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QUALITY CONTROL AND HYPOGLYCEMIC EFFECT EVALUATION OF DRUGS WITH *BAUHINIA FORFICATA* LINK. (FABACEAE)

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ABSTRACT

The objective of the present study was to analyze the quality of commercially available samples of the plant B. forficata and compare their hypoglycemic effects with those of in naturasamples. To that end, three samples of B. forficata were acquired from commercial sourcesand subjected to quality control tests to examine their adequacy for human consumption. The samples were found to contain potentially pathological microorganisms (Klebsiella sp. and Aspergillus sp.), disqualifying them from use due to their potential negative effects on public health. To compare the hypoglycemiceffects of the commercial samples with an in natura standard, hyperglycemia was induced in 36 male rats of the Wistar line (60 days old) by the administration of a hyperlipidicdiet for 60 days (from 60 to 120 days of life), except in the control group (G1). Hyperglycemia was confirmed at 120 days of life. The animals were subsequently randomly divided into five groups of six animals each. The G1 group served as the control (n=6), the G2 group received an aqueous extract (AqE) of B. forficata derived from a standard sample (in natura), groups G3, G4 and G5 received AqE from B. forficata samples 1, 2 and 3 respectively during 30 days. Group 6 did not receive any treatment withplant extracts and was returned to a normocaloric diet after the induction of hyperglycemia. Blood collections were made at 60, 90, 120 and 150 days of life; an oral test of glucose tolerance was made at 150 days. Our results indicated that the hyperlipidicdiet induced hyperglycemia, and that the standard in naturasample of B. forficataexerted a greater hypoglycemiceffect than the commercial samples.

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INTRODUCTION

Medicinal plants are used throughout the world in empirical manners to treat diverse illnesses. In Brazil, a country with an incalculable biodiversity, those medicinal uses are very evident and numerous studies there have investigated the effectiveness of medicinal plants in combating chronic diseases such as Diabetes Mellitus (DM) (Lino *et al* 2004). Although the medical sciences have developed a number of pharmaceuticals to treat DM, many individuals still rely on *in natura* plants to treat and prevent diabetes due to the low levels of collateral effects associated with those plants, as well as their low cost. Within that context, there are many plants with pharmacological potential that still require careful studies to certify their ability to control glycemia without negative side effects (Negri 2005; Volpato 2002). The genus *Bauhinia* (family Leguminosae) is principally encountered in tropical regions and comprises approximately 300 plant species used as popular medicines in many parts of the world, including Africa, Asia, and Central and South America (Achenbach 1988). Phytochemical and pharmacological studies indicate that those plants contain many bio-active substances, principally glycoside steroids, triterpenoids, lactones, and flavonoids (Cechinel-Filho 2000). In Brazil, plants of the genus Bauhinia are commonly known as "Pata-de-vaca" or "Unha-de-boi". The leaves, stems, and roots of *Bauhinia*

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species, especially *B. manca*, *B. rufescens*, *B. forficata*, *B. cheitantha,* and *B. splendens*, are widely used there (and in other countries) in the form of teas or other phytotherapeutic preparations to treat infirmities such as infections and diabetes (Achenbach 1988; Gupta 1995; Teske *et al* 1995). The leaves of *B. forficata* (a small tree, approximately 20 m tall) represent the plant part most commonly used in popular medicine to combat Type II DM due to the facility of harvesting them– as they are native to numerous regions of Brazil. The aqueous extracts of the leaves demonstrate hypoglycemic activity, being effective in reducing blood levels of triglycerides, total cholesterol, and LDL. The flavonoid kaempferol present in that plant functions to stimulate beta-pancreatic cells to release insulin into the bloodstream (Engel *et al* 2008). The present study was designed to: 1) evaluate the quality of commercially available samples of *B. forficata* (including anatomical identifications of the samples, microbiological analyses, and measures of contaminating materials); 2) analyze the hypoglycemic effects of the aqueous extracts of commercial and *in natura* samples of *B. forficata* on rats exposed to hypercaloric diets.

