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Research Article

Hypoglycemic Effect of *Kalanchoe pinnata* (Lam) Pers. Leaf Extract

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ABSTRACT

Introduction: *Kalanchoe pinnata* (Lam) Pers (Crasulaceae) is a succulent ornamental plant. In Costa Rica, the leaves are used as a coadjuvant treatment for *Diabetes Mellitus* based on traditional knowledge of natural remedies. Moreover, there are some studies mentioning its use for *Diabetes Mellitus* as medicinal plant in several countries. This research aimed to demonstrate the antidiabetic properties of hydroalcoholic extracts of *K. pinnata* leaves through phytochemical screening, alpha amylase inhibition and rodent models. **Methods:** Crude extracts of *K. pinnata* leaves were prepared by infusion and decoction using water:ethanol (70:30) as a solvent. The extracts prepared by decoction (LAED, lyophilized-water:ethanol-decoction) and by infusion (LAEI, lyophilized-water:ethanol-infusion) were analyzed by Folin-Ciocalteu, HPLC and capacity of inhibition of α-amylase activity. To determine hypoglycemic activity in rats, extracts were administered orally at doses of 250, 500 and 750 mg/Kg and blood sugar levels were monitored over a four hours period using a glucometer. **Results:** A significant reduction ($p < 0.05$) in blood glucose was observed after one hour in rats treated with 500 mg/Kg of LAED extract. Treatment with 750 mg/Kg LAEI induced a statistically significant reduction in blood sugar at 90, 180 and 240 min, showing that the glucose-lowering effect of this extract was greater at a higher concentration. **Conclusions:** This study confirmed the hypoglycemic effect of *K. pinnata* extracts in the acute phase in rats and supports the use of this Crassulaceae as a home remedy.

Key Words: Antidiabetic activity, Diabetic, Extract, *Kalanchoe pinnata*.

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INTRODUCTION

Diabetes Mellitus (DM) is a global health problem. It is the most common of the endocrine disorders and is characterized by chronic hyperglycemia caused by a relative or absolute lack of insulin secretion or insulin activity. According to the World Health Organization (2016),[1] the diabetic population is projected to increase to 300 million or more by the year 2025. In Costa Rica, 8743 new cases of *Diabetes mellitus* were reported in 2014, representing a rate of increase of 183.17 cases per 100000 inhabitants.[2] In the same year, there were 720 deaths from this disease, representing 15.08 deaths per 100000 inhabitants, primarily among women older than 75 years of age.[3]

The recommended treatment for diabetes includes oral medications or subcutaneous insulin injections, as well as diet modification and exercise. However, some of the medications can cause secondary effects in patients. Modern synthetic prescription medications and insulin for effective treatment of diabetes are scarce, especially in rural areas, since they are expensive and have important adverse effects. There is not ideal medication for the treatment of *Diabetes mellitus* that can control blood sugar without secondary effects such as hypoglycemia and weight gain, and also reduce cardiovascular morbimortality and maintain the integrity and normal functioning of pancreatic cells. Development of complementary and alternative approaches to diabetes management, such as the isolation of phytochemicals with antihyperglycemic activity, is imperative. For this reason, many patients resort to home remedies or medicinal plants for diabetes treatment or to complement prescribed medications.[4]

Medicinal plants have been known for thousands of years and are appreciated as rich sources of therapeutic agents for the prevention of disease and other ailments.[5] *Kalanchoe pinnata* (Lam) Pers. (Synonym *Bryophyllum pinnatum)* is a succulent plant native to Madagascar and found in South America, India, and the Caribbean.[6] Leaves of this plant are consumed raw or prepared as infusions or decoctions as alternative medicine for diabetes.[7,8] Other therapeutic properties attributed to this plant include antibacterial activity against *Staphylococcus*, *E. coli*, *Shigella*, *Bacillus* and *Pseudomonas,*[9] and anticancer,[10] antiparasitic,[11] antiallergic, and anti-inflammatory properties.[12] This research aimed to demonstrate the antidiabetic properties of hydroalcoholic extracts of *K. pinnata* leaves through phytochemical screening, alpha amylase inhibition and rodent models.

