© 2011 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 10 (4): 369 - 378
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Metabolites extraction optimization in *Tamarindus indica* L. leaves.

[Optimización de la extracción de metabolitos de las hojas de *Tamarindus indica* L.]

Julio César ESCALONA-ARRANZ[a]; Jesús RODRÍGUEZ-AMADO[a]; Renato PÉREZ-ROSÉS[b]; Javier CAÑIZARES-LAY[a]; Gustavo SIERRA-GONZÁLEZ[c]; Humberto MORRIS-QUEVEDO[d]; Irina LICEA-JIMÉNEZ[a]

[a]Pharmacy Department, Oriente University. Santiago de Cuba. Cuba. [b]Departament Farmacología i Química Terapéutica. Universitat de Barcelona. Spain. [c]Finlay Institute. Ciudad de la Habana. Cuba. [d]Centre for Studies in Industrial Biotechnology. Oriente University, Santiago de Cuba. Cuba.

****Contactos | Contacts: Jesús RODRÍGUEZ-AMADO E-mail address: jimmy@cnt.uo.edu.cu***

Abstract

A central composite 2^3 full factorial design was used to study the combined effect of ethanol concentration and moistening time on total phenol (TP), flavonoid (TF) and carbohydrates (TC) content, as well as over total soluble substance (TSS) and ashes content (TA) in the *Tamarindus indica* L. leaves extraction process. Desirability function (0.8377) suggest the 71.73% ethanol concentration and 92.37 minutes of moistening time as the best combination variables, predicting values of 25.972 and 2.678 mg/mL for TP and TF; whereas for TSS and TA was predicted 18.417 and 0.566% respectively. The TC variable was discarded, because was impossible to find an statistical strong model to describes this variable. The theoretic values predicted were contrasted with new experimental data (n=5) by a hypothesis contrast test and examining the absolute error in the prediction. Results show a good adjustment to the prediction for TP, TSS and TA variables, meanwhile TF exhibit a slight deviation to the predicted value (absolute error over 10%). Nevertheless, this study offers a combination of variables when it is intended to prepare a tamarind extract that afford a high extractive capacity and, as a consequence, a larger probability to exhibit some kind of pharmacologic activity

Keywords: Total phenol, total flavonoids, total carbohydrates, total soluble substances, total ashes, response surface, moistening time, vegetative stage, flowering time and fruiting time.

Resumen

Un diseño factorial con compuesto central 2^3 fue empleado en la evaluación de la influencia de la concentración de etanol y el tiempo de humectación sobre el contenido de fenoles (TP), flavonoides (TF), carbohidratos (TC) sólidos (TSS) y cenizas totales (TA) en el proceso de extracción de hojas de *Tamarindus indica* L. Valores de 71.73 % de etanol y de 92.37 minutos de tiempo de humectación fue la combinación de factores sugerida por la función deseabilidad (0,8377), la cual predice concentraciones de 25,972 y 2,678 mg/mL para las variables TP y TF; y 18,417 y 0,566% para TSS y TA respectivamente. La variable TC fue descartada ante la imposibilidad de encontrar modelos predictivos con significación estadística. Estos valores teóricos fueron comparados por contraste de hipótesis y cálculo del error absoluto de la predicción con los obtenidos por réplicas del experimento (n=5). Los resultados mostraron un buen ajuste a la predicción para las variables TP, TSS y TA, mientras que la variable TF muestra una pequeña desviación con un error absoluto ligeramente superior al 10%. A pesar de ello, el presente estudio ofrece una combinación de variables a la cual se logra una alta capacidad extractiva en hojas de *Tamarindus indica* L., ofreciendo una mayor probabilidad de actividad farmacológica.

Palabras Clave: Fenoles totales, flavonoides totales, carbohidratos totales, sólidos totales, cenizas totales, superficie respuesta, tiempo de humectación, estado vegetativo, floración y fructificación.

Recibido | Received: March 21, 2011.
Aceptado en versión corregida | Accepted in revised form: June 6, 2011.
Publicado en línea | Published online: July 30, 2011.
Este artículo puede ser citado como / This article must be cited as: Julio César ESCALONA-ARRANZ; Jesús RODRÍGUEZ-AMADO; Renato PÉREZ-ROSÉS; Javier CAÑIZARES-LAY; Gustavo SIERRA-GONZÁLEZ; Humberto MORRIS-QUEVEDO; Irina LICEA-JIMÉNEZ. 2011. Metabolites extraction optimization in *Tamarindus indica* L. leaves. Bol Latinoam Caribe Plant Med Aromat 10(4): 369 – 378.

