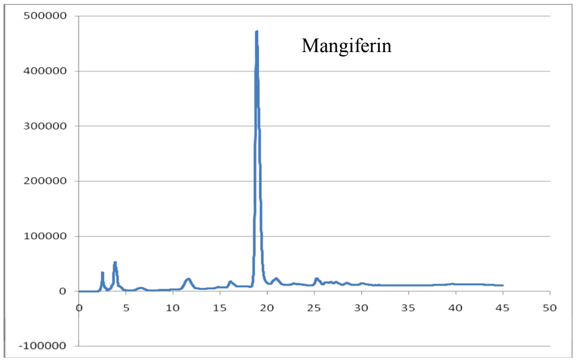

Figure 1. LC chromatogram of an extract of *Mangifera indica* L. leaves. Mangiferin a majority compound.

Optimization of MAE conditions by BBD

A precise choice of solvent is necessary for obtaining an optimal extraction procedure. The best solvent employed for MAE is establishment by the solubility of the chemical market, by the interaction that is creation between solvent and plant matrix, and the end by the microwave absorbing properties of the solvent. 10 g of *Mangifera indica* leaves were extracted with different volume of solvent at different microwave power. The solvent choice was water, as the polar solvent was found to increase mangiferin extraction (unpublished values of results). The influence of different factors such as the power, the time and the solvent volume/ vegetal material over the yield of mangiferin extraction in a MAE process was evaluated. The results of the experimental design appear in the Table 2.

The yield ranged from 34.11% (100.63 mg of mangiferin) to 62.16% (183.38 mg of mangiferin). The major result of yield achieved is near to 63% and is superior to the report where the yield of mangiferin is 31.1% (Venkatesh et al., 2010).

Table 2. Results obtained from the MAE tests. Experimental Box-Behnken response surface design.

Exp	Time (min)	Rate (solv vol / mat veg) (mL/g)	Power (W)	Mass (mg)	Yield (%)
1	1	15	540	105.98	35.92
2	3	20	540	183.38	62.16
3	1	20	720	109.89	37.25
4	1	15	900	100.63	34.11
5	3	10	540	119.07	40.36
6	5	15	540	121.10	41.05
7	1	10	720	103.14	34.96
8	5	10	720	157.85	53.51
9	3	10	900	156.32	52.99
10	5	20	720	157.28	53.32
11	3	20	900	149.58	50.70
12	5	15	900	179.27	60.77
13-15	3	15	720	140.69 ±15.29	47.69 ±5.19

* average of three experiment expressed as X±SD.

It is known that the microwave power and exposure time are two factors, which eject effects of considerable magnitude. A mixture of low or little power with extensive exposure time may be a sensible approach. The concentration of mangiferin and the extraction efficiency were found to increase when the microwave power improve until 900 W. In the experiments when was necessary elevated the microwave power from 540 to 900 W, electromagnetic energy was transmitted to the extraction vessel quickly and this enhanced the extraction efficiency of mangiferin.

Also is considering a critical factor the volume of the extracting solvent. The quantity of the solvent volume must be enough to ensure that the vegetal material is always completely absorbed in the solvent during the entire extraction time. In the literature is referred many reports concerning the volume of solvent to be used with respect to the amount of vegetal material. In this case, the diminished of solvent volume/ vegetal material rate increased the extraction of mangiferin. This behavior has been reported previously in the literature by other authors (Wang and Weller, 2006). Generally, a higher relation of solvent volume to vegetal material may be very effective in conventional extraction methods such as agitated tank. Conversely, in MAE a higher ratio may diminish the yield of recovery. This behavior is probably due to an inadequate stirring and mixture of the solvent into the microwaves (Wang and Weller, 2006).

The time, as in other extraction process, is other parameter whose influence needs to be taken into account. Normally, if the extraction time is increased, the mass or quantity of metabolites extracted is increased, although in sometimes there is the risk that chemical degradation may take place. In our investigation the concentration of mangiferin first increased for time near to 4.5 min and then decreased at 5 min. Analogous results were observed in the withdrawal of flavonoid from *Radix astragali* (Xiao et al., 2008). In this work, during smaller irradiation time, the mangiferin content increased with microwave power. Nevertheless, when the vegetal material and the water (as the solvent) were heated beyond 20 s, the mangiferin content diminished. This behavior was observed for all microwave powers studied. Is possible that, due to the high temperature involved in the extraction process, the degradation of mangiferin can be occurs (Yan et al., 2010). Therefore, very high irradiation time is not appropriate for mangiferin extraction using microwave technique.

