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Short Communication

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Influence of Extractive Parameters on the Preparation of a Solution from *Psidium guajava* L.

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***SUMMARY*. The aim of this work is evaluate the influence of extractives parameters on tannin content of solutions obtained from leaves of *P. guajava* and the antimicrobial activity of the best extract solution. The type of extraction, drug proportion and alcohol concentration were studied following a factorial design and the responses evaluated were tannin content and dry residue. The tannins content was assayed by spectrophotometric method at 271nm using casein as precipitant agent and the dry residue was determined by gravimetric method. The statistical analysis demonstrated that only the alcohol concentrations have significant influence on tannins content, but on the dry residue both factors (drug and alcohol proportion) were important. In accordance of the results, the best extractive method was decoction on reflux during 15 min using alcohol concentration 50 % (v/v) as solvent and this extract solution shows a promising antimicrobial activity.**

INTRODUCTION

Psidium guajava L. commonly known as guava plant, belongs to the Myrtaceae family, and is native of Tropical America. Its leaves are traditionally used in folk medicine for treating fevers, diarrhea, gastrointestinal disorders and as a tonic [1]. The pharmacological studies confirm its anti-inflammatory and analgesic activity [1]. The anti-diarrheic effect of guava leaf products is related to its content of quercetin. This flavonoid could be responsible for acetylcholine inhibition [2,3]. According to pre clinic studies, no side effects were observed in the use of guava leaf extracts, and the DL50 of aqueous extract was higher than 20 g/kg [4]. Other activities related with this medicinal plant are their high level of antibacterial activity, mainly against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* [5]. Concerning the chemical composition, the main constituents are tannins, flavonoids, triterpens and volatile oils [2,3]. According to researches the tannin content may be responsible for antibacterial and antioxidant activities [6].

However, the lack of studies regarding extractive solutions from medicinal plants prevent that *Psidium guajava* and other vegetable raw materials be submitted to reproductive and effective extractive processes, thus decreasing the quality, effectiveness and safety of the final product [7]. In accordance with the type of the utilized solvent and extractive method, different kinds of substances are extracted and consequently the biological activity may be altered.

The factorial design is a statistic tool used for process optimization in a rapid and economic way, as well as maximization of the final product quality. Besides, the mathematical model enables reliable results [8]. Aiming to obtain a standardized extractive solution, the goal of this work was to evaluate the influence of extractive parameters on the preparation of an extractive solution of *P. guajava*.

KEY WORDS: Psidium guajava, Extractive solution, Tannin, Factorial design, Antimicrobial activity.

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MATERIALS AND METHODS

Plant material

The leaves of *Psidium guajava* were collected in Natal, Rio Grande do Norte, Brazil in August 2004 and authenticated by the Botany Department of The University of Rio Grande de Norte, Brazil.

Raw material treatment

The leaves were dried for seven days on circulating air oven at 40 ± 5 °C temperature. After drying the leaves were knifed by a knife mill (1 mm mesh) and stored in polystyrene bottle. The raw material was characterized by granulometric analysis [9], using sieves of 210, 300, 420, 500, 600, 710 e 1000 µm, and loss of drying by gravimetric method [10].

Extractive solution preparation

Selection of extractive method

Three extractive methods with different proportions of drug were evaluated following an experimental design. In order to evaluate the different extractive methods two factorial designs 2^2 were elaborated. In the first design, the analyzed factors were extractive method (infusion and decoction) and drug proportion (5.0% and 7.5%). In this experiment, the extractive solution was prepared using water as extractive solvent during 15 min of extraction. After the extraction time the solution was cooled, filtered and the volume of water lost during extraction was replenished. In the second design, the analyzed factors were extractive method (maceration and decoction) and drug proportion (5.0% and 7.5%). In this case, the extractive solvent used was ethanol (50% v/v). The decoction was prepared within 15 min of extraction and the maceration within 5 days of extraction. After the extraction the solution was filtered and the lost volume of solvent was then replenished. The factorial designs 2^2 were performed with replication (n = 3) and the dependent variables analyzed was the tannin content of the extractive solutions.

Selection of drug proportion and solvent extraction

A 3^2 factorial design was performed to verify the influence of drug proportion and alcohol concentration on the tannin content and dry residue of the extractive solution (Table 1).

