

*Full Length Research Paper*

## Nutritional characteristics of guava leaves and its effects on lipid metabolism in hypercholesterolemic rats

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Received 3 February, 2014; Accepted 15 August, 2014

The objective of this study was to evaluate antioxidant substances, dietary fiber and antioxidant activity of guava leaves, and evaluate their amelioration hypercholesterolemia in rats. The leaves were dried and crushed into granulated powder. The active ingredient of the leaf powder was extracted by an ethanol/acetone mixture and called ethanol/acetone extract flour. Dietary fiber, vitamin C, beta-carotene, phenolic compounds and antioxidant activity were determined in the flours. Thirty (30) rats were used in the present study and categorized into: hypercholesterolemic control, non-hypercholesterolemic rats treated with ethanol/acetone extract flour, hypercholesterolemic rats treated with ethanol/acetone extract flour, non-hypercholesterolemic rats treated with leaf flours, and hypercholesterolemic rats treated with leaf flours. The animals were subjected to experimental hypercholesterolemia and treatment with flours for 42 days. At the end of the treatment, liver weight index *versus* body weight index, total hepatic lipids, C reactive protein, total cholesterol and fractions were evaluated. The treated hypercholesterolemic animals showed a reduction in total and fractionated cholesterol levels, but no difference was observed in the ratio liver weight index *versus* body weight index, total hepatic lipids and C reactive protein. It is concluded that guava leaves are a significant source of dietary fiber, phenolic compounds, vitamin C, beta-carotene, besides presenting antioxidant activity and hypocholesterolemic potential.

**Key words:** *Psidium guajava*, leaves, antioxidant, dietary fiber, hypocholesterolemia.

### INTRODUCTION

Much of the world's population use various parts of plants in therapy for the control and prevention of diseases. Several people use the empirical knowledge about

medicinal plants as the only therapeutic resource. In this context, plants that are popularly used for therapeutic purposes, but lack scientific evidence of their effects, are

of great value in the search for the development of new drugs of proven efficacy and safety (Balbino and Dias, 2010).

In recent decades, cardiovascular diseases have been the main cause of mortality in developed and developing countries. The increase in the incidence of those diseases is due to changes in nutritional patterns and physical inactivity, enabling an increase in the prevalence of hyperlipidemia, considered one of the main risk factors, due to the elevation of plasmatic levels of total cholesterol and fractions, associated to the decrease in high-density lipoprotein (HDL) level (Bruckner, 2008).

The excess cholesterol stimulates the production of reactive oxygen species, contributing to endothelial dysfunction, low-density lipoprotein (LDL) modification and reduction in the antioxidant defense system, such as the decrease in the activities of superoxide dismutase and glutathione peroxidase (Lester et al., 2009).

Many plants are used in the control and prevention of cardiovascular diseases. It is believed that its effect is due to the presence of antioxidants and dietary fiber, resulting in the inactivation of reactive species and cholesterol absorption, respectively (Beling et al., 2007).

Guava leaves have several antioxidant compounds, such as ferulic acid, quercetin, gallic acid, caffeic acid and ascorbic acid, which may play an important role in the body. Among its highlights are the following effects: hypoglycemic action, *in vitro* antioxidant activity, anti-inflammatory, antimicrobial, and hepatoprotective effects on hemostasis and blood coagulation (Jimenez et al., 2001; Thaipong et al., 2006; Gutiérrez et al., 2008; Deguchi and Miyazaki, 2010).

Thus, the objective of this study was to evaluate the contents of phenolic compounds, dietary fiber and antioxidant activity of the leaves of Pedro Sato guava, as well as its effect on plasma levels of total cholesterol and fractions, hepatic lipids and C-reactive protein.

## MATERIALS AND METHODS

### Sample collection and preparation

The leaves of Pedro Sato guava (*Psidium guajava*) were harvested, washed under running and distilled water and, soon afterwards, they were placed in forced-air ovens for drying for five days, at  $\pm 35^{\circ}\text{C}$ . After drying, the leaves were ground in a Wiley type mill and the guava leaf flour was stored in hermetically sealed flasks under refrigeration. Then, ethanol/acetone (70/30, v/v) were added to the guava leaf flour during 24 h, followed by filtration. The supernatant was collected, submitted to evaporation and lyophilized (Rufino et al., 2007), and this new flour was called ethanol/acetone extract flour.

The leaves were identified by the College of Agriculture Lavras Herbarium, where the voucher specimen was deposited and received voucher number 26277.

