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FULL PAPER

Antioxidant and hypoglycemic effect of *Vasconcellea candicans* (A. Gray) A. DC. in albino mice: a native fruit of the Peruvian flora

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Abstract

"Mito", "Andean Papaya" and "kerko" are the popular names of *Vasconcellea candicans* (A. Gray) A. DC. In some regions in Peru, this species is a shrub tree, endemic to the Peruvian Andes and Ecuador. This study aimed to evaluate the antioxidant and hypoglycemic effect of the ethanolic extract of *Vasconcellea candicans* fruit on alloxan-induced hyperglycemia in albino mice. *Vasconcellea candicans* fruits were collected in Uruiza, Lucanas-Ayacucho, Peru. Phytochemical analysis was carried out to confirm chemical groups, and antioxidant activity *in vitro* was measured using two methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid) diammonium salt radical cation (ABTS). In the pharmacological evaluation, hyperglycemia was induced with alloxan using doses of 170 mg/kg in albino mice, animals with a glucose level of more than 250 mg/dL were included in the treatment. Animals were randomized into five groups (I: Alloxan 170 mg/kg; II, III, and IV: *Vasconcellea candicans* extract: 100, 300, and 500 mg/kg body weight; and V: glibenclamide 5 mg/kg). The phytochemical analysis confirmed the presence of flavonoids, alkaloids, terpenes, saponins, and phenolic compounds. It was observed that at doses of 100, 300, and 500 mg/kg, blood glucose was reduced by 43.6%, 60.8%, and 70.5 %, respectively, after 16 days of treatment. The ethanolic extract had an IC_{50} = 19.6±0.5 µg/mL and 5.05±0.01 µg/mL against DPPH and the ABTS radical, respectively. In conclusion, the ethanolic extract of the *Vasconcellea candicans* fruit was demonstrated to be effective at 500 mg/kg following 14 days of treatment in mice.

1. Introduction

Diabetes mellitus (DM) is a term used to describe a group of metabolic illnesses characterized by chronic hyperglycaemia caused by abnormalities in insulin secretion, insulin action, or both (Tseng, 2021). Type 1 diabetes (T1-DM), Type 2 diabetes (T2-DM), particular forms of diabetes-related to various causes, illnesses of the exocrine pancreas, drug- or chemical-induced diabetes, and gestational diabetes mellitus are now defined by the American Diabetes Association (Tan *et al.,* 2019). Hyperglycemia might develop, causing acute symptoms and metabolic abnormalities. Chronic consequences produced by sustained hyperglycemia, such as retinopathy, neuropathy, renal disease, and cardiovascular disease, are the leading causes of morbidity in diabetes (Kabir *et al.,* 2021). Fortunately, continuous glycemic management can help many patients to avoid persistent problems. There are numerous therapeutic approaches for hyperglycemia, in which several targets are involved in glucose regulation (Duan *et al.,* 2021).

Around the world, herbal medications and plant components with low toxicity and no adverse effects are popular therapeutic choices for treating this disease. Herbal therapy can be used as an adjutant in diabetes

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patients to complement conventional treatments due to its multiple mechanisms of action (Kooti *et al.,* 2016). Although those medicinal plants have been used as a primary form of healthcare for a long time, they have not been fully used as acceptable drugs in the management of diabetes due to a lack of understanding of their chemical profile, standardized preparation procedure, potential side effects, and ambiguity regarding the most effective form and dosage of administration (Yeung *et al.,* 2020).

On the other hand, the Vasconcellea genus is the largest of the six genera of the Caricaceae family, and its distribution extends throughout the Andes Mountains in South America, with a concentration of species in Ecuador, where 16 species live at 3500 meters above sea level (m.a.s.l.). *Vasconcellea candicans*, known as "kerco" or “mito” (Figure 1), is a native species in Peru and southern Ecuador (Gutiérrez and Cruz, 2016), where it grows from 0 to 3000 m.a.s.l. Adapted to sub-xerophytic places, in the mountains and also in coastal hills, it has been known since pre-Hispanic Peru times and has very important phytogenetic potential (Acosta-Villalba *et al.,* 2015). The fruits have pleasant and very fragrant flavours when consumed fresh. Ripe fruits are used as a digestive, as well as to treat liver pain, and the latex of the plant is used to treat warts. *Vasconcellea candicans* is a typical shrub in the Peruvian highlands (Gutiérrez *et al.,* 2017). To date, there is no scientific information regarding its medicinal use in the preclinical phase. Hence, the main objective of this study was to determine the antioxidant activity and the hypoglycemic effect of the ethanol extract of *Vasconcellea candicans* fruit against alloxan-induced hyperglycemia in albino mice.

