

## Ethnopharmacological and preclinical study of diuretic activity in medicinal and food plants used by Cuban population

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**Abstract:** In Cuba, there exists about 179 medicinal plants which are known because of their diuretic properties. To evaluate the diuretic activity attributed to six medicinal plants used by the Cuban population: *Ocimum basilicum* L., *Parthenium hysterophorus* L. (medicinal plants), *Justicia pectoralis* Jacq., *Plectranthus amboinicus* (Lour.) Spreng., *Allium cepa* L. and *Citrus aurantium* L. (medicinal and food plants). Aqueous extracts were prepared from the dry drug of the first four plants mentioned above, and in the case of *A. cepa* and *C. aurantium* natural juice were taken for the study. Eight Sprague-Dawley male rats were taken randomly and eight homogeneous groups were formed: group 1, received 20 mg/kg of furosemide (reference drug); group 2, received NaCl (0.9%), and groups from 3 to 8 received doses of 400 mg/kg BW, based on extracts determination of total solids, this dose volume was completed with physiological saline solution to achieve an hydrosaline overcharge in a constant total administration volume of 40 ml/kg PV, in all experimental groups as described in literature, using in all of the cases an 16 G intragastric cannula. Rats were placed in metabolic cages, where urinary excretion during the ½, 1, 2, 3, 4, 6 and 24 hours was measured and diuretic activity and action were later mathematically calculated. There was observed an increase of the urine volume in treated groups in relation with the negative control group. Urinary excretion, action and diuretic activity were superior in the experimental groups corresponding to *O. basilicum* L. and *A. cepa* and similar to reference diuretic drug. This research allowed us to conclude preliminarily that from the six studied plants, *O. basilicum* and *A. cepa* exerts best diuretic activity (moderated).

**Keywords:** diuretics, medicinal plants, rats.

### دراسة حول استخدام الادوية التقليدية العرقية وتأثير نشاط مدر البول للنباتات الطبية والغذائية المستخدمة لدى الشعب الكوبي

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**الملخص:** يوجد بكوبا حوالي 179 نباتا طبيا معروفا بأنه يسبب إدرار البول. ولتقييم نشاط مدر البول لستة نباتات طبية تستخدم بواسطة الشعب الكوبي وهي كالآتي: *Ocimum basilicum* L., *Parthenium hysterophorus* L. (medicinal plants), *Justicia pectoralis* Jacq., *Plectranthus amboinicus* (Lour.) Spreng., *Allium cepa* L. and *Citrus aurantium* L. عمل محاليل مائية مستخلصة من الأربع نباتات الأولى بعد تجفيفها وطحنها. وأيضا تم استخلاص محاليل مائية من العصير الطبيعي للنباتان الآخران. وقد تم عمل مجموعات عشوائية من ذكر الفئران براغ دوالي ووضع ثمانية فئران في كل مجموعة من الثماني مجموعات المتجانسة التشكيل وأخذت المجموعة الأولى المجموعة 1، تلقى 20 ملغم / كغم من فوروسيميد (المخدرات المرجعية)؛ مجموعة 2، تلقى كلوريد الصوديوم (0.9 ٪)، ومجموعات من 3 إلى 8 تلقت جرعات من 400 مغ / كغم من وزن الجسم، استنادا إلى استخلاص المواد الصلبة الكلية وتم تكملة الحجم النهائي للمحلول بالمحلول الفسيولوجي المحلي لتحقيق hydrosaline overcharge للكمية الثابتة المأخوذة والتي تقدر ب 40 مل/كجم في كل المجموعات التجريبية كما وضعت في الأبحاث السابقة وتستخدم في جميع الحالات intragastric cannal ووضعت الفئران في أقفاص التمثيل الغذائي حيث تم قياس عملية إدرار البول خلال 1/2، 1، 2، 3، 4، 6، 24 ساعة ولاحقا تم إدرار البول. ولوحظ هناك زيادة في حجم البول في المجموعات المعاملة بالمقارنة مع مجموعة المراقبة السلبية. إفراز البول وتأثير نشاط مدرار البول كان عاليا جدا في المجموعات المعاملة بواسطة *Ocimum basilicum* L. , *Allium cepa* L. وكان مشابهة للأبحاث السابقة. وهذه الدراسة المبدئية للبحث تبين إن من دراسة 6 أنواع من النباتات تبين أن مستخلص من *O. basilicum* and *A. cepa* أفضل مدر للبول.

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## Introduction

Generally, it is estimated that 64.0% of the world's population uses non-industrialized medicinal plants, either whole or parts thereof in the form of infusions. In technologically advanced countries like the United States, 60% of the population commonly uses medicinal plants to combat certain diseases and in Japan there is more demand for herbal preparations as official drug. Humans use many species of flora in food and medicine. These species contribute significantly to food and health, especially in developing countries. It is also estimated that the traditional and modern medicine uses about 50000-70000 species of plants. The availability of natural resources threatens the revenue from the wild harvest, health and welfare of the people who depend on them (Macías, 2009).

By definition, diuretics are drugs that bring about an increase in urinary volume as well as in the electrolyte output. Due to this they are used to regulate both volume and composition of the milieu intérieur in different affections like high blood pressure, heart failure, nephrotic syndromes among other indications. Historically, the classification of diuretics has been a medley of ideas like: place of action (loop diuretics), efficiency (high ceiling diuretics), chemical structure (thiazide diuretics), similarity of action to other diuretics (diuretics similar to thiazides), the effects upon the potassium excretion (potassium-sparing diuretic), and others (Florez, 2003; Rang et al., 2008).

There is a wide therapeutic stock of synthetic drugs that belong to each of these pharmacological groups. In spite of this, a considerable amount of decoctions and infusions of medicinal plants are used by Cuban population to this end. This is an aspect that has been transmitted from generation to generation through an ethnomedical approach (Beyra et al., 2008; Boffill et al., 2008).

About 179 plants have been reported in Cuba to be used by the population as diuretics. The plants belong to 78 different families, evidencing that the selection is not the result of a chemotaxonomic criterion (Bofill et al., 2008).

The diuretic effectiveness of this kind of medicinal plants needs to be experimentally proved, because diuresis could be influenced by the form of administration (infusion or decoction) which implies the consumption of a great amount of liquids that can provoke an increase in the amount of urine excreted without a true evidence of a diuretic action. Only 17 plants (9%), from the above mentioned total (179) have been experimentally proved; thus, to validate scientifically this effect is of vital importance taking into account the search for new diuretics potentially less toxic derived from plants that could be considered of higher security for patients (Bofill et al., 2008).

Several preclinical studies have been carried out in our country to assay the diuretic action of the following plants: *Achyranthes aspera* L. (rabo de gato), *Xanthium strumarium* L., (guizaso de caballo) (Jimenez et al., 1999), *Lepidium virginicum* L. (mastuerzo) (Carvajal, 1986), *Orthosiphon aristatus* Blume (té de riñon) (León et al., 1996), *Rosmarinus officinalis* L. (romero), *Bryophyllum pinnata* Lam (siempreviva) (Pérez et al., 2006), *Peperomia pellucida* L. H. B. K. (corazón de hombre) (Iglesias., 2002), *Tamarindus indica* L. (tamarindo) (Martínez et al., 2007), *Boldoa purpurascens* Cav. (nitro blanco) (González et al., 2007), where a significant increase of urinary excretion was evident.

Although the mechanism of diuretic action of these plants is not elucidated yet, researchers have stated that it could be as result of an increase in the renal circulation which implies higher glomerular filtration. Glomerular filtration does not require energy allowance because the driving force is the arterial pressure of the filtrating membrane; for that reason, when the renal blood flow increases as consequence of diuretic use from vegetable origin, glomerular filtration will increase. This kind of diuretic is known as water diuretics (Cárces, 1999).

The traditional use of Cuban plants as diuretics has been described by Roig (1974) and recent ethnomedical surveys have shown that are still in use, but the diuretic action has

not been experimentally proved. The scope of our study was to evaluate the in vivo biological activity of five of those plants by using the same preparation that population traditionally does, to experimentally analyze the diuretic activity attributed.

### Materials and Methods

An ethnopharmacological study to know the plants that have not been submitted yet to preclinical studies was first carried out (Abreu and Cuellar, 2008). Later a preclinical study was executed to evaluate the diuretic action of five plants identified through ethnomedical surveys. Plant identification was carried out by a qualified specialist and a sample of each specimen was conserved at the Central University of Las Villas herbarium (Méndez, 2007).

According to the traditional use reports (Table 1), *Ocimum basilicum* L., *Parthenium hysterophorus* L., *Justicia pectoralis* Jacq., *Plectranthus amboinicus* (Lour.) Spreng., leaves were dried at 40°C in a laboratory oven (Labor Muzerimipari, Typ LP-114, Hungary). Aqueous extracts were prepared from the dry drug of the first four plants mentioned previously, and in the case of the *Allium cepa* and *Citrus aurantium* L. from their natural juice.

The day before to each administration, 30% decoctions were prepared as follows: 30g of drug were mixed with 100 mL of distilled water in a glass container with its lid which was placed in a heater (TEHTNICA, MM.510c, Czech Republic) to reach the boiling state and keeping it 5 min, afterwards the container was cooled at room temperature and then filtered using filter paper 11 cm diameter. Total solids were determined for each decoction as described in literature (Miranda and Cuellar, 2000).

The animal model guidelines used for the verification of the diuretic activity was the method described by (Lipschitz et al., 1943) modified by (Kau et al., 1984). This test has been considered as a standard method and it has been widely used to assess diuretic activity of both natural and synthetic potentially diuretic drugs.

Wistar male albino rats weighing 180-220g were brought from the National Center for Laboratory Animal Production (NCLAP) and received with the corresponding genetic quality as well as the sanitary and hygienic certificates. During 5 days they were quarantined for their acclimatization to the experimental conditions (temperature: 19-22°C and relative humidity 40%, dark/light cycles: 12/12h). Animal's food was also acquired in NCLAP with the corresponding quality certificate and drinking water was the same used for human consumption and the access to both was *ad libitum* (Boffill et al., 2008).

Animals were distributed at random to 7 experimental groups: 5 treated, 1 positive control (furosemide, 20 mg/kg, QUIMEFA, Cuba) and 1 negative control (NaCl, 0, 9%, QUIMEFA, Cuba). Treated groups received a dose of 400 mg/kg BW based on the above mentioned total solid calculations. Dose selection was based on previous research (Boffill, 2006) that uses several dose levels reaching to non-dose dependent results where the generality of the best diuretic action moved around 400 mg/kg BW, considering also that our study is intended to preliminarily demonstrate the presence of diuretic activity. It is considered that diuretic action of plant extracts extends generally for 6 to 8h which leads us to select 6h as experimental time termination; furosemide was selected as positive control.

Animals were starved since the day before at 3:00pm as well as water was removed the following day at 7:00am. After the administration of substances (8:00am), the animals were placed in metabolic cages (TECNIPLAST, model 3700M071) (Figure 1). All the substances were orally administered by gavage using a 16 G intragastric cannula. Dose volume were completed with physiological saline solution up to a total constant administration volume of 40ml/kg BW as described in the guidance used (Lipschitz et al., 1943; Kau et al., 1984).

Urinary excretion was then quantified after ½, 1, 2, 3, 4, 5 and 6h. Later the urinary excretion and diuretic action were calculated to

be used in the final equation of diuretic activity which numerical results were classified as follows: high:  $\geq 0.90$ , Moderated:  $0.89-0.70$ , low:  $0.69-0.50$  and null:  $< 0.50$  (Pérez et al., 2011).



**Figure 1. Metabolic cages for rats.**

Once the experiment was concluded, bioethical euthanasia method was employed. (Figure 2). Urinary elimination, diuretic action, and diuretic activity were calculated according to the formulae proposed by the authors (Pérez et al., 2011). Eqs. [1], [2] and [3]. SPSS statistic package for Windows (8.0 version) was used to process the results. Standard deviations and means of each studied parameter in each experimental group were determined and compared using the Kruskal Wallis and Mann Whitney tests.

Urinary excretion = (urine excreted / phisioiological solution administered) x 100 [1].

Diuretic action = urinary excretion from treated group / urinary excretion from control group [2].

Diuretic activity = diuretic action of extract / diuretic action dependence the watery extract on drugs [3].



**Figure 2. Bioethical euthanasia method.**

## Results and Discussion

The ethnopharmacological study allowed to identify 21 plants as the most used as diuretics, of which only 12 (57%) had been preclinically tested. From the non preclinically tested we choose the 5 most reported by surveyed population: *O. basilicum*, *P. hystrophorus*, *J. pectoralis*, *P. amboinicus*, *A. cepa* and *C. aurantium* (Pérez et al., 2011).

The preclinical study showed that during the first 2h after the administration, urine volume had not significant increases in almost any studied plant. Urinary excretion, action and diuretic activity were superior in the experimental groups corresponding to *Ocimum basilicum* and *Allium cepa* (Table 1).

In general terms none of the plants showed diuretic activity in the first 2h after the administration of substances. At 1h *O. basilicum* started to show this effect and the rest of plants started at 2 h, then it can be considered as an irregular diuretic activity starting, differing from furosemide which pharmacological activity lasts at short time (1/2h) (Florez, 2003; Rang et al., 2008).

Eliminated urine volume at each evaluation time is shown in Table 1, where can be appreciated that at 6h *O. basilicum* ( $43.21 \pm 12.68$  mL/Kg) and *A. Cepa* ( $36.87 \pm 12.92$  mL/Kg) showed best results, which means a remarkable diuretic activity, higher than furosemide at the same period ( $54.40 \pm 14.06$  mL/Kg).

**Table 1. Volume of urine excreted (arithmetic mean  $\pm$  standard deviation, n= 64).**

| Treatment                        | Volume of urine excreted     |                                 |                                |                                |                                |                                |
|----------------------------------|------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                                  | ½ h                          | 1 h                             | 2 h                            | 3 h                            | 4 h                            | 6 h                            |
| NaCl 0,9 %<br>(Negative Control) | 0.56 $\pm$ 1.58              | 4.74 $\pm$ 4.40                 | 15.54 $\pm$ 4.18               | 22.99 $\pm$ 9.00               | 27.69 $\pm$ 8.79               | 32.37 $\pm$ 9.53               |
| Furosemide<br>(Control positivo) | 17.68 $\pm$ 6.19             | 31.55 $\pm$ 10.81               | 41.73 $\pm$ 15.35              | 46.92 $\pm$ 17.28              | 48.63 $\pm$ 17.27              | 54.40 $\pm$ 14.06              |
| <i>J. pectoralis</i>             | 1.73 $\pm$ 2.39 <sup>b</sup> | 8.10 $\pm$ 5.98 <sup>b</sup>    | 12.16 $\pm$ 6.17 <sup>b</sup>  | 19.10 $\pm$ 5.56 <sup>b</sup>  | 20.25 $\pm$ 5.81 <sup>b</sup>  | 23.13 $\pm$ 5.73 <sup>b</sup>  |
| <i>C. aurantium</i>              | 1.78 $\pm$ 2.46 <sup>b</sup> | 11.86 $\pm$ 5.55 <sup>ab</sup>  | 21.76 $\pm$ 4.57 <sup>ab</sup> | 26.95 $\pm$ 5.47 <sup>b</sup>  | 29.96 $\pm$ 7.53 <sup>b</sup>  | 34.61 $\pm$ 6.55 <sup>b</sup>  |
| <i>P. amboinicus</i>             | 2.83 $\pm$ 6.32 <sup>b</sup> | 10.64 $\pm$ 9.51 <sup>b</sup>   | 20.22 $\pm$ 7.64 <sup>b</sup>  | 24.86 $\pm$ 7.46 <sup>b</sup>  | 28.41 $\pm$ 5.00 <sup>b</sup>  | 30.72 $\pm$ 6.20 <sup>b</sup>  |
| <i>P. hysterothorus</i>          | 1.81 $\pm$ 2.50 <sup>b</sup> | 17.50 $\pm$ 3.72 <sup>b</sup>   | 15.41 $\pm$ 7.67 <sup>b</sup>  | 23.19 $\pm$ 7.54 <sup>b</sup>  | 20.90 $\pm$ 11.29 <sup>b</sup> | 35.36 $\pm$ 8.25 <sup>b</sup>  |
| <i>O. basilicum</i>              | 2.23 $\pm$ 5.38 <sup>b</sup> | 14.34 $\pm$ 9.43 <sup>b</sup>   | 28.61 $\pm$ 13.64 <sup>a</sup> | 34.70 $\pm$ 11.15 <sup>a</sup> | 35.89 $\pm$ 11.76 <sup>a</sup> | 43.21 $\pm$ 12.68 <sup>a</sup> |
| <i>A. cepa</i>                   | 1.16 $\pm$ 2.14 <sup>b</sup> | 14.00 $\pm$ 15.22 <sup>ab</sup> | 21.39 $\pm$ 12.37 <sup>b</sup> | 27.88 $\pm$ 12.47 <sup>a</sup> | 31.99 $\pm$ 13.03 <sup>a</sup> | 36.87 $\pm$ 12.92 <sup>a</sup> |

a: Statistical meaning NaCl 0,9 % (Negative control); b: Statistical meaning Furosemide (Positive control). Mann-Whitney test.

In the particular case of *O. basilicum*, statistical differences in relation to NaCl ( $p < 0,05$ ) began after 1/2 h of being administered. In *A. Cepa* these differences are not appreciated until 1 h later, however this plant provoked the higher volume of urine eliminated. Urine volumes at 6 h in negative and positive control groups coincide with those reported by (Masereel et al., 1993).

*P. hysterothorus* was the third most important plant with an action that resembles furosemide, reaching, after 6h of administration, a diuresis level of 35.36 $\pm$ 8.25 mL/Kg. It is important to note that *J. pectoralis* was the unique plant that statistically differed

from NaCl ( $p < 0.05$ ) during the first 2 h of administration, however it showed a moderated diuretic activity (0.4) (Table 2).

Diuretic activity represents an indicator of effectiveness in respect of diuretics. Thus the indicators which were calculated (Table 2) helped to evaluate all plants, in a qualitative way, according to the urinary elimination, its action and diuretic activity. According with the results, only four plants showed an interesting diuretic activity ( $\geq 1$ ): *P. hysterothorus*, *O. basilicum*, *A. cepa* and *C. aurantium*, being more marked in the first two; all this data was also statistically corroborated.

**Table 2. Urinary excretion, diuretic activity and action 6 h post-administration.**

| Treatment                              | Urinary excretion (%) | Diuretic Action | Diuretic Activity |
|----------------------------------------|-----------------------|-----------------|-------------------|
| NaCl 0,9 % (Negative control)          | 32.45                 | -               | -                 |
| <i>Justicia pectoralis</i>             | 57.8                  | 0.7             | 0.4               |
| <i>C. aurantium</i>                    | 86.5                  | 1.07            | 0.6               |
| <i>P. amboinicus</i>                   | 76.8                  | 0.9             | 0.5               |
| <i>P. hysterothorus</i>                | 90.9                  | 1.12            | 0.66              |
| <i>O. basilicum</i>                    | 108.02                | 1.33            | 0.79              |
| <i>A. cepa</i>                         | 103.0                 | 1.27            | 0.75              |
| Furosemide (Positive control) 20 mg/Kg | 111.85                | 3.44            | -                 |

The ethnopharmacological study showed coincident results with those achieved in similar investigations which concluded that there were no scientifically validated

references on its use and that its phytochemical composition is not already known (Beyra et al., 2008; Boffill et al., 2008).

In the preclinical study when we compare the urine volumes excreted after 6h of being administered the plant decoction with better diuretic results (*O. basilicum*: 43.21±12.68 mL/Kg *P* and *A. cepa*: 36.87±12.92 mL/Kg) shown to have lower activity than those reported for *Rhoeo spathacea* (Sw.) Stearn (47,98 ±8,26 mL/Kg), *Costus cylindricus* Jacq. (52,89±9,57 mL/Kg), *Capraria biflora* L. (42,71± 8,10 mL/Kg), *Carica papaya* L. (54,08±10,23 mL/Kg), and *Bidens pilosa* L. (50,22± 7,72 mL/Kg) (Boffill et al., 2006) and lower than those of *Boldoa purpurascens* Cav. (58,2±5.74 mL/Kg) (Pérez et al., 2008).

Several metabolites in medicinal plants are responsible of diuretic action, although the contribution level of each one to total diuretic activity is not always clear. The main active metabolites that can intervene on diuretic actions are essential oils, flavonoids, saponosids, and potassic salts, but some of these substances could be more active on the glomerular level than on the tubule, provoking an increase in renal circulation and rising, this way, glomerular filtration rate and primary urine formation, thus, the final effect could be an aquaresis. However, potassic salts could originate a diuretic effect as result of an osmotic process (Arteche, 1998; Peris, 1995).

Specifically, the diuretics activity of *P. hysterothorus*, *O. basilicum*, *A. cepa* could be related to the presence of some of these metabolites (flavonoids and potassic salts). Previous phytochemical studies of its leaves as well as the analysis by chromatography showed the presence of several types of flavonoids (Shena et al., 2001; Skaltsa and Philianos, 1990).

According to revised literature, some flavonoids have shown diuretic effect, several isoflavonoids like genistein and daidzeine have been reported to cause inhibition of the Na<sup>+</sup>-K<sup>+</sup>-2CL<sup>-</sup> cotransporter as well as an increase of natriuresis and kaluresis (Mareck et al., 1991). Also the flavonoid crisine showed a significant increase of urine flow, glomerular filtration, and Na<sup>+</sup>, K<sup>+</sup> excretion (Jouad, 2001). Recently, seven methoxy-flavonoids were actively bounded to A1 adenosine receptor, provoking

antagonism and as a result diuresis and sodium excretion.

*A. Cepa*, the second most active plant, contains some metabolites (potassic salt, flavonoids) which could be associated with its diuretic effect.

No plants were identified with high diuretic activity. *J. pectoralis* activity was nil and the species *O. basilicum* *A. cepa* and *P. hysterothorus* strain, showed moderate activity in that order of importance. *C. aurantium* and *P. amboinicus* showed low activity but showed no statistical difference with the negative control.

In general terms it is also necessary to pay attention to the dose behavior of studied plants, as most of them show a non-dose dependent effect and, for instance, studies with aqueous extracts of *B. pilosa* and *C. cylindricus* produced minor urine excretion at a dose of 800 mg/kg BW compared with 400 mg/kg BW (Boffill, 2008).

## Conclusions

Ethnomedical information showed wide use of 5 plant species as diuretics by Cuban population which have not experimentally tested. All studied plants exerted high diuretic activity, except *J. pectoralis* which diuretic activity was null. *O. basilicum*, *A. cepa* and *P. hysterothorus* produced a highly significant increase of urine volumes in relation to the negative control (moderate diuretic activity), urinary excretion and diuretic action and activity was very similar to furosemide. *P. amboinicus* and *P. hysterothorus* plants showed low activity.

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