MATERIALS AND METHODS

Botanical material: The standard (*in natura*) sample of *B. forficata* was identified and collected by specialists in that plant family on Campus I of the Iguaçu University, in Nova Iguaçu, Rio de Janeiro, Brazil (Figure 1 a – d). The commercially available samples of the same species were acquired from a pharmaceutical store in the city of Nova Iguaçu; the samples were denominated 1, 2 and 3 (Figure 2 a – c).

Analyses of contaminating materials: To determine the presence of contaminants in the commercial samples, they were spread on a clean surface and visually examined using a magnifying glass; any foreign materials (insects and dirt) were removed using a pair of forceps. The contaminating materials were weighed using a high precision balance to calculate the percentage of foreign material in relation to the total mass of material described on the packaging (Brasil 2010).

Microscopic analyses: To analyze the microscopic characteristics of the material, the leaves of *B. forficata* from each sample (including the standard sample) were hydrated and subsequently transversely sectioned freehand or using a Ranvier microtome. The sections were then stained with safranin-astra blue (Bukastsch 1972) and semi-permanent slides prepared using 50% glycerin (Berlyn *et al* 1976) for viewing under a light microscope.

Microbiological analyses: The microbiological analyses used salt mannitol agar, sabouraud dextrose agar, and cetrimide agar media. All of the commercial samples were tested in the three media, with the Petri plate being divided into four quadrants and seeded with plant parts (using sterilized swabs to spread the material over the plates). The plates were subsequently incubated at 37ºC for 48 hours to promote bacterial growth, and for 120 hours for fungal growth. Gram staining was used to identify the bacteria. The fungal samples were cleared using 20% potassium hydroxide (KOH) and examined and identified using a light microscope (Moura *et al* 2000).

Animals: Thirty-six male rats (Wistar line), 60 days old, from the Biotherium at Iguaçu University (UNIG) were used. The animals were maintained during the experimental period at a constant temperature of 23 ± 1ºC, under an artificial 12/12 photoperiod, with free access to food and water.

Ethical aspects: All of the procedures used here were performed according to the principles of animal experimentation and followed the norms established by the Brazilian Regulations of Animal Experimentation. Licensing (protocol: PEBIO/UNIG N.º 007/2017) was obtained from the Commission of Ethics for Animal Use (CEUA).

Diets: The normocaloric diet (ND) consisted of a commercial rat diet (Nuvilab®), containing by weight: 19.0% protein, 56.0% carbohydrate, 3.5% lipids, 4.5% cellulose, and 5% vitamins and minerals, totaling 17.03kJ/g. (Estadella *et al* 2004). The hyperlipidic diet (HD) consisted of a mixture of hypercaloric foods in the following proportions: 15g of the standard ration (Nuvilab®), 10g of dried peanuts, 10g of milk chocolate, and 5g of "maisena" crackers. Those ingredients were ground, mixed, and offered in the form of pellets containing (by weight): 40% protein, 48.0% carbohydrates, 20.0% lipids, 4.0% cellulose, and 5.0% vitamins and minerals. The energetic content of the hyperlipidic diet was 21.40 kJ/g. (Estadella *et al* 2004).

Aqueous extracts of *B. forficata* leaves: The aqueous extract of the standard (*in natura*) sample of *B. forficata* was prepared from an infusion of leaves (150g leaves / liter of water). The commercial samples were prepared using the entire contents of their packages (50g material / 500 ml of water) as recommended on the labels. The extracts were then filtered and concentrated under vacuum to obtain the Aqueous Extracts (AqE) used in the experiments. The extracts were divided into aliquots and maintained in a freezer until needed (Oliveira *et al* 2018).

