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Impact of different extraction methods on the quality of *Dipteryx alata* extracts

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Article

Abstract: This study aimed to impact of different extraction methods on the quality of *Dipteryx alata* Vogel, Fabaceae, extracts from fruits. The major compounds found were the lipids 38.9% (w/w) and proteins 26.20% (w/w). The residual moisture was 7.20% (w/w), total fiber 14.50% (w/w), minerals 4.10% (w/w) and carbohydrate 9.10 % (w/w). The species studied has great potential in producing oil, but the content and type of fatty acids obtained is dependent on the method of extraction. The Bligh & Dyer method was more selective for unsaturated fatty acids and Shoxlet method was more selective for saturated fatty acids. The tannin extraction by ultrasound (33.70 % w/w) was 13.90% more efficient than extraction by decoction (29 % w/w).

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Introduction

Medicinal plants are recognized as important sources of novel biomolecules and in recent years, there has been an increasing interest in its use for the prevention and treatment of various diseases (Heinrich & Gibbons, 2001). However, the lack of quality is a problem that is overshadowing the potential benefits of various herbal products (Hinneburg & Neubert, 2005; Hui 2002; Wang et al., 2003).

Actually regulatory agencies as FDA (Food and Drug Administration) and Anvisa (Brazilian Health Surveillance Agency) require that herbal extracts are standardized to ensure safety and efficacy of medicines (Brazilian Pharmacopoea, 2010; FDA, 2010).

The extraction is an important step because the choice of extraction conditions may determine the quality and the yield of the constituents (Hinneburg & Neubert, 2005). Selection of an appropriate extraction method will depend upon the kind of herb and also the compounds required (Wang et al., 2003).

The study of new herbal species may lead the inappropriate choice of extraction methods, which is responsible for many analysis mistakes. The use of more than one extraction method is required to determine the phytochemical profile of a plant. However, any extraction methods have chemical or physical limitations. Thus, the choice of the appropriate extraction method for each kind compounds must be considered (Cechinel-Filho & Yunes, 1998).

Conventional extraction methods such as heating, boiling or refluxing can be made use for extraction of natural products; the disadvantages are the loss of substances of interest due to hydrolysis, oxidation and ionization during the extractive process. In recent years, some new extraction methods have been employed for the extraction of natural products from herbals, including ultrasound-assisted extraction (Zhang et al., 2011) microwave-assisted extraction (Hayat et al., 2009) supercritical fluid extraction (Oliveira et al., 2009) and accelerate solvent extraction (Wang & Lan, 2011). Through shear forces created by ultrasonic cavitation, cell walls are broken mechanically, improving the material transfer. Besides, there is no chemical relationship in the ultrasound-assisted extraction, which can possible chemical degradation in metabolites of interest (Wang & Weller, 2006).

During the extraction of compounds of interest, several factors must be considered, such as particle size, solvent polarity, acidity of de medium, agitation system, method of extraction, temperature and contact time (Soares et al., 1998).

The evaluation of the influence of several factors simultaneously is hard, when not using the correct techniques. Currently several statistical models have been employed to solve this problem, among them the Box-Behnken design and Response Surface Methodology (RSM), allow for the studies on factors and its multiple interactions, and is getting highlights for biological system studies (Freitas et al., 2012; Moreira et al., 2007; Wang & Lan, 2011; Weuster-Botz, 2000; Zafar et al., 2010).

In this sense, the present study aimed to evaluate the influence of the extraction method over the quality of extracts of fruit pulp from *Dipteryx alata* Vogel, Fabaceae, extracts. *D. alata*, popularly known as "baru" or "combaru", have great interest by the population, due many properties such as: cicatrizing, anti-rheumatic, tonic, regulating menstruation and antiophidian (Ferraz et al., 2012; Nazato et al., 2010; Puebla et al., 2010). Besides, the seeds are rich in saturated fatty acid and its pulp contains large amount of tannins (Almeida, 1998).

Material and Methods

Plant material

Fruits of *Dipteryx alata* Vogel, Fabaceae, were collected in Caldazinha, Goiás, Brazil (16° 42' 50" S 49° 00' 07"). The species was identified by Dr. José Realino de Paula and a voucher was deposited in the Herbarium of the Universidade Federal de Goiás under code number 27.806. The pulp (obtained from the pericarp) and the almonds of baru were pulverized in a knife mill and stored in sealed vials.

Chemical characterization of almonds Dipteryx alata

Determinations of residual moisture, proteins, lipids, total fiber, ash and carbohydrate were performed in triplicate samples according AOAC (1998). Extraction of fatty acids were carried out by two different methods: i) by Shoxlet, and ii) Bligh & Dyer (1959).