### Characterization of guava leaves

The content of soluble and insoluble dietary fiber was determined through the Sigma Total Dietary Fiber Kit. The results were expressed in  $\text{g } 100 \text{ g}^{-1}$  sample (Association of Official Analytical Chemists - AOAC, 2005). Phenolic compounds were extracted with 50% methanol at a rate of 3 g sample diluted with 250 mL of 50% methanol at reflux for three consecutive times at  $80^{\circ}\text{C}$  and the extracts were combined, evaporated to 25 mL (Goldstein and Swain, 1963) and measured using the Folin-Denis reagent (AOAC, 2005). The results were expressed in mg of tannic acid  $\text{g}^{-1}$  dry material (DM).

The antioxidant activity was determined by the colorimetric methods 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Thaipong et al., 2006) and beta-carotene/linoleic acid (Rufino et al., 2007), using the extracts obtained for the determination of phenolic compounds. The vitamin C content was determined by the colorimetric method (Strohecker and Henning, 1967). The content of beta-carotene was determined by the colorimetric method (Nagata and Yamashita, 1992).

### Biological analysis

The experiment was conducted according to the ethical principles for animal experimentation adopted by the Brazilian School of Animal Experimentation (COBEA), approved in 11/11/2010 by the Animal Research Ethics Committee of University Federal of Alenas, protocol number 326/2010.

The experiment was conducted over six weeks, using 30 male Wistar rats (*Rattus norvegicus*), with initial body weight of  $400.00 \pm 50.00$  g. The animals were kept in individual cages at  $21^{\circ}\text{C}$ , light/dark cycle of 12 h, with access to distilled water *ad libitum*. The rats were randomly divided into six groups with five rats each: NC: non-hypercholesterolemic control; HC: hypercholesterolemic control; NE: non-hypercholesterolemic rats treated with ethanol/acetone extract flour; HE: hypercholesterolemic rats treated with ethanol/acetone extract flour; NF: non-hypercholesterolemic rats treated with leaf flours; HF: hypercholesterolemic rats treated with leaf flours.

The rats in the non-hypercholesterolemic groups received a commercial diet (Biobase Bio-tec Ratos e Camundongos) during the six weeks of experiment. The rats in the hypercholesterolemic groups received the same diet, but with cholesterol (0.5%) and eolic acid (0.25%). For the preparation of the diet, the commercial food was triturated, and cholesterol and cholic acid were added. The mixture was moistened with water, shaped and taken to a ventilated oven at  $35^{\circ}\text{C}$  for two days (Rocha et al., 2012).

The guava leaf flour and ethanol/acetone extract flour was administered to the rats by gavage, once a day for 42 days, at a dosage of  $50 \text{ mg Kg}^{-1}$  body weight, while the control groups (non-hypercholesterolemic and hypercholesterolemic control) received water by the same administration method. Dosage choice was based on the study performed by Gutiérrez et al. (2008), who observed hepatoprotective, hypotensive, anti-inflammatory and

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**Table 1.** Chemical characterization and antioxidant activity of guava leaves.

Parameter	Leaf flour	Ethanol/acetone extract flour
Soluble dietary fiber (g 100 g <sup>-1</sup> DM)	4.10 ± 0.39	ND
Insoluble dietary fiber (g 100 g <sup>-1</sup> DM)	57.84 ± 1.23	ND
Vitamin C (mg 100 g <sup>-1</sup> DM)	200.01 ± 6.96	ND
Beta-carotene (mg 100 g <sup>-1</sup> DM)	12.80 ± 0.64	ND
Phenolic compounds (mg g <sup>-1</sup> DM)	130.05 ± 6.71	369.93 ± 13.80
Antioxidant activity (IC <sub>50%</sub> mg mL <sup>-1</sup> )	0.02 ± 0.00	0.01 ± 0.00
Antioxidant activity (inhibition %)	82.93 ± 0.99	82.81 ± 6.66

ND, Not detected. DM, dry matter.

analgesic effects.

At the beginning of the experiment and three days before euthanasia, the rats in the control groups (non-hypercholesterolemic and hypercholesterolemic control) were analyzed by puncture of the tail vein for total cholesterol, in order to confirm the induction of hypercholesterolemia.

At the end of the experiment, the rats remained under fasting for 12 h, and were then anesthetized with sodium thiopental (35 mg kg<sup>-1</sup>). The blood was removed by the heart puncture technique, and the liver was dissected and separated.

Total and fractionated cholesterol levels were determined in the blood samples using the Labtest<sup>®</sup> enzymatic-colorimeter kit. Non-HDL cholesterol levels were determined by the difference between the total and HDL cholesterol levels. The ratio liver weight *versus* body weight was determined dividing the weight of the whole liver by the body weight of the animal. Hepatic lipids were determined by the methodology proposed by AOAC (2005). C-reactive protein levels were determined in the blood serum of the rats by the turbidimetric method using the Human<sup>®</sup> kit.