Experimental groups

The animals were divided into six groups (n = 6): G1 (Control) was fed with ND during the entire experiment, without the ingestion of the AqE of *B. forficata;* groups G2, G3, G4, G5 and G6 were fed with HD between 60 and 120 days of life, and then with ND until 150 days. With hyperglycemia established, groups G2, G3, G4 and G5 were treated with the AqE of *B. forficata* by forced-feeding between 120 and 150 days of their lives according to the following regime: G2 standard (*in natura*) sample; G3 sample 1; G4 sample 2; G5 sample 3. The G6 (hyperglycemic control) did not receive any treatment with an AqE.

Accompanying blood glucose levels: Glycemia monitoring was performed after 12-hours of fasting; blood was collected from the caudal vein of the animals and deposited on disposable test strips that were read by a glucose meter (G-TECH*)* at 60, 90, 120 and 150 days of life.

Oral tests of glucose tolerance: Oral glucose tolerance tests (OGTT) were performed with animals during the last week of the experimental period, after 12-hours of fasting. The first blood collection was performed by making an incision in the extremity of the animal's tail (time 0). A solution of 50% glucose (to prepare a dose of 2 g/kg body weight) was administered to the animals by forced-feeding, and blood samples were collected after 30, 60, 90 and 120 minutes to determine glucose levels. A single incision at the tail extremity

was sufficient to collect all of the blood samples required. Blood glucose concentrations were determined using a glucose meter (G-TECH) and a glycemia curve was plotted using GraphPad Prism 6.0 software for Windows.

Statistical analyses: Bivariate Variance Analysis (ANOVA), followed by the Bonferroni post-test was used to analyze and compare glycemia levels and glucose tolerance tests. The results were expressed as means and were considered significant when $p<0.05$. The analyses were performed and the graphs plotted using GraphPad Prism 6.0 software for Windows.

RESULTS AND DISCUSSION

In relation to quality analyses, all of the commercial samples were found to contain excessive quantities of foreign materials. The standard limits in Brazil for contaminating materials in plant remedies are determined according to the "Farmacopéia Brasileira V" (Brasil 2000), and the contaminants found here were consistently above the permitted upper limit of 2%. Of the three samples tested, sample 1 contained insects, while samples 2 and 3 contained sand and other plant parts (in addition to leaves), thus failing the quality analyses.

Table 1. Microbiological analisys

Growthmedium	Sample 1	Sample 2	Sample 3
Salt manitol	+	+	-
Saubouraud	+	+	+
Cetrimide	-	-	-

(+)presence of microorganisms.
(-) absence of microorganisms.

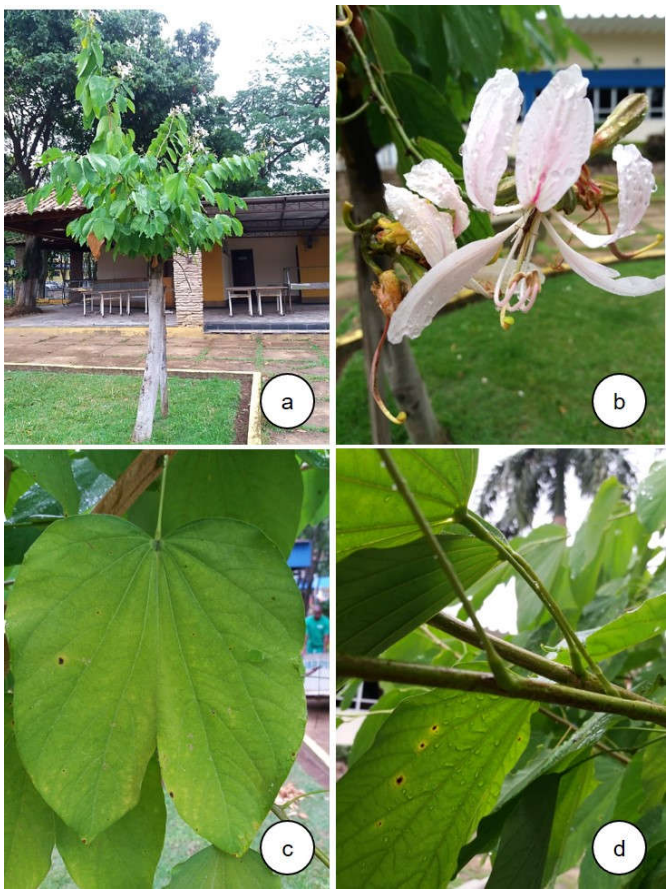

Figure 1. a-d – Standard sample of *B. forficata.* a – *Habitus*. b – Flower detail. c – bilobed leaf. d – Stem and leaf insertion