Fatty acids were analyzed on Gas chromatography (GC-MS) model Varian 3900 A, fitted with a capillary column CP WAX 30 m and was used under the following conditions: carrier gas, nitrogen with a flow rate of 2.5 µL.min^{-1}; column temperature, 5 µmin hold for 90 °C, 90 to 250 °C at 5 °C.min^{-1}, 30 µmin hold at 280 °C; injector temperature, 240 °C; volume injected, 2 µL. The MS operating parameters were as follows: ionization potential, 70 µeV; ion source temperature, 280 °C; quadrupole 100 °C, speed 2000µ amu/s. All the samples used for method development and validation were prepared volumetrically.

Extraction of tannins

Ultrasound-assisted extraction (UAE) was performed in an ultrasonic cleaner (USC-4800, 50 kHz, 200 W Instrument Company, Brazil). All chemical reagents used in experiments were of analytical grade. The ethanol/water volume ratios used for extraction were 5, 50 and 95%. Dried and ground fruit pulp of baru was submitted to UAE from 5 to 15 min. Particle sizes of the powder were 150, 250 and 350 mesh.

Tannin contents were quantified by protein precipitation using the Hagerman & Butler (1978) adapted by Waterman & Mole (1987). This method uses Bovine Serum Albumine (BSA) solution 1 mg/mL in 0.2 M acetate buffer, and pH 4.9. After extraction, the resulting solutions were complexed with BSA and centrifuged. The precipitate was then dissolved in a solution of sodium dodecyl sulfate/triethanolamine. The tannins reacted with ferric chloride ($FeCl_3$), the absorbance was read at 510 nm. The calibration curve was prepared with tannic acid (Vetec) at the dilutions: 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. The correlation coefficient calculated for this curve was 0.9959.

The UEA was compared with the extraction method proposed in the Brazilian Pharmacopoeia (2010).

Experimental design

UEA of the tannin contents of the *D. alata* fruit pulp were performed as prescribed by the Box-Behnken design shown in Table 1. Three extraction characteristics (or factors, in statistical terminology) were investigated at three levels: i) extraction times of 5, 10, and 15 min, ii) particle sizes of 150, 250, and 350 µm, and iii) ethanol/water volume ratios of 5, 50, and 95% (v/v). As a result, seventeen runs were obtained by combinations of the three levels of these three factors. The experimental runs were randomized in order to satisfy the statistical requirement of independence of observations.

A second-order polynomial regression model was used to express the yield as a function of the independent variables as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j \quad \text{(Eq 1)}$$

Where y represents response variable (tannin content), β_0 is a constant, β_i, β_{ii}, and β_{ij} are the linear, quadratic and interactive coefficients, respectively. The terms x_i and x_j are the levels of the independent variables. These parameters were extraction time (x_1), particle size (x_2), and ethanol/water volume ratio (x_3). Calculations were carried out using Design-Expert® version 8.0.7.1 (Stat-Ease, Inc. Minneapolis, MN, USA).

In order to verify the predictive capability of the model, optimum conditions were established by Response Surface Methodology (RSM) and comparisons between the predicted results and the practical values were carried out by experimental rechecking using those presumed optimal conditions.

Results and Discussion

In the almonds the majority compounds found were the lipids 38.90% (w/w), and proteins 26.20% (w/w). The residual moisture was 7.20% (w/w), total fiber 14.50% (w/w), minerals 4.10% (w/w) and carbohydrate 9.10 % (w/w).

The lipids yield was influence by extraction method, the Bligh &Dyer (1959) method was able to extract 10.50% more lipids than Shoxlet method (Table 1). Tables 2 and 3 presents the extraction method Bligh &Dyer (1959) was able to enhance the extraction unsaturated fatty acids and decrease the presence of saturated fatty acid.

Table 1. Code and level of factors chosen for the trials.

Factor	Levels		
	-1	0	+1
Extraction time, x_1 (min)	5	10	15
Particle size, x_2 (µm)	150	250	350
Ethanol/water, x_3 %(v/v)	5	50	95

The chemical composition of the oil was also affected by the extraction method (Figures 1 and 2). The majority unsaturated fatty acids were oleic (46.50%) and linoleic (24.20%), while the samples obtained by Blingh & Dyer contained 94.70% more linolenic acid than samples obtained Shoxlet method.

The increase in efficiency extraction is due the characteristics of the extractor solvents. The Shoxlet method uses hexane, and this solvent is more efficient to extraction of apolar acids, whereas methanol and chloroform used in Blingh & Dyer method extrais fatty acids neutral, polar and apolar (Shahidi & Wanasundara, 1998).