### Statistical analysis

A completely randomized design in a 2 x 2 + 2 factorial outline was used; being two forms of extract preparation (leaf flour and ethanol/acetone extract flour), two diet types (hypercholesterolemic and non-hypercholesterolemic) and two additional treatments (hypercholesterolemic control and non-hypercholesterolemic control), totaling six treatments with five repetitions. The statistical analysis was conducted using the Sisvar program (Ferreira, 2000), and the means were compared by the Tukey test (p≤0.05).

## RESULTS AND DISCUSSION

Table 1 shows the contents of dietary fiber, antioxidant compounds and the antioxidant activity of guava leaf flour and ethanol/acetone extract flour. Guava leaf flour is shown as a good source of soluble (4.10 g 100 g<sup>-1</sup> dry matter - DM) and insoluble fiber (57.84 g 100 g<sup>-1</sup> DM). Dietary fiber was not detected in the ethanol/acetone extract flour. Dietary fiber prevents cholesterol absorption in the intestine, enhancing its excretion as bile salts, reducing therefore, cholesterol levels in the plasma (Rique et al., 2002; Mello and Laaksonen, 2009). The FDA recommends the consumption of 25 g dietary fiber

per day on a 2,000-calorie diet; thus, guava leaves prepared in the form of flour are a rich source of this nutrient.

In the leaf flour, antioxidant substances such as vitamin C (200.01 mg 100 g<sup>-1</sup> DM), beta-carotene (12.80 mg 100 g<sup>-1</sup> DM) and phenolic compounds (130.05 mg 100 g<sup>-1</sup> DM) were also found. On the other hand, only phenolic compounds (369.03 mg 100 g<sup>-1</sup> DM) were reported for the ethanol/acetone extract flour, and these contents are higher than those found in guava leaf flour.

Beta-carotene acts as a lipophilic antioxidant and, together with vitamin C and phenolic compounds (hydrophilic antioxidants), it forms a strong defense against free radicals, by acting in different cell compartments.

Regarding antioxidant activity, guava leaf flour and the ethanol/acetone extract flour showed antioxidant activity by both methods, and the antioxidant activity of the ethanol/acetone extract flour was higher than that of the leaf flour by the DPPH method, and similar by the beta-carotene/linoleic acid method. These results can be explained by the higher contents of phenolic compounds found in the ethanol/acetone extract flour (Table 1), which resulted in a greater antioxidant activity by the DPPH method.

Several studies have shown that vegetables are sources of dietary fiber and antioxidants, and a diet rich in vegetables has a positive influence on plasma lipids and antioxidant activity (Leontowicz et al., 2001; Leontowicz et al., 2002; Salgado et al., 2008).

### Biological analysis

Table 2 shows the contents of lipids, the liver weight/body weight ratio and the levels of C-reactive protein, with no statistical difference (p≤0.05) between the treatments for liver fat content and the liver weight/body weight ratio, when compared to their respective controls.

C-reactive protein is a protein synthesized by the liver in response to cytokines, which shows active inflammation. From Table 2, it is observed that there was

**Table 2.** Levels of hepatic lipids, liver weight/body weight ratio and C-reactive protein<sup>1</sup> in *Wistar* rats treated with guava leaves.

Treatment	Hepatic lipid (%)	Liver weight x body weight	PCR <sup>1</sup> (mg L <sup>-1</sup> )
NC <sup>1</sup>	3.56 <sup>b</sup>	0.023 <sup>b</sup>	0.2 <sup>c</sup>
HC <sup>2</sup>	13.32 <sup>a</sup>	0.030 <sup>a</sup>	1.0 <sup>ab</sup>
NE <sup>3</sup>	2.83 <sup>b</sup>	0.023 <sup>b</sup>	0.6 <sup>bc</sup>
HE <sup>4</sup>	12.46 <sup>a</sup>	0.030 <sup>a</sup>	1.0 <sup>ab</sup>
NF <sup>5</sup>	3.59 <sup>b</sup>	0.025 <sup>b</sup>	1.2 <sup>ab</sup>
HF <sup>6</sup>	13.36 <sup>a</sup>	0.031 <sup>a</sup>	1.4 <sup>a</sup>

Means followed by the same letter in the columns do not differ by the Tukey test ( $p \leq 0.05$ ); <sup>1</sup>non-hypercholesterolemic control; <sup>2</sup>hypercholesterolemic control; <sup>3</sup>non-hypercholesterolemic rats treated with ethanol/acetone extract flour; <sup>4</sup>hypercholesterolemic rats treated with ethanol/acetone extract flour; <sup>5</sup>non-hypercholesterolemic rats treated with leaf flours; <sup>6</sup>hypercholesterolemic rats treated with leaf flours.

**Table 3.** Total serum cholesterol, triglycerides, HDL and non-HDL cholesterol in *Wistar* rats treated with guava leaves.