Figure 1. *Vasconcellea candicans* (A. Gray) A. DC. fruits collected

2. Materials and methods

2.1 Taxonomic classification

The plant was classified in the Herbarium of the Natural History Museum of the Universidad Nacional Mayor de San Marcos as *Vasconcellea candicans* (90-USM-2020) according to the APG IV (2016).

2.2 Plant material

Fruits of *Vasconcellea candicans* were collected in Uruiza, Otoca district, Lucanas province, Ayacucho department, located at 3019 m.a.s.l. in February–March 2020. Optimal fresh fruits in a good state of ripeness were used for the extraction. Subsequently, fruits were washed with drinking water to eliminate contaminating residues, and then the mesocarp was separated from the epicarp manually and fragmented to a suitable size.

2.3 Obtention of the ethanolic extract of Vasconcellea candicans

To obtain the ethanolic extract, 2000 g of fruits (only pulp) of *Vasconcellea candicans* was weighed, which was subjected to fragmentation using a blender, previously sterilized and macerated in 3000 mL in 96% ethanol for 7 days and stored in a dark place, shaking 2–3 times a day. The blend was then filtered and concentrated on a rotary evaporator. At the end of the process, 40 g of the constant weight of dark brown ethanolic extract with a characteristic smell of the fruit was obtained. It was stored in an amber glass flask and refrigerated until further use.

2.4 Phytochemical screening of the ethanolic extract.

To carry out phytochemical analysis, 20 mL of the ethanolic extract of *Vasconcellea candicans* was used and diluted with 96% ethanol. The colouration and precipitation reactions were confirmed to determine the presence of characteristic functional groups (Herrera-Calderon *et al.,* 2017a).

2.4.1 Shinoda reaction

To recognize the presence of flavonoids in a plant extract, an aliquot of the extract was mixed with 1 mL of concentrated hydrochloric acid and a piece of metallic magnesium. A red colour confirms the presence of flavonoids.

2.4.2 Gelatin reaction

R total of 0.5 mL of extract was taken, and 0.5 mL of 0.5% aqueous gelatin solution was added. For the determination of tannins, the reaction is positive if turbidity or precipitate appears.

2.4.3 Reaction with ferric chloride ($FeCl_3$)

A total of 0.5 mL of extract was taken, and 1-2 drops of 0.5% aqueous ferric chloride solution were added. For the determination of free phenolic groups, the reaction is

positive if an intense blue, black, or green colour appears.

2.4.4 Reaction for alkaloids

In all, 2 mL of the sample was placed in a test tube and brought to dryness, then the residue was dissolved with 5 mL 1% HCl by heating slightly to 50°C. Approximately 0.5 mL of the solution was taken and added with the corresponding reagent for Mayer, Wagner and Dragendorff tests. For Mayer reaction, the reaction is considered positive if a white precipitate appears. For Dragendorff reaction, the reaction is considered positive if an orange precipitate appears. For Wagner reaction, the reaction is considered positive if a brown or dark brown precipitate appears.

2.4.5 Lieberman–Burchard reaction

A total of 1 mL of the sample was taken, and 1 mL of acetic anhydrous and 2 drops of concentrated sulfuric acid were added. For the determination of terpenoids, the reaction is positive if a blue-green or orange colour appears.

2.4.6 Foam test

An aqueous solution of the sample was subjected to vigorous shaking for 30 s. Foaming confirms the presence of saponins.

2.5 Antioxidant activity

The determination of antioxidant activity was assayed using the methodology of inhibition of organic radicals (Herrera-Calderon *et al.,* 2017b). Different concentrations of the extract were reacted with two radicals, 2.2-diphenyl-1-picrylhidrazyl (DPPH) and acid 2.2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic ($ABTS^{+}$).