The lipids have a wide hydrophobicity range, it is practically impossible to use a single solvent for extracting fatty acids. The neutral lipids form covalently bond and it can be extracted by apolar solvents. The polar lipids form forces electrostatic and hydrogen bonds with proteins, and to breaking this kind of bonds is required polar solvents as methanol (Shahidi & Wanasundara, 1998).

Extraction methods involving heat as Soxhlet can increase the peroxidation and hydrolysis reactions, thus cold extraction methods with Bligh & Dyer are more suited to preserve the lipid profile (Yehuda et al., 2002).

The baru oil like olive oil are rich in unsaturated fatty acids, mainly oleic, linoleic and linolenic acids. In humans, linoleic and linolenic are necessary to maintain the integrity of cell membranes, brain function, transmission of nerve impulses, participate in the transfer of atmospheric oxygen, hemoglobin synthesis and division cell. They are called essential fatty acids because they are not synthesized naturally by human body, then it must be obtained by feed (Yehuda et al., 2002; Youdim et al., 2000).

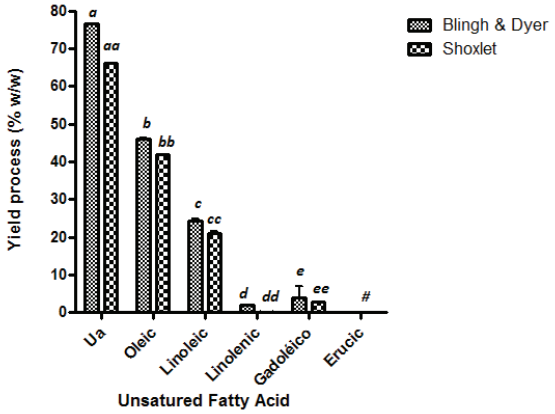

Figure 1. Difference profile of unsaturated fatty acids extracted by Blingh & Dyer and Shoxlet. The experiments were performed in triplicate, and the bars represent the mean±SD. The difference between the averages were calculated by *t-test* (*a, b, c* and *d* with *p* less than 0.05) (# t-test is not applicable because it was not possible to compare the two averages).

The UEA yield (UY %) values are summarized in Table 4 and analysis of variance (ANOVA) in Table 5. This analysis showed that the primary effects X_1, X_2 ,X_3 and second order interaction X_1^2, X_2^2, X_3^2 are significant effect on UY of tannins.

The theoretical optimized conditions calculated by RSM are, 10 min to extraction time, 150 µm to particle size and 42 % of alcohol degree (Figure 1). The theoretical content of tannins obtained in these conditions was 33.0 % (w/w). In rechecking extraction the tannins yield was 33.70±0.02 (% w/w), it confirmed the capacity prediction of RSM (Eq 2). The UY % was 13.90% higher than the tannin content obtained by decoction 29±0.01 (% w/w) according to the Brazilian Pharmacopoeia (2010). This result proves the superiority capacity of tannins extraction by UEA.

Table 2. Profile of unsaturated fatty acids found in almonds *Dipteryx alata*.

EM	Ua % w/w	Oleic	Linoleic	Linolenic	Gadoleic	Erucic
B&D	76.5±0.04	46.5±0.30	24.2±0.70	1.9±0.07	3.9±0.03	0.02±0.001
Sh	66.2±0.01	42.0±0.20	21.1±0.40	0.1±0.06	2.8±0.05	0

EM: Extraction Method; B&D: Blingh & Dyer; Sh: Shoxlet; Ua: unsaturated fatty acids.

Table 3. Profile of saturated fatty acids found in almonds *Dipteryx alata*.

EM	Sa % w/w	Arachidic	Heneicosanoic	Behenic	Lignoceric	Palmitic	Estesric
B&D	23.2±0.1	1.64±0.6	0.01±0.001	4.22±0.1	6.1±0.3	5.42±0.02	5.69±0.1
Sh	33.1±0.9	3.81±0.3	0.01.0±0.01	5.59±0.2	9.3±0.04	7.21±0.01	6.9±0.01

EM: Extraction Method; B&D: Blingh & Dyer; Sh: Shoxlet; Yp: yield process; Ua: unsaturated fatty acids.

