

**DIURETIC ACTIVITY OF FIVE MEDICINAL PLANTS
USED POPULARLY IN CUBA**

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Summary

Introduction: diuretics are drugs capable of increase levels of urine, so they are useful in the treatment of diseases related with the retention of fluids. In Cuba it is attributed diuretic action to 179 vegetable species based on its traditional use but only a few of them has been experimentally valuated. The objective of this investigation was to experimentally proof the diuretic action because of its traditional use attributed to: *Bidens alba* (romerillo), *Carica papaya* (fruta bomba), *Rhoeo spathacea* (SW) Stearn (cordován), *Costus cylindricus* Jack (caña de la India) and *Capraria biflora* (esclaviosa) and to evaluate the toxic potential at a single dose of those more pharmacologically effective. Materials and Methods: Primary phytochemical characterization was made to all extracts; diuretic effect was carried out in S/D rats (180 – 220 g BW) by measuring the urine volume every hour in the first 6 hours and later at 24 hours. Positive control was furosemide (20 mg/kg BW). The aqueous extracts were administered at 400 mg/kg BW and those who exerted similar diuretic effect to that of furosemide at doses of: 200, 400 and 800 mg/kg BW. Results: *Bidens pilosa* and *Costus Cylindricus* showed the best diuretic effects but with higher K⁺ and Na⁺ excretion compared to positive control ones. It was proved that *Bidens pilosa* and *Costus cylindricus* are non toxic after single dose administration. Conclusions: it was determined that all the extracts show diuretic effect outstandingly those of *Bidens alba* and *Costus cylindricus*.

Introduction

The diuretics are drugs that act on the kidney and are able to increase the volume of urine excreted, reason why are used in affections such as: cardiac failure, chronic and moderate insufficiencies cardiac, acute oedema of the lung, nephritic oedema syndrome, arterial hypertension, among other.(1,2). In Cuba to 179 vegetal species diuretic actions is traditionally attributed, but only a little of them have been valued in an experimental form (3-5). For that reason the objective of this work was to verify experimentally the diuretic action attributed to *Bidens pilosa* L (romerillo), *Carica papaya* (papaya) *Rhoeo spathacea* (SW) Stearn (Cordován), *Costus cylindricus* Jack (caña de la India) and *Capraria biflora* (esclaviosa), as well as the toxic potential in a single dose to those being most pharmacologically effective (6).

Methods

Referring data to the vegetal material: The used vegetal material in the investigation was collected in the UBPC "Octubre Victorioso" of the municipality of Santa Clara taking data such as: the date and hour of collection, the name of the collector and phenological state of the plant and identified by the Botanist and samples were selected for their conservation in the herbarium. The collected plants were:

Scientific name	Common name	Family	Employed part
<i>Rhoeo spathacea</i> (Sw) <i>Stearn</i>	Cordován	Commelinaceae	aerial part
<i>Carica papaya</i>	Fruta bomba	Caricaceae	seeds
<i>Costus cylindricus</i>	Caña de la India	Gramineaceae	aerial part
<i>Bidens pilosa</i>	Romerillo	Asteraceae	aerial part
<i>Capraria biflora</i>	Esclaviosa	Scrophulariaceae	leaves

Preparation of extracts:

After verifying the absence of strange matters in the collected material it was dried in a stove at 33 °C and reduced to fine dust by means of blades (Retsch 6 mbh 5657 type SR-2 with a sieve of 2,0 mm). The watery extracts were elaborated in the Provincial Medicine Laboratory by the percolation method. Each extract was characterized registering No. of Lot, content, average, organoleptic characteristics, refractive index, pH, total density and total solids as well as the primary phytochemical characterization was made.

Biological model and experimentation conditions:

The study was made in healthy male Sprague Dawley rats, with a weight between 180-220g. The same ones were put for a period of 5 days to their adaptation to the experimental conditions (temp:19 - 22°C and relative humidity: 50%). The animals were lodged in T-4 boxes with bottom of grid at a rate of 5 animals by cage. The selection of the animals to the groups was at a random form. 7 experimental groups of 8 rats settled down each one, the group I negative control (NaCl 0.9%), the group II positive control (furosemide 20 mg/ Kg BW), group III extract of *Carica papaya*, the IV extract of *Rhoeo spathacea*, the V extract of *Bidens pilosa*, the VI extract of *Costus cylindricus* and the VII the extract of *Capraria biflora*. All the extracts of the plants to study were administered at a dose of 400 mg/Kg BW, considering total solids. The volume administered to all the groups was 40 mL/Kg BW. The animals were privates of food from 3:00 pm of previous day and the water was suppressed to them at 7:00 am of the following day. The administration was made by means of intragastric cannula beginning at 8:00 am. The rats were placed in metabolic cages and the urine volume was registered to ½, 1, 2, 3, 4, 5, 6 and 24 hours postadministration. At 24 hours it was collected accumulated urine and determinations of Na⁺ and K⁺ were made. Additionally blood by means of retroorbital puncture was extracted and the concentrations of Na⁺ and K⁺ were also determined. Finally the animals were sacrificed by means of anaesthesia with ether. The aqueous extracts which exerted the best diuretic effect were administered at doses of: 200, 400 and 800 mg/kg BW in order to study dose response relationship and also acute toxicology study was carried out to these extracts, the biological model was Sprague Dawley rats, by the method of the classes (guideline No. 423 OCDE). Three groups were utilized, the group 1 and 2 received 2,000 mg / Kg BW of the two more pharmacologically effective extracts and the group 3 functioned as negative control. Each group had 10 animals (5 females and 5 males). The statistical processing of the results was made by the statistical package SPSS for Windows, version 11.5. The averages and standard deviations of each one of the variables evaluated in each experimental group were compared using the Test of Mann Whitney and Kruskal Wallis.

Results

The phytochemical characterization threw that the flavonoids are in all the extracts being minimum in the seeds of *Carica papaya* and maximum in *Bidens pilosa* and *Costus cylindricus*, whereas the alkaloids were in a greater proportion in the seeds of *Carica papaya*. Although present in all the extracts the tannins its amount was smaller. There were reached diverse diuretic levels of action and renal excretion of ions as showed in the Table 1 and Table 2.

Table 1. Volumes of urine in the experimental groups at 1, 3, 6 and 24 hours

Group	Volumen of urine mL/Kg			
	1 hour	3 hours	6 hours	24 hours
Negative control	0	14,16 ± 7,52	30,61 ± 5,74	62,54 ± 5,43
Furosemide	28,23 ± 11,47 (a)	50,15 ± 16,51 (a)	53,92 ± 16,55 (a)	80,19 ± 12,42 (a)
<i>Rhoeo spathacea</i>	22,10 ± 9,35 (a)	41,85 ± 8,67 (a)	47,98 ± 8,26 (a)	71,48 ± 7,81 (a)
<i>Carica papaya</i>	17,47 ± 8,25 (a) (b)	36,16 ± 9,45 (a) (b)	54,08 ± 10,23 (a)	77,09 ± 11,56 (a)
<i>Bidens pilosa</i>	9,36 ± 3,38 (a)(b)	30,94 ± 7,56 (a)(b)	50,22 ± 7,72 (a)	88,81 ± 7,69 (a)
<i>Costus cylindricus</i>	23,32 ± 7,79 (a)	40,52 ± 8,65 (a)	52,89 ± 9,57 (a)	78,04 ± 10,69 (a)
<i>Capraria biflora</i>	8,01 ± 4,05 (a) (b)	32,25 ± 6,69 (a) (b)	42,71 ± 8,10 (a)(b)	69,49 ± 6,06 (b)

N =8, (a) Signification whit the negative control, (b) Signification whit the furosemide

Table 2. Na⁺ K⁺ concentrations in mEq/L of urine at 24 hours

Group	N	Na ⁺ Concentration mEq/L	K ⁺ Concentration mEq/L
Negative control	7	1,61 ± 0,46	0,53 ± 0,14
Furosemide	8	1,98 ± 0,58	0,67 ± 0,25
<i>Carica papaya</i>	8	1,28 ± 0,02(a)(f)	2,75 ± 0,14 (c)(f)
<i>Rhoeo spathacea</i>	8	1,59 ± 0,37	1,43 ± 0,28(c)(f)
<i>Bidens pilosa</i>	8	2,35 ± 0,49(b)	1,34 ± 0,49(c)(f)
<i>Costus cylindricus</i>	8	2,09 ± 0,77	1,62 ± 0,39(c) (e)
<i>Capraria biflora</i>	8	2,05 ± 0,66	1,37 ± 0,48(c)(f)

n =8, a) Signification whit the negative control p< 0.05; (b) Signification whit the negative control p< 0.01; (c) Signification whit the negative control p< 0.001 ; (d) Signification whit the furosemide p< 0.05; (e) Signification whit the furosemide p< 0.01; (f) signification whit the furosemide p< 0.001

In all the groups the concentrations of ions in blood stayed within the normal values (Na^+ between 139-150 mmol/L and K^+ between 3.6-8.4 mmol /L).

The aqueous extracts which exerted the best diuretic effect were *Bidens pilosa* and *Costus cylindricus*, but there were no dose response relationships because the excretion of urine of those groups treated at the dose of 800 mg/Kg was lower than that of 400 mg/Kg BW. These results are similar to reported by Jiménez et al (5).