In Table 3 are given the results of analysis of variance (ANOVA) realized. The time is the parameter with significant influence in the process ($p <0.05\%$) to take account the statistical results. Not significant effects were achieved for the rest the variables (solvent volume/vegetal material rate and radiation power) respect to the mangiferin concentration. The ANOVA of the regression equation showed that the values of the determination coefficient (R^2) were 0.8869. This result suggested that has an elevated high degree of correlation between the observed and predicted values. Additionally, a low value of coefficient of the variation (CV 5.19%) showed an extraordinary degree of precision and a good reproducibility of the experimental values. The coefficients of the regression models together the response to the operating variables and their interactions are showed in Table 4.

Table 3. Results of ANOVA showing the significance of the sources on the dependent variable (mangiferin yield).

Source	Sum of squares	Degree freedom	Mean square	F value	Significance
A:time	551.286	1	551.286	32.87	0.0004
B:rate	58.374	1	58.374	3.48	0.0991
C:power	45.5058	1	45.5058	2.71	0.1381
AA	136.303	1	136.303	8.13	0.0214
AC	115.885	1	115.885	6.91	0.0302
BC	145.082	1	145.082	8.65	0.0187
Error	134.159	8	16.7699		
Corrected Total	1186.59	14			

Table 4. Coefficients of the regression equation.

	Yield of mangiferin
constant	-33.7623
A: time	2.44911
B: rate	5.35825
C: power	0.08771
AA	-1.51058
AC	0.01495
BC	-0.00669

$R^2 = 88.6938$ %

The p values were used to check the significance of each coefficient, which in turn may indicate the pattern of the interaction between the variables. The Pareto□s graphic show that time was the most significant single factor, which affect the mangiferin yield (Figure 2). To take account the graphic of principle interactions, the content of mangiferin increased until a maxim value and then diminished. Probably due to the elevated value of the temperature obtaining in the extraction process, which might will be caused damage of mangiferin. Water has the maximum value of the dielectric constant of common solvents used in the pharmaceutical industry. For these reason the dissipation factor is significantly inferior to other solvents. So, the velocity at which water absorbs the microwave energy is more elevated respect to the rate at which the system can dissipated the heat. This phenomena explain the “superheating” effects, which occur when water existing in the vegetal material. This phenomenon (localized superheating) can have positive or negative repercussion, depending on the vegetal matrix. In some circumstances it can increase the diffusivity of analyte in the raw vegetal material. In other cases, the powerful heating can origin the degradation for the thermo degradable compound (Wang and Weller, 2006; Mandal et al., 2007; Jain et al., 2009).

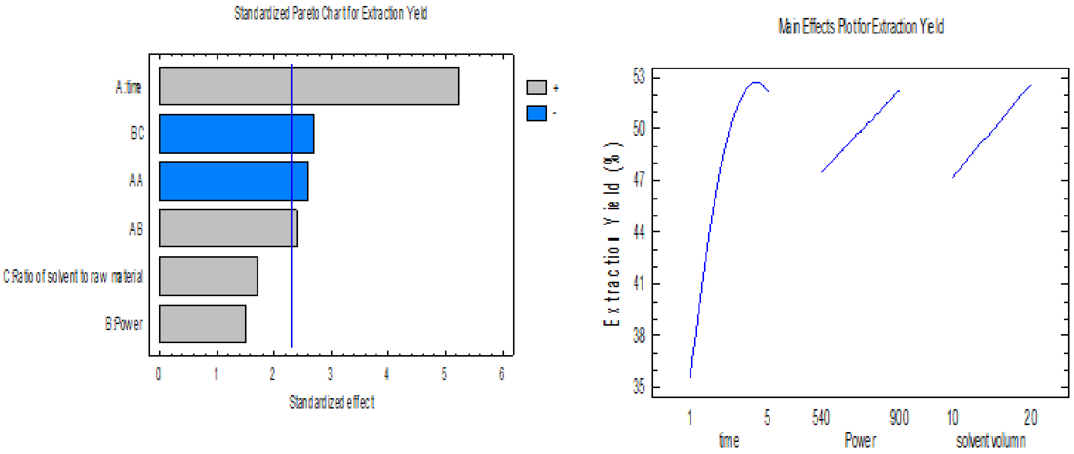

Figure 2. Pareto□s graphic and principle effects from yield of mangiferin.

