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EFFECTIVENESS OF PROCESSES OF DRYING OF LEAVES OF PSIDIUM GUAJAVA L. IN FUNCTION OF LEVELS OF FLAVANOLS, PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY

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Abstract

Leaves of *Psidium guajava* L. (guava) in brazilian communities are used in cases of diarrhea, dysentery, flatulence and abdominal pain. There is no standardization of organ used, how to use or dosage of plant. In the communities, the traditional population reports the use "in natura" of leaves and bark in teas (infusion and decoction) and beverages. With the expansion of phytotherapy in Brazil, the vegetable drug (dried leaves of guava) is available in the herbalists, without having standardized quality and/or determination of minimum levels of biomarkers. Seeking to establish a standard that meet the brazilian market of medicinal plants, this study aimed to validate methodologies for natural and artificial drying of the leaves of guava at different stages of maturity – young, in primary development and adult, fully expanded – relating them to of the quantification secondary compounds and antioxidant properties. In the botanical standardization was made macroscopic description of the leaves. In the processes of drying, 5 g of fresh leaves (young and adult) were exposed to artificial drying - DMW (microwave), DCAO (circulating air oven), DCO (conventional oven) and natural (DTL - drying thin layer) to constant weight, constituting treatments (TDMW1, TDMW2, TDCAO1, TDCAO2, TDCO1, TDCO2, TDTL1 and TDTL2). After the drying process, in all treatments, methanol extracts were made by maceration. In dried extracts of treatments, the flavonoids identified qualitatively by TLC (finger print) e was quantified the contents of phenolic compound, flavanols and antioxidant activity for validation of drying processes. All analyzes were performed with four replicates and the data from the experiments were subjected to ANOVA and mean comparison test (Tukey) at 5 % significance level. The stage of leaf development did not influence the drying processes, for residual humidity. The organoleptic characteristics (color, odor, flavor) don't changes in any treatment. The highest yield of extracts of dried leaves (both stages) was obtained in artificially dried process (microwave - DMW). In the TLC, flavanols and tannins were detected in all treatments. The levels of phenolics, in the development stages, did not differed in function of artificial and natural drying, thus being both effective processes. The extracts obtained after drying in circulating air oven (DCAO) had higher yield of flavanols for young and mature leaves. The content of flavanols of extracts of young leaves was greater than that of mature leaves, when compared against the same methodology drying and the antioxidant activity of extracts from young leaves of guava obtained after drying in a conventional oven were superior to all other extracts, confirming that the phytomedication obtained with young leaves should have better pharmacological effect. In function on the results obtained, the best drying process was in a ventilated oven for young leaves and mature leaves, in terms of biocompounds's preservation.

Keywords: biocompound, drying process; natural drying.

Introduction

Psidium guajava L. (Myrtaceae), guava is tree small, with less than 3 m, rustic, native of tropical America, specially of Brazil and Antilhas [1]. The stem is tortuous, slick, with ritidoma. Leaves are simples, texture tough, of 8-12 cm long by 3-6 cm wide [2].

The leaves of *P. guajava* are popularly employed in gastrointestinal disorders, practice originally inherited from Aztec medicine in Mexico [3]. The infusion or decoction, prepared with fresh leaves or dried, are indicated for diarrhea, dysentery, flatulence and abdominal colics, in Brazil [4, 5].

The major bioactive compounds already isolated from guava leaves were tannin, essential oils, flavonoids, triterpenoids and sesquiterpenoids [6, 7]. The monograph *P. guajava* in the Brazilian Pharmacopoeia defines the leaves as part to be used in pharmacological preparations and as "markers" are considered tannins, flavonoids and essential oils.

In studies of antifungal and antimicrobial activities were comproved that guava leaf extracts (*P. guajava* L.) had inhibitory action on the growth of yeast strains (*C. albicans* and *C. tropicalis*) [8, 9]. Methanol, acetone and N,N-dimethylformaldehyde extracts of leaves of *P. guajava* were able to inhibit the growth "in vivo" of the bacteria gram negative *Pseudomonas* sp; *Escherichia coli*; *Klebsiella* sp. and *Proteus* sp [10]. However, these studies do not specifically mentioned which portions of leaves were used if were young or adult leaves.

Due to the use of guava leaves, “in natura” or as "vegetable drug", it is proposed studies for facilitate the obtaining of vegetal drug, with phytochemistry quality, based in the quantification of phenolics, flavonoids and antioxidant potential, in sheets in two stages of maturation (young and adult), after drying processes natural and artificial.