The method of extraction was decoction during 15 min. The experiment was realized with replication (n = 4) on the central point.

Factors	Level	
	Coded	**Natural**
D: Drug proportion % (w/V)	-1	2.5
	0	5.0
	+1	7.5
E: Ethanol concentration % (V/V)	-1	00.0
	0	50.0
	+1	100.0

Table 1. 3^2 factorial design.

Analysis of tannin content

The tannin content analysis was performed considering the capability of precipitation with protein. For determination of total polyphenol (TP), a diluted aliquot of extractive solution (ES) was analyzed on spectrophotometer at 271 nm using water as compensation solution. For determination of non tannin fraction (NTF) an amount of 0.150 g of casein (Merck) was stirred with 10.0 mL of the extractive solution (ES) during one hour. After filtration, the assay proceeded as described for the total polyphenols content determination. The total tannin content (TTC) was expressed as gram of Gallic Acid per 100 g of extractive solution according to the equations [**1**], [**2**] and [**3**]. The results represent the mean of three determinations.

$$TP = \frac{A_1 \cdot DF}{m \cdot A^{1\%}_{1cm}} \quad [\mathbf{1}]$$

$$NTF = \frac{A_2 \cdot DF}{m \cdot A^{1\%}_{1cm}} \quad [\mathbf{2}]$$

$$TTC = TP - NTF \quad [\mathbf{3}]$$

where: TP = total polyphenols (g%); NTF = non tannin fraction (g%); TTC = total tannin content (g%); A = absorbance (A.U.); DF = dilution factor; m = drug weight (g) and $A^{1\%}_{1cm}$ = specific absorption of gallic acid.

Dry residue determination

The dry residue was determined by gravimetric method using an oven at 105 °C [11].

Statistical Analysis

The statistical analysis was performed by STATISTICA® 6.0. The data were analyzed by ANOVA and a quadratic model was tested to determine the relation between the dependent variables (tannin content and dry residue) and independent variables (drug proportion and alcohol concentration) [8].

Antimicrobial Assay

The paper disc diffusion method for antibiotic susceptibility testing was used [12]. Six different standard strains (*Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25922, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* DAUFPE16 e *Candida albicans* ATCC 10231) was used in this assay. Paper discs of 6 mm diameter were prepared with 25 µL of extractive solution of *P. guajava* leaves prepared by decoction using 5.0% (w/v) of drug:solvent and ethanol 50 % (v/v) as solvent and 25 µL of ethanol 50% (v/v) as a control, the paper discs were dried at 37 °C before use. The medium used was Mueller-Hinton Agar (Biobrás-Brazil) and the bacterial broth suspension was streaked evenly on the surface of a medium with a cotton swab. Subsequently the paper discs were placed on the surface of the agar with flamed forceps and gently pressed down to ensure the contact. Plates were incubated at 37 °C overnight. After 24 h of incubation, the inhibition zone diameters were measured with calipers. A reading of more than 6 mm indicated growth inhibition.

RESULTS AND DISCUSSION

The raw material of *Psidium guajava* used in this research presented a particle mean diameter of 476.47 µm and on drying loss of 12.62 %. These proprieties are important for the standardization of the extractive process, since particles mean diameter has an influence on the extraction efficiency and the loss on drying is important for conservation of the raw material [7].

The popular preparation of *Psidium guajava* leaves generally use hot water to obtain the infusion used with medicinal purpose [1]. Therefore, infusion and decoction with water as solvent and two different proportions of drug were selected as extractive methods to evaluate the extractive capability of tannins. Considering this experiment (Figure 1A), the statistical analysis demonstrated that on the tannin content only the extractive method was a significant factor (p = 6.1x10^{-3}), the drug proportion, in this case, was not an important factor (p = 0.72), however there was a significant interaction between these two factors (p = 2.2x10^{-5}). Considering other extractive methods (maceration and decoction) using ethanol 50% as solvent (Figure 1B) the statistical analysis revealed that both factors (extractive method and drug proportion) was significantly important (p < 0.001) and there was significant interactions (p = 0.0002) between the factors.

Figure 1 showed that in both cases the decoction method was more efficient to extract the tannins and depending on the extractive method the increase of the drug proportion seems to decrease the tannin content. This fact could be justified by the saturation of solvent with the increase of drug proportion.