2.6 Pharmacological study

2.6.1 Experimental animal

In all, 30 male Balb/C albino mice with an average weight of 20-25 g, six weeks of age, purchased from the National Center for Biological Products of the National Institute of Health (Chorrillos, Peru), were used for the pharmacological study. Animals were acclimatized for a period of 15 days with free access to balanced food and water and were kept at an average temperature of 20±2° C and a 12/12-hour light/dark cycle. Institutional and International ethical standards and procedures were considered.

2.6.2 Induction of experimental diabetes

Hyperglycemia induction was carried out according to the method described (Issa and Bule, 2015) with dose modification of Alloxan: Experimental diabetes was induced in groups: I, II, III, IV, and V by intraperitoneal injection of alloxan monohydrate using a dose of 100 mg/kg of body weight after 12 hrs of fasting. Alloxan was dissolved in 0.9% NaCl at a concentration of 20 mg/mL. Then, it was applied immediately. After 48 hrs, hyperglycemia was verified by means of determination of glucose in the blood, mice with blood glucose levels >250 mg/dL were included in this study.

2.6.3 Distribution of the experimental and treatment groups

Animals were randomly distributed into five groups, made up of six animals each:

Group I: Alloxan 170 mg/kg + Distilled Water (10mL/Kg)

Group II: Alloxan 170 mg/kg + Ethanolic extract of *Vasconcellea candicans* 100 mg/kg.

Group III: Alloxan 170 mg/kg + Ethanolic extract of *Vasconcellea candicans* 300 mg/kg.

Group IV: Alloxan 170 mg/kg + Ethanolic extract of *Vasconcellea candicans* 500 mg/kg.

Group V: Alloxan 170 mg/kg + Glibenclamide 5 mg/kg

The ethanolic extract of *Vasconcellea candicans* was administered orally twice a day, for a period of 16 days. Glibenclamide at a dose of 5 mg/kg was administered orally every 24 hrs according to the specific dosage of the drug.

2.6.4 Determination of glucose (Glucose oxidase method)

The blood sample was extracted from each of the mice through an incision of the apex of the animal's tail, discarding the first drop and receiving the next one on the Prestige Easy (Nipro®) test strip. For analysis with a Nipro® digital glucometer, glucose was determined once a day every 3 days until the 16 days of treatment had been completed (McMillin, 1990).

2.7 Statistical analysis

To interpret the research results, the results obtained for each group were compared by performing statistical procedures such as mean values (X)±standard error (SE) using the SPSS version 22.0 statistical package. The analysis of variance (ANOVA) and Duncan's multiple comparisons test were used to assess significant differences between treatment groups. At $p<0.05$, the results were judged to be statistically significant.

3. Results

3.1 Phytochemical study of the ethanolic extract of Vasconcellea candicans

The results of the qualitative phytochemical analysis are presented in Table 1. It is noted that only tannins were not identified using the gelatin test, which was confirmed when an aqueous solution of the extract was reacted with 1% gelatin. We used three types of reactions to determine alkaloids in order to confirm and avoid false-positive reactions. A positive reaction was achieved for phenolic compounds and flavonoids but was less abundant. Additionally, steroidal saponins were confirmed using a foam test and Lieberman Burchard reaction.

Table 1. Phytochemical analysis of the ethanolic extract of *V. candicans* fruit

Secondary Metabolite	Test	Results
Tannins	Gelatin reaction	-
Phenolic compounds	Ferric Chloride ($FeCl_3$)	+++
Alkaloids	Dragendorff reaction	+++
Alkaloids	Wagner reaction	+++
Alkaloids	Mayer reaction	+++
Flavonoids	Shinoda reaction	+
Terpenoids	Lieberman Burchard reaction	+++
Saponins	Foam test	++

Note: absence (-), mild presence (+), moderate (++), intense (+++)

3.2 Antioxidant activity of the ethanolic extract of Vasconcellea candicans

Antioxidant activity was determined using DPPH and ABTS organic radicals. Figure 2 shows that the ethanolic extract had good concentration-dependent activity, i.e., half of the inhibitory concentration, IC_{50} = 19.6±0.5 µg/mL; for the ABTS method, the IC_{50} was 5.05±0.01 µg/mL. The concentrations of Trolox tested in the antioxidant study had better results compared to the ethanol extract, and the results were statistically significant ($P \leq 0.001$), except at 50 µg/mL for the DPPH test.