Table 2. Blood glucose levels (mg/dL) on days 60, 90, 120 and 150 days of age

	60		90		120		150	
Groups	MED	SD	MED	SD	MED	SD	MED	SD
G 1	105,2	±4,2	94,7	±5,6	82,2	±7,6	80,8	±9,8
G2	101,2	±10,4	131,0	±3,3	192,5	±6,5	87,8	±8,2
G3	99,7	±7,3	128,0	±4,2	197,7	±10,8	115,5	±4,3
G4	99,7	±3,7	139,8	±2,9	208,8	±6,5	120,0	±5,0
G5	99,3	±5,0	119,2	±3,5	183,8	±4,6	118,5	±6,8
G6	98,8	±11,2	138,5	±10,1	174,0	±11,6	207,7	±10,6

MED: Medium
SD: Standard deviation

In terms of the microscopic analyses, investigations of the anatomical characteristics of all the samples were compared with data available in the literature. Comparisons of samples 1, 2 and 3 with the standard sample showed that sample 3 demonstrated large quantities of long pluricellular tector hairs although it demonstrated similarity to the standard sample in terms of the organization of its vascular bundles and the presence of druses. Samples 1 and 2 demonstrated complete anatomical compatibility with the standard sample (Figure 3 a - d). In terms of microbiological analyses, all of the commercial samples were considered substandard (Table 1). All three samples demonstrated *Aspergillus* sp. growth (Figure 4) – a fungal species that constitutes a significant risk to human health as it can produce mycotoxins such as aflatoxin (a known carcinogen) (Araújo *et al* 2000) and cause fungal pneumonia, an inflammatory process of the pulmonary parenchyma with a difficult etiological diagnosis (José Roberto Azevedo de Oliveira, Federal University of Rio de Janeiro state - Brazil, personal communication). Samples 1 and 2 demonstrated bacterial growth of the genus *Klebsiella* sp.

Figure 2. a-c – Macroscopics aspects of the comercial. Sample 1 (a); Sample 2 (b); Sample 3 (c)

