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Phytochemical Characterisation and *in vivo* Antilithiatic Activity of the Stems of *Caesalpinia bahamensis* (Brasilete)

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Phytochemical Characterisation and *in vivo* Antilithiatic Activity of the Stems of *Caesalpinia bahamensis* (Brasilete)

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 brazilin 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. Brazilin and protosappanin B were proposed as chemical markers for this plant species.

Keywords: *Caesalpinia bahamensis*; brazilin; protosappanin B; antilithiatic activity; homoisoflavonoids

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 et al. 2019a) 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 et al. 2019b). 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-

methyl-protosappanin B (**1a**) (Figure 1A) 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 1B) and isoprotosappanin B (**2b**) according to previously published NMR assignments (Zhao et al., 2016). Compound **3** was identified as brazilin (**3**) (Figure 1C) 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-methyl-protosappanin 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* $[M-H]^-$ 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 $[M-H]^-$. Tentative identification of these compounds revealed the presence of homoisoflavonoids with an elemental composition of $C_{16}H_{16}O_6$ and of $C_{17}H_{18}O_5$. The presence of homoisoflavonoids in *C. bahamensis* was reported for 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, antitumoral, 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 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 et al. 2019b). Recently, Zheng 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 polarized 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 anti-lithiatic 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.

Funding

This work was supported by Vlaamse Interuniversitaire Raad - Universitaire Ontwikkelingssamenwerking (VLIR-UOS) Project under Grant number ZEIN2016PR418 – 75155.

Conflict of interests

The authors declare not conflict of interests

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