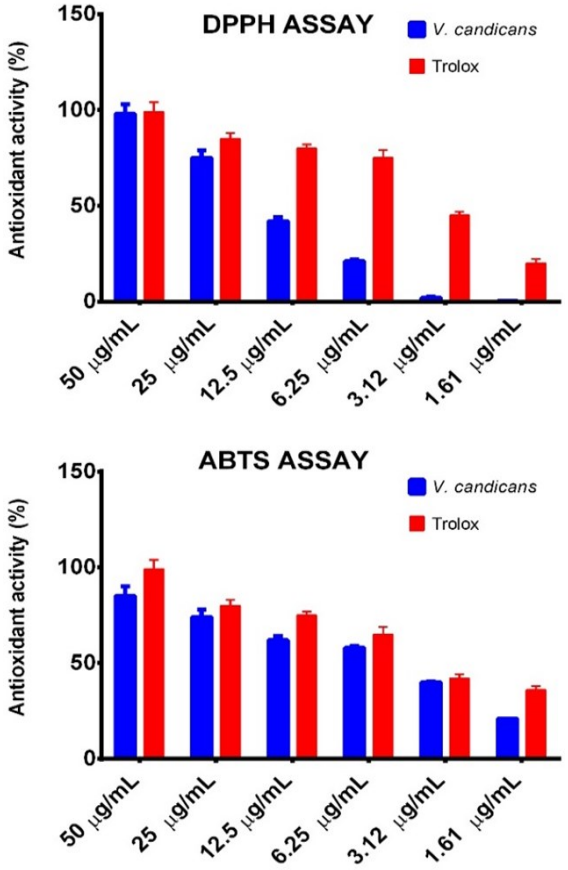

Figure 2. Antioxidant activity of the ethanolic extract of *V. candicans.*

3.3 Hypoglycemic activity of the ethanolic extract of Vasconcellea candicans

In Tables 2 and 3, better results of efficacy are observed in the group treated with an extract at a dose of 300 mg/kg. However, the Duncan test suggests that there is no significant difference between the glycemic averages of the groups treated with extract at doses 500 mg/kg and Glibenclamide 5 mg/kg. However, no significant differences were found between the glucose values of groups III and IV treated at doses of 300 and 500 mg/kg. All groups treated with the extract and drug control had significant differences from the alloxan group.

Table 2. Hypoglycemic activity of *V. candicans* in albino mice

Experimental group	Glucose values (mg/dL), n = 6						
	Time						
	Basal	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16
I: Control 10 mL/kg (distilled	400.0±10.2	378.7±10.5	395.3±9.8	384.0±8.5	397.2±10.5	401.8±20.5	395.3±10.5
II: Extract 100 mg/kg +Alloxan	350.0±15.2	200.8±10.0	190.8±8.0	178.5±10.5	158.5±10.0	186.2±10.2	198.8±5.0
III: Extract 300 mg/kg + Alloxan	389.3±20.2	130.7±11.5[a]	155.5±15.0[a]	151.0±12.5[a]	122.0±10.5	139.0±9.0[a]	130.8±10.0[a]
IV: Extract 500 mg/kg + Alloxan	371.3±15.2	130.2±10.5 [a]	121.0±10.2	122.8±10.0[a]	127.0±11.0	89.7±9.5[a]	109.0±7.5
V: Glibenclamide 5 mg/kg + Alloxan	349.2±10.5	116.0±9.5	122.3±9.0	140.0±8.5	121.7±7.0	169.2±10.6	127.0± 7.0

Blood glucose levels of experimental groups (Group III, and IV) were compared to glibenclamide group (Group V). Values are presented as mean±SD. Values with a superscript are significantly different ($P \leq 0.05$) from group V using one-way ANOVA followed by Duncan's test.

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Table 3. Variation percentage of the hypoglycemic effect of *V. candicans* in albino mice.