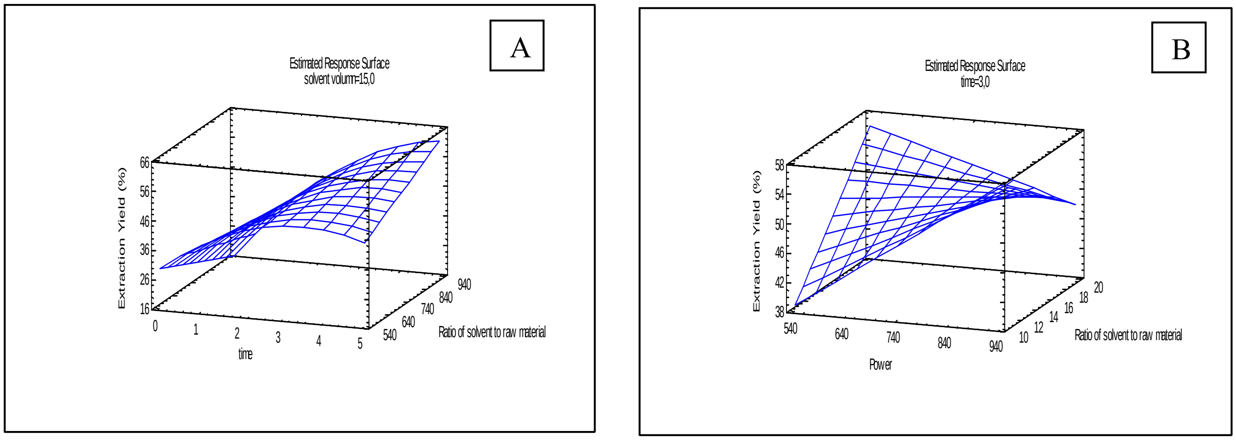

Figure 3. Estimated response surfaces for yield variable. A) Volume solvent/vegetal material rate 15 mL/g, B) Radiation time 3 min.

Two independent response surface plots are shown in Figure 3. The shapes of the plots indicated whether the common interactions between the independent variables were significant statically or not. It permit concluded that the optimal MAE conditions of mangiferin from *Mangifera indica* leaves were as: microwave power 900 W, irradiation time 5 min., and relation of solvent to raw material 10, for to obtain a 63.22% of yield of mangiferin. This value is superior to achieve in other previous report (Venkatesh et al., 2010). However successive stage will be evaluated if is necessary to extract all the mangiferin present in the vegetal matrix.

Comparison with conventional extraction method

The conventional isolation of mangiferin from *Mangifera indica* L. involves reflux extraction, using two times the amount of solvent (water). The best experimental conditions for MAE were compared to RE parameters. The MAE yield obtain was similar respect to RE which 63.22 ± were 2.1% and 64.18 ± 1.29% respectively. Moreover, significant advantages due to the low consumption of solvent and extraction time show that MAE provides a very good and consistent extraction method of bioactive metabolite from natural plants (Jain et al., 2009; Mandal et al., 2007; Chan et al., 2011).

Conclusion

An efficient method of MAE was evaluated for the extraction of mangiferin from *Mangifera indica* L. leaves with enhanced yield. A BBD design was satisfactorily employed to optimize extraction parameters, in this work. Results showed that microwave power 900 W, extraction time 5 min., and ratio of solvent to raw material 10:1 were the best conditions to extract mangiferin from mango leaves. The best value of yield achieved was 63.22%. This value is superior to obtain in other previous report. Successive stage will be evaluated if is necessary to extract all the mangiferin present in the vegetal matrix. When compared to the conventional extraction method (RE) the MAE is more efficient method with both lower solvent consumption and time. The yield obtained in both methods are similar, to take values near to 64%. No significant statistical differences were found (p= 0.5639) between conventional and non-conventional extraction methods.

References

Camel, V. 2000. Microwave-assisted solvent extraction of environ- mental samples. TrAC Trends Anal. Chem. 19:229–248.

Chan, C. H., R. Yusoff, G. Ngoh and F. W. L. Kung. 2011, Microwave-assisted extractions of active ingredients from plants. J. Chromatogr. A. 1218:6213–6225.

Dean, J. R. 2010. Extraction Techniques in Analytical Sciences. Wiley, New York, pp. 167–183.

Hou, Y., S. Fan, H. Zhang, Y. Gu, X. Yu and B. Li. 2010. Pharmacokinetic study of mangiferin in rat plasma and retina using high-performance liquid chromatography. Mol. Vis. 16:1659-1668.