INTRODUCTION

Tamarindus indica L. or tamarind, as it is commonly known, is a medium-sized tree belonging to the *Caesalpinaceae* family. Every part of the tree (wood, root, leaves, bark and fruits) has some value in the subsistence of rural people, and a number of commercial applications are well known. Its fruits are eaten fresh or processed, being valuable as food not only for their good ratios Proteins:Carbohydrates/Fat:lipid but also for their good levels of vitamins, fiber and minerals; mainly Potassium, Phosphorus and calcium (Shankaracharya, 1998). Due to their acidic taste fruits are also used for seasoning or spicing, like curries, chutneys and sauces in many cultures. Seeds have a gel-forming substance used as stabilizer in ice cream, mayonnaise, cheese and as an ingredient in a number of pharmaceutical products, meanwhile, the bark is used in tanning industry and the wood is used for making furniture, wheels, mallets, tools and other many products (Leakey, 1999; El Siddig *et al*. 2006).

As the fruits, tamarind leaves are also edible and are used to make curries, salads, stews and soups in many countries, but especially in times of scarcity, even when their protein ratios (4.0-5.8%) (Duke, 1981) are not too far from those reported in fruits (2.0-7.1%) (Ishola *et al*., 1990). Tamarind leaves are also rich in fat, fibre, and some vitamins such as thiamine, riboflavin, niacin, ascorbic acid and β-carotene. Flavonoids and other polyphenols have also been found in tamarind leaves (Chitra, 1999; Dehesa *et al*., 2006). Furthermore, it has been reported the presence of essential oils (Pino *et al*., 2002) and a variety of other products (Imam *et al*., 2007).

Leaves are frequently used in the treatment of hepatic disorders due to their high content of antioxidant polyphenols (Vipul *et al*., 2009; Dehesa *et al*., 1995; Jouyex *et al*., 1995). More recently, numerous scientific papers have made reference to their activity as antimicrobial agents (Meléndez y Carriles, 2006; Lans, 2007; Shankar *et al*., 2005), and again, polyphenols and flavonoids are quoted as responsible for this antimicrobial activity. The main part of these papers makes use of hydroalcoholic extracts to demonstrate the measured activity. Recent papers suggest the participation of other kinds of metabolites in these pharmacological actions (Escalona et al. 2010) and the phytochemical richness including their mineral composition suggest that a more complex mechanism should be involved.

Response surface methodology (RSM) (Gabrielson *et al*., 2002) is a technique for the modelling and analysis of problems in which a response (dependent variable) is influenced by several independent variables (factors) with the objective to optimize the response. RSM is one of the most applied techniques not only in the improvement of existing products, but also in the design, development and formulation of new products in many industrial areas. It defines the effect of the independent variables, alone or in combination, on the processes modelled. This combination of mathematical and statistical techniques also offers the possibility to rank in different level, the interaction of these dependent variables, allowing the researcher to define the important grade of each variable and therefore their hierarchic influence in the optimized response (Ohene-Afoakwa *et al*., 2007).

By this way, it's expected the design of a new tamarind leaves formulation that contains not only high levels of the desirable phenol and flavonoid composition, but also other organic and inorganic compounds that can improve the pharmacological activities reported for tamarind leaves. Thus, the aim of this paper is: to evaluate the influence of the ethanol concentration and the moistening time in the total phenol (TP), total flavonoid (TF), and total carbohydrates concentration (TC) as well as total soluble substances (TSS) and total inorganic material measured as ashes (TA) variables; in order to select the best combination of these factors in the tamarind leaves metabolites extraction process. Once obtained, this factors combination will be used to prepare formulations in three different steps of the vegetative stages of plant life, in order to evaluate the variation of the levels of those variables (TP, TF, TC, TSS and TA) along two years.

MATERIALS AND METHODS

Plant material

Tamarind leaves were collected in March 2007 from a tamarind tree population in Santiago de Cuba, eastern part of Cuba. A voucher specimen registered as 052216 was deposited at the herbarium of the biology department, University of Oriente, Cuba. Leaves were sun dried and later milled to facilitate the extraction procedure. For the dynamic accumulation study were chosen: June 2007 and 2008 as months in which the tamarind tree population was in vegetative status, November 2007 and 2008 in flowering time, and March 2008 and 2009 in fruiting time.

Extraction conditions

All sample combinations were treated under the same conditions, excepting the two independent variables modeled. Conditions were: 50 grams of sun dried leaves (residual humidity below 10% by the stove method), milled (MLK, Russia), passed across of a 5mm of mesh light sieve and moistened with a solvent equivalent of 1mL for each gram of sun dried leaves. Moistened leaves were extracted afterwards by percolation during four days, and concentrated up to fluid extract using a vacuum evaparation system (KIKA WERKE, Germany) under 42ºC of temperature. Once optimized the process, the predicted conditions by the mathematical model were fixed in order to obtain the different fluid extracts for the evaluation of the dynamic accumulation process.