Experimental group	Variation of Glucose values (%), n = 6					
	Time					
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16
Extract 100 mg/kg +Alloxan	-42.2	-45.4	-47.4	-52.9	-44.1	-43.6
Extract 300 mg/kg + Alloxan	-66.3	-59.7	-61.1	-68.5	-63.9	-60.8
Extract 500 mg/kg + Alloxan	-64.6	-67.2	-66.8	-65.6	-75.7	-70.5
Glibenclamide 5 mg/kg + Alloxan	-66.2	-63.7	-59.0	-64.7	-51.2	-63.3

In Table 3, it is noted that high variation percentages in regard to the decrease in blood glucose levels were found in the groups treated with 100, 300, and 500 mg/kg, standing at 43.6%, 60.8%, and 70.5%, respectively, following 16 days of treatment. However, as is shown in Table 3, there are no significant differences between day 1 and day 16, but these values were constant during treatment with the pharmacological substances.

4. Discussion

Diabetes mellitus is a chronic disease that can be controlled with diet, oral hypoglycemic agents, and insulin, so the contribution of the present investigation using *Vasconcellea candicans* is interesting for the management of diabetic patients. Hyperglycemia was induced using alloxan as a toxic reagent. Several chemical agents are cytotoxic to beta cells of the pancreas, while alloxan and streptozotocin have been systematically investigated and are widely used to induce diabetes in experimental animals (Radenković *et al.,* 2016). Mortality linked to the use of alloxan is variable and linked to multiple factors capable of changing the effects of the drug and the sensitivity of the host, considering the hydration status of the drug, route of administration, diet, time fasting, and the weight of the animal (Gargouri *et al.,* 2016). The action of alloxan in the pancreas is preceded by its rapid absorption by pancreatic beta cells. On the other hand, a reduction process occurs in the presence of different reducing agents, such as reduced glutathione (GSH), cysteine, ascorbate, and sulfhydryl groups (-SH) bound to proteins. Alloxan reacts with two -SH groups at the glucokinase sugar-binding site, resulting in disulfide bond formation and enzyme inactivation (Lenzen, 2007). Dialuric acid is generated during the reduction of alloxan, which is then re-oxidized back to alloxan, initiating a redox cycle for the formation of reactive oxygen species (ROS) and superoxide radicals ($O^{2\bullet-}$). Ferric ions are released from ferritin by superoxide radicals, which then reduce them to ferrous and ferric ions. In the presence of superoxide dismutase, superoxide radicals also decay to form hydrogen peroxide (H_2O_2). As a result, highly reactive hydroxyl radicals are formed according to the Fenton reaction in the presence of ferrous metals and H_2O_2, producing severe cellular damage (Rahman *et al*., 2021).

Our result indicates that the fruit of the *Vasconcellea candicans* presented phenolic compounds, alkaloids, flavonoids, triterpenes, and saponins. Flavonoids play an essential role in protecting against oxidative damage phenomena, due to their anti-free radical properties, and are mainly directed toward hydroxide and superoxide radicals, and highly reactive species, thus blocking the deleterious action of alloxan on beta cells (Akter *et al*., 2021)

The ethanolic extract at doses of 100 and 300 mg/kg reduced blood glucose by 43.6% and 60.8%, respectively, after 16 days after administration. However, doses at 500 mg/kg reduced blood glucose by 70.5% in the same period and were thus the most effective doses tested in mice. Meanwhile, Glibenclamide at a dose of 5 mg/kg reduced blood glucose by 63.3% 16 days after administration.

The flavonoids present in the ethanolic extract may be responsible for the hypoglycemic effect since they have been shown to participate in the initial stages of insulin in the liver and muscles in rats *in vivo*, and one of their mechanisms of action is that they bind the receptors of insulin, potentiate the activity of the enzyme tyrosine kinase, an essential enzyme for the final biological effects of insulin, including reducing hyperglycemia (Testa *et al.,* 2016). There are also clinical studies that implicate flavonoids in the prevention of diabetes, alluding to their anti-inflammatory, antithrombotic, antioxidant, and anticancer properties. However, it remains to be verified whether other constituents present in the ethanolic extract may be involved in the observed effects (Al-Ishaq *et al.,* 2019).

It is concluded that the extract administered at a dose of 500 mg/kg was more effective than the other treatments at the end of the study period. Phytochemical characterization of the ethanol extract by liquid chromatography-mass spectrometry (LC-MS) is needed in order to confirm the bioactive molecules responsible for the hypoglycemic and antioxidant effects.

Conflicts of interest

The authors declare no conflicts of interest in this work.

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