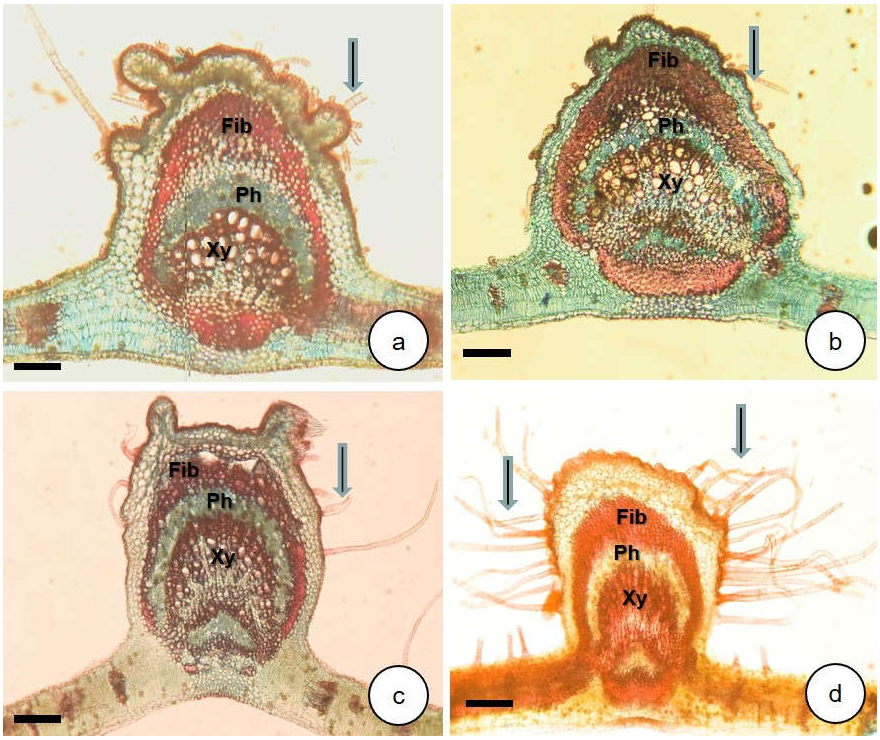

Figure 3 a-d. Anatomical comparison of the midrib of standard sample (a) and comercial samples 1, 2 and 3 (b, c, d respectively). Note the vascular pattern is similar between samples, however the sample 3 (d) is disapproved for possess excessive amount of trichomes (arrows). Xy = xylem; Ph= Phloem; Fb = Fibers. Scale bar = 100 µm

According to Araújo *et al* (2000), *Klebsiella* sp. is commonly found in plant material as it is common in the environment and not necessarily of fecal origin; it can nonetheless offer risks to consumer health as it is capable of antibiotic resistance (being classified as a superbacteria) (Podschun *et al* 1998). No microorganism growth was observed in the cetrimide medium.

Figure 4. Microbiological control. *Aspergillus* sp. *Hyphae* septate hyaline and conidiophore with vesicles, phalides and conidia (arrow). Scale bar = 20 µm

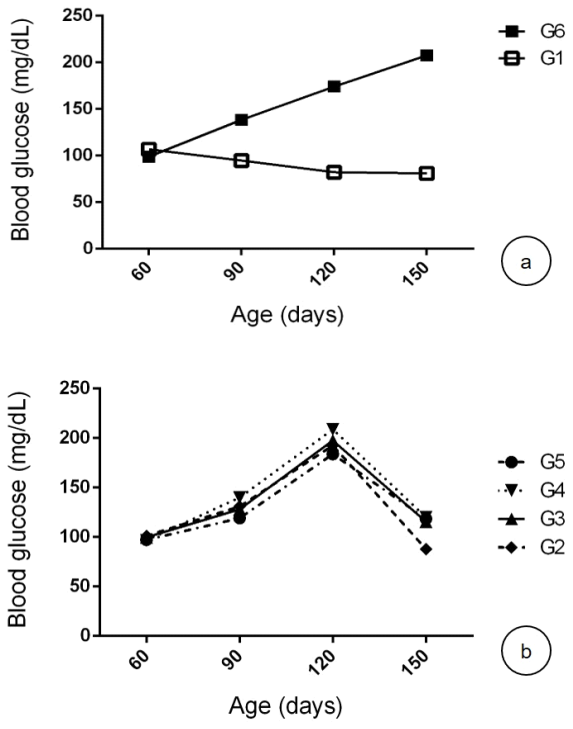

Figure 5 a-b. Concentration of blood glucose in 60, 90, 120 and 150 days. a) Note the difference between the glucose levels in G1 (control) and G6 (Hyperglycemic control). b) Note that G2 (standard sample) presents a more significative reduction of glycemy after treatment with AqE *B. forficata.* Values represent mean. ANOVA bivariate, followed post test Bonferroni, p<0,05

Analyses of hypoglycemic effects

Our results indicated that HD, provided between 60 - 120 days, induced hyperglycemia (Figure 5a – b; Table 2), as was observed by Oliveira *et al* (2018). The rat groups treated with the AqE of *B. forficata* showed significant reductions in glycemia at 150 days of life (Figure 5b). Oliveira *et al* (2018) likewise observed glycemiareductions with the use of AqE of *B. forficata* (derived *in natura*). The hypoglycemic properties of *B. forficata* are due to the presence of flavonoids – specifically kaempferol (Trojan-Rodrigues *et al* 2012), which, according to Zanata *et al* (2008), stimulates glucose absorption. We observed, however, significant differences between the hypoglycemic effects of the standard sample and commercially available samples (Figure 5b).

