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Standardization of extracts from *Momordica charantia* L. *(Cucurbitaceae)* by Total Flavonoids Content Determination

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SUMMARY. The quality of vegetable extracts is influenced by parameters such as: extraction method, extracting liquid, vegetable matter granulometry and plant:solvent ratio. The standardization of these parameters in obtaining extracts is of great importance in order to guarantee their quality. The spectrophotometric trial based on the formation of complexes with aluminum chloride was applied to evaluate the flavonoids contents of *Momordica charantia* L., which showed significant differences between the extraction methods, extracting liquids and plant:solvent ratios. The extractive solution which presented the greatest level of flavonoids was prepared by maceration, with ethanol 70º GL, vegetable matter granulometry up to 710 µm and plant:solvent ratio 1.5:10.

RESUMEN. "Estandarización de extractos de *Momordica charantia* L. *(Cucurbitaceae)* por medio de la determinación del contenido de flavonoides totales". La calidad de los extractos vegetales es influenciada por parámetros tales como el método de extracción, el líquido extractor, granulometría del material vegetal y proporción planta-solvente. La uniformidad de esos parámetros en la obtención de extractos es de gran importancia para garantizar la calidad de los mismos. El ensayo espectrofotometrico basado en la formación de complejos con el cloruro de aluminio fue aplicado en la dosificación de flavonoides de *Momordica charantia* L., lo cual evidenció diferencias significativas entre los métodos de extracción, líquidos extractores e proporciones planta-solvente. La solución extractiva que presentó el mayor tenor de flavonoides fue preparada por maceración, con etanol 70º GL, granulometría del material vegetal de hasta 710 µm y en la proporción planta-solvente 1,5:10.

INTRODUCTION

Momordica charantia L. *(Cucurbitaceae)* is a creeping plant native of Asia and found throughout the world. It has numerous uses in popular folk medicine. Its leaves and roots serve as anti-rheumatic, anti-inflammatory, antiseptic and anti-diabetic remedies in Brazil [1,2]. In Guatemala, Caribe, Japan and India it has been used in inflammation, diabetes and stomach problems [3] .

Due to the climatic variations it arises the necessity of validation of quality control technics in order to characterize and quantify the most important compounds of the plant or the active substances. This makes possible the standardization of the vegetable specie, intermediate product as well as phytomedicine with a concentration of active substances within a straight interval, which may be used as a reference of quality by the industry [4].

Moreover, for the development of a phytopharmaceutical product which meets all legal criteria, the standardization of extractive solutions represents an indispensable step [5,6].

The technological transformation of vegetable drugs is required in the use of extraction operations in order to remove the substances or active fractions of interest in these vegetable drugs, using a solvent or a mixture of solvents which are both technologically appropriate and toxicologically safe [7].

The lack of studies concerning standardization of extractive solutions from medicinal plants has caused *Momordica charantia* L., as

KEY WORDS: *Momordica charantia* L., Phytopharmaceutical , Standardization, Total Flavonoids.
PALABRAS CLAVE: Estandarización, Flavonoides totales, *Momordica charantia* L., Fitoterápico.

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well as a lot of Brazilian plants, to be submitted to extractive processes which could compromise quality, yield, efficiency and even the safety of the intended usage [8,9].

The main factors which affect efficiency of the extraction process are related to the vegetable drug (quantity, granulometry), extracting solvent or mixture of solvents (selectivity or quantity) and extraction conditions (plant:solvent ratio, agitation, extraction time and temperature) [10].

The total flavonoids contents in extractive solutions represents an important parameter in evaluating the efficiency of the extractive process, and is frequently based on the complexation of the flavonoids with aluminum chloride [11].

The objective of the present study is to select the best extractive process conditions, evaluating the influence of the extraction method, extracting solvent, granulometry of the vegetable drug and plant:solvent ratio, on the total flavonoids contents found in *Momordica charantia* L. extractive solutions, as a first step in the development of a phytomedicine according to the brazilian law [12].

MATERIAL AND METHODS

Vegetable Drug

The leaves were dried in an oven at 45 ± 1 °C, for 7 days. The dry matter was ground in a Multpratic FAET food processor and strained through cloth with openings of 1000; 710; 500; 210; 125 and 88 µm. This was performed at 60 vibrations per second for 15 minutes, using a straining device (BERFEL).

Extractive solutions preparation

The vegetable drug was classified under two granulometric categories: Fraction I (ø < 210 µm) and Fraction II (210 < ø < 710 µm) and extracted by maceration and percolation, with 1000 mL of distilled water and/or ethanol at 70° GL, at plant:solvent ratios: 0.5:10; 1.0:10 and 1.5:10 (w/v), origining 24 extractive solutions. The macerate was prepared at room temperature and sheltered from light, for seven days, with daily agitation. The percolate was also prepared at room temperature and sheltered from light, with dripping speed of 45 drops per minute. Figure 1 shows the process used in the preparation of the extractive solutions.

Determination of Flavonoid Content [13]

A quantity of 15.0 g. of each extractive solution prepared as described above was weighed

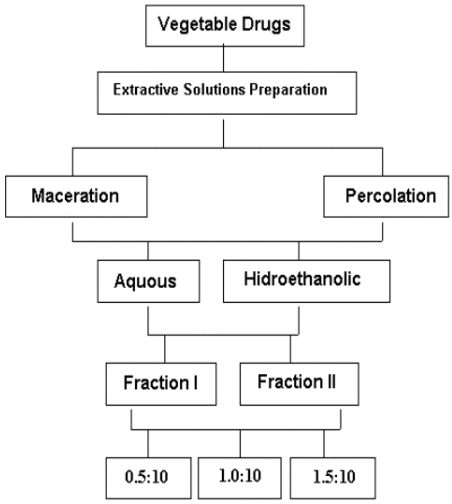

Figure 1. Scheme for the preparation of the *Momordica charantia* L. extractive solutions.

and transferred to a 100.0 mL round-bottomed flask, adding 1.0 mL of 0.5% urotropine solution (Quimibrás, Brasil), 20.0 mL of acetone R (QEEL, Brasil) and 2.0 mL of hydrochoric R acid (Quimex, Brasil) and warmed on a heating pad (Quimis® Q-321A), remaining under reflux for 30 minutes. Two 20.0 mL portions of acetone were added, under reflux, for 10 minutes. After cooling to room temperature, the solutions were filtered through cotton, to a volumetric flask, and filled with acetone (Mother Solution- MS). In a separation funnel were added 40.0 mL of acetone mother solution (MS), 20.0 mL of distilled water and extracted with 15.0 mL of ethyl acetate R (Quimibras, Brasil). The procedure was repeated 3X 10.0 mL. The fractions of ethyl acetate were pooled and washed in a separation funnel with two 50.0 mL portions of distilled water and placed in a 50.0 mL volumetric flask, and filled with ethyl acetate (Stock Solution- SS).

An aliquot of 2.0 mL of aluminum chloride 2% (CPQ, Brasil) was added to 10.0 mL of ethyl acetate SS, diluted to 25.0 mL with acetic acid 0,5% methanolic solution (v/v) (Sample Solution- SoS). At the same time, 10.0 mL of ethyl acetate SS were diluted to 25 mL with acetic acid 0.5% (v/v) (Comparative Solution- CS). The spectrophotometer analysis was carried out in an Ultraviolet/visible equipment (CARY 1E-VARIAN) at 425 nm after 30 minutes. The results were expressed in percentage of total flavonoids, calculated as quercetin, from the average of six determinations, using the equation below:

$$C = \frac{A.DF}{A^{1\%}.m.(100 - t)}$$

where: A = absorbance read; m = mass of the extracted solution in grams; DF = dilution factor (31250); $A^{1\%}$ = specific absorption of $AlCl_3$-quercetin (500) complex; t = drying loss.

Variance Analysis (ANOVA) and Multivaried Variance Analysis (MANOVA) were used for statistical analysis, followed by Tukey and Ducan tests, allowing for multiple comparisons among the levels of the factors which were considered different in the Variance Analysis [14].

RESULTS AND DISCUSSION

Table 1 shows significant differences between extraction methods (maceration and percolation), between the solvents (distilled water and ethanol 70 °GL) and among the plant:solvent ratios, as well as in the interactions between the solvents and the remaining factors, which suggests that the correct use of these factors can cause significant influence on the yield of flavonoid extraction.

It can be observed in Figures 2 and 3 that the utilization of ethanol at 70° GL leads to an increase in the total flavonoids contents, which can be attributed to the difference of the dielectric constant of the solvents, resulting in a greater extraction capacity of ethanol at 70° GL [15,16].

The granulometry of the vegetable drug is considered to be one of the determining factors for the homogeneity and reproductibility of the extractive process. In comparison with the entire drug, the grated or ground matter obviously presents a greater contact surface with the extracting liquid, besides possessing a larger proportion of cells whose walls are ruptured, providing greater exposure of the cellular contents to the solvent [10].

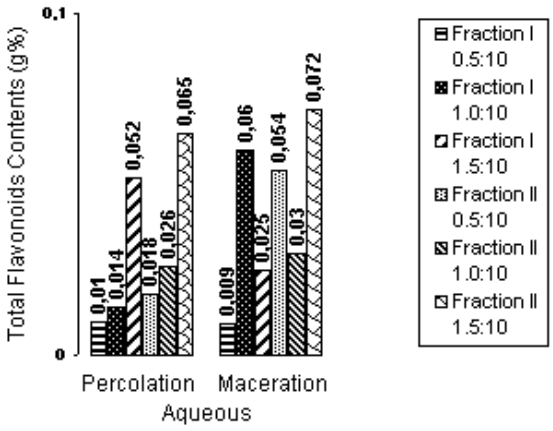

Figure 2. Total flavonoids contents in aquous extractive solutions of *Momordica charantia* L.

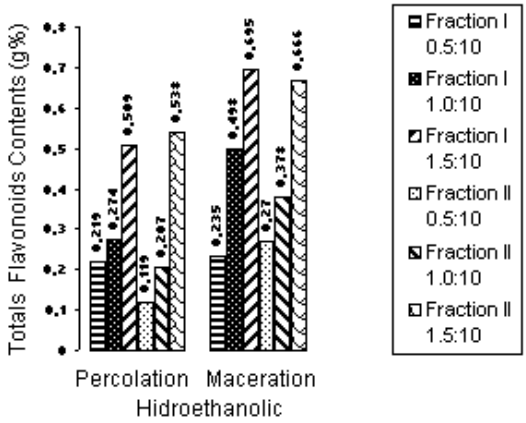

Figure 3. Total flavonoids contents in hidroethanolic extractive solutions of *Momordica charantia* L.

Fractions I and II, analyzed separately, were not significantly different in relation to the flavonoids contents (Table 1). The same was observed when the effect of the interaction of this factor with the extraction methods was eval-

Sources of Variation	Degrees of Liberty	Average Square	F
extraction method	1	0.2247	103.41*
solvent	1	4.3450	1999.96*
granulometry	1	0.0064	2.94
plant:solvent ratio	2	0.5578	256.76**
extraction method x solvent	1	0.1639	75.42*
extraction method x granulometry	1	0.0006	0.27
solvent x granulometry	1	0.0292	13.45*
extraction method x plant:solvent ratio	2	0.0113	5.18**
solvent x plant:solvent ratio	2	0.4030	185.49**
granulometry x plant:solvent ratio	2	0.0136	6.27**
Erro	129	0.0022	

Table 1. Analysis of Multivaried Variance for the total flavonoid dosage in extractive solutions of *Momordica charantia* L. *Significant for Fcrit = 3.91 (0.05;1:α), ** Significant for F_{crit} = 3.07 (0.05;2:α).

Sources of Variation	Degrees of Liberty	Average Square	F
extraction method	1	0.2247	5.2999*
granulometry	1	0.0064	0.1507
plant:solvent ratio	2	0.5578	13.1594**
extraction method x granulometry	1	0.0006	0.0140
extraction method x plant:solvent ratio	2	0.0113	0.2657
granulometry x plant:solvent ratio	2	0.0136	0.3212
extraction method x granulometry x plant:solvent ratio	2	0.0144	0.3407
Error	132	0.0424	

Table 2. Analysis of Multivaried Variance for ethanol solvent at 70° GL with relation to extraction method, granulometry and plant:solvent ratio. *Significant for Fcrit = 3.91 (0.05;1:α), ** Significant for F_{crit} = 3.07 (0.05;2:α).

	Maceration 0.5:10	Maceration 1.0:10	Maceration 1.5:10	Percolation 0.5:10	Percolation 1.0:10	Percolation 1.5;10
Averages	0.14213	0.24135	0.36575	0.09160	0.12994	0.29069
Maceration 0.5:10		0.08963	0.00026	0.41934	0.83480	0.01497
Maceration 1.0:10	0.08963		0.04304	0.01729	0.07100	0.39863
Maceration 1.5:10	0.00026	0.04304		0.00001	0.00013	0.19916
Percolation 0.5:10	0.41934	0.01729	0.00001		0.51197	0.00149
Percolation 1.0:10	0.83480	0.07100	0.00013	0.51197		0.01023
Percolation 1.5:10	0.01497	0.39863	0.19916	0.00149	0.01023	

Table 3. Duncan test for ethanol solvent at 70° related to the extractive process and plant:solvent ratio p (α = 5%).

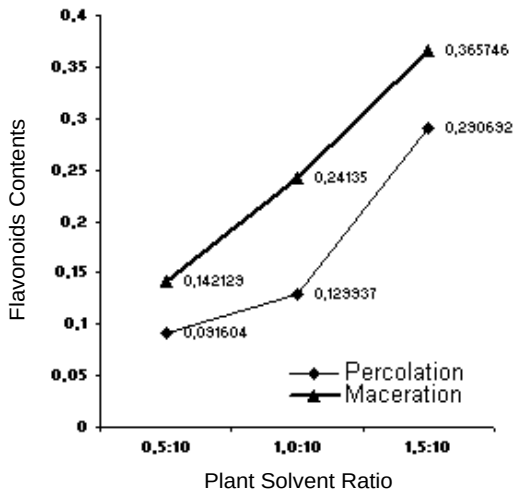

Figure 4. Flavonoids contents as a function of the extractive method and plant:solvent ratio.

uated. However, statistically significant differences were observed in relation to the solvents and the plant:solvent ratios. Thus, taking into consideration only the granulometry factor, particles up to 710 µm should be used, to obtain a good extractive yield.

Concerning to the extraction methods, percolation yielded flavonoid levels below to those obtained from maceration. Despite being a dynamic method, this result can be explained because the use of a limited volume of solvent, which could lead to saturation of the extracting liquid or due to the reduced contact time of the solvent with the vegetable drug, thus prevent its depletion.

The Multivaried Variance Analysis for extracts obtained with ethanol 70° GL, demonstrated that there are significant differences between

the methods of extraction and among the plant: solvent ratios (Table 2).

The total flavonoids contents was directly proportional to the increase in plant:solvent ratio, which benefit the production of extracts richs in flavonoids, which are imputed the pharmacological activity of the plant (Figure 4 and Table 3).

It can be observed that extraction by maceration, at the plant:solvent ratio 1.5:10, led to a greater yield in flavonoid extraction, with significant differences in relation to the other combinations, except for the percolation method, at the same plant:solvent ratio.

CONCLUSIONS

The importance of standardization in the preparation of extractive solutions can be observed, with the objective of achieving better yields and guaranteeing quality of the extractive solution.

The extracting solvent was the main factor responsible for variations in total flavonoid content, and the use of ethanol 70° GL gave the best results.

The conditions selected for obtaining an extractive solution with greater yield on flavonoid extraction were: maceration with ethanol 70° GL, granulometry up to 710 µm and plant:solvent ratio 1.5:10. Under these conditions, the selected extractive solution contains 0.666g% of total flavonoids, expressed as quercetin.

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