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Pharmacognostic study of the stem of *Caesalpinia bahamensis* and characterization of its aqueous and hydroalcoholic extracts

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Abstract
Caesalpinia bahamensis Lam. is a medicinal plant widely used by the Cuban population to treat hepatic and renal diseases, peptic ulcer and diabetes. However, the scientific knowledge on this species is limited. The aim of this study is to determine the quality parameters and the preliminary chemical composition of the stem of *Caesalpinia bahamensis* Lam. and its aqueous and hydroalcoholic extracts. The quality parameters of the stem of *C. bahamensis* and its aqueous and hydroalcoholic extracts were determined according to WHO recommendations. The chemical composition of the extracts was studied by phytochemical screening and their TLC and UV profiles. Flavonoids and phenolic compounds were quantified by the aluminium trichloride and Folin-Ciocalteu methods, respectively. In this study, the morphological characteristics and physicochemical parameters of the stem of *C. bahamensis* and its extracts were determined for first time. Flavonoids were higher for the hydroalcoholic extract and phenols for the aqueous extract.

Keywords: *Caesalpinia bahamensis*, flavonoids, phenols, TLC profile, UV profile, IR profile

Introduction
The use of medicinal plants dates from the beginnings of humanity, when people had no other effective therapeutic resources for the treatment of their diseases. This knowledge was transmitted through legends, pictographs and various monographs until our days [1]. According to data from the World Health Organization (WHO), 80% of the world's population uses plants as a remedy to cure their diseases [2]. On the other hand, it is known that around 20% - 30% of the medicines available in the market are derived from natural products [3]. Due to the wide use of natural products, pharmacognostic studies are required to ensure the quality of the plants used in the production of phytotherapeutics, thus guaranteeing their safety and efficacy [4]. *Caesalpinia bahamensis* Lam. (Leguminosae) is a medicinal plant used by the Cuban population to treat renal and hepatic diseases, diabetes and peptic ulcers [5]. The diuretic effect of aqueous extract has been evaluated in rodents with similar results to furosemide, a well-established diuretic drug [6]. In *in vitro* studies, the dichloromethane extract of the bark of *C. bahamensis* showed cytotoxic effects against SK-Mel-28 (human melanoma), MDA-MB-231 (human mammary adenocarcinoma) and 5637 (human bladder carcinoma) cells [7] and the acetone extract showed poor antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* [8]. In addition, the chloroform, ethyl acetate and methanol fractions of the stem showed antioxidant activity in FRAP and DPPH method [9]. The non-polar fraction of a methanolic extract has been analyzed by gas chromatography - mass spectrometry (GC-MS), and a series of fatty acids, terpenoids and phytosterols have been identified [10], but apart from this, the scientific information about this species is limited.
In the present study, the morphological characteristics and the quality parameters of the stem of *Caesalpinia bahamensis* and its hydroalcoholic and aqueous extracts are reported for the first time. Also, the preliminary chemical composition of both extracts were determined and flavonoids and phenol were quantified, being this the first step for the development of a future herbal formulation.

Materials and Methods
Plant material
Stems of *C. bahamensis* were collected in March 2017 at Cañada Arroyón, Artemisa, Cuba. It was identified in the National Botanical Garden of Cuba, where a voucher specimen

(No. 85369) was deposited. The materiel was dried in an oven at 40 ^{0}C during seven days and milled until the size of the particles was less than 2 mm.

Morphological study
The macro-morphological characteristics were determined by visual inspection. To identify the micro-morphological characteristics, the milled drug was decolorized with 10% sodium hypochlorite and colorized with 1% safranin in water. Observations were done with an optical microscope Novel with a coupled camera.

Physicochemical parameters of the drug
Residual humidity, total ashes, soluble ashes in water, non-soluble ashes in 10% hydrochloric acid and extractive substances in water, 30% ethanol, 50% ethanol and 80% ethanol were determined. The residual humidity was determined by the azeotropic method. All assays were done according to well-established official methods and procedures [11].

Preparation of the extracts
One kg of dried and powdered stem material was macerated with 15 L of distillated water for seven days at room temperature in the dark, filtered and the aqueous extract was obtained. The ethanol 80% extract was obtained using the same procedure.

Physicochemical parameters of the extracts
Organoleptic properties, total solids, refraction index and relative density were determined according to the methods described by Miranda & Cuéllar (2000) [12].

Phytochemical study
Phytochemical screening
The phytochemical screening was carried out through the assays of Dragendorff [alkaloids], Wagner [alkaloids], Baljet [lactones and coumarins], Liebermann-Burchard [triterpenes and steroids], Fehling [reducing sugars], foaming [saponins], ferric trichloride [polyphenols], ninhydrin [amino acids], Bornträger [anthraquinones], and Shinoda [flavonoids]. Color changes of the extracts by applying the mentioned reagents were observed [12].

Thin Layer Chromatography (TLC) profile
The TLC profile was established on silica gel F_{254} plates (Merck) using chloroform / methanol (9:2) as mobile phase. UV 254 nm, UV 366 nm, visible light and vanillin 2% in sulphuric acid followed by applying heat were used to detect spots.

Ultraviolet (UV) profile
Ultraviolet spectra of the extracts were recorded from 200 nm to 400 nm on a X Lambda 35 UV-Vis spectrophotometer.

Quantification of flavonoids and phenols
Total phenolic content
The total amount of phenolic compounds was determined by the Folin– Ciocalteu reagent [13]. A total of 200 µL of extract was dissolved with 10 mL of Folin–Ciocalteu solution and 8 mL of saturated sodium carbonate solution. After 90 min, the absorbance was recorded at 765 nm with an UV–Vis spectrophotometer. Gallic acid was used as standard.

Total flavonoid content
The total amount of flavonoids was determined using the aluminum trichloride ($AlCl_3$) reagent [13]. A volume of 1.5 mL (1 mg/mL) of extract was added to an equal volume of a 2% $AlCl_3$ solution. The mixture was vigorously shaken, and the absorbance was recorded at 367 nm after 10 min of incubation with an UV–Vis spectrophotometer. Quercetin was used as standard.

Statistical analysis
For the physicochemical parameters, the mean and standard deviation were determined. The Student t-test was used to compare the content of phenols and flavonoids of the extracts.

Results
Morphological analysis of the drug
The stem of *C. bahamensis* has a compact and cylindrical form. The internal surface is stringy and orange (Figure 1A), and the external surface is wrinkled and gray (Figure 1B). By microscopic inspection, it is possible to observe acute and elongated fibres composed of sclerenchyma cells and highly lignified secondary walls (Figure 1C). On the other hand, fibres and scarified or woody xylem vases are observed, typical of fibrous and compact stems (Figure 1D).

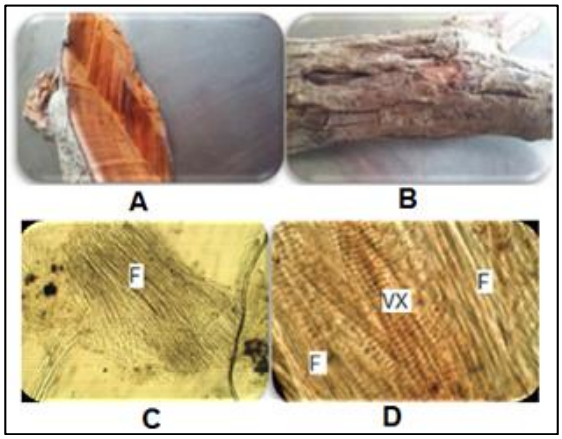

Fig 1: Macro and micro morphological characteristic of the stem of *C. bahamensis*

Physicochemical parameters of the drug
Some quality parameters of the plant material are listed in Table 1. Total ashes constitute a base to determine the purity and identity of drugs. The highest value established by WHO for this parameter is 5% [11]. On the other hand, insoluble ashes in hydrochloric acid are indicative of the presence of heavy metals in the drug and the established values in the Chinese Pharmacopeia are below 1.5% [14]. Our results suggest that the plant material does not contain heavy metals, or only a little quantity. The residual humidity below 10% does not permit the growth of micro-organisms and prevents degradation of the drug [12]. Finally, the results for the soluble constituents suggest that the highest extraction rate of secondary metabolites from this drug is in ethanol 50% and 80%; for this reason, ethanol 80% was used as extraction solvent and compared with aqueous extract in basis to it traditional use.

Physicochemical parameters of the extracts
The parameters pH, total solids, relative density were higher in the aqueous extract, while the parameter refractive index was higher in the hydroalcoholic extract. Both extracts

showed a slightly acid pH, indicative of the presence of phenolic acids in the samples [Table 2].

Table 1: Quality parameters of the stem of *C. bahamensis*

Parameter	Values (Mean ± SD)
Total ashes (%)	1.33 ± 0.81
Water-soluble ashes (%)	0.04 ± 0.00
Acid-insoluble ashes [in HCl 10%] (%)	0.01 ± 0.00
Moisture content (%)	8.01 ± 1.04
Water soluble constituents (%)	4.60 ± 0.33
Ethanol (30%) soluble constituents (%)	6.16 ± 1.32
Ethanol (50%) soluble constituents (%)	7.72 ± 0.72
Ethanol (80%) soluble constituents (%)	7.37 ± 0.57

Table 2: Physicochemical parameters of the extracts of the stem of *C. bahamensis*

Parameters	Aqueous extract	Hydroalcoholic extract
pH	5,37 ± 0.01	5,27 ± 0.01
Total solids [%]	0.63 ± 0.13	0.35 ± 0.002
Refraction index	1.3283 ± 0.0004	1.3567 ± 0.0001
Relativity density [g/mL]	1.0052 ± 0.0035	0.8724 ± 0.0001

Phytochemical study of the extracts

Phytochemical screening

The presence of lactones, coumarins, triterpenes, steroids, reducing sugars, phenolic compounds, quinones, saponins and flavonoids were evidenced for both extracts. The results of the Dragendorff, Mayer and Wagner assays were negative, indicating the absence of alkaloids in the extracts obtained from the stem of *C. bahamensis*.

TLC profile

Figure 2 shows the chromatogram obtained by TLC. Three spots can be observed in visible light: two of them near to the solvent front and the other near to the application point. They showed fluorescence under UV light at 254 nm, indicative of chromophores groups. Also, fluorescence at 366 nm was observed, indicating the presence of conjugated groups. When the spots were revealed with vanillin, they developed a pink colour. In general, this comportment is associated with phenolic compounds and flavonoids.

UV profile

Both extracts showed bands at 282 nm and 445 nm. This band has been associated with the presence of flavonoids [Figure 3].

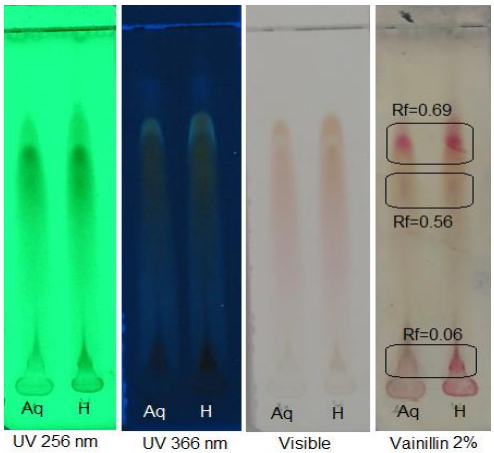

Fig 2: TLC profile of the extracts of the stem of C. bahamensis

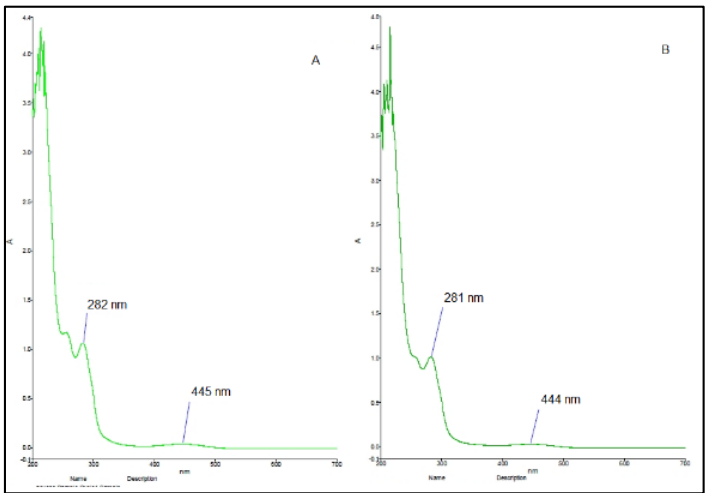

Fig 3: UV profile of the aqueous (A) and hydroalcoholic (B) extracts of the stem of *C. bahamensis*

Total phenolic and flavonoid content

Phenols and flavonoids were quantified (Table 3). The hydroalcoholic extract contained a higher quantity of flavonoids than the aqueous extract, but this was the opposite for the phenolic compounds.

Table 3: Content of phenols and flavonoids of the extracts of the stem of *C. bahamensis*

Parameters	Aqueous extract	Hydroalcoholic extract
Content of phenols [mg/mL]	148.43 ± 8.01a	7.07 ± 0.12b
Content of flavonoids [mg/mL]	0.24 ± 0.03c	0.57 ± 0.007d

Differents letters indicate significative differences ($p<0.05$)

Discussion

Pharmacognosy is the science that studies the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs of natural origin, and it also includes the search for new drugs from natural sources [15].

The establishment of the quality parameters of drugs guarantees the safety and efficacy of the finished herbal medicine [16], and avoids the adulteration or substitution of the plants used as raw materials [4].

Caesalpinia bahamensis Lam. (Leguminosae) is a medicinal plant used by the Cuban population to treat renal and hepatic diseases, diabetes and peptic ulcers [5]. In earlier studies, this species showed diuretic, antimicrobial, antioxidant and cytotoxic activity [6-9]. In addition, 74 compounds have been identified by GC/MS from the stem of the plant [10]. Despite the fact that studies carried out on this species are scarce, the preliminary results are encouraging, converting it into a potential source for the development of herbal medicines, which requires knowledge of its quality parameters and phytochemical composition.

The quality parameters of the stem of *C. bahamensis* and its aqueous and hydroalcoholic extracts were evaluated for the first time in this study. The macromorphological and micro morphological characteristic of the stem permitted to observe the presence of fibres and scarified or woody xylem vases, which corresponds to the woody and compact structure of the drug. The values of the evaluated physical-chemical parameters were between the established general quality limits for medicinal plants.

In this study, the aqueous and hydroalcoholic extract of the stem of *C. bahamensis* were obtained. The aqueous extract

was chosen in correspondence with the traditional use of the plant while the hydroalcoholic extract was selected because it had the highest content of extractables. The quality parameters of both extracts were determined and compared.

The highest percentage of soluble constituents was observed in the 50% and 80% ethanol extracts. However, the level of total solids in the aqueous extract was higher than in the hydroalcoholic extract. In this study, a predominance of polar compounds in the extracts was observed through it TLC profiles. This comportment can explain the higher quantity of total solids obtained from the aqueous extract. On the other hand, the determination of soluble constituents requires a stir system increasing the yield of extraction, which can explain an increase in the content of soluble constituents in hydroalcoholic mixtures of solvents. The influence of these two factors can explained these differences [17-18].

The presence of flavonoids and phenols in the extracts was tested by the Shinoda and aluminium trichloride assays. Also, the TLC profile showed spots related to flavonoids and phenolic compounds. The UV profile showed bands at 282 nm and 445 nm, corroborating the presence of flavonoids in the extracts [19-20]. This suggest that flavonoids and phenols can be used as markers in the quality parameters of the extracts.

Based on these results the amount of phenols and flavonoids in the extracts was determined. The quantity of flavonoids was higher in the hydroalcoholic extract, but this was not the case for the phenols. The hydroalcoholic mixtures have been used to obtain flavonoid-rich fractions. On the other hand, a higher content of phenols in the aqueous extract suggest the presence of glycosylated phenols, which are more soluble in water than non-glycosylated phenols [21-22].

Conclusion

The quality parameters of the stem of *Caesalpinia bahamensis* and its aqueous and hydroalcoholic extracts were evaluated being within the general limits established for medicinal plants. The content of flavonoids was higher in the hydroalcoholic extract while in the aqueous the phenolic compounds predominated. The results presented in this study have not been previously reported for this species.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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