Antioxidant, Antimutagenic, and Antidiabetic Activities of Edible Leaves from *Cnidoscolus chayamansa* Mc. Vaugh

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**ABSTRACT:** The methanolic extract of *Cnidoscolus chayamansa* leaves from Mexico was screened for antioxidant and antimutagenic properties by the DPPH, ABTS, iron chelating, and Kado microsuspension assays, respectively. The hypoglycemic effect was also studied. Total phenolic and flavonoid contents as well as HPLC identification and quantification of protocatechuic acid and rutin were also carried out. The *C. chayamansa* leaves extract contained 71.3 ± 1.7 mg gallic acid equivalent/g extract and 42.6 ± 3.7 mg (+)-catechin equivalent/g extract of total phenols and flavonoids, respectively. Concentrations of 0.242 ± 0.001 mg/g and 2.00 ± 0.097 mg/g were found for protocatechuic acid and rutin, respectively. The extract was capable of scavenging DPPH and ABTS \(^{+}\) radicals in a concentration dependent manner. The extract was not toxic to TA100 and TA98 strains at the concentrations tested; moreover, the extract at a concentration of 1000 μg inhibited 24% and 39% the mutagenicity induced by 4-nitro-O-phenylenediamine and sodium azide, respectively. An acute hypoglycemic effect in diabetic rats was observed.

Practical Application: *C. chayamansa* has been proposed as an herbal medicine to treat diabetes; however, the reported results are not conclusive and further studies need to be performed. Despite this fact, chaya leaves can be commercialized as tea in a dried presentation since the dried leaves conserve high polyphenol contents.

Keywords: antimutagenic, antioxidant, *Cnidoscolus chayamansa*, hypoglycemic, secondary metabolites

**Introduction**

*Cnidoscolus chayamansa* Mc. Vaugh. Euphorbiaceae family, commonly known as “chaya,” is a domesticated plant highly valued by the population of rural communities in Central and South of Mexico as an ornamental and medicinal plant, and food (Ross-Ibarra and Molina-Cruz 2002). Since pre-Columbian time, chaya leaves and young shoots have been consumed. Often, the leaves are boiled to prepare dishes and blended raw to prepare drinks. The *C. chayamansa* leaves are an important source of protein, β-carotene, vitamins, ascorbic acid, calcium, and iron (Kuti and Kuti 1999; Sarmiento-Franco and others 2003). Phytochemical studies concerning the chemical composition of this plant have allowed the isolation or identification of cyanogentic glycosides, amentoflavone, kaempferol, and quercetin glycosides (González-Laredo and others 2003; Kuti and Konuru 2004). Raw and cooked ethanol/acetone/water/acetic acid extracts of chaya leaves demonstrated antioxidant activity (Kuti and Konuru 2004) and the chaya tea diminished the glucose levels of diabetic rabbits (Kuti and Torres 1996). We report here the antioxidant, the antimutagenic and hypoglycemic properties of the methanolic extract of leaves from *C. chayamansa* collected in Queretaro, Mexico.

**Materials and Methods**

**Chemicals**

Solvents were either analytical or HPLC grade and were obtained from Baker (Mallinkrodt Baker Inc., Phillipsburg, N.J., U.S.A.), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin Ciocalteu reagent, ferrozine, ferric chloride, FeSO\(_4\), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate (K\(_2\)S\(_2\)O\(_8\)), aluminum chloride (AlCl\(_3\),H\(_2\)O), acetic acid, gibbenclamide, sodium acetate, (+)-catechin, gallic, protocatechuic, caffeic, and rosmarinic acid, quercetine, naringenine and rutin were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

**Plant material**

Leaves of *C. chayamansa* were collected at the young stage, from Queretaro in August 2004. Mature leaves are almost three-lobed, with entire to slightly dentate margins while young leaves are often entire, lacking of any distinct lobes (Ross-Ibarra and Molina-Cruz 2002). A voucher specimen of the plant was deposited at the Ethnobotanical Collection of the Herbarium of Queretaro “Dr. Jerzy Rzedowski” (QMEX) located at the School of Natural Sciences, Univ. of Queretaro. Air-dried, ground plant material was extracted by maceration; 500 g were extracted with 1 L of a solvent mixture of hexane-acetone 1 : 1 at room temperature for 5 days twice. Thereafter, the material was extracted with methanol at the same conditions. The chaya methanolic extract was evaporated to dryness in vacuum, and stored at 4 °C for later use.