Material and Methods

Harvest of material

Guava leaves in maturation stages distinct - young, in development primary, and, adult, fully expanded - from to organic cultivation, were collected before the start of flowering, in the month of September, in the municipality of Conselheiro Lafaiete (20 39’36”S, 43 47’9”O), Minas Gerais, Brazil and transported to University Federal de Ouro Preto. Exsiccate (voucher specimen) of nursery material was deposited in the Herbarium José Badini, in the Intitute of Sciences and Biological of the Federal University of Ouro Preto, under the number OUPR 27242. The leaves collected were subjected to processing post-harvest where were segregated in young leaves or in budding, collected from the first to the third node, counted from the apex of the branch (first stage of development - figure 1) and mature leaves fully expanded leaves, collected below the third node (second stage of development – figure 1a). The macroscopic characterization of leaves was carried out with the naked eye, when necessary, with the aid of magnifying glass (increased 5x), according to the parameters described by Hickey [11] and Oliveira & Akisue [12]. Then there were made procedures for weighing and drying methods.

Evaluation of methods of drying

In assessing the efficiency of drying methods on the two stages of maturation, leaves were distributed in eight treatments with four replications. The treatments were appointed according to the method of drying employed and the stage of maturation of the leaf, being TDMW 1 and 2 related to drying in microwave, TDCAO 1 and 2 oven with air circulation; TDCO 1 and 2 conventional oven; and TDTL 1 and 2, thin layer, where 1 and 2 are the stages 1 (young leaf) and stages 2 (adult leaf), respectivelty. In treatments TDCAO and TDCO, 5g of fresh leaves were weighed, placed in packages of multilayered paper, and then, they were put into in oven (Marconi) for 72 hours, the temperature of 40 ºC, with regular weighing at 24 hour intervals, until maintenance of constant weight. In treatments TDMW, 5g of fresh leaves were accomodated in paper packaging multilayered and taken to the appliance microwave (Panasonic) for a period of 20 seconds, in 17 intervals in average power of 800W. At each interval, leaves were weighed again. This procedure was done until constant weight (total time of 340 seconds). In treatments TDTL, 5g of fresh leaves were placed on filter paper in a thin layer, non-overlapping, and the ambient temperature of 25 ºC. Every 24 hours were weighed, up constant weight, in total time of 120 hours. The residual water content was calculated on the basis of loss of mass of fresh leaves in to drying process.

Preparation of the methanolic extracts

The dry leaves of guava, obtained from drying process, were submitted to maceration using methanol PA (Vetec). The leaves remained in contact with solvent for 72 hours, and then the solvent was filtered, dried in rotary evaporator at reduced pressure and temperature of 40 ºC.

The yield of extracts was calculated on the basis in weight dry mass and of dry extract (w/w).

Determining the content of phenolic compounds

The method used was Jayaprakasha [13]. The extracts of treatments (0.2 mg) were added to 1.0 mL of reagent Folin-Ciocalteu (1:10) and 0,8 mL sodium carbonate 7.5%. The solutions are homogenized and incubated at 30 ºC during 30 minutes. The absorbance was mensured at 765 nm in spectrophotometer. Standard curve was constructed using as reference standard, tannic acid. The results were expressed in mg/g of tannic acid.

Determining the content of flavanols

The method consisted in adding 2 mg of the methanolic extract of treatments in 2,5 mL vanillin 0.5% (Sigma) and 2.5 mL HCl 4% (Vetec) The solutions were homogenized and stored, protected from light, for 20 minutes and then, the reading of the treatments was made in spectrophotometer at 500 nm (Femto). The standard curve was constructed using as reference substance catechol (Sigma). The results were expressed in mg/g of catechol.

Antioxidant activity

The antioxidant activity was made second Singh [14]. Concentrations of 400 ppm of each treatment were diluted in metanol PA and added to 5 mL of DPPH 0.1 Mm. 50 ppm of BHA, substance of reference with antioxidant, were used in the comparison in the same conditions. The control was prepared using DPPH 0,1Mm and methanol was used as blank.

The tubes were mixed in vortex and the reading was held in time 0 and 30 minutes in a spectrophotometer at 517 nm. The percentage of antioxidant activity was calculated using the formula:

% AAT = (CONTROL ABS – (Sample ABS/ CONTROL ABS)) X 100

Screening preliminary phytochemical by thin layer chromatography (TLC)

The treatments were analyzed by TLC (finger print) on silica gel G plates (0, 25 mm thick, Sigma-Aldrich), employing two mobile phases according Wagner & Bladt [15]. 10 µg of extract was solubilized in 100 µL of methanol for application in CCD. The determination of tannins was used chloroform: metanol: n-propanol: water (5:6:1:4 v/v) as mobile phase and as developer was used ferric chloride in methanol 1%. The analysis by TLC was also made with the mobile phase ethyl acetate: formic acid: acetone: water (100:11:11:27 AM v/v) and reveled with vanillin hydrochloric acid for viewing of flavonoids. The Rf's were calculated and compared to the bibliography of reference and standards of rutin, quercetin and tannic acid.

Experimental design

All the analyzes of the extratcts of treatments (TDMW1, TDMW2, TDCAO1, TDCAO2, TDCO1, TDCO2, TDTL1 and TDTL2) were made with four repetitions and the date of the experiments were submitted to ANOVA and test of media (Tukey), at 5% significance level by SAEG program UFV.

Discussion

Macroscopic description of young leaves fully expanded leaves of Psidium guajava L.

Young leaves are simple, entire, slightly asymmetrical, mucronadas, velvety or pubescente, elliptic or lanceolate, with approximately 0.8 to 2 cm wide and 4 to 6 cm in lenght, mucronulate apex and base obtuse. Leaf blades discolored being green adaxial epidermis with reddish or pinkish margins and light green abaxial, covered by numerous simple trichomes. Pinnate veins where standard of veins is of type camptodromous brochidodromous. Petiole short, fragile, coated by simple trichomes, with 0.2–0.5 cm long, cylindrical and grooved (Figure 1).

Adult leaves or fully expanded leaves are simple with entire margins, asymmetric, or little asymmetric, slightly revolute, coriaceus, oblong or ellipticla or oval-sghaped, with approximately 6 to 12cm in lenght and 3 to 7 cm wide, mucronolate apex, base obtuse. Leaf blades are discolores with adaxial epidermis dark green and brownish green abaxial epidermis and may have pores translucent, and/ or presence of trichomes simple.

Adaxial Epidermis glabra, coated by a thin waxy layer. Pinnate veins, where primary and secondary veination are protruding on the abaxial surface and printed in adaxial face. The standard of veins is of type camptodromous brochidodromous, where the anastomosis veins occurs since the base of the sheet forming a series of arches next to the edge. Petiole short (0.5–0.7 cm) long, cylindrical and grooved (Figure 1).

Evaluation of methods of drying

The drying process is crucial stage in industries that use raw materials of plant origin in surveys of plants with pharmacological and biologic interest. Drying processes carried out improperly may compromise the quality of manufactured products and change the efficiency of medicines.

The dry mass and H_2O content should be controlled during the drying process since cannot occur physical changes (organoleptic) and chemical losses. Thus, Infusions, decocções and macerations should be prepared from leaves in good state of conservation, avoiding future complications with harmful effects or undesirable. The table 1 shows results of the average values of H_2O loss, fresh and dry mass, in leaves of *P. guajava.* The water content in young leaves had average of 59.87 ± 1.84 %, the highest value was in TDTL (thin layer = 62.03 ± 0.91 %). For the leaves aduls, the average content of water was 59.52 ± 2.29 %. The higher percentage of water was in TDTL (61.01 ± 3.8 %).

The drying methods did not differ at 5% of significance, in water loss as a function of the two stages of maturation (young and adult). The organoleptic characteristics (color, smell, taste) have not changed in any of the treatments. The drying of plant is required to prevent degradation, chemical changes and losses of content of active ingredients during storage. Thus for the drying of the leaves of *P. guajava*, in the four drying methods employed, in the predetermined conditions of this test, maintained the organoleptic qualities.

Those results, allows us to infer, that it is feasible to make the drying of young and adult leaves of guava by natural processes or artificial, without that have losses of quality and constitutional, and further, with the preservation of vegetable raw material quality.

Yield of extracts

The yield of the extracts are shown in table 1. The methodology of natural drying in thin layer (TDLT) had the lowest yield of dry extract, therefore, its not indicated,as ideal method in obtaining bioextratos in guava.

The highest yields in the extracts were in TDMW, in both stages of maturation. The best methodology of drying of leaves guava was in microwave (TDMW). TDMW had efficacy in function of the best preservation of the material, in less time spent. Tne other method efficient was ventilated oven (TDVO), in young leaves; and in conventional oven (TDCO) in both stages of leaf development. The importance of linking methods of drying the yield of dry extract is directly related to the yield of secondary compounds and active mass for the manipulation, and then to obtain phytotherapics. There is no data in the scientific literature on influence of methods of drying of leaves guava and yield of extracts. (Table 1)

Determation of phenolic

The phenolic include huge range of substances, among them the phenolic acids and flavonoids, which, by its chemical constitution, have antioxidant properties. Thus, at high concentration of phenolic compounds in a plant extract ensures the same, broad use in diseases whose pathogenesis is promoted by oxidative stress.

From the ajusted linear equation ($\hat{y}$ = 0.075x – 0.0097; r^2 = 0.9871) were calculated the concentration of phenolics (mg/g) in the treatments. The results showed no significant difference between the drying methodologies (Table 2). The drying methods did not interfered significantly in levels of phenolic, indicating that there was no losses of biocompounds by heating and that the natural drying process was effective in maintenance of phenolic compounds. Drying processes incorrect may cause losses in the contents of the active principle or the chemical marker, compromising the whole productive chain of phytotherapic or functional foods.

Determination of flavanols

Medicinal plants and herbs are used for thousands of years in folk medicine. The flavanoids include flavanols, are substance active present in the most plants, are known by the pronounced antioxidant activity, responsible for protective function antiaging cellular and in the treatment of degenerative diseases mediated by oxidative stress.

From the ajusted linear equation ($\hat{y}$ = 0.00005x + 0.0086; R^2= 0.9903) the values of concentration of flavanols were calculated in all treatments of extract of guava leaves obtained in function of the drying processes (Table 2).

The average levels of favanols (mg/g) in the eight treatments differed statistically as the drying method. The TDCAO had the highest yield of flavanols in both developmental stages (young and mature leaves) (TDCAO1 = 0.3320 ± 0.01015 mg/g; TDCAO2= 0.2645 ± 0.0071 mg/g). In young leaves, in all treatments, the content of flavanols was superior to that adult leaves, in all drying processes. The TDLT had the lowest content of flavanols in extracts, that were in TDLT1 = 0.0795 ± 0.0045 mg/g and TDLT= 2 0.0520 ± 0.0043 mg/g.

Artificial drying in a circulating air was more feasible to provide for greater conservation of the active principles. The preservation of bioactive ensures better therapeutic efficacy, whether as the medicinal plant or "vegetable drug" in infusions and deccotos, whether in developing phytomedication.

Antioxidant Activity

The result of the antioxidant activity is showed in Table 2. The antioxidant activity most promissing was in TDCAO1 at time zero – T0 = 79.25 ± 1.69% and at time T30 = 99.66 ± 2.20 %. This result demonstrates that of high flavanols concentration may be responsible for the antioxidant activity of the extracts of guava leaves young people, since the, in TDCAO1 obtained the highest antioxidant activity as well as the largest concentration of flavanols. The flavonoids act as antioxidants in inactivation of free radicals in cellular compartments lipophilic and hydrophilic. These compounds have the ability to donate hydrogen atoms, and, therefore, inhibit the chain reactions caused by free radicals.

Screening preliminary phytochemical by thin layer chromatography (TLC)

The thin layer chromatography (TLC) is important methodology in preliminary analyzes of vegetable extracts. In the thin layer chromatography performed in all treatments, were detected tannins (Figure 2a) that after application of developer (ferric chloride 1 %), in TLC were visualized as bands slightly blue with Rf's = 0.4; 0.5; 0.58; 0.92, values compatible to this compound class when compared to bibliography of reference Wagner & Bladt [15]. It is interesting to highlight that the tannins are important pharmacologically and can be little stable in some drying techniques, due to the high temperature and duration of the process.

In all treatments also were detected flavonoids in chromatographic profile, that were visualized with yellow bands with Rf's = 0.23; 0.39; 0.65; 0.76, compatible with flavonoids (Figure 2b) cited by reference literature Wagner & Bladt [15].

In neither the drying methods there was of bioactive loss by overheating. Thus, TLC analyses allowed us to conclude that TDCOA and TDMW treatments are qualitatively superior to others by presenting more structured bands. (Figure 2)

Conclusion

On the basis of the results obtained, the best drying process was in a ventilated oven for young leaves and in mature leaves, in relation to the preservation of biocompounds or chemical markers.

This study showed that the content of flavanols and antioxidant activity in extracts from young leaves was higher than that of the extracts from leaves In maturation more advanced (adult stage), this corroborates reports of folk medicine, in which the use of young leaves of guava is widely disseminated.

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Figure 1. Stages of the leaves of *P. guajava* (guava). **A**. Adult leaves. **B**. Young leaves. Source: personal file.

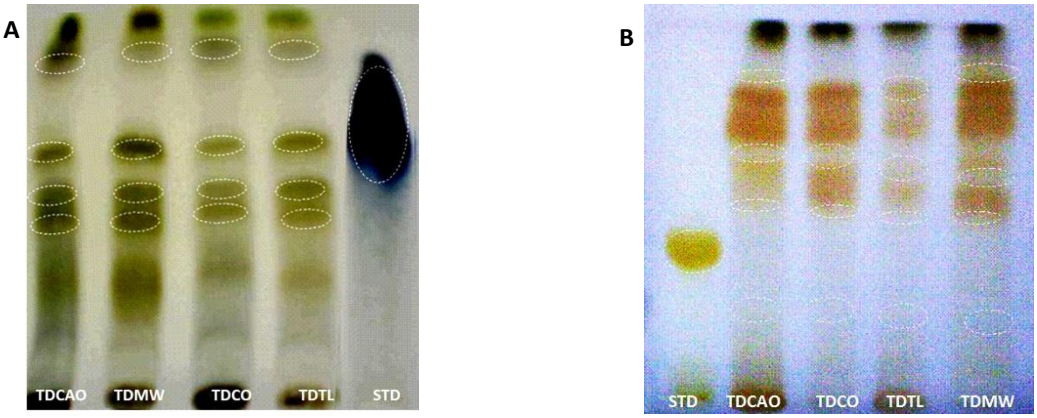

Figure 2A. Chromatography thin layer (TLC). 2A. Chromatography for identification of tannins in guava leaf extracts after revelation with ferric chloride 1% and standard of tannic acid (STD). **2B.** Chromatography for identification of flavonoids in guava leaf extracts after revelation with vanillin methanolic 5% and standard of rutin (STD) (Source: personal file).

Table 1. Mean level of fresh mass (FM), dry mass (DM) in grams (g), percentage of loss of H_2O (PW %) and yield of extracts (YExt) in leaves of *Psidium guajava* in maturation stages – young (1) and fully expanded (2) – in the treatments of drying – ventilated oven (TDCAO), microwave (TDMW), conventional oven (TDCO) and thin-layer (TDTL).

TREATMENTS	MEANS			
	FM(g)^{ns}	DM (g) ^{ns}	PW (%)^{ns}	YExt (%)*
TDCAO1	5.0292	2.1112	58.02	0.37 **AB**
TDCAO2	5.1375	2.1360	58.42	0.21 **B**
TDMW1	5.2095	2.0985	59.71	0.54 **A**
TDMW2	5.1097	2.1390	58.13	0.42 **AB**
TDCO1	5.2315	2.1012	59.82	0.32 **AB**
TDCO2	5.3567	2.1065	60.61	0.31 **AB**
TDTL1	5.2437	1.9918	62.03	0.21 **B**
TDTL2	5.1252	1.9970	60.01	0.18 **B**
CV (%)	3.243	3.69	6.02	34.2

^{ns}- Not significant at 5% probability by Tukey test at 5 %.
* - Significant at 5% probability.
The medium followed by the same letter do not differ statistically by the Tukey test at 5% probability.

Table 2. Mean level of phenolic (FC), flavanol (FLAV) (mg/g) and antioxidant activity at time zero – ATT0' and 30 minutes - ATT30' (%) in leaves of *Psidium guajava* in maturation stages – young (1) and fully expanded (2) – in the treatments of drying – ventilated oven (TDCAO), microwave (TDMW), conventional oven (TDCO) and thin-layer (TDTL).

TREATMENTS	MEANS			
	CF ^{ns}	FLAV*	ATT0' *	ATT30' *
TDCAO1	29.05	0.3320 **A**	79.25 **A**	99.66 **A**
TDCAO2	28.9	0.2645 **AB**	73.4954 **ABC**	98.95 **AB**
TDMW1	30.75	0.2420 **AB**	78.70 **AB**	99.59 **A**
TDMW2	30.1	0.1420 **BC**	70.28 **BC**	97.09 **ABC**
TDCO1	28.55	0.1645 **BC**	68.40 **CD**	96.52 **BC**
TDCO2	28.9	0.0755 **C**	66.38 **CD**	96.04 **BC**
TDTL1	27.0	0.0795 **C**	61.29 **D**	95.37 **C**
TDTL2	26.15	0.0520 **C**	61.17 **D**	94.77 **C**
CV (%)	3.01	25.96	5.37	1.18

$^{ns.}$ Not significant at 5% probability
* Means followed by the same letter do not differ statistically by the Tukey test at 5% probability.