MATERIALS AND METHODS

Plant material

Kalanchoe pinnata (Lam) Pers. plants were collected in Garabito, Puntarenas (9”51´05”N, 84°38´48”W), Costa Rica. Permits for collection and bioprospection were obtained from the National Commision for Biodiversity CONAGEBIO (R-CM-ITCR-0014-

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2018-OT). The collected plant material was identified at the National Herbarium of the National Museum of Costa Rica (DHN: 060-2017). Plants were transferred to a greenhouse for leaf propagation.

Preparation of extracts

Leaves were washed with antibacterial soap and water and dried by lyophilization (L) (-20°C, 50 bar for 14 h). After drying, leaves were pulverized using a laboratory knife mill (IKA®, Germany) and stored in the dark.

Extracts of the dried leaf powder were prepared by infusion (I) or decoction (D) at a ratio of 1:10 (w/v) using water:ethanol (70:30) (AE, acronym in Spanish) as the extraction solvent. Each sample was extracted five times. The extracts were centrifuged, lyophilized and protected from light.

Phytochemical screening

Total polyphenols were quantified using the Folin-Ciocalteu method.[13] The phenolic compounds kaempferitrin® and kaempferol® were quantified by HPLC (High Performance Liquid Chromatography), Agilent Technologies. Detection was achieved at 280 nm at 30 °C with a flow rate of 0.3 mL/min, using 5 µL of extract. Phase A consisted of 99.9% water and 0.1% formic acid. Phase B was 50% methanol and 50% acetonitrile. The gradient phase was 88% A and 12% B, and the final phase was 35% A and 45% B.

A-amylase inhibition assay

The inhibitory effect of the extracts on the enzyme α-amylase was determined using the EnzChek® Ultra Amylase Assay Kit (Thermo Fisher Scientific E33651) with some modifications. Briefly, different concentrations of the extracts were incubated with 2 mU of *Bacillus sp*. α-amylase (A-6380-Sigma), at room temperature for 30 min. Incubation of α-amylase without extracts were used as positive control. After incubation of the enzyme and the extracts, 10 ug of the DQ starch substrate were added to the mixture and incubated for 30 min at room temperature. The fluorescence was measured in the Synergy® plate reader adjusted for absorption at 480/20 nm and emission at 528/20 nm.

Hypoglycemic activity: Oral starch tolerance model

Drugs

Glibenclamide 5 mg (Lisan® Laboratories, San José, Costa Rica). Two tablets were dissolved in 20 ml of distilled water for oral administration at a dose of 7.5 mg/Kg.

Animals

Male Sprague-Dawley rats weighing between 180 and 220 g were obtained from the Biological Assays Laboratory Bioterium of the University of Costa Rica. The experimental protocol was approved by the University Bioethics Committee (CICUA-053-17). Rats were maintained under standard conditions of temperature 22 ± 2 °C, with light/dark cycles of 12 h and food and water *ad libitum*.

Hypoglycemic activity: Oral starch tolerance model

Rats were subjected to a 12 h fasting period prior to oral starch tolerance testing. Each rat was weighed to determine the amount of extract to be administered. Groups of 7 animals received the following treatments: hydroalcoholic extracts LAED (lyophilized-water:ethanol-decoction) and LAEI (lyophilized-water:ethanol-infusion) at 250, 500 and 750 mg/Kg body weight dissolved in water, water (negative control) and glibenclamide (positive control). Thirty minutes after treatments were applied; each rat was fed 2g of starch per kg of body weight. All treatments were administered orally. Blood glucose was measured in fasting rats (time 0) and 30 minutes after treatments were administered (time 1). Further glucose measurements were made 30, 60, 90, 120, 180 and 240 min after starch ingestion. Blood samples were obtained by scratching the tip of the tail using a scalpel. Blood glucose levels were quantified with an Accu Chek Performa ® glucometer. The change ratios of blood glucose levels were calculated for each animal using the following formula: 100 + 100× (postdrug blood glucose level – predrug blood glucose level) / (predrug blood glucose level).