**Determination of total phenolic and flavonoid contents**

The total phenolic content of the extracts was determined according to the Folin–Ciocalteu colorimetric method (Dewanto and others 2002). The appropriate dilutions of the extracts were oxidized with 250 μL of 1N Folin–Ciocalteu reagent. After 5 min, 1.25 mL of a 20% Na\(_2\)CO\(_3\) solution were added to neutralize for 2 h. The absorbance was measured against a prepared blank at 760 nm. Results are expressed as milligram of gallic acid equivalents per gram of extract. The total flavonoid content was determined by
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the method described by Liu and others (2002). The appropriate dilutions of the extracts were mixed with 75 μL of a 5% NaNO₂ solution. After 6 min, 150 μL of a 10% AlCl₃, 6H₂O solution was added, and the mixture was allowed to stand for another 5 min. Then, 0.5 mL of 1M NaOH was added, and distilled water was added for a total volume of 2.5 mL. The solution was well mixed, and the absorbance was measured against a prepared blank at 510 nm. The results are expressed as milligram of (++)-catechin equivalents per gram of extract. All data are reported as means ± SD of triplicate analysis.

HPLC analysis
Plant extract was filtered through a 0.2 mm filter membrane and 20 μL were injected in triplicate into a reversed phase column (Zorbax Eclipse XDB-C18, 60 Å, 5 μm, 250 × 4.6 mm), using a Waters high-performance liquid chromatography (HPLC) system (Waters Corp., Milford, Mass., U.S.A.), which consisted of a quaternary pump (model 600), a photodiode array detector (model 996), an in-line vacuum degasser (MetaChem Technologies Inc.), and a Rheodyne injector (7493). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium™ software program (Waters). The mobile phase was composed of solvent A (acetonitrile) and solvent B (acetic acid 0.0125 N). The elution was as follows: isocratic conditions from 0 to 2 min with 5% A and 95% B, gradient conditions from minute 2 to 5 starting with 5% A and ending with 15%, gradient conditions from minute 5 to 20 starting with 15% A and ending with 50%, gradient conditions from minute 20 to 25 starting with 50% A and ending with 5%, isocratic conditions from 25 to 35 min with 5% A and 95% B. The flow rate was 1 mL/min, the absorbance was set at λmax 280 nm and 20 μL of sample were injected. Quantification was carried out by external standardization with protocatechuic, caffeic, rosmarinic acid, quercetin, naringenin, and rutin. Full standard curves were constructed with protocatechuic acid and rutin.

Antioxidant and metal quelating capacities

**DDPH method.** Antiradical activity (ARA) was determined using the stable radical DPPH, according to the method reported by Fukumoto and Mazza (2000). All reactions were conducted in 96 well microplates. A 20 μL aliquot of a methanolic solution of the extracts at various concentrations (10, 100, 500, 1000, 2500, 5000 μg/mL) was mixed with 200 μL of 150 μM of DPPH in 80% methanol. The controls contained all the reaction reagents except the extract or positive control substances (Trolox and gallic acid). After 30-min incubation at ambient temperature in darkness, the resultant absorbance was recorded at 730 nm at 0 and 6 min in a Spectra Max Tunable Microplate Reader (Molecular Devices Co.). The percentage of absorbance inhibition was calculated according to the Eq. (1). The radical scavenging activities for the DPPH and ABTS results were expressed as the median inhibitory concentration (IC₅₀) values and TEAC. The IC₅₀ was calculated from the log-dose inhibition curve obtained by a nonlinear regression algorithm (Prism, 4.0, GraphPad) and the TEAC value was calculated employing a Trolox calibration curve and expressed as micromol Trolox per gram extract.

**Metal chelating activity.** The chelating activity of Fe²⁺ was obtained according to the method described by Hinneburg and others (2006). Briefly, 200 μL of the extract was added to 100 μL of 2 mM aqueous FeCl₃ and 900 μL of methanol. After 5 min, 400 μL of 5 mM ferrozine were added. The absorbance at 562 nm was recorded after 10 min. The iron quelation activities were calculated following the Eq. (1) and expressed as μmol Na₂EDTA/g extract.

**Mutagenicity and antimutagenicity testing.** The Kado microsuspension assay was used for testing mutagenic and antimutagenic activity (Kado and others 1983; Kado and others 1986). The tester strains, TA100, TA98 and the microsomal fraction S9 were purchased from Molecular Toxicology Inc. (Annapolis, Md., U.S.A.). Sodium azide (Na₃N₅) and 4-nitro-O-phenylenediamine (4-N-O-P) were used as positive control for TA100 and TA98 tester strains, respectively. For TA100 strain, 0.01 mL Na₃N₅ (40 μg/mL), 0.01 mL extract solution (125, 250, 500, and 1000 μg extract/tube), and 0.005 mL extract solution (125, 250, 500, and 1000 μg extract/tube) + 0.005 mL of Na₃N₅ (40 μg/mL) were evaluated. While for TA98 0.01 mL of 4-N-O-P (50 μg/mL), 0.01 mL the extract solution (125, 250, 500, and 1000 μg extract/tube), and 0.005 mL extract solution (125, 250, 500, and 1000 μg extract/tube) + 0.005 mL of 4-N-O-P (50 μg/mL) were tested. Samples were tested in triplicate for each independent experiment performed.