The toxicity study to the plants that exerted the best diuretic effect (*Bidens pilosa* and *Costus cylindricus*) showed neither symptoms nor signs of toxicity and the body weight gain were normal and no alterations in the anatomopathologically analyzed organs were observed in treated groups at a dose of 2000 mg/Kg BW.

Discussion

In the negative control group the diuresis began passed 2 hours of the administration, showing low volumes of urine excreted until completing 62.54 mL/ Kg at 24 hours. The values of excreted Na^+ and K^+ in urine were equally low. On the contrary, in the group dealt with furosemide (positive control), the beginning of the diuretic action was at 30 minutes agreeing with previous reports (1). A final volume of 80.19 mL/Kg was reached being significantly different from the obtained in the negative control group ($p < 0.05$). These results corroborate the powerful diuretic effect of furosemide (2).

In group III dealing with the watery extract of the seeds of *Carica papaya* the beginning was also at 30 minutes postadministration, but the volume was smaller (1.22 mL /Kg), differing significantly from the values obtained with furosemide ($p < 0.05$) being reached a total volume of 77.09 mL/Kg. this results was similar to the one with the root of this plant (7) perhaps associated with the alkaloids contents in this extract. Similarly it was obtained an increase of the excretion of Na^+ in the urine significantly superior to the one registered in the negative control group ($p < 0.05$) and very highly significantly superior compared with furosemide group. The K^+ concentration in urine, was very highly significantly superior compared with negative control and furosemide groups ($p < 0.001$), but the concentration of both ions in blood were in the normal level.

Group IV, which was administered with the watery extract of *Rhoeo spathacea*, showed a furosemide like behaviour ($p > 0.05$), reaching a total volume 71.48 mL /Kg. the beginning of action was at 1 hour, this result can be attributed to the high contents of flavonoids and saponins in this extract. The Na^+ concentration in urine did not differed significantly from the positive control group ($p > 0.05$), instead was very highly significantly superior those of K^+ ($p < 0.001$). There were obtained blood Na^+ and K^+ values in the normal rank.

In the group V, treated with *Bidens pilosa*, diuresis started 1 hour postadministration but in other times was similar to furosemide. It was reached 88.81 mL/kg of total excreted volume of urine in 24 hours, superior to positive control. This result can be attributed to the high contents of flavonoids in this extract (8), in a similar way there were high urine levels of Na⁺ that was highly significantly superior compared to negative control ($p < 0.01$) and high levels of K⁺ which was very highly significantly superior compared to negative control and furosemide ($p < 0.001$), although serum levels were normal.

In the case of group VI, receiving *Costus cylindricus*, diuresis was very rapid (30 min postadministration) reaching a volume of 9.89 mL/Kg similar to furosemide ($p > 0.05$). The total excreted volume was 78.04 mL/kg. This result can be attributed also to the high contents of flavonoids (9) and saponins in this extract. Urine Na⁺ concentration was superior to that of negative control nevertheless there were no significant differences between them. Referring to urine K⁺ there were significantly superior to those of negative control ($p < 0.001$) and furosemide ($p < 0.01$); serum values of both ions stayed in the normal rank.

Group VII, which was administered with the watery extract of *Capraria biflora* diuresis started 1 hour postadministration. All the time diuresis was lower than furosemide. It was reached 69.49 mL/Kg of total excreted volume of urine in 24 hours, significantly inferior to furosemide. There were high urine levels of Na⁺ and K⁺, being only this last one very highly significantly superior compared to negative control ($p < 0,001$), although serum levels were normal.

All the extracts studied present diuretic effect, but the watery extracts of *Bidens pilosa* and *Costus cylindricus* showed the best diuretic effects, what could be associated to their high content of flavonoids in these plants, both extracts promote high levels of Na⁺ and K⁺ in urine. There are correspondence between the volume of urine and the concentration of Na⁺, this aspect is logical because the mechanisms of action of great diuretics drugs is to decrease the tubular reabsorption of this ion, it produces the dragging of the osmotic equivalent of water, other explanation that can support this, is the high ions concentration in this medicinal plants (7). All the plant produced high concentration of K⁺ in urine. From all the extracts the most powerful in excreting K⁺ was *Carica papaya*.

The results obtained in the acute toxicology prove that both plants *Bidens pilosa* and *Costus cylindric* are non toxic after an administration of a single limit dose, coinciding with another toxicological study with medicinal plant (10).

Conclusions

All the evaluated extracts showed diuretic effects after an administration of a 400 mg/kg BW dose. The extracts of *Bidens pilosa* and *Costus. cylindricus* showed the best diuretic properties and also superior urine excretions of Na⁺ and K⁺ compared to negative and positive control. The diuretic effect of both extracts behaved in a non dose-response way and none of them exerted acute toxicity.

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