Statistical analysis

Treatments in the *in vivo* test (LAEI and LAED extracts at 750 mg/Kg, 500 mg/Kg and 250 mg/Kg) were compared at each sampling time. Results were analyzed by One-way ANOVA and the Fisher test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Phytochemical screening

Lyophilization conserved the appearance and color and the concentration of chemical compounds of interest. The LAEI and LAED fractions contained high concentrations of polyphenols (1.467 mg and 0.898 mg EQ of gallic acid/mL) and flavonoids (0.08 and 0.05 mg EQ quercetin/mL), respectively, and identify Kempherol and Kapheritrin by HPLC. This information is consistent with that reported by Tadera *et al,* (2006),[14] who found that naringenin, kaempferol, luteolin, myricetin and quercitin were potential inhibitors of porcine alpha-amylase. The presence of inhibitory compounds in the LAEI and LAED extracts suggests their potential as diabetes treatments.

A-amylase inhibition

Inhibition of enzyme activity by LAED and LAEI extracts was greater than 90% (97% and 92.7%, respectively) at an extract concentration of 100 µg/mL (Figure 1).

Hypoglycemic activity

Treatment with LAED 500 mg/Kg resulted in significantly lower ($p<0.05$) blood glucose levels than in negative control (water) treatments 60, 90, 120 and 180 min after starch ingestion. Moreover, the lowest (250 mg/Kg) and highest (750 mg/Kg) concentrations of LAED extracts induced a reduction in glucose levels 180 and 240 minutes after starch ingestion, respectively (Figure 2A).

On the other hand, the effect of LAEI 250 mg/Kg was significantly different from negative control at 90 minutes, after which the effect was similar to water. However, at 750 mg/Kg, blood glucose was significantly lower at 90, 180 and 240 minutes, showing that at a higher concentration the hypoglycemic effect was greater (Figure 2B).

DISCUSSION/CONCLUSIONS

Inhibition of the intestinal enzyme α–amylase may represent an important therapeutic approach for the treatment of Type 2 diabetes. Inhibition of this enzyme impedes carbohydrate digestion and slows glucose absorption, thereby reducing the increase in postprandial glucose in the plasma.[14,15] Some phenolic acids and flavonoids known for their antioxidant properties have been associated with enzyme inhibition and may be candidates for control of starch digestion and postprandial glycemia.[15,16]

Blood sugar was significantly lower in rats treated with glibenclamide (7.5 mg/Kg) than in rats treated with extracts or water (negative control) at all evaluation times (from 30 to 240 min). This response was expected, as glibenclamide is a commercial medicine used for diabetes control. It would be interesting to determine the effect of the natural

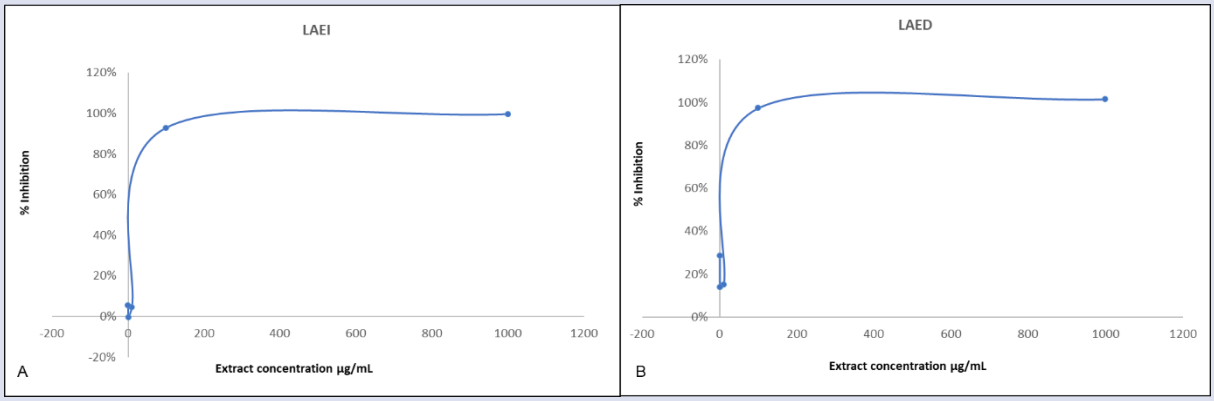

Figure 1: Percent inhibition of α-amylase by extracts of *Kalanchoe pinnata* LAED (lyophilized-water:ethanol-decoction) and LAEI (lyophlized-water:ethanol-infusion, acronym in Spanish).