According to these experiments it was demonstrated that decoction was the best tannin extractive method independent of the solvent used. However, the hydroalcoholic solvent seems to be more efficient than water to extract the tannins, since the extractive solution obtained by decoction with ethanol 50% v/v presented a higher tannin content (5.13 ± 0.02 g%) than decoction with water (2.71 ± 0.12 g%).

Thus, the statistical design 3^2 was developed to verify the influence of two factors (drug proportion and alcohol concentration) on the tannin content and the dry residue of the extractive solution.

The statistical analysis showed that on the tannin content only the alcohol concentration (p = 0.00014) was significantly important. The mathematical model proposed (equation [**4**]) was able to describe 98.2% of the experiments (r^2 = 0.98195).

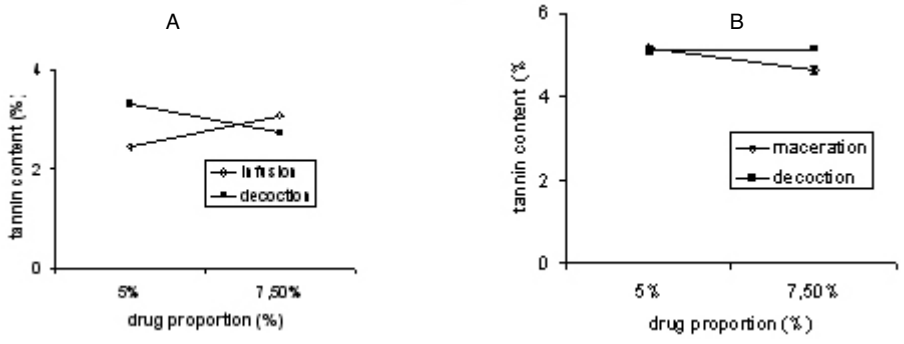

Figure 1. Tannin content in different extractive method, using water (**A**) and ethanol 50% (**B**) as solvent.

$$TT = 4.450 - 0.250 \cdot D + 0.002 \cdot D^2 + 0.0090 \cdot AC^2 + 0.002 \cdot D \cdot AC \quad [4]$$

Where: TT = total tannin (g%) D = drug proportion (g) and AC = Alcohol concentration (%).

From the analysis of response surface it is possible to observe an optimum concentration of alcohol (Fig. 2) with 50%, where there was a maximum extraction of tannin.

Concerning the dry residue both drug proportions ($p < 0.000$) and alcohol concentration were statically significant ($p < 0.000$). The response surface (Fig. 3) showed that the alcohol concentration had a quadratic influence on the dry residue where the optimum concentration was 50% of alcohol. On the other hand, the drug proportion had practically a linear influence where higher drug proportion meant higher dry residue.

The mathematical model proposed (equation [**5**]) was able to describe 97.5% of the experiments ($r^2 = 0.97582$).

$$DR = 0.0515 + 0.2101 \cdot D - 0.0101 \cdot D^2 + 0.0146 \cdot AC - 0.0001 \cdot AC^2 + 0.0006 \cdot D \cdot AC \quad [5]$$

Where: DR = dry residue (g%) D = drug proportion (g) and AC = Alcohol concentration (%).

The statistical analysis indicated that the best conditions to prepare the extractive solution from *P. guajava* leaves is by decoction using alcohol 50% (v/v) as solvent. This solution presented 5.13 g% of tannins and 1.30 g% of dry residue. Therefore, it was selected to analyze the antimicrobial activity.

Table 2 summarizes the results of antimicrobial activity of standardized *P. guajava* leaves extract. The data indicate a complete inhibition for all tested microorganism, except *P. aeruginosa* when no activity was shown. These results are in accordance with the literature that reveals hydroalcoholic extract from *P. guajava* with high antimicrobial activity [13,14].

Microorganism	**Inhibition zone diameter (mm)**
P. aeruginosa ATCC 27853	-
S. aureus ATCC 29213	13
S. aureus ATCC 25922	14
B. cereus ATCC 11778	12
B. subtilis DAUFPE 16	12
C. albicans ATCC 10231	10

Table 2. Antimicrobial activity of *P. guajava* leaves extract in disk zone inhibition.