Treatment	Cholesterol (mg dL <sup>-1</sup> )	Triglyceride (mg dL <sup>-1</sup> )	HDL cholesterol (mg dL <sup>-1</sup> )	Non-HDL cholesterol (mg dL <sup>-1</sup> )
NC <sup>1</sup>	46.26 <sup>d</sup>	40.06 <sup>ab</sup>	14.98 <sup>b</sup>	31.22 <sup>c</sup>
HC <sup>2</sup>	96.70 <sup>a</sup>	50.78 <sup>a</sup>	8.80 <sup>c</sup>	87.90 <sup>a</sup>
NE <sup>3</sup>	50.40 <sup>cd</sup>	38.14 <sup>b</sup>	19.99 <sup>a</sup>	30.90 <sup>c</sup>
HE <sup>4</sup>	84.78 <sup>ab</sup>	29.84 <sup>b</sup>	16.10 <sup>ab</sup>	70.04 <sup>b</sup>
NF <sup>5</sup>	51.60 <sup>cd</sup>	35.44 <sup>b</sup>	17.01 <sup>ab</sup>	32.22 <sup>c</sup>
HF <sup>6</sup>	67.46 <sup>bc</sup>	38.74 <sup>b</sup>	13.01 <sup>bc</sup>	54.28 <sup>b</sup>

Means followed by the same letter in the columns do not differ by the Tukey test ( $p \leq 0.05$ ); <sup>1</sup>non-hypercholesterolemic control; <sup>2</sup>hypercholesterolemic control; <sup>3</sup>non-hypercholesterolemic rats treated with ethanol/acetone extract flour; <sup>4</sup>hypercholesterolemic rats treated with ethanol/acetone extract flour; <sup>5</sup>non-hypercholesterolemic rats treated with leaf flours; <sup>6</sup>hypercholesterolemic rats treated with leaf flours.

a significant difference ( $p \leq 0.05$ ) between the animals of the NC group with the HC group, that is, there was the formation of an inflammatory process that can progress to atherosclerosis, which demonstrates that none of the treatments was efficient in reducing the synthesis of C-reactive protein by the liver.

In Table 3, it is possible to observe that the HC group had serum levels of total cholesterol significantly ( $p \leq 0.05$ ) higher than the ones of the NC group, indicating hypercholesterolemia. The values of the HC group (96.70 mg dL<sup>-1</sup>) are close to those of other studies with rats that developed hypercholesterolemia (Machado et al., 2003; Rocha et al., 2012). Among the treatments, only guava leaf flour (HF group) decreased total cholesterol levels significantly ( $p \leq 0.05$ ), compared to the HC group, with a reduction of 30.24%.

A hypercholesterolemic diet induces an increase in lipid accumulation in hepatocytes, leading to a fatty liver, which can be demonstrated in this study. The significant reduction in cholesterol levels provided in this study by guava leaf flour may be due to the presence of dietary fiber found in this flour (Table 1). Several studies show that soluble fibers cause a reduction in blood cholesterol levels. Some studies show that the reduction in serum

cholesterol in rats was caused by soluble fibers that bind to cholesterol, leading to its elimination, thus reducing its absorption by the liver (Camire and Dougherty, 2003; Rocha et al., 2012).

For triglycerides, the results show that the treatments guava leaf flour (HF) and ethanol/acetone extract flour (HE) significantly ( $p \leq 0.05$ ) decreased triglyceride levels, compared to the HC group, with a reduction of 41.24 and 23.71% for the HF and HE groups, respectively.

The highest reduction in triglyceride levels caused by the extract flour of guava leaves can be probably justified by the higher content of phenolic compounds and antioxidant activity shown in this extract.

For HDL cholesterol, only the HE group caused a significant ( $p \leq 0.05$ ) increase in HDL levels, compared to the HC group, with an increase of 83.95%. The treatments guava leaf flour (HF) and ethanol/acetone extract flour (HE) significantly ( $p \leq 0.05$ ) decreased the levels of non-HDL cholesterol, compared to the HC group, with a reduction of 38.25 and 20.32% for HF and HE groups, respectively.

The animals in the groups that received only the NE and NF commercial diet were statistically ( $p \leq 0.05$ ), similar to the NC group in relation to serum levels of total chole-

terol, triglycerides, HDL and non-HDL cholesterol.

The reduction of non-HDL cholesterol is important, since several studies have shown that a reduction in total cholesterol is not an efficient measure to reduce cardiovascular mortality associated with cardiovascular disease patients or prevent their emergence (Liu et al., 2000; Magalhães et al., 2002).

## Conclusion

Guava leaves (Pedro Sato cultivar) constitute a significant source of dietary fiber, phenolic compounds, vitamin C, beta-carotene, and they have antioxidant activity. These leaves present a hypocholesterolemic potential, since they cause a reduction in serum levels of total cholesterol, triglycerides and non-HDL cholesterol.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors would like to thank CAPES, CNPq and FAPEMIG for the financial support.

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