**Experimental animals.** Wistar rats (280 to 300 g) for diabetes studies were obtained from Harlan, S.A. All animals were fed on standard pellet diet and water ad libitum. The animals were housed at 23 to 24°C with a 12 h light–dark cycle (light period from 8:00 a.m. to 8:00 p.m.). The rats used in the present studies were maintained in accordance with guidelines of the Univ. of Queretaro.

**Experimental induction of diabetes in rats.** Wistar rats were injected intraperitoneally with streptozotocin (STZ) at a dose of 45 mg/kg body weight dissolved in 0.1 M citrate buffer (pH 4.5). After 1 wk, rats with blood glucose of 180 to 240 mg/dL were used for the experiment. Blood samples were taken from tail vein and the glucose concentration was measured with a glucometer (ROCHE).

**Fasting blood glucose levels in diabetic rats.** Diabetic rats were divided into 3 groups of 6 rats—group 1: control (water), group 2: chaya extract (10, 40, and 70 mg/kg bw), and group 3: glibenclamide (3 mg/kg bw). Animals were fasted overnight and intragastrically treated with the methanolic extract; blood glucose concentrations were evaluated at 0, 60, 120, 180, and 240 min after treatment. To evaluate the acute effect of chaya, nondiabetic and diabetic rats were used. The animals were divided into 6 groups as follows: nondiabetic groups: control (water) and group treated with chaya extract (70 mg/kg bw); diabetic groups: control (water), animals treated with chaya extract (70 mg/kg), glibenclamide (3 mg/kg), and insulin (3 U/100g). The rats were treated daily with chaya extract and glibenclamide by intragastric administration and insulin was injected subcutaneously. After 3 d of treatment, fasting blood glucose levels were quantified.
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Statistical analysis
Experimental values are given as means ± SD. Statistical significance was determined by one-way variance analysis (ANOVA). Differences at P < 0.05 were considered to be significant. A Tukey test for comparison of multiple means was used.

Results and Discussion
Total phenols and flavonoids in C. chayamansa. The extraction with hexane and acetone was performed to remove the non-polar components and most of the pigments. Since phenolic substances have been shown to be responsible for the biological activities of plant extracts (Soobrattee and others 2005), the total phenol and flavonoid contents of the methanolic extract were investigated, 71.3 ± 1.7 mg gallic acid equivalent/g dried extract and 42.7 ± 3.7 mg (+)-catechin equivalent/g dried extract were obtained for total phenols and flavonoid, respectively. Kuti and Konuru (2004) reported 1.2 ± 0.02 and 0.7 ± 0.28 mg chlorogenic acid equivalent/g fresh weight and 67.1 ± 5.4 and 58.3 ± 5.8 μg of flavonol (kaemferol and quercetin) /g fresh weight for raw and cooked C. chayamansa, respectively. The contents, in the present study, are higher than the reported values by Kuti and Konoburo since we are reporting the data base on grams of dried extract. Although, the phenolic content of C. chayamansa is lower compared with reported data for the leaves of Camellia sinensis, Eucalyptus globules and Flex paraguarensis (200, 113, 202 mg gallic acid equivalent/g, respectively), the content is higher compared with Mentha Canadensis, Ocimum basilicum, Rosmarinus officinalis, and Salvia officinalis (51.5, 36.4, 50.7, 53.2 mg gallic acid equivalent/g, respectively) (Shan and others 2005; Anesini and others 2008; Dudonné and others 2009). Regarding the HPLC analysis, only protocatechuic acid (0.242 ± 0.001 mg/g) and rutine (2.00 ± 0.097 mg/g) were identified and quantified by comparing the retention time with standards, and calculating the concentration from the respective calibration curves.

Antioxidant activity
Free radicals play an important role in chronic diseases related to oxidative stress, such as diabetes, cancer, and cardiovascular pathologies (Willcox and others 2004; Soobrattee and others 2005). Therefore the free radical scavenging properties of the extract were determined by the DPPH and ABTS assays, where both radicals derivatives. The ARA, TEAC, and IC50 values for the extract are shown in Table 1. The methanolic extract was capable of scavenging DPPH and ABTS radicals in a concentration dependent fashion; however, the extract demonstrated different capacity to scavenge the radicals as can be observed in Figure 1. At the maximum concentration the extract demonstrated different capacity to scavenge the radical (

It has been suggested that the antioxidant capacity of phenolic compounds such as flavonoids is also performed through the chelation of transition metal ions involved in free radicals production (Mira and others 2002; Leopoldini and others 2006). Therefore the chelation properties of the extract were determined by the Fe2+ chelating assay. A moderate chelating activity was detected at the concentration of 5000 μg/mL corresponding to 36.92 ± 0.85 mg Na2EDTA/g extract.

