Diuretic activity of *Sambucus nigra* L. ssp. *palmensis* (Link) R. Bolli, an endemic Canary Islands species

Sandra Dévora Gutiérrez¹, Felipe Hernández-Luis², Domingo Martín-Herrera¹, Chaxiraxi C. Morales Marrero¹ & Susana Abdala¹

¹Departamento de Medicina Física y Farmacología, Facultad de Farmacia, Universidad de La Laguna, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.

²Departamento de Química, Facultad de Ciencias, Universidad de La Laguna, San Cristóbal de La Laguna, Santa Cruz de Tenerife, Spain.

Abstract: To determine the diuretic activity of *Sambucus nigra* L. ssp. *palmensis* (Link) R. Bolli (SP), SP was evaluated in adult female Swiss mice. Urinary excretion volume was measured and the concentration of sodium, potassium, chloride, pH and specific conductance of 3 doses of aqueous extract (35.0, 52.2 and 70.0 mg kg⁻¹) were determined. SP (70.0 mg kg⁻¹) produced a higher urinary excretion (6.41 mL) and diuretic index (15%) than hydrochlorothiazide (HCTZ) (6.27 mL and 12%, respectively). The saluretic index indicates a lower sodium excretion than HCTZ (13%) and inversely proportional to the dose (8% - 5%). The same occurs with potassium excretion (0.0172 - 0.0162 mEq.K⁺/100 g/6 h), which achieves a lower value than the control group (0.0166 mEq.K⁺/100 g/6 h), suggesting potassium retention. These results support the use of this plant species as a diuretic in Canary folk medicine.

Keywords: *Sambucus palmensis*; Diuretic power; Diuretic activity; Medicinal plant; Hydrochlorothiazide.

Resumen: Determinar la actividad diurética de *Sambucus nigra* L. ssp. *palmensis* (Link) R. Bolli (SP). SP fue evaluada en hembras adultas de ratones Swiss. Se midió el volumen de excreción urinaria y se determinó la concentración de sodio, potasio, cloruro, el pH y la conductividad específica de 3 dosis de extracto acuoso (35.0, 52.2 y 70.0 mg kg⁻¹) were determined. SP (70.0 mg kg⁻¹) produjo una excreción urinaria (6.41 mL) e índice diurético (15%) superior a hidroclorotiazida (HCTZ) (6.27 mL y 12%, respectivamente). El índice salurético indica una excreción de sodio inferior a HCTZ (13%) e inversamente proporcional a la dosis (8% - 5%). Lo mismo ocurre con la excreción de potasio (0.0172 - 0.0162 mEq.K⁺/100 g/6 h) que alcanza un dato inferior al del grupo control (0.0166 mEq.K⁺/100 g/6 h), lo que sugeriría retención de potasio. Estos resultados apoyan el uso de esta especie vegetal como diurético por la medicina popular canaria.

Palabras clave: *Sambucus palmensis*; Poder diurético; Actividad diurética; Planta medicinal; Hidroclorotiazida
INTRODUCTION
Cardiovascular diseases, such as hypertension or stroke, are some examples of non-communicable diseases associated with the ingestion of large amounts of sodium. Data published by the World Health Organization indicate that much more sodium is consumed in the world than is needed for physiological activity (Elliott & Brown, 2007), which may be one of the reasons why hypertension already affects one billion people worldwide and is a risk factor for heart attacks and strokes. In addition, it is estimated to produce nine million deaths per year (WHO, 2013). One of the key treatments for this disease and others, such as congestive heart failure, ascites or pulmonary oedema, is diuretics, either alone or in combination with other drugs. Diuretics work at kidney level by promoting the loss of fluid from the body. In order to be clinically effective, these compounds must induce sodium loss, which is achieved by interfering with the re-absorption of ions and water through the walls of the renal tubules (Wright et al., 2007; Al-Saikhan & Ansari, 2016; Titko et al., 2020).

For many years, there has been growing interest in the health benefits of natural products. In this context, there are numerous published articles stating that plants, or the active compounds derived from them, may be useful as mild diuretic agents (Mariano et al., 2018; Tenfen et al., 2019; Cecchinel-Zanchett et al., 2020; Boeing et al., 2021). Different species of Sambucus have long been used in folk medicine for the management of various diseases. One such species is Sambucus palmensis (‘Saúco’), an endemic species distributed in four of the seven Canary Islands (La Palma, La Gomera, Tenerife and Gran Canaria), belonging to the Viburnaceae family and which has been used in traditional Canarian medicine for its properties as diuretic, antioxidant, purgative, diaphoretic, emollient, analgesic and anti-inflammatory, purifying, sweaty or antipyretic (Pérez de Paz & Hernández Padrón, 1999; Cruz Suárez, 2007; Abdala et al., 2014). Several active ingredients, isolated from flowers and fruits, could be responsible for these properties, in particular: essential oils, flavonoids, organic acids, anthocyanosides, mucilages, pectins, tannins, sugars, citric acid and vitamin C (Cruz Suárez, 2007).

Until now, diuretic activity has been estimated determining urine excretion volume and concentrations of Na+, K+ and Cl-. In two recent studies a new index, diuretic power (DP) was defined by our research group (Hernández-Luis et al., 2014a; Hernández-Luis et al., 2014b). Unlike other previous parameters, this index makes it possible to determine the possible diuretic properties of a drug, measuring only the specific conductivity of the urine, which is a function of the total concentration of ions present, and the total volume excreted. Its usefulness was demonstrated when it was applied to urine samples from mice or rats obtained after the administration of infusions of native Canarian plants and hydrochlorothiazide, as a reference drug.

So, the objective of this research is to indicate the therapeutic potential of the aqueous extract of Sambucus palmensis as a diuretic, quantifying both the volume of total urine excreted and the urinary specific conductance, and the usefulness of the DP, our new index described.

MATERIALS AND METHODS

Plant material
Aerial part of Sambucus palmensis (Sambucaceae) was collected in La Palma, Canary Islands and authenticated by the Department of Plant Biology, University of La Laguna (Tenerife, Spain), where certificate specimens have been deposited (TFC46328).

Sambucus palmensis (SP) aqueous extract.
Aerial part of SP was dried in an oven at 40°C for 4 days. The dried plant was cut and ground to powder by mechanical milling. Then an aqueous extract was prepared adding 100 mL of boiling distilled water to 10 g of dried plant. After 1 hour of extraction, it was filtered and lyophilized, obtaining 140 mg of a dry extract with a yield of 2.8%.

The LD50 of SP aqueous extract was found to be above 140.0 mg kg−1 orally. To evaluate the diuretic activity, the doses employed were 35.0 mg, 52.5 mg and 70.0 mg per kg body weight (bw). The extract suspended in distilled water was administered orally through a gastric gabage to laboratory mice at a rate of 1 mL per 40 g of mouse weight.

Experimental animals
The study was performed, after approval by the Ethics Committee for Animal Research, La Laguna University (CEIBA2017-0237). Healthy adult female Swiss mice, weighing between 20 - 30 g, were used. The animals were supplied by the Central Animal Laboratory of La Laguna University and were housed in standard environment conditions (temperature 28°C; 12/12 h light/dark cycle and approximately 50 to 55% relative humidity). Before the experiment, the mice were kept fasting for 5 hours with access to water ad libitum.

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Determination of diuretic activity
The animals were divided into 5 batches of 16 animals each and all received an oral overload of normal saline (5% bw). Then three groups of mice received orally 0.5 mL/100 g bw of SP aqueous extract at doses of 35.0, 52.5 and 70.0 mg kg\textsuperscript{-1} bw, respectively; another group of mice received 0.5 mL kg\textsuperscript{-1} bw of hydrochlorothiazide (HCTZ), as reference drug, all dissolved in deionized water. Finally, the 5\textsuperscript{th} group (or control group) received orally the same amount of volume (0.5 mL kg\textsuperscript{-1} bw) of deionized water.

Subsequently after administration, each batch was divided into groups of 4 and located in metabolic cages. The urine was collected in a measuring cylinder and the excreted millilitres were measured at 6 hours (Abdala et al., 2012) (Figure N\textsuperscript{°} 1). The total excretion of urine was determined according to the body weight of the animal and has been expressed as ml/100 g/6 h. At the end of the experience, the urine samples were frozen until the time of the electrolytic determinations.

Analytical method
Na\textsuperscript{+} and K\textsuperscript{+} concentrations were obtained using a flame atomic absorption spectrophotometer Varian Mod. SpectrAA - 220FS, while Cl\textsuperscript{-} concentration was measured potentiometrically with an ISE-Cl (Orion 9417B) and a reference electrode Ag/AgCl double union (Orion 90-02) connected to an Ionometer Orion/901.
Specific electrical conductivity was determined on urine thermostated at 25.00 ± 0.05\textdegree{}C, with an YSI 3200 Conductivity Instrument with conductivity cell YSI 3252. The system was previously calibrated with the following three standard solutions of KCl in conductivity water, supplied by the manufacturer: 0.053% (10\textsuperscript{3} ± 0.50% µS cm\textsuperscript{-1} at 25\textdegree{}C), 0.582% (10\textsuperscript{4} ± 0.25% µS cm\textsuperscript{-1} at 25\textdegree{}C) and 6.532% (10\textsuperscript{5} ± 0.50% µS cm\textsuperscript{-1} at 25\textdegree{}C).

Indexes
Diuretic index (DI)
This index is the ratio between the volume of urine excreted by each of the batches and the volume of urine excreted by the control batch, which has only been given water.
Diuretic power (DP)
This novel parameter is obtained by relating the conductivity of each urine sample with the concentration of NaCl that would correspond according to that measured conductivity. Once the concentration of each electrolyte is determined, the milliequivalents (mEq) are calculated and related to those obtained by the control group in order to obtain our DP value as explained by Hernández Luis et al. in 2014.

Statistical analysis
Values are expressed as mean ± standard deviation of mean (SD). The results have been calculated by analysis of variance (ANOVA) followed by unpaired Student’s t-test. Values of p less than 0.05 were considered significant. The software used was Microsoft Excel version 2010.

Preliminary phytochemical screening
A qualitative phytochemical screening of the aqueous extract was carried out using standard procedures to identify the presence of flavonoids (cyanidin reaction and staining reaction in alkaline medium), tannins (staining reaction with ferric chloride and precipitation reactions with salt gelatine and ammoniacal zinc acetate) and cyanogenetic glycosides (staining reaction with picrosated paper) (Villar del Fresno, 1999).

RESULTS
Urine excretion and diuretic power (DP)
Table N° 1 shows the urinary volume and its corresponding diuretic index (DI). It is observed that there is a slight dose-dependent increase in the accumulated urinary volume with Sambucus palmensis aqueous extract (AE-SP) (6.06, 6.16 and 6.26 mL/100 g/6 h). The higher dose of AE-SP (70.0 mg·kg⁻¹) showed an urinary volume similar to that obtained with the reference drug, HCTZ, (6.27 mL/100 g/6 h) and approximately 14% higher than that of the control group.

The same table also presents the results of the specific conductivity (κ) necessary to obtain the diuretic power (DP). It is observed that this index was increased in all batches, in comparison with the control group, being the result obtained for the major dose of AE-SP (70.0 mg·kg⁻¹) very similar to that recorded by HCTZ.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg kg⁻¹)</th>
<th>Nº of animals</th>
<th>V (ml/100 g/6 h)</th>
<th>κ (mS cm⁻¹)</th>
<th>DI</th>
<th>DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>16</td>
<td>5.59±0.15</td>
<td>7.60±0.58</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>HCTZ</td>
<td>10.0</td>
<td>16</td>
<td>6.27±0.29**</td>
<td>10.29±0.27***</td>
<td>1.12</td>
<td>1.54</td>
</tr>
<tr>
<td>AE-SP</td>
<td>35.0</td>
<td>16</td>
<td>6.06±0.14**</td>
<td>9.94±0.27***</td>
<td>1.08</td>
<td>1.44</td>
</tr>
<tr>
<td>AE-SP</td>
<td>52.5</td>
<td>16</td>
<td>6.16±0.17**</td>
<td>10.11±0.38**</td>
<td>1.10</td>
<td>1.48</td>
</tr>
<tr>
<td>AE-SP</td>
<td>70.0</td>
<td>16</td>
<td>6.26±0.21**</td>
<td>10.30±0.19**</td>
<td>1.14</td>
<td>1.53</td>
</tr>
</tbody>
</table>

DI = urine volume of test group/urine volume of control group
Excreted urinary volume (V) and urine specific conductivity (κ) are expressed as the mean ± standard deviation. **p<0.01 vs control; ***p<0.001 vs control
Table No. 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg kg(^{-1}))</th>
<th>(mEq.Na(^+)/100 g/6 h)</th>
<th>(mEq.K(^+)/100 g/6 h)</th>
<th>SI(_{Na^+})</th>
<th>SI(_{K^+})</th>
<th>Na(^+)/K(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.1037±0.0078</td>
<td>0.0166±0.0011</td>
<td>1.00</td>
<td>1.00</td>
<td>6.25</td>
</tr>
<tr>
<td>HCTZ</td>
<td>10.0</td>
<td>0.1176±0.0096***</td>
<td>0.0188±0.0001***</td>
<td>1.13</td>
<td>1.13</td>
<td>6.26</td>
</tr>
<tr>
<td>AE-SP</td>
<td>35.0</td>
<td>0.1121±0.0057*</td>
<td>0.0172±0.0099***</td>
<td>1.08</td>
<td>1.04</td>
<td>6.52</td>
</tr>
<tr>
<td>AE-SP</td>
<td>52.5</td>
<td>0.1114±0.0093**</td>
<td>0.0168±0.0098**</td>
<td>1.07</td>
<td>1.01</td>
<td>6.63</td>
</tr>
<tr>
<td>AE-SP</td>
<td>70.0</td>
<td>0.1092±0.0024**</td>
<td>0.1062±0.0056*</td>
<td>1.05</td>
<td>0.98</td>
<td>6.74</td>
</tr>
</tbody>
</table>

Urinary ions excretion is expressed as the mean ± standard deviation.

\(*p<0.05\) vs control; \(**p<0.01\) vs control; \(***p<0.001\) vs control.

SI\(_{Na^+}\): concentration of excreted sodium in the test group/concentration of excreted sodium in the control group.

SI\(_{K^+}\): concentration of excreted potassium in the test group/concentration of excreted potassium in the control group.

Excretion of electrolytes

Regarding ionic excretion (Table N° 2), it was observed that excreted sodium decreased as the dose increased, from 0.1121 mEq.Na\(^+\)/100 g/6 h with the 35.0 mg kg\(^{-1}\) dose to 0.1092 mEq.Na\(^+\)/100 g/6 h with the 70.0 mg kg\(^{-1}\) dose. These data are lower to those obtained by HCTZ (0.11176 mEq.Na\(^+\)/100 g/6 h) and slightly higher than the amount of sodium excreted by the control group (0.1037 mEq.Na\(^+\)/100 g/6 h).

With regard to potassium excretion, in a similar manner than that observed with sodium excretion, potassium urine content decreases as the dose of extract increases from 35.0 mg kg\(^{-1}\) to 70.0 mg kg\(^{-1}\) (0.0172-0.0162 mEq.K\(^+\)/100 g/6 h), but with the particularity that these values are not only lower than those obtained by the HCTZ (0.0188 mEq.K\(^+\)/100 g/6 h) since the dose of 70 mg kg\(^{-1}\) also obtained a lower data than the control group (0.0166 mEq.K\(^+\)/100 g/6 h). So, this dose, produced retention of potassium instead of excretion.

The saline index (SI) for sodium and potassium, which relate the amount excreted by each ion to the negative control, is also shown in Table N° 2. These indexes indicate that an animal treated with HCTZ excretes 13% more sodium and potassium than an animal treated with placebo (control group). It is also observed that lots treated with AE-SP excrete sodium, but with values lower than HCTZ and inversely proportional to the dose (8% at 35.0 mg kg\(^{-1}\), 7% at 52.5 mg kg\(^{-1}\) and 5% at 70.0 mg kg\(^{-1}\), with respect to the control group). Finally, it is observed that the excretion of potassium in batches treated with AE-SP, also follow a pattern of excretion inversely proportional to the dose, becoming the excretion of this ion at dose of 52.5 mg kg\(^{-1}\) similar to that of the control group (1% with respect to the control group) and lower than this one at a dose of 70.0 mg kg\(^{-1}\) (2% less excretion with respect to the control group).

The natriuretic index, which is the relationship between sodium and potassium concentration, increases as the dose of AE-SP is incremented and, with the three doses of AE-SP, data are considerably higher than those obtained with both the control group and the reference drug because of the particular difference in excretion between sodium and potassium mentioned above.

Preliminary phytochemical screening

Preliminary qualitative phytochemical tests carried out by our research group on AE-SP revealed the presence of flavonoids, tannins and cyanogenic glycosides.

DISCUSSION

In the clinic, the use of diuretic active compounds is frequent and necessary in patients suffering from high blood pressure, fluid retention due to renal, hepatic or cardiac conditions, or in cases of electrolyte alterations. These agents act by promoting the excretion of urine which facilitates the reduction of the volume of fluid through the cardiovascular system. In general, this urinary excretion is associated with the loss of sodium, by inhibition of its absorption at the level of the renal tubes (Lahlou et al., 2006), which is ultimately responsible for the diuretic action.

The results of this research are in line with those obtained in other studies of other species of *Sambucus* that indicate diuretic activity (Beaux et al., 1999; Wright et al., 2007). The potential diuretic...
The diuretic power of SP aqueous extract is related to the results obtained with conventional parameters to measure diuresis (DI). Thus, the three doses of the aqueous extract of SP increased the diuretic power as the dose increased, obtaining, at the highest dose, a value practically equal to that of the reference drug (HCTZ), analogous to that described with the diuretic index. This fact strengthens the use of this index as a measure of the diuretic effect of a substance.

**CONCLUSIONS**

The data obtained in this work allow us to conclude that *Sambucus palmensis* aqueous extract, especially in doses of 52.5 and 70.0 mg kg\(^{-1}\), shows a significant diuretic effect in mice. Therefore, these results support the use of this plant species as a diuretic by Canarian folk medicine. For these reasons, the next step in the *Sambucus palmensis* study will be the isolation, characterization, and diuresis testing of the main active compounds of the plant, which could lead to an advance in the development and knowledge of diuretic pharmacotherapy.
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