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Comparative pharmacognostic study of leaves, stems, and roots of *Urera baccifera* (L.) Gaudich. ex Wedd.

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The quality control of drugs is necessary to guaranty the safety and efficacy of natural products. In this sense, the present investigation is led to compare the pharmacognostic parameters and preliminary phytochemical composition of the leaves, stems and roots of *Urera baccifera*, a medicinal plant with gastroprotective, anti-inflammatory, analgesic, and antimicrobial activities. The morphological characteristics and physicochemical parameters were evaluated for three organs, according to well-established methods for vegetal drugs. In addition, phytochemical screening was made. In microscopical analysis, different types of crystals were observed in each organ. Also, the leaves showed the highest percentage of total ashes and soluble constituents. Water was the better extraction solvent. The micromorphological characteristics and physicochemical parameters were reported for the first time for this species. The hydroalcoholic extract of three organs showed the presence of lactones, triterpenes, flavonoids, amino acids, phenols and sugar reducing. Based on the results, the leaves are proposed for the development of future phyto drugs from *U. baccifera*, in contrast with the ethnomedical information, where the roots are used.

Keywords: Leaves, Micromorphology, Quality parameters, Roots, Stems, *Urera baccifera*

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Introduction

The use of plants for medicinal purposes dates back to the very origins of human history when people had no other effective therapeutic resources to treat their diseases. This knowledge has been transmitted through legends, pictographs, and various monographs[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 on the market are derived from natural products[3]. The traditional knowledge about medicinal plants is the first clinical evidence on the efficacy of herbal medicine; however, scientific studies are necessary to corroborate the ethnobotanical information[4]. 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[5]. Therefore, pharmacognostic studies evaluating the quality parameters of drugs are required to ensure their safety and efficacy[6].

In Cuba, the roots of *Urera baccifera* (L.) Gaudich. ex Wedd. has been traditionally used as diuretic and antilithiatic[7]. In pharmacological studies, diuretic, antiproliferative (Ovarian Carcinoma Cell Lines), antimicrobial, antiviral, and gastroprotective activities of leaves and roots have been demonstrated[8-11]. These activities have been related to the presence of flavonoids and phenols in the drug[12]. In phytochemical studies, diosmetin and apigenin glucuronides have been identified as majoritarian compounds of the hydroalcoholic extract of the leaves by Ultra Performance Liquid Chromatography tandem Electrospray Ionisation-Mass Spectrometry (UPLC-ESI-MS)[9].

Taking into consideration that the use of leaves is more feasible than the use of roots in the production of herbal medicine because it guarantees the conservation of the species, although, in Cuba, the roots are used in traditional medicine, there is scientific evidence that supports the use of the leaves

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in several pharmacological activities. The present research compares the pharmacognostic parameters and preliminary phytochemical composition between roots, leaves, and stems of *U. baccifera* to obtain pharmacognostic information about the species growing in Cuba, especially in the leaves.

Materials and Methods

Plant collection and identification

U. baccifera was collected in March 2017 at Capellanía, Artemisa, Cuba. It was identified in the National Botanical Garden of Cuba, where a voucher specimen (HFC 89704) was deposited. The leaves, stems, and roots were separated and manually cut into small pieces with the help of scissors. They were dried in an oven at 40 ^{o}C to constant weight. The drying time and percentage of water loss were determined in the process.

Morphological characterization

Macroscopic characterization

The macro-morphological characteristics of the leaves, stems, and roots were determined by visual inspection. The length and width of the blade were measured in one hundred leaves with the help of a graduated ruler.

Microscopic characterization

A pinch of the powdered crude drug was decolourized with 10% sodium hypochlorite and placed on a glass slide. After staining with 1% safranin in water, it was fixed with glycerin and observed under an optical microscope with a camera attached[13].

Physicochemical parameters

Residual humidity, total ashes, soluble ashes in water, non-soluble ashes in 10% hydrochloric acid, and soluble constituents 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[14].

Phytochemical screening

The phytochemical screening was carried out on the leaves, stems, and roots of *U. baccifera*. The drug was successively extracted with ether, ethanol, and water by maceration for 48 hours. The assays Sudan (fatty compounds), Dragendorff (alkaloids), Wagner (alkaloids), Mayer (alkaloids), Baljet (lactones and coumarins), Liebermann-Burchard (triterpenes and steroids), Fehling (reducing sugars), foaming (saponins), ferric trichloride (polyphenols), ninhydrin (amino acids), Resines (resins), Antocianidins (antocianidins), Mucilages (mucilages), Foaming (saponins), Kedde (cardiotonics), Bornträger (anthraquinones) and Shinoda (flavonoids) were made for each fraction. Colour changes of the extracts by applying the mentioned reagents were observed[15].

Thin-layer chromatographic profile

The Thin-layer chromatographic (TLC) profile of aqueous extract was established on silica gel F254 plates (Merck) using butanol/ acetic acid/ water (4:1:5) as mobile phase. UV 254 nm and anisaldehyde were used as revelators. The aqueous extract was chosen considering the highest values of soluble constituents obtained in the evaluation of physicochemical parameters. The retention factor (Rf) was calculated for each spot.

Statistical analysis

Values were expressed as mean/standard deviation. Statistical analysis was performed with SPSS 18.0. For multiple comparisons, one-way ANOVA was used followed by Duncan post hoc test. Values of P <0.05 were considered statistically significant.

Results and Discussion

Morphological characteristic

The leaves were simple, petiolate, and inserted in an opposite decussate pattern. The petiole was long (2.47/0.91 cm). The blade was symmetrical with an ovate to cordate shape, slightly scarious consistency and warty surface. The length and width were 16.08 and 11.73 cm, respectively. The adaxial surface showed an intense green colour with the presence of stinging hairs and the abaxial surface showed a light green colour. The apex was acuminate, the base was cordate and the margin was slightly serrate. The venation pattern was pinnate. The stems showed a fibrous and cylindrical form. The external surface was a reddish-green colour. The internal surface was cream-greenish colour with a fibrous texture and small striae. The roots showed a fibrous and cylindrical form too, but, the external surface was reddish-brown colour and rough texture, while, the internal surface was a light cream colour and fibrous (Fig. 1).

In general, the macroscopic characteristics match with previous reports[16], however, the texture, venation, and colour of the leaves and the macroscopic characteristics of the roots were described for the first time in this study.

By microscopic inspections of the leaves, epidermal cells, anomocytic stomata, and needle-shaped calcium oxalate crystals were identified. These crystals were observed in the stems and roots as well but in a rosette form. In fact, the genotoxic effect of the extracts in the leucocyte culture has been attributed to the presence of these crystals in the drug[17]. In addition, spindle cells and helical xylem vessels were common structures for the three organs studied. Spindle cells appear next to medullary rays as a set of pointy cells, almost isodiametric and stratified (Fig. 2). These structures have been reported as part of vascular cambium[18-19]. Helical xylem vessels have been reported as a resistance mechanism to water deficit during growth of the plant[20] and spindle cells participate in plant division cell[21].

In a previous study, the microscopic analysis of pistillode in flowers of *U. baccifera* was made[22], but, in leaves, stems, and roots there is no previous report. In this sense, this research makes a novel contribution because the micromorphological characteristics were reported for the first time for *U. baccifera*. In addition, the morphological characteristics described in this research can be used to avoid adulteration for future analysis[23].

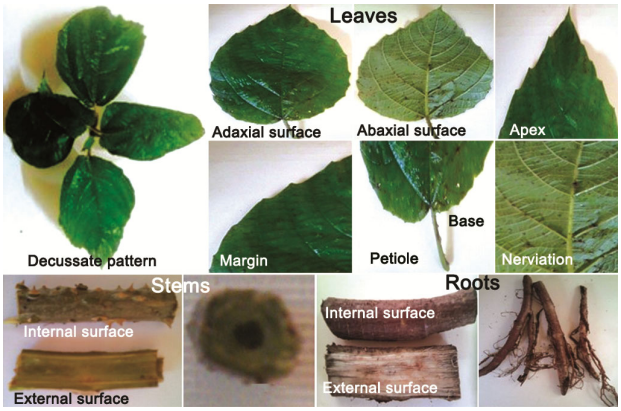

Fig. 1 — Macromorphological characteristics of the leaves, stems and roots of *Urera baccifera*.

Physicochemical parameters

Some quality parameters of the plant material are listed in Table 1. Determination of ashes is a constant parameter for the plant and can be used to set a standard for screening. It establishes the inorganic content and adulterant in crude drugs, such as salts, metals, and silica[24]. The values established in the Chinese Pharmacopeia for total ashes and acid-insoluble ashes are 15 and 2%, respectively[25]. In the present research, the leaves of *U. baccifera* showed slightly elevated acid-insoluble ashes values (2.87%), this result can be related to the presence of silica or heavy metal in the drug[26]; therefore, future analysis of heavy metal in the leaves is suggested due to the toxicity associated to these compounds on human health[27]. The control of moisture content is an important parameter to avoid the microbial growth and degradation of bioactive compounds. Also, this parameter is related to the drying process[28]. The values obtained below 10% is in correspondence with the values established in Chines Pharmacopeia[25]. The results of the samples did not show significant differences with respect to this parameter, therefore, the drying process was efficient. The drying time of the stems and roots (168 hours) was higher than the leaves (96 hours). These results were in correspondence with the percentage of lost water calculated for each organ; leaves (62.36%), stems (83.53%), and roots (80.17%). Finally, the determination of soluble constituents is useful in measuring the amount of chemical constituents in drugs extractable by a chosen solvent. It helps to also suggest the chemical nature of the contained constituents

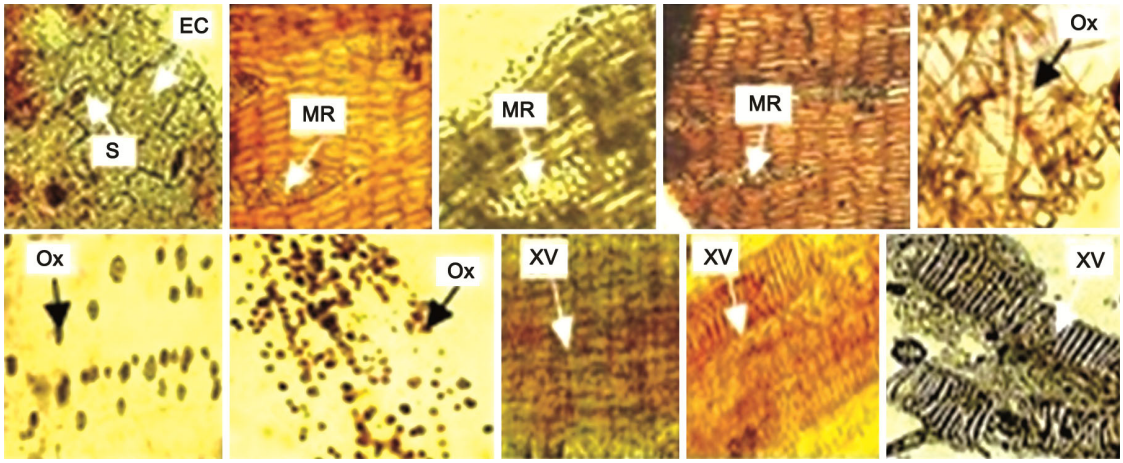

Fig. 2 — Micromorphological characteristics of the powder of leaves, stems and roots of *Urera baccifera*.

Table 1 — Physicochemical parameters of the leaves, stems, and roots of *Urera baccifera*

Parameter	Leaf	Stem	Root
Totalash	9.84/0.07a	7.27/0.14 b	7.16/0.04 b
Water-soluble ash	3.77/0.19 c	2.25/0.04 d	2.14/0.02 d
Acid-insoluble ashes [in HCl 10%]	2.87/0.06 e	1.45/0.05 f	1.64/0.06 f
Moisturecontent	8.18/0.06 g	8.30/0.02 g	8.50/0.06 g
Water soluble constituents	15.73/0.05 h	7.42/0.04 i	8.62/0.03 j
Ethanol (30%) soluble constituents	15.38/0.03 h	7.38/0.04 i	8.83/0.07 j
Ethanol (50%) soluble constituents	9.88/0.08 k	6.15/0.05 m	6.11/0.02 m
Ethanol (80%) soluble constituents	7.89/0.06 n	5.46/0.02 o	4.72/0.03 p

*The values are expressed as medium/standard deviation. Different letters are indicative of significant differences (P <0.05) between groups (n=3).

Table 2 — Phytochemical screening of leaves, stems and roots of *Urera baccifera*

Assay	Metabolite	Hydroalcoholic extract		
		Leaves	Stems	Roots
Sudan	Fatty compounds	++	+	+
Baljet	Lactones and coumarines	+	+	+
Lieberman-Burchard	Triterpenes and steroids	++	+	+
Dragendorff	Alkaloids	-	-	-
Mayer	Alkaloids	-	-	-
Wagner	Alkaloids	-	-	-
Foaming	Saponins	-	-	-
Resins	Resins	-	-	-
Ninhydrine	Amino acids	++	+	+
Fehling	Sugar reducing	++	+	+
Shinoda	Flavonoids	++	+	+
$FeCl_3$	Phenols	++	+	+
Borntrager	Quinones	-	-	-
Antocianidines	Antocianidines	+	+	-
Mucilages	Mucilages	-	-	-
Kedde	Cardiotonics	-	-	-

*(-) negative result, (+) positive result, (++) very positive result

and the choice of the most suitable solvent for extraction and phytochemical studies[28]. In this research, the highest values of soluble constituents were obtained in the leaves. In addition, the better extraction solvent was water, suggesting the presence of a high amount of polar metabolites in the plant. Therefore, the aqueous extract of the leaves is recommended for future pharmacological and phytochemical studies. These results differ from the traditional use in Cuba, where the roots are the organ used as diuretic and antilithiatic[7]. However, in other countries, the leaves have been used to treat problems on the skin, urinary infections, and to reduce inflammation[17].

Phytochemical screening

In phytochemical screening, the three organs showed the presence of lactones, triterpenes and steroids, amino acids, sugar reducing, flavonoids, phenols, saponins, fatty compounds and antocianidines. Alkaloids, resins, quinones, mucilages and cardiotonics were not detected in this study. However, qualitative differences were observed between leaves, stems, and roots. According to colour changes observed, leaves showed the highest concentration of all metabolites (Table 2). In a previous study, the presence of flavonoids, saponins, steroids, terpenes, and phenols was reported[12]. In fact, the majoritarian compounds of the leaves and roots of *U. baccifera* were reported as diosmetin and apigenin, two flavonoids related with several biological properties demonstrated for this plant[9-10]. On the other hand, the highest concentration of phenols and flavonoids in the leaves was corroborated in previous studies by quantitative methods[8].

Thin Layer Chromatography profile

Fig. 3 shows the chromatogram obtained by TLC. Four spots were observed showing fluorescence under UV light at 254 nm, indicative of chromophores groups. One of them, close to the front of solvent and the other close to the point of application (Fig. 3a).

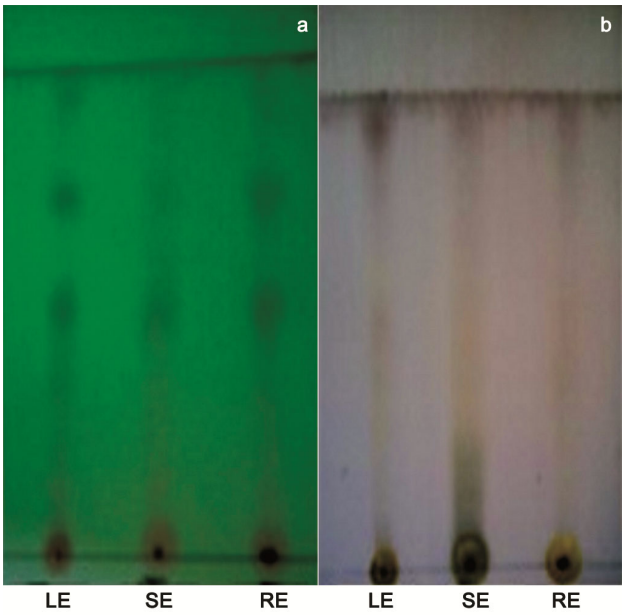

Fig. 3 — Thin Layer Chromatography (TLC) profile of the aqueous extract of leaves, stems and roots of *Urera baccifera*, a) Plates observed under UV light at 254 nm, b) Plates reveled with anysaldheyde, LE: Leaf extract; SE: Stem extract; RE: Root extract.

The retention factor (Rf) values calculated for the other two spots were 0.51 and 0.83, matching with the Rf reported for apigenin 7-O-β-glucopyranoside and diosmetin[29], two majoritarian flavonoids reported for *U. baccifera*. In addition, when the spots were revealed with anisaldehyde, they took the reddish-brown and yellow colour, associated with the presence of phenolic compounds, especially flavonoids (Fig. 3b). The conditions used for the TLC profile allowed identification of the main flavonoids reported for *U. baccifera*, using the retention factor. Therefore, the established conditions for the TLC profile can be used in the quality control of the drug. However, it is necessary to compare with standards to corroborate these results.

Conclusion

In microscopical analysis, leaves, stems, and roots of *U. baccifera* showed similar structures. However, the leaves showed the highest values of physicochemical parameters and more richness of metabolites in phytochemical screening with respect to stems and roots. On the other hand, the better solvent for the extraction was water and hydroalcoholic solution at 30%. Therefore, the scientific evidence justifies the use of the aqueous extract of the leaves of *U. baccifera* growing in Cuba as raw material for the development of future herbal medicine, contrasting with traditional use, where the roots are used. This is the first report about the micro-morphological characteristics and quality parameters of each organ.

Conflict of interests

The authors declare no conflict of interest

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