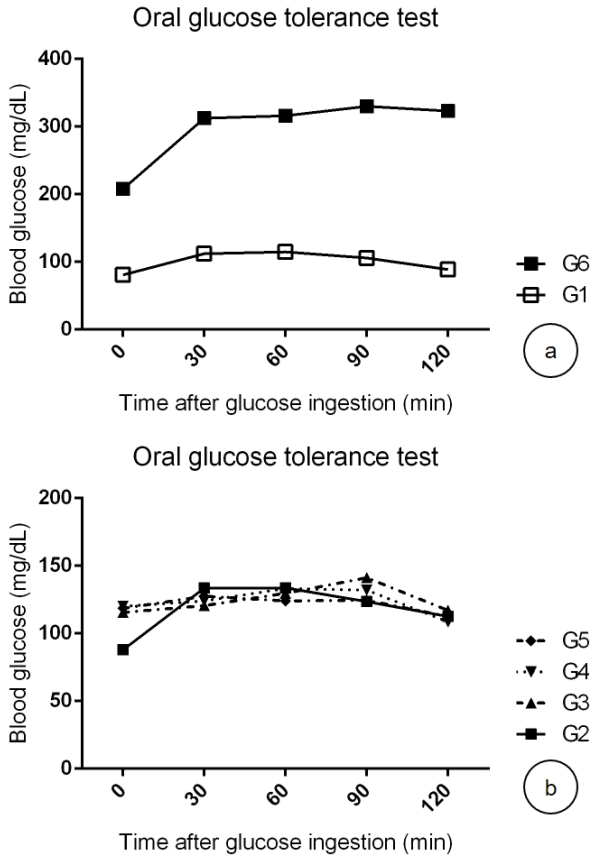

Figure 6 a-b. Concentration of blood glucose during oral glucose tolerance test. a) Note the difference between the glucose levels in G1 (control) and G6 (Hyperglycemic control) what indicates that glucose metabolization is damaged in hyperglycemic rats. b) Note that all groups restore the glucose metabolization after treatment with AqE *B forficata.* Values represent mean. ANOVA bivariate, followed post test Bonferroni, p<0,05

The standard sample significantly reduced glycemia levels to those observed in the control group at 150 days of life (Table 2); no significant differences in glycemia level reductions were observed between the three commercially available samples tested (Figure 5b). No other comparative study of this type was encountered in the scientific literature, and it will be necessary to determine if the many other plant remedies used in popular folk medicine can provide effective treatments for human ailments, and if commercial preparations have adequate sanitary properties and medicinal qualities. The present study therefore evidenced that the treatments with AqE from *B. forficata* resulted in the reestablishment of normal glucose metabolism during OGTT. Those results contrast, however, with other studies examining complementary therapies that were not successful in reducing the area under the glucose curve during OGTT (Moura *et al* 2000). Our results also evidenced that the rat group maintained on a HD and that did

not receive any treatment with AqE from *B. forficata* (G6) demonstrated significant alterations in blood glucose metabolism (Figure 6a).

Conclusion

Based on the results of the present study, which was designed to evaluate consumer security in terms of the therapeutic use of the medicinal plant *B. forficata*, commercially available samples of that species were found to contain pathogenic microorganisms and unacceptable levels of foreign materials. Our study also demonstrated that a HD was capable of inducing hyperglycemia, and that an AqE of *B. forficata* can reduce blood glucose levels – although the hypoglycemic effect of the *B. forficata* extract (*in natura*) reduced glycemia more effectively than commercially available samples.

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