Huie, C. W. 2002. A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. Anal. Bioanal. Chem. 373:23–30.

International Conference on Harmonization (ICH). Text on Validation of Analytical Procedures. 1995.

Jain, T., V. Jain, R. Pandey, A. Vyas and S. S. Shukla. 2009. Microwave assisted extraction for phytoconstituents - An overview. Asian J. Res. Chem. 2:19-25.

Jatoi, S. A., A. Kikuchi, S. A. Gilani, K. N. Watanabe. 2007. Phytochemical, pharmacological and ethnobotanical studies in mango ginger (*Curcuma amada* Roxb. Zingiberaceae). Phytother. Res. 21:507–516.

Kullu, J., A. Dutta, D. Constales, S. Chaurari and D. Dutta. 2013. Experimental and modeling studies on microwave-assisted extraction of mangiferin from *Curcuma amada*. 3 Biotech 4:107-120.

Mandal, V., Y. Mohan and S. Hemalatha. 2007. Microwave assisted extraction – an innovative and promising extraction tool for medicinal plant research. Pharmacog. Rev. 1(1):7-18.

Núñez, A. J. and L. Rastrelli. 2010. Chemical composition of the mango stem bark extract (*Mangifera indica* L.), In: V. K. Gupta (Ed.), pp. 433-56. Comprehensive Bioactive Natural Products, Stadium Press, Houston, USA.

Núñez, A. J., H. T. Velez-Castro, J. Aguero-Aguero, J. Gonzalez-Gonzalez, F. Naddeo, F. De Simone and L. Rastrelli. 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. J. Agric. Food Chem. 50:762-766.

Núñez, A. J., E. Paéz, D. Amaro, J. Acosta, J. Agüero, R. Capote, M. R. Gárcia, I. G. Morales, O. García, G. Garrido, G. Martínez and M. A. Morales. 2002a. Composiciones farmacéuticas a partir del extracto de *Mangifera indica* L. Patente No. 1814, Octubre, Oficina Cubana de la Propiedad Industrial.

Núñez, A. J., M. D. Durruthy Rodríguez, E. Rodríguez Balseiro, L. Nieto González, V. Nicolais and L. Rastrelli. 2007. Comparison of major and trace element concentrations in 16 varieties of Cuban mango stem bark (*Mangifera indica* L.). J. Agric. Food Chem. 55(6):2176-2181.

Padmapriya, K., A. Dutta, S. Chaudhuri and D. Dutta. 2012. Microwave assisted extraction of mangiferin from *Curcuma amada*. 3 Biotech 2:27-30.

Pérez, J. C. 2012. Extracción asistida por microondas de los lípidos de las semillas de *Cucurbita pepo* L., Tesis en opción al título de Licenciado en Química, Universidad de La Habana, Facultad de Química.

Romanik, G., E. Gilgenast, A. Przyjazny and M. Kaminski. 2007. Techniques of preparing plant material for chromatographic separation and analysis. J. Biochem. Biophys. Methods 70:253-261.

Ronghua, D., G. Jun and B. Kaishun. 2004. High-performance liquid chromatographic method for the determination and pharmacokinetic study of mangiferin in plasma of rats having taken the traditional chinese medicinal preparation Zi-Shen Pill. J. Chromat. Sci. 42:February.

Rusakova, S., V. I. Glyzin, S. I. Kocherga, L. F. Libizova and A. A. Ananieva. 1985. Process for preparing mangiferin. Patent No FR2486941.

Venkatesh, P., V. Soumya, K. Hariprasath and P. Hari. 2010. Microwave assisted extraction of Mangiferin from *Mangifera indica* L. IRJP. 1:209-212.

Wang, L. and C. L. Weller. 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol. 17:300-312.

Xiao, W, L. Han and B. Shi. 2008. Microwave-assisted extraction of flavonoids from *Radix Astragali*. Sep. Purif. Tech. 62:616–620.

Xue-Lian, B., Y. Tian-Li, Y. Ya-Hong and Z. Hua-Wei. 2010. Optimization of microwave-assisted extraction of polyphenols from apple pomace using response surface methodology and HPLC analysis. J. Sep. Sci. 33:3751–3758.

Yan, M. M., W. Liu, Y. J. Fu and Y. G. Zu. 2010, Optimisation of the microwave-assisted extraction process for four main astragalo-sides in *Radix astragali*. Food Chem. 119:1663–1670.