CONCLUSION

Among the extractive methods studied the decoction presented the greatest efficiency. The solvent type was the factor that presented the most significant influence on the tannin content and dry residue of extractive solution from *P. guajava* leaves. The concentration of alcohol had a quadratic influence on the tannin content in the P. guajava extract and the maximum extractions occurred when using ethanol 50% (v/v), independent of drug proportion. Thus, the standardized conditions to guajava leaves were 5% (w/v) of vegetal drug and alcohol 50% (v/v) as solvent by decoction during 15 minutes. This extractive solution presented a promising antimicrobial activity.

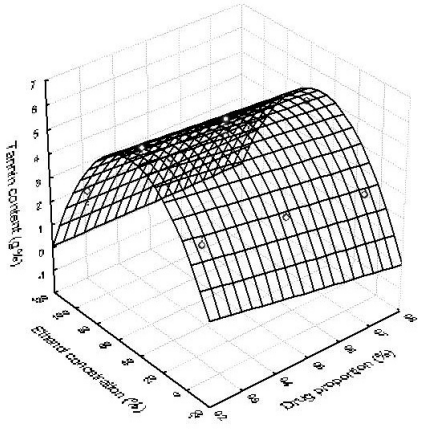

Figure 2. Response surface to tannin content.

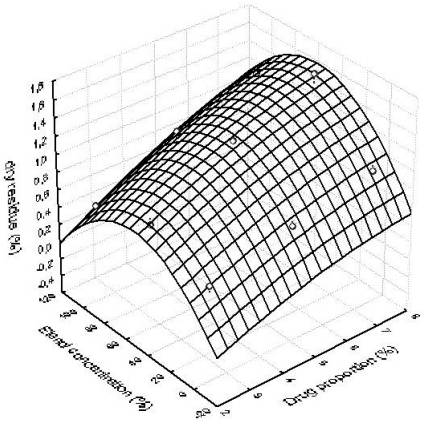

Figure 3. Response surface to dry residue.

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REFERENCES

1. Olajide, O.A., S.O. Awe & J.M. Makinde (1999) *Fitoterapia* 70: 25-31.
2. Lutterodt, G.D. (1989) *J. Ethnopharm.* **25**: 235-47.
3. Re, L., S. Barocci, C. Capitani, C. Vivani, M. Ricci, L. Rinaldi, G. Paolucci, A. Scarpantonio, O.S. León-Fernández & M.A. Morales (1999) *Pharm. Res.* **39**: 239-45.
4. Jaiarj, P., P. Khoohaswan, Y. Wongkrajang, P. Peungvicha, P. Suriyawong, S. Saraya, & O. Ruangsomboon (1999) *J. Ethnopharm.* **67**: 203–12.
5. Abdelramin, S.I., A.Z. Almagboul, M.E.A. Orner & A. Elegami (2002) *Fitoterapia* **73**: 731-735.
6. He, Q. & N. Venant (2004) *Jzus.* **5**: 676-83.
7. List, P.H. & P.C. Schimdt (1989) *"Phytopharmaceutical Technology".* Boca Raton, New York.
8. Montgomery, D.C (1991) *"Diseño y análisis de experimentos".* Iberoamérica, México.
9. Voigt, R (2005) "Pharmazeutische Technologie". 10. überarb. Aufl., Ullstein Mosby, Berlin.
10. The United States Pharmacopeia (2000) 24. rev. ed., Rockville: United States Pharmacopeial Convention, Easton: Mack.
11. Hartke, K. & E. Mutschler (1987) *"Deutsches Arzneibuch-9 Kommentar".* Wissenschaftliche, Stuttgart.
12. Bauer, A.W., E. Kirby, E.M. Sherris & M. Turk (1966) *Am J Clin Path.* **45**: 493-6.
13. Carvalho, A.A.T., M.C.C. Sampaio, F.C. Sampaio, A.F.M. Melo, K.X.F.R. Sena, A.A. Chiappeta & J.S. Higino (2002) *Acta Farm. Bonaerense* **21**: 255-8.
14. Sanches, N.R, D.A.G. Cortez, M.S. Schiavini, C.V. Nakamura & B.P.D. Filho (2005) *Braz Arch Biol Tech.* **48**: 429-36.