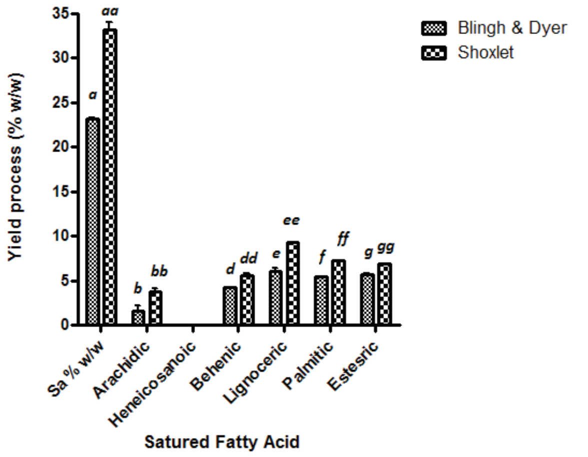

Figure 2. Difference profile of saturated acids (Sa) extracted by Bligh & Dyer and Shoxlet. The experiments were performed in triplicate, and the bars represent the mean±SD. The difference between the averages were calculated by *t-test* (*a, b, d, e, f* and *g* with *p* less than 0.05).

The increasing in extraction by ultrasound-assisted method is attributed to effects of acoustic cavitations produced in the solvent (Waksmundzka-Hajnos et al., 2004; Zhang et al., 2009). The ultrasonic wave also exerts a mechanical effect, allowing greater penetration of solvent into herbal matrix, increasing the contact surface between solid and liquid phase, resulting the solute diffuses from solid phase to the solvent (Zhang & Liu, 2008). Several authors have reported the efficiency of ultrasound-assisted extraction of foods and bioactive compounds (Santos et al., 2010; Zhang & Liu, 2008).

Table 4. 3^3 Box-Behnken factorial design matrices and result of UAE.

Run	x_1 (min)	x_2 (µm)	x_3 % (v/v)	UY % (w/w)
1	10	350	95	13.44
2	15	250	95	21.6
3	5	250	5	22.6
4	10	250	50	32.64
5	10	250	50	29.59
6	10	250	50	30.76
7	15	350	50	19.9
8	10	250	50	29.71
9	15	150	50	30.45
10	10	150	95	25.19
11	5	350	50	15.85
12	15	250	5	27.06
13	5	250	95	14.91
14	10	350	5	15.38
15	10	250	50	30.71
16	5	150	50	29.28
17	10	150	5	29.12

Table 5. Summary of factor effects and significances (p) ANOVA.

Source	*SQ*	*df*	*F*-Value	*p*-value Prob>F
Model	671.7	9	30.2	< 0.0001
X_1	33.4	1	13.5	0.0078
X_2	305.9	1	123.8	< 0.0001
X3	45.2	1	18.3	0.0037
$X_1 \cdot X_2$	2.0	1	0.83	0.3899
$X_1 \cdot X_3$	1.2	1	0.50	0.5009
$X_2 \cdot X_3$	0.9	1	0.40	0.5467
X_1^2	38.5	1	15.6	0.0055
X_2^2	60.3	1	24.4	0.0017
X_3^2	157.3	1	63.7	< 0.0001
Residual	17.2	7	-	-
Lack of Fit	11.3	3	2.52	0.1965
Pure Error	5.9	4	-	-

SQ: Sum of Squares; *df* :Degrees of freedom.

In the extraction processes there are multiple independent variables interacting with responding factors. It is important optimization studies for cost reduction, process time, energy, raw materials and therefore environmental impacts. The results showed that in the tannin extraction from baru pulp is possible obtain increasing tannins yield and savings of 58% of ethanol (Figure 3).

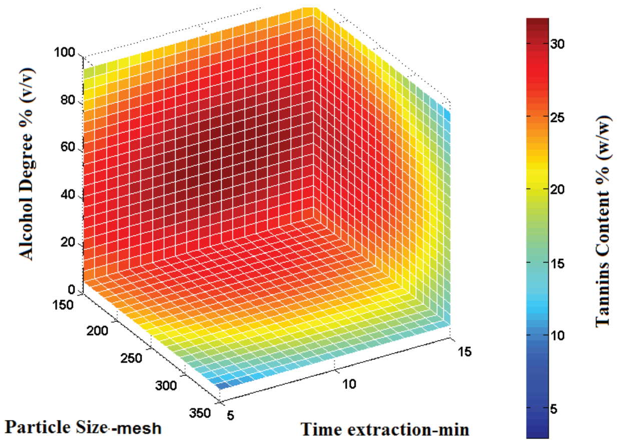

Figure 3. Response surface for the UAE of tannins content from *Dipteryx alata.*

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Authors contributions

FSM contributed in drafting of the article, analysis of fatty acid almonds and interpretation of results; LLB contributed to the optimization of extraction process of tannins and interpretatiing the results; JRP in the identification of the plant material and the evaluation of the chemical composition of almonds. ECC contributed in the interpretation of results and drafting of the manuscript.

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