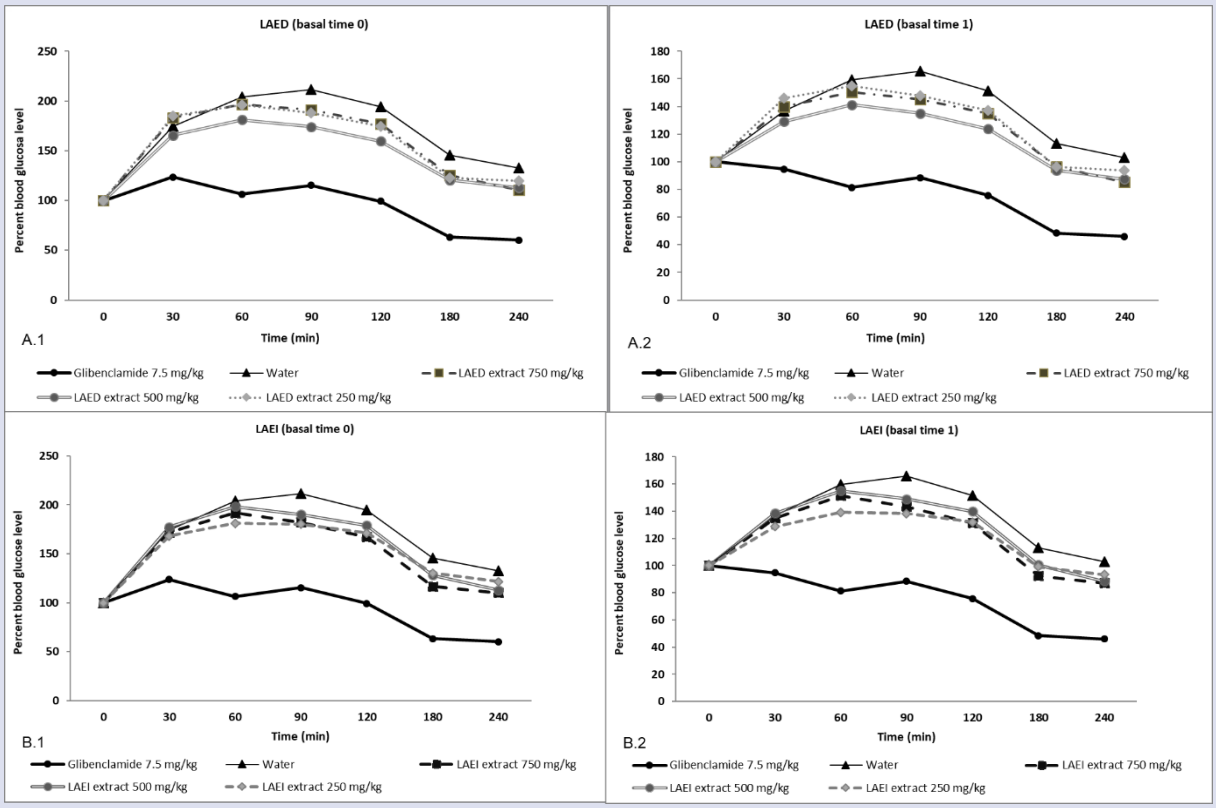

Figure 2: Hipoglycemic activity of hydroalcoholic extracts of *K. pinnata* leaves at different doses in rats (n=7). (A) Percentage reduction of blood glucose measured at time 0 (fasting glucose) and after administering the extract (basal time 1) of LAED. (B) Percentage reduction of blood glucose measured at time 0 (fasting glucose) and after administering the extract (basal time 1) of LAEI.

extract administered jointly with glibenclamide in order to evaluate the coadjuvant effect of this plant species.[17]

Although the effect of the extracts did not surpass that of glibenclamide, blood sugar was reduced at most of the extract concentrations and evaluation times (Figure 2).

Ojewole (2005)[7] obtained similar results with aqueous extracts of *B. pinnatum* at 400 mg/Kg. Blood glucose levels were significantly lower relative to controls one and two hours after administration of the extracts, with a peak reduction at two and four hours. Ogbonnia *et al* (2008)[18], using hydroalcoholic mixtures of extracts of *B. pinnatum* and *T. africana* (500 mg/Kg body weight), reported lowered blood glucose levels in diabetic rats after 120 minutes. Results of this study provide evidence of the hypoglycemic effect of *K. pinnata* in traditional home preparations of infusions or decoctions and support its possible use as a natural coadjuvant for enhanced diabetes control. Therefore, it would be interesting to evaluate the antihyperglycemic activity of *K. pinnata* in diabetic animals.

We choose LAEI and LAED fractions contained high concentrations of polyphenols (1.467 mg and 0.898 mg EQ of gallic acid/mL) and flavonoids (0.08 and 0.05 mg EQ quercetin/mL). In addition, was greater than 90% in α-Amylase Inhibition.

This study confirmed the hypoglycemic effect of *K. pinnata* in the acute phase. The effects of the extracts on postprandial glucose in plasma were evaluated during a four hours period following starch ingestion.

Although the extracts did not elicit the same response as the commercial drug glibenclamide, the extracts LAED (500 mg/Kg) and LAEI (750 mg/Kg) induced a positive response in the reduction of blood glucose levels after starch ingestion. The LAED (500 mg/Kg) extract showed a more constant glucose reduction over time.

These results show the enormous pharmacological potential of *K. pinnata* and the importance of further research. It will be important to evaluate the effect of these extracts administered jointly with glibenclamide to determine their function as coadjuvants to potentiate or enhance the response to prescription medications.

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ABBREVIATION

CENIBiot: Centro Nacional de Innovaciones Biotecnológicas; **CIB:** Centro de Investigación en Biotecnología; **INIFAR:** Instituto de Investigaciones Farmacéuticas; **ITCR or TEC:** Instituto Tecnológico de Costa Rica; **LAED:** Lyophilized-water:ethanol-decoction; **LAEI:** Llyophilized-water:ethanol-infusion.

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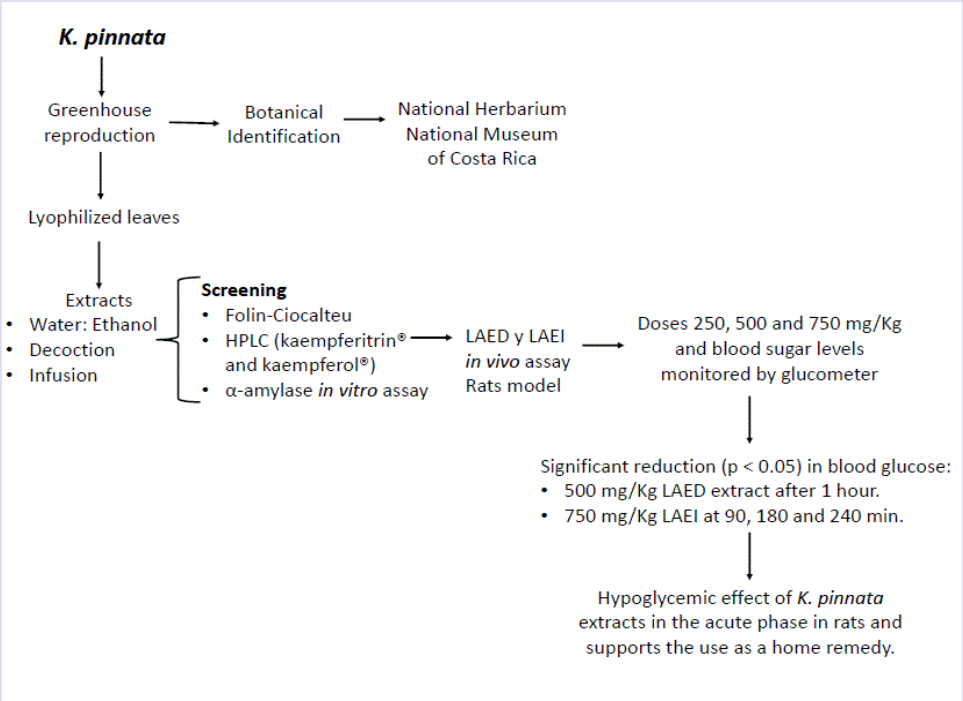
GRAPHICAL ABSTRACT



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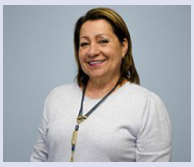

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