ABSTRACT
The aim of this work was to identify the main chemical constituents and to evaluate the antilithiatic activity of the aqueous and hydroalcoholic extracts of stems of *Caesalpinia bahamensis* Lam. Fractionation and isolation of constituents from the hydroalcoholic extract was carried out by flash chromatography and semi-preparative liquid chromatography. The antilithiatic activity of the aqueous and hydroalcoholic extracts was evaluated in Wistar rats, where kidney stones were induced by ethylene glycol and ammonium chloride. Creatinine, calcium, and oxalate levels were evaluated and histological analysis was carried out. The homoisoflavonoids protosappanin B, 10-methyl-protosappanin B and brazилиn were isolated and the antilithiatic activity of the aqueous and hydroalcoholic extracts was demonstrated by the reduction of the concentration of calcium and oxalate in urine compared to the lithiasis group. It was corroborated by histological analysis. Brazилиn and protosappanin B were proposed as chemical markers for this plant species.
1. Introduction

Renal lithiasis is a health problem of high incidence, prevalence and recurrence rates around the world (Cano et al. 2015). For the treatment of renal stones minimally invasive surgery is used. It is effective to break the calculi, but it does not reduce recurrence rates. On the other hand, many drugs have been used, such as thiazide diuretics, potassium citrate and non-steroidal anti-inflammatory drugs (NSAIDs); but they are only used for preventing or treating the symptoms (Alelign and Petros 2018). For these reasons, many studies have been focused on understanding the mechanism involved in renal lithiasis, and the development of an herbal medicine as a new drug for the treatment and prevention of this pathology and its recurrences is a promising approach.

*Caesalpinia bahamensis* is a medicinal plant traditionally used in Cuba to treat renal and hepatic diseases, diabetes, and peptic ulcers (Roig 2012). However, experimental studies to support the traditional knowledge are lacking. In pharmacological studies, the diuretic (Felipe et al. 2020), cytotoxic (Setzer et al. 2015) poor antimicrobial (Abreu et al. 2017) and antioxidant (Felipe, Hernández, et al. 2019) activities have been demonstrated. Chemically, 74 compounds have been identified in the non-polar fraction of a methanolic extract of the species, using gas chromatography - mass spectrometry (GC-MS). In this study, fatty acids, terpenoids, and phytosterols were reported as the major compounds of this fraction (Felipe et al. 2017). In addition, the chemical composition of both extracts was similar according to HPLC analysis. However, the total yield and quantity of the flavonoids was higher for the hydroalcoholic extract (Felipe, Gutiérrez, et al. 2019). Apart from this, the scientific information about this species is limited. This study reports the isolation and identification of the main constituents of stems of *Caesalpinia bahamensis*, and the evaluation of the in vivo antilithiatic activity in a rat model of an aqueous and hydroalcoholic extract of stems of *Caesalpinia bahamensis*, according to its traditional use in Cuba.

2. Results and discussion

Three homoisoflavonoids were isolated and identified by 1D and 2D NMR spectroscopy and mass spectrometry according to published data. This class of compounds has a complicated stereochemistry; they exist as an inseparable mixture of two conformers, which explains the doubling of signals as observed in the NMR spectra (Zhao et al. 2016). Compound 1 was identified as a mixture of the enantiomers 10-methylprotosappanin B (1a) (Figure 1) and iso-10-methyl-protosappanin B (1b) in agreement with previously published data (Zhao et al. 2016). Compound 2 was identified as a mixture of the enantiomers protosappanin B (2a) (Figure 1) and isoprotosappanin B (2b) according to previously published NMR assignments (Zhao et al. 2016). Compound 3 was identified as brazilin (3) (Figure 1) according to previously published NMR data (Nirmal et al. 2015).

The hydroalcoholic extract was analysed by UPLC-UV-MS. In the obtained chromatogram (Figure S1) the major peaks could be attributed to the isolated compounds brazilin (7.23 min) and (iso-)protosappanin B (7.45 min), while (iso-)10-methylprotosappanin B (9.50 min) is a minor constituent of the extract. Mass spectrometric
analysis of the peak with retention time of 8.20 min revealed the presence of two compounds with an $m/z \ [\text{M-H}]^-$ value of 303.0865 and 333.0971. The pseudomolecular ion of the peak at Rt 8.65 min also showed a value of $m/z \ 333.0971 \ [\text{M-H}]^-$. Tentative identification of these compounds revealed the presence of homoisoflavonoids with an elemental composition of $\text{C}_{16}\text{H}_{16}\text{O}_6$ and of $\text{C}_{17}\text{H}_{18}\text{O}_5$. The presence of homoisoflavonoids in $C. bahamensis$ was reported for the first time in this study; however, these compounds have been isolated before in other species of the genus $Caesalpinia$ (Baldim et al. 2017).

Medicinal plants are composed of a wide variety of chemical compounds related to external factors such as environmental conditions and age of the plant. For this reason, it is necessary to identify chemical markers in order to guarantee their quality, efficacy and safety (Indrayanto 2018). In the present study, three homoisoflavonoids were isolated from the hydroalcoholic extract of the stems of $Caesalpinia bahamensis$ for the first time. Among them, brazilin and protosappanin B were identified as the major compounds. In biological studies, protosappanin B has been studied as anti-inflammatory (Mueller et al. 2016) and anti-tumoral drug (Yang et al. 2016, 2019). For brazilin, various activities have been demonstrated, such as anticoagulant, antimicrobial, antioxidant, anti-tumoral, hypoglycaemic and hepatoprotective (Nirmal et al. 2015). Therefore, protosappanin B and brazilin were proposed as a chemical marker for the quality control of the stems of $C. bahamensis$ and its preparations.

In addition, the antilithiatic activity of the aqueous and hydroalcoholic extracts of the stems of $C. bahamensis$ was evaluated for the first time. The increase of the calcium and oxalate concentration in urine of the lithiasis group compared to the healthy control group demonstrated the effect of the ethylene-glycol and ammonium chloride solution on the formation of renal stones. In contrast, after administration of aqueous and hydroalcoholic extracts of the stems of $C. bahamensis$ at dosage of 200 mg/kg, a reduction of these parameters was observed, demonstrating their antilithiatic activity (Figure S2). Despite both extracts showed similar antilithiatic activity, the effect on the elimination of calcium oxalate of the hydroalcoholic extract was significantly higher than the aqueous extract. A previous comparative analysis of these extracts demonstrated that flavonoids are present in a larger amount in the hydroalcoholic extract compared to aqueous extract (Felipe, Gutiérrez, et al. 2019). Recently, Zeng et al. (2018) described the role of flavonoids in the treatment of renal lithiasis. The authors explained the reduction of oxalate in urine by the capacity of some flavonoids to inhibit the synthesis of oxalate, which also may have been the case in our experiment. Also, the absence of red spots in the renal tubules of the kidney sections stained with Von Koss (Figure S3) and the absence of white spots under polarised light microscopy (Figure S4) in rats of the groups treated with the extracts corroborated these results. Other doses were tested in a preliminary study (data not shown), at dose of 100 and 10 mg/kg with a significant antilithiatic effect at the dose of 100 mg/kg. At dose of 10 mg/kg and below, no antilithiatic effect was obtained.

On the other hand, the lithiasis and the treated groups showed a higher plasmatic creatinine level compared to the control group, indicating renal damage (Figure S5). On the kidney sections stained with hematoxylin/eosin signs of renal damage were
observed, such as loss of the morphology of the Bowman Capsule and renal tubules (Figure S6). Therefore, in our experimental conditions, the extracts of the stems of *C. bahamensis* did not show effect on renal damage produced by renal stones. In previous studies on natural products using the ethylene-glycol induced urolithiasis method, extracts have been administered longer than 28 days (Kumar et al. 2016; Patel and Shah 2017). In the present study, the rats were treated only for seven days, which may explain the absence of these effects.

### 4. Conclusion

Three homoisoflavonoids were isolated for first time from *Caesalpinia bahamensis*; two of them, protosappanin B and brazilin, were proposed as a chemical marker for the quality control of the plant material and its extracts. The aqueous and hydroalcoholic extracts of the stems of *Caesalpinia bahamensis* showed antilithiatic activity in a lithiasis model in Wistar rats.

### Disclosure statement

The authors declare not conflict of interests

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### References

Abreu OA, Sánchez I, Barreto G, Campal AC. 2017. Poor antimicrobial activity on seven Cuban Plants. J Pharm Negative Results. 8(1):11–14. [http://dx.doi.org/10.2174/0976-9234.204910](http://dx.doi.org/10.2174/0976-9234.204910).

Alelign T, Petros B. 2018. Kidney stone disease: an update on current concepts. Adv Urol. 2018: 3068365..

Baldim JL, Conceição MF, Chagas-Paula DA, Ghilardi JH, Gomes M. 2017. Homoisoflavonoids from *Caesalpinia* spp.: a closer look at chemical and biological aspects. In: Goncalo J. Flavonoids: from biosynthesis to human health. Geneva: IntechOpen. [http://dx.doi.org/10.5772/67723](http://dx.doi.org/10.5772/67723).

Cano R, Carrasco J, Pérula LA, Jiménez C, Olaya I, Criado M, Requena MJ. 2015. Prevalence of renal stones in Andalusian population: results of PreLiRenA study. Actas Urol Esp. 39(1):26–31. [https://doi.org/10.1016/j.acuroe.2014.11.012](https://doi.org/10.1016/j.acuroe.2014.11.012).

Conn H. 1962. Biological stain. In: Animal tissue techniques. Geneva: NY Biotech Publication.

Felipe A, Gutiérrez YI, Scull R, Noa AC, Beverly D, Fubert K, Pieters L, Delgado R. 2019. Pharmacognostic study of the stem of *Caesalpinia bahamensis* and characterization of its aqueous and hydroalcoholic extracts. J Pharmacogn Phytochem. 8(3):3079–3083.

Felipe A, Hernández I, Gutiérrez YI, Scull R, Carmenate LM, Pieters L, Rodeiro I, Delgado R. 2019. Phytochemical study and antioxidant capacity of three fractions from the stem of *Caesalpinia bahamensis* Lam. J Pharm Pharmacogn Res. 7(1):12–20.

Felipe A, Marrero D, Scull R, Cuéllar A, Gutiérrez YI. 2017. Composición química de una fracción apolar del extracto metanólico de *Caesalpinia bahamensis* Lam. Rev Cienc Farm Aliment. 3(2):1–8.
Felipe A, Núñez CR, Gutiérrez YI, Scull R, Noa AC, Foubert K, Pieters L, Delgado R. 2020. Diuretic activity and acute oral toxicity of Caesalpinia bahamensis Lam. extracts (Brasilete). Int J Pharm Phytopharmacolog Res. 10(3):65–69.

Indrayanto G. 2018. Recent development of quality control methods for herbal derived drug preparations. Nat Prod Commun. 13(12):1599–1606.

Kumar D, Niranjan P, Alok S, Kulshreshtha S, Dongray A, Dwivedi S. 2016. A brief review on medicinal plant and screening method of antilithiatic activity. Int J Pharmacogn. 3(1):1–9. http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(1).1-9.

Mueller M, Weinmann D, Toegel S, Holzer W, Unger FM, Viernstein H. 2016. Compounds from Caesalpinia sappan with anti-inflammatory properties in macrophages and chondrocytes. Food Funct. 7(3):1671–1679.

Nirmal NP, Rajput MS, Prasad RGSV, Ahmad M. 2015. Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: a review. Asian Pac J Trop Med. 8(6):421–430. http://dx.doi.org/10.1016/j.apjtm.2015.05.014.

Patel KM, Shah SK. 2017. Evaluation of antiurolithiatic activity of Lawsonia inermis Linn. in rats. Int J Pharm Sci Nanotech. 10(1):3616–3622.

Roig JT. 2012. Plantas medicinales, aromáticas o venenosas de Cuba. 2nd ed. La Habana: Ciencia y Técnica.

Setzer MC, Schmidt J, Moriarity DM, Setzer WN. 2015. A phytopharmaceutical survey of Abaco Island, Bahamas. Am J Essent Oils Nat Prod. 3(1):10–17.

Yang X, Zhao L, Zhang T, Xi J, Liu S, Ren L, Zheng Y, Zhang H. 2019. Protosappanin B promotes apoptosis and causes G1 cell cycle arrest in human bladder cancer cells. Sci Rep. 9(1):1048–1058. http://dx.doi.org/10.1038/s41598-018-37553-z.

Yang X, Ren L, Zhang S, Zhao L, Wang J. 2016. Antitumor effects of purified Protosappanin B extracted from Lignum Sappan. Integr Cancer Ther. 15(1):87–95.

Zeng X, Xi Y, Jiang W. 2018. Protective role of flavonoids and flavonoid-rich plant extracts against urolithiasis: a review. Food Sci Nutr. 59(7):1–44. http://doi.org/10.1080/10408398.2018.1439880.

Zhao MB, Cai CQ, Tu PF, Tang L. 2016. Conformational analysis and NMR assignments of dibenzoxocins from Sappan lignum. Magn Reson Chem. 54(7):601–605.