Mutagenicity and antimutagenicity testing
To determine the concentration of Na3 and 4-N-O-P for the antimutagenic assays in TA100 and TA98, a dose-response curve was obtained for each mutagen (Na3 μg/tube: 30, 60, 125, 250, 500, 1000 and 4-N-O-P μg/tube: 0, 50, 100, 250, 500, 750, 1000). The antimutagenic effect of the methanolic extract of leaves from chaya (Cnidoscolus chayamansa) collected in Queretaro on sodium azide and 4-Nitro-O-phenylendiamine mutagenicity in TA100 and TA98.

<table>
<thead>
<tr>
<th>Strain/mutagen</th>
<th>Extract (μg)</th>
<th>Revertants/plate</th>
<th>Inhibition (%)</th>
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</thead>
<tbody>
<tr>
<td>TA100/Na3</td>
<td>0</td>
<td>1567 ± 24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1241 ± 22</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1173 ± 31</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1039 ± 27</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>957 ± 40</td>
<td>39</td>
</tr>
<tr>
<td>TA98/4-N-O-P</td>
<td>0</td>
<td>510 ± 18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>408 ± 17</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>402 ± 26</td>
<td>21</td>
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<tr>
<td></td>
<td>500</td>
<td>389 ± 19</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>389 ± 23</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 1 — Antioxidant activities of the methanolic extract of leaves from chaya (Cnidoscolus chayamansa) collected in Queretaro.

<table>
<thead>
<tr>
<th>Assay</th>
<th>ARA /%</th>
<th>TEAC μmol/g extract</th>
<th>IC50 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>13.75 ± 0.6</td>
<td>144.68 ± 13.7</td>
<td>ND</td>
</tr>
<tr>
<td>ABTS</td>
<td>52.07 ± 6.6</td>
<td>214.18 ± 29.4</td>
<td>1693 ± 1.2</td>
</tr>
</tbody>
</table>

*Data obtained for 1000 μg/mL.
Results are the average of 3 independent experiments ± SD.
ND = not determined.

Table 2 — Antimutagenic effect of methanolic extract of leaves from chaya (Cnidoscolus chayamansa) collected in Queretaro on sodium azide and 4-Nitro-O-phenylendiamine mutagenicity in TA100 and TA98.

Results are the average of 3 independent experiments ± SD. Different letters mean a statistical difference (Tukey test, α = 0.05). Na3 = 100 μg/mL, and 4-N-O-P = 50 μg/mL.

Figure 1 — % Inhibition compared with concentration of extract curves of antioxidant effect for DPPH and ABTS assays.

Figure 2 — Hypoglycemic effect of chaya extract (70 mg/kg) administered intragastrically to diabetic rats. Data are means ± SEM of 6 rats per treatment. *P < 0.01, significantly different from the diabetic control.
Antidiabetic effect

Currently, there is an interest of natural hypoglycemic compound sources considering the adverse side effects of the available oral hypoglycemic drugs (Burt and others 2009; Solomon and others 2009). Therefore, the hypoglycemic effect of C. chayamansa extract was evaluated through the STZ-induced experimental diabetes in rats. The chaya methanolic extract was intragastrically administered to diabetic rats at doses of 10, 40 and 70 mg/kg body weight to evaluate the hypoglycemic effect. There was no observed hypoglycemic effect of chaya extract at 10 and 40 mg/kg. However, the chaya extract at dose of 70 mg/kg decreased the fasting blood glucose concentration in the experimental time frame; being statistically significant lower than the control group at 120 (11%) and 180 (18%) min (P < 0.01). A similar effect was observed when glibenclamide was administered (Figure 2). These results suggest that chaya extract probably act by stimulating the insulin secretion of the few surviving β-cells of the pancreas from animals with 240 mg/dL glucose levels under fasting conditions. The results obtained in this study are according with the reported by Kuti and Torres (1996), who demonstrated that oral administration of chaya tea, diminishes the blood glucose levels of the diabetic rabbits up to 26%.

Conclusions

The methanolic extract of leaves from C. chayamansa showed a high content of phenolic compounds including protocatechuic acid and rutine. The extract was capable of scavenging DPPH and ABTS*+ radicals in a concentration dependent fashion, although the antioxidant activity was poor. The antimutagenic evaluation demonstrated that the extract was not toxic to TA100 and TA98 strains at the concentrations tested, and showed a 39% and 24% average of antimutagenic effect on 4-nitro-O-phenylenediamine and sodium azide mutagenicity, respectively. The chaya extract exhibited a hypoglycemic effect after 120 min of administration. C. chayamansa has been proposed as an herbal medicine to treat diabetes; however, the results are not conclusive and further studies need to be performed.

References

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