Experimental design

A central composite 2^2 full factorial design (4 runs, 4 star points and two center points) was set up using Statgraphics Plus 5.0.1 software (StatEase Co. Minneapolis USA). Ethanol concentration (X_1) and moistening time (X_2) were used as independent variables. The experiment evaluates the influence of the independent variables and its possible interactions in the response variables total phenols (TP), total flavonoids (TF), total carbohydrates (TC), total soluble substances (TSS) and total ashes (TA) content. The sample combinations generated from the software are shown on Table 1.

Table 1.

Process variables and their levels used in the design proposed to optimize the metabolites extraction process in *T. indica* leaves.

		Variable Levels				
Independent variable	Code	-1.414	-1	0	1	1.414
Ethanol-water concentration (%)	X_1	55.86	60	70	80	84.14
Moistening time (min)	X_2	17.57	30	60	90	102.43

The optimization process: A stepwise multiple regression analysis was conducted on the design to relate the ethanol concentration and the moistening time on the TP, TF, TC, TSS and TA in tamarind leaves extraction. In order to obtain the ideal independent variables combination that reaches the highest possible levels of the four dependent variables evaluated, a combined variable was analyzed. This artificial function (known as desirability) results from the combination of the different hierarchic levels of four previously dependent variables examined each one, individually. Phenol and flavonoid concentrations were ranked with 3, total soluble substances with 2, and carbohydrates concentration and total mineral (ash) content with 1. The response surface models were generated and presented as three-dimensional plots.

Results validation

In order to demonstrate the capacity of the design to describe the modeled process, a new extraction process was developed fixing the conditions defined in the optimized response. Five replicas of this experiment were conduced and statistically compared with the predicted values given by the model. It was also computed the predicted absolute errors for each dependent variable using the equation $e_r = [(P-O)/O]*100$ where e_r, P and O are the absolute error (%), the predicted value by the convenience function and the experimental value observed respectively.

Analytical methods

Total phenolic content: was estimated using the Folin-Ciocalteu method a relatively straightforward procedure that is useful for determining the total phenolic content of an extract (Ricco *et al*., 2010; Vogel et al., 2010; Cervantes-Cardoza *et al*., 2010; Ricco et al., 2011; García Rodríguez et al., 2011).

In brief, the extract was dissolved in water and an aliquot of this solution was added to 2 ml of Folin reagent; after 2 min, 1 ml of 20% sodium carbonate (Na_2CO_3) was added. Fifteen minutes later, the absorbance was measured at 700 ηm. The total phenol content was expressed as tannic acid equivalents.

Total flavonoid content: Was determined utilizing Aluminum Chloride ($AlCl_3$) (Quettier-Deleu *et al*., 2000). Briefly, a final volume of extract solution was adjusted to 10 ml with absolute ethanol. Subsequently, 1 ml of 2% $AlCl_3$ was added and then the absorbance of a sample solution was measured at 430 ηm. Data were given as quercetin equivalents.

***Total carbohydrates content*:** were determined by the phenol–sulfuric acid method according to Dubois *et al*., (1956). Briefly, the extract was dissolved in water and an aliquot of this solution was added to 500 µL of an aqueous phenol 5% (w/v) solution and 2.5 mL of fuming sulfuric acid. After heating by 20 minutes at 80 °C and chilling, absorbance was measured at 490 ηm. The total carbohydrates content was expressed as glucose equivalents. Measurements for total phenol, total flavonoids and total carbohydrates were developed using a CECIL CE7-200 UV-visible spectrophotometer.

Total soluble substances determination: The total soluble substances content of the samples was determined heating at 105 °C. In porcelain capsules previously tared, 5 mL of the extract were placed in an oven dried at 105 °C during 6 h. The sample was cooled and weighed, and the total soluble substances content quantified and expressed in percentage. The experiment was conducted in triplicate and the mean value reported.

Total ash determination: The ash content of the samples was determined using the procedure as outlined in the AOAC approved method 14.41 (AOAC, 1990). The crucibles were heat dried at 600°C in a muffle furnace for 30 min and cooled in desiccators. The crucibles were weighed and the initial weight noted. Accurately, 2 g sample were weighed and ashed at 600 °C in the muffle furnace overnight. The sample was then cooled and weighed and the percentage ash determined. The experiment was conducted in triplicate and the mean value reported.

Statistical analysis

The statistical analysis and graphical presentations were carried out using Statgraphics 5.1 (Graphics Software Systems, STCC, Inc., Rockville, MD, USA). The significant probability was set at $p \leq 0.05$. For validation analysis, a hypothesis contrast test was developed.

RESULTS AND DISCUSION

The design matrix and variable combinations in the experimental runs generated by the software, as well as the computed values for each dependent variable are shown in Table 2, meanwhile Table 3 presents the coefficients that modified the polynomial equation and their statistical parameters obtained for each response variable. In all cases the polynomial equation:

$$VR = a_o + a_1X_1 + a_2X_2 + a_3X_1^2 + a_4X_2^2 + a_5X_1X_2$$

satisfies the quadratic dependences of each dependent variable. In this equation a_n (n = 0, …, 5) are the polynomial coefficient, meanwhile X_1 and X_2 are the independent variables ethanol concentration and moistening times respectively. The values of these coefficient describes that for all dependent variables, the linear influences of the independent variables and especially ethanol concentration results more important that any quadratic term.

Table 2.
Design matrix and compute values for each answer variables in the experimental design

Runs	X_1 (%)	X_2 (min)	TP (mg/mL)	TF (mg/mL)	TC (mg/mL)	TSS (%)	TA (%)
1	70.00	60.00	25.24	2.474	43.80	17.05	0.55
2	84.14	60.00	19.32	2.919	18.25	12.70	0.23
3	70.00	102.43	26.07	2.533	49.48	19.27	0.55
4	60.00	90.00	23.88	2.256	52.54	18.87	0.75
5	70.00	17.57	17.56	1.627	30.15	12.34	0.21
6	55.86	60.00	23.19	1.656	67.32	16.89	0.81
7	80.00	90.00	24.47	3.001	22.69	16.81	0.48
8	80.00	30.00	21.63	2.221	19.03	13.33	0.18
9	70.00	60.00	25.86	2.378	44.10	16.78	0.51
10	60.00	30.00	21.05	1.876	44.14	14.29	0.38

Table 3.
Coefficients of the polynomial equation and their statistical parameters for each response variable considered in the extraction process.

Factor/Variable	***TP***	***TF***	***TC***	***TSS***	***TA***
Constant	-71.59200	-1.3357	0.7323	-1.2237	0.54805
X_1	2.52484	0.0587	1.8431	1.2937	-0.00474
X_2	0.28139	0.0047	0.9396	0.14090	0.01896
X_1^2	-0.018422	- 0.0003	-0.0225	-0.0099	-0.00006
$X_1 * X_2$	0.00001	0.0003	-0.0039	-0.0001	-0.00006
X_1^2	-0.00174	-0.0002	-0.0041	-0.0005	-0.00008
$\mathbf{r^2}$	0.8143	0.9431	0.9381	0.9405	0.9515
Durbin-Watson (p-valor)	0.4533	0.4028	0.2443	0.3984	0.0824
Lack of fit (p-valor)	0.1481	0.2497	0.0221	0.1374	0.2457

$X_1 \rightarrow$ Ethanol percent $X_2 \rightarrow$ Moistening time TP→ Total Phenol TF→ Total Flavonoid
TC→ Total Carbohydrates TSS→ Total Soluble Substances TA→ Total Ashes

In order to check the statistical quality of the models and indeed their possibility to offer reliable information about the extraction process, three statistical parameters were considered; the determination coefficient (r^2), the Durbin-Watson (DW) test and the lack of fit test.

The determination coefficient (r^2), is an indicator of how well the model explain the data. Values over 0.8 should be considered as good. When DW p-value is it is lesser than 0.05, reveal statistical evidences that another model can explain better the variability of the dependent variable. The last statistical quality criteria considered in this equation is the lack of fit. This statistical test determines if the regression model adequately fits the data or whether a more complicated model should be used instead. When the p-value <0.05, the current model is not adequate to describe the observed data at the 95 percent confidence level and is necessary to include additional terms for the predictor variables.

In general sense, the model obtained (see table 3) for each response variable accomplishes the defined statistical requirements with the exception of lack of fit p-value in TC. This fact forces us to not to consider this response variable in future analyses. Nevertheless, from the data (see Table 2) is inferred that total carbohydrate extraction is enhanced when lower ethanol concentration and higher moistening times are considered. This fact suggests that in tamarind leaves the major quantities of carbohydrates are free or joined to relative hydro-soluble substances.

Effect of the independent variables on each one of the response variables.

Total phenol content: The maximum predicted value for TP content in the experiment was 26.373mg/ml and should be reached using ethanol 68.54% and 81.26 min of moistening time. This performance can be confirmed graphically by the response surface plots (Figure 1) which show a maximum around this point. Depending of the molecular size, the number of hydroxyl groups, and if are free or conjugated with other substances; phenols can be water soluble and water insoluble. The results obtained in this experiment sign that the tamarind leaves phenol constituents have an intermediary level of water solubility and that mixtures of ethanol and water should be employed to obtain high extraction yields. This agrees with the solubility of the majority of polyphenols isolated in tamarind leaves as apigenin, luteonin, caffeic acid and others (Chitra, 1999).

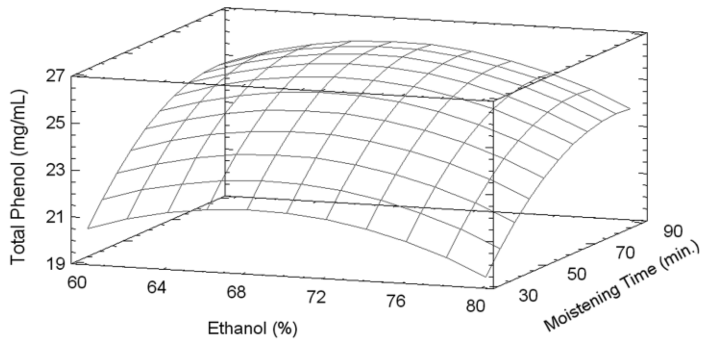

Figure 1

It's also graphically observed that, for lower values of moistening times, the ethanol concentration has a minor curvature, but when higher values are considered the curvature becomes more pronounced, evidencing the influence of the quadratic factors in the equation. This behavior is in agreement with the proved fact that moistening time, when appropriated volume of solvent is used; open the pores of the dried drugs until a point in which the extraction process suffers an inflection. This point seems to be close to the 80 minutes for the specific case of tamarind leaves phenol constituents.

Total flavonoid content.

In this case, a good quadratic model was found to describe the behaviour of the flavonoids extraction in the experiment (see table 3). As this model describes, the maximum flavonoid content predicted (3.2362 mg/mL) is reached when the extreme values of ethanol percent (84.14) and moistening time (102.43 min.) are combined (see figure 2). The increase of flavonoid content with the ethanol concentration is an expression of the solubility of these compounds in medium-polar solvents. This preference for medium-polar solvents occur when the flavonoids have not high levels of glycosidation; even when certain flavanone and chalcone glycosides still have difficulty to be dissolved in methanol, ethanol, or alcohol–water mixtures (Andersen and Markham, 2006). In the specific case of tamarind leaves flavonoids (Dehesa *et al*., 2006), only vitexin, isovitexin, orientin and isoovitexin are glycosides, but with only one unit of saccaride, therefore a little influence over the total polarity of the molecule should be expected. This relative poor affinity for polar solvents could be also interpreted when an analysis of moistening time is realized. In this case, even for longer moistening times, the extraction of flavonoid seems to not to reaches a complete extraction, suggesting that a deepest study must be developed.

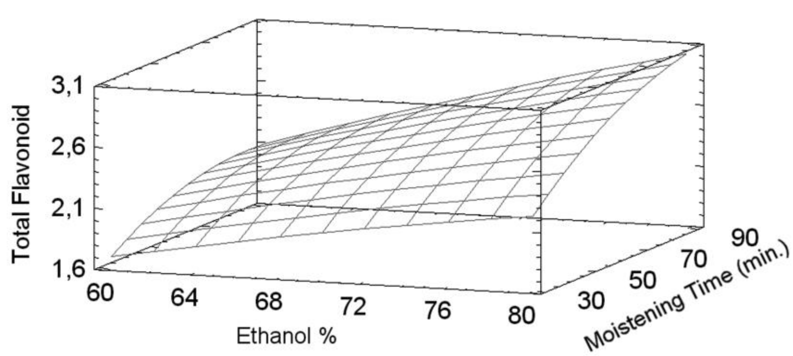

Figure 2.
Response surface plot estimated for total flavonoids in the experimental design

However, it's also clear that this observation is suitable when flavonoid concentration is the only variable to be considerate, but in studies like this one in which multiple response factors are analyzed, this single variable should fit up to the requirements of the rest.

Total soluble substances content:
The response surface plot generated for this variable is presented in Figure 3. When ethanol concentration has higher influence in total soluble substances (close to 60%) the moistening time show a linear increment on the variable modeled. On the opposite side, when ethanol concentrations are close to 80% a slight curvature is perceptible, and total soluble substances values decrease when long moistening times are considered. This fact occur because of, at lower ethanol concentration (high amount of water), the dried drugs can be soaked easily, the pores get wider, solvent can penetrate deeper inside the drug and can be extract a higher amount of water soluble substances. The mathematical model predicts the optimal value of total soluble substances content equal to 19.19% when combine 64.81% of ethanol concentration and 102.43 minutes of moistening time.

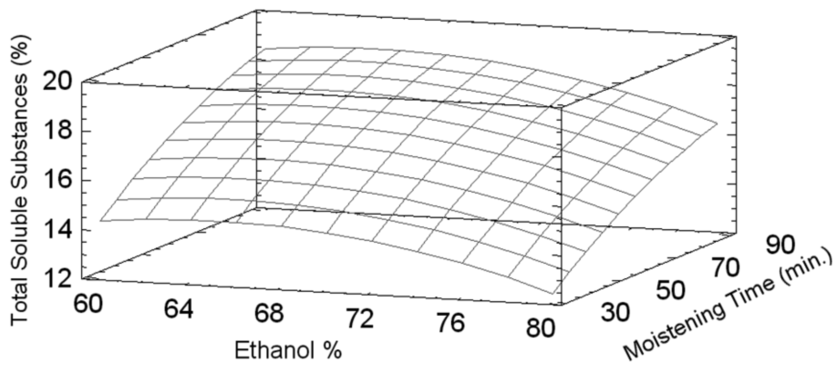

Figure 3.
Response surface plot estimated for total soluble substances in the experimental design

Total ash content:
The response surface plot generated, show a decreasing trend of mineral content when high ethanol concentrations are considered (Figure 4). This was an expected result, attending to the polar characteristics of the majority plant mineral compounds. On the other hand, moistening time shows a slight curvature that becomes more important in the way that ethanol concentration diminishes. By this way, the optimal value predicted by the model (0.8408%) is reached when the 55.86% of ethanol concentration and 93.44 minutes of moistening time are combined. As well as flavonoid compounds, this variable deserves a wider range of ethanol concentration analysis, but as we declared before, it must be realized only when a particular study about tamarind inorganic substance is going to be considered.

In general sense and attending to experimental results obtained for each one of response variables analyzed, becomes evident the fact that the extraction out of every type of metabolite demand of particular conditions (ethanol concentration and moistening times) to achieve its bigger extraction. Still when for all of the response factors the tendency is the need of elevated moistening times, for TA and especially for TP, excessive moistening times disfavor the extraction process of suchlike metabolites. On the other hand, ethanol concentration variable seems to be critical, because of the ethanol concentration necessary to maximize the extraction of TF and TA constitute the extreme values considered in the experiment for the aforementioned response variables (84.14% for TF and 55.86% for TA).

For this reason, acquire relevance the use and interpretation of the “desirability function”, (Montgomery, 1984) as a way for a process optimization, which is able to model the complex interactions among the independent variables in order to maximizing the outcomes values of the dependent variables.

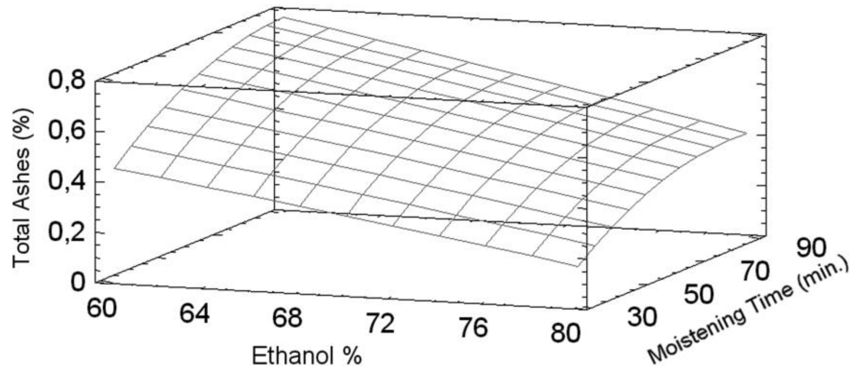

Figure 4.
Response surface plot estimated for total ashes in the experimental design

Process optimization.

In this function, the procedure transforms the four outcome variables into desirability scores that could range from 0.0 for undesirable to 1.0 for total desirability. The obtained value of the desirability function was 0.8377. Since this value is close to the unit, the function seems to predict with a good precision the interactions between the independent variables in the extraction process. This desirability value is reached when 71.73 percent of ethanol and 92.37 minutes of moistening time is combining. Table 4 show the maximum experimental values achieve in the 10 experimental runs for each dependent variable individually and the predicted values by the desirability function. In this case, only TA is distant of the maximum observed value, the rest of the predictions for each factor are close to their maximum values, being an indicative of good prediction of the desirability function.

The suggested ethanol concentration (71.73), occupies a medium position between the opposite extreme values suggested for TF and TA, and at the same time this ethanol concentration is relatively close to those defined as optimal for TSS and TP. Also it is close to the central point defined in the design. On the other hand, the optimal moistening time, is around 92 minutes, close to the +1 variable level and in accordance to the tendency (for the four response variables) to maximize the extraction when relative high values of moistening times are considered.

The response surface graphic (Figure 5) shows how when ethanol concentration is taken into account, lower moistening times have less influence in the curvature around 70% than when higher times are considered. This is an important evidence of the interaction that is established between both independent variables considered in the modeled extraction process, and how they can affect the metabolites extraction procedure.

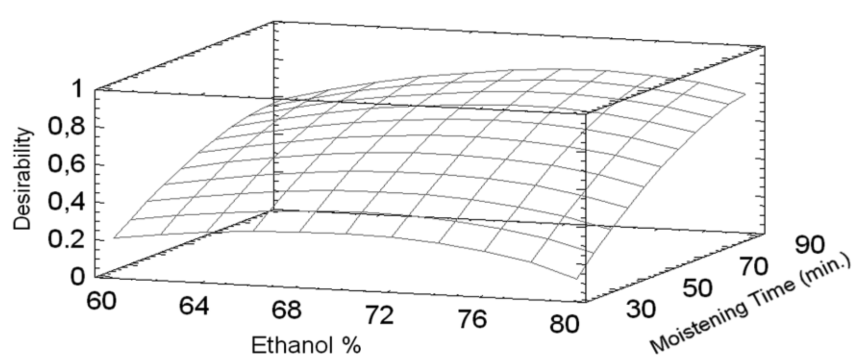

Figure 5.

Response surface plot estimated for the desirability function in the experimental design employed to optimize the metabolites extraction process in *Tamarindus indica* L. leaves.

Results validation.

The mean values obtained once the optimal conditions were considered (72% of ethanol concentration and 92 minutes of moistening time) for each dependent variable are displayed also in Table 4. Three of the dependent variables exhibit no statistically significant differences related to the predicted values when the optimal conditions described by the desirability function were fixed. Total flavonoid content variable fulfill this condition but only when statistical probability is increased to 99 %. This performance it's also revealed when considers the absolute error. While than the TP, TSS and TA variables have an absolute error under 1,5%, for TF this parameter reach it values close to 11%. This behavior can be explained applying the inferences deduced from TF model: flavonoid extraction has a great dependence to high ethanol concentration, and this point (72%) is too far from those defined as optimal in model 2 (84,14%). Nevertheless, and according to the results in this experiment obtained, we consider that the optimal conditions predicted by the design used describe with an acceptable precision the tamarind leaves metabolites extraction process.

Table 4. Observed and predicted values in the optimization and validation (n=5) of the metabolites extraction process in *Tamarindus indica* L. leaves.

Dependent variable	Highest experimental value	Predicted value by desirability function	Validation observed value (S. deviation)
TP (mg/mL)	26,070	25,972	25,687 (±0,337)
TF (mg/mL)	3,001	2,678	2,409 (±0,141)[a]
TSS (%)	19,270	18,417	18,158 (±0,926)
TA (%)	0,810	0,566	0,574 (±0,034)

[a] p-Valor = 0,01299

CONCLUSIONS

Tamarind leaf extracts have been reported as curatives in many parts of the world. Phenols and flavonoids metabolites have been suggested as the most important compounds in order to justify their main ethnobotanical uses, but recent papers give active participation on the pharmacological activities of the tamarind leaves to other types of metabolites. By this way, this central composite 2^3 full factorial design was aimed to optimize the effect of two factors (ethanol concentration and moistening time) on the dependent variables related to the chemical composition of tamarind leaves. The theoretic and practical results presented in this paper confirm that it's possible to arrive at a good combination of these factors in which a balance of the chemical composition variables taken into account is reached. By this way, this study offers a combination of variables when it is intended to prepare a tamarind extract that afford a high extractive capacity and, as a consequence, a larger probability to exhibit some kind of pharmacologic activity.

REFERENCES

Andersen ØM, Markham KR (Edit). 2006. **Flavonoids, Chemistry, Biochemistry and applications.** Pp 13-24. Boca Raton, FL, USA: CRC Press.

AOAC. 1990. **Official methods of analysis.** 15th ed. Washington, DC. Association of Official Analytical Chemists.

Cervantes-Cardoza, V, Rocha-Guzmán NE, Gallegos-Infante JA, Rosales-Castro M, Medina-Torres L, González-Laredo RF. 2010. Actividad antioxidante de extractos de semilla de tres variedades de manzana (*Malus domestica* Borkh -Rosaceae-). **Bol Latinoam Caribe Plant Med Aromat** 9: 446 - 456.

Chitra A. 1999. Post Infectional Changes in Flavonoids and Phenolic Acids by Fungal Pathogens in *Tamarindus indica* L. **Res J Chem Envirom** 3: 305 - 317.

Dehesa MA, Jauregui O, Cañigueral S. 2006. Estudio por HPLC-MS/MS de compuestos fenólicos presentes en las hojas de *Tamarindus indica* L. **Revista de Fitoterapia** 6: 79 - 82.

Dehesa MA, Escalona-Arranz JC, Padilla S. 1995. Evaluación Preclínica de Extractos Flavonólicos de las hojas de *Tamarindus indica* L. **Etnomedicina** 1: 157 - 168.

Dubois M, Gilles KA, Hamilton JK, Rebber PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. **Anal Chem** 28: 350 - 351.

Duke JA. 1981. **Handbook of legumes of world economic importance.** Pp 228 - 230, New York, USA. Plenum Press.

El-Siddig K, Gunasena HPM, Prasad BA, Pushpakumara DK, Ramana KVR, Vijayanand P, Williams JT. 2006. **Tamarind, *Tamarindus indica*.** Pp 13 - 27, Southampton, U.K: Southampton Centre for Underutilized Crops.

Escalona-Arranz JC, Péres-Rosés R, Urdaneta-Lafitta I, Camacho-Pozo MI, Rodríguez-Amado J, Licea-Jimenez I. 2010. Antimicrobial activity of extracts from *Tamarindus indica* L. leaves. **Pharmacog Mag** 6: 242 - 247.

Gabrielson J, Lindberg N, Lundtedt T. 2002. Multivariate methods in pharmaceutical applications. **J Chemiometric** 16: 141 – 160

García-Rodríguez RV, Zavala-Sanchez MA, Susunaga-Notario AC, Pérez-Gutierrez S. 2011. Anti-inflammatory evaluation and antioxidant potential of Senna crotalarioides and Penstemon roseus **Bol Latinoam Caribe Plant Med Aromat** 10: 23 - 29.

Imam S, Azhar I, Mohtasheemul MH, Ali MS, Waseemuddin A. 2007. Two triterpenes lupanone and lupeol isolated and identified from *Tamarindus indica* Linn. **Pak. J Pharm Sci** 20: 125 - 127.

Ishola MM, Agbaji EB, Agbaji AS. 1990. A chemical study of *Tamarindus indica* (Tsamiya) fruits grown in Nigeria. **J Sci Food Agric** 51: 141 - 143.

Joyeux M, Mortier F, Fleurentin J. 1995. Screening of antiradical, antilipoperoxidant and hepato-protective effects of nine Plant Extracts used in Caribbean Folk Medicine. **Phytoter Res** 9: 228 - 230.

Lans C. 2007. Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine. **J Ethnobiol Ethnomed** 3: 102 - 109.

Leakey R. 1999. Potential for novel food products from agroforestry trees: A review. **Food Chem** 66: 1 - 14.

Meléndez PA, Carriles VA. 2006. Antibacterial properties of tropical plants from Puerto Rico. **Phytomedicine** 13: 272 - 276.

Mongomery DC. 1984. **Design and analysis of experiments.** Pp 367, John Willey. NY. USA.

Ohene-Afoakwa E, Simpson-Budu A, Bullock-Merson A. 2007. Response surface methodology for studying the effect of processing conditions on some nutritional and textural properties of bambara groundnuts (*Voandzei subterranea*) during canning. **Int J Food Sci Nutr** 58: 270 - 281

Pino JA, Escalona-Arranz JC, Licea I, Pérez R, Agüero J. 2002. Leaf oil of *Tamarindus indica* L. **J Essent Oil Res** 14: 187 - 188.

Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, Cazin JC, Bailleul F, Trotin F. 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and four. **J Ethnopharmacol** 72: 34 - 42.

Ricco RA, Wagner ML, Portmann E, Reidss C, Llesuy S, Gurni AA, Carballo MA. 2010. Análisis de polifenoles, actividad antioxidante y genotoxicidad en especies argentinas de *Lippia* y *Aloysia* (Verbenaceae). Bol **Latinoam Caribe Plant Med Aromat** 9: 388 - 396.

Ricco RA, Wagner ML, Gurni AA. 2011. Dinámica de polifenoles de "Cedrón" (Aloysia citrodora Palau Verbenaceae-) en relación al desarrollo foliar. **Bol Latinoam Caribe Plant Med Aromat** 10: 67 - 74.

Shankar EM, Subhadra N, Usha AR. 2005. The effect of methanolic extract of *Tamarindus indica* Linn. on the growth of clinical isolates of *Burkholderia pseudomallei*. **Ind J Med Res** 122: 525 - 528.

Shankaracharya NB. 1998. Tamarind: Chemistry, technology and uses - A critical appraisal. **J Food Sci Tech** 35: 193 - 208.

Vipul VD, Sunil AN, Nachiket SD, Shashikant RP. 2009. An overview of *Tamarindus indica* Linn: Chemistry and pharmacological profile. **Pharmacology online** 3: 809 - 820.

Vogel H, Razmilic-Bonilla I, Polanco-González X, Letelier-Muñoz ME. 2010. Effect of different provenances and production conditions on antioxidant properties in Buddleja globosa leaves. **Bol Latinoam Caribe Plant Med Aromat** 9: 333 - 342.