

EVALUATION OF *Rhizophora mangle* BARK COMING FROM THE WESTERN AREA OF CUBA FOR BIOMEDICAL DESTINATION

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ABSTRACT: Cuban mangroves constitute the 5% of the country surface and they are distributed in the whole coast of the island. One of the species represented is *Rhizophora mangle* L. (red mangrove). From the bark of this plant, new drugs for human and veterinary use have been obtained. All the areas studied in the western region showed values of soluble substances (>14.5 mg/ml); ash (< 12%); total tannins (>6 mg/ml) and percentage of polymerized polyphenolic compounds (PPC) (>90%). *Rhizophora mangle* bark can be collected in every season. It is stable for more than one year when being stored in polyethylene sacks in a fresh place at room temperature ($25 \pm 5^\circ\text{C}$) with a relative humidity between 70-90%.

(Key words: *Rhizophora mangle* L; red mangrove; .CIKRON; stability)

EVALUACIÓN DE LA CORTEZA DE *Rhizophora mangle* L. CON FINES BIOMÉDICOS PROCEDENTES DE LA ZONA OCCIDENTAL DE CUBA

RESUMEN: Los manglares cubanos constituyen el 5% de la superficie del país y se encuentran distribuidos en todo el litoral de la isla, una de las especies representada es *Rhizophora mangle* L. (mangle rojo) y a partir de la corteza de esta planta, se han obtenido nuevos medicamentos para uso humano y veterinario. Todas las zonas estudiadas en la región occidental mostraron valores de sólidos solubles totales (>14.5 mg/ml); ceniza (< 12%); taninos totales (>6 mg/ml) y porcentaje de compuesto polifenólico polimerizado (>90%) La corteza de *Rhizophora mangle* L. puede recolectarse en cualquier época del año y es estable por más de un año cuando se almacena en sacos de polietileno en un lugar fresco a temperatura ambiente ($25 \pm 5^\circ\text{C}$) con una humedad relativa entre 70-90%.

(Palabras clave: *Rhizophora mangle* L; mangle rojo; CIKRON, estabilidad)

INTRODUCTION

Mangrove forests constitute the most important formation of Cuba and they represent the 26% of the total surface of forests. The mangrove areas of the country reach approximately 532400 ha, representing the 4.8% of the surface. The main mangrove species are *Rhizophora mangle* L. (red mangrove), *Avicennia germinans* (black mangrove), *Laguncularia racemosa* (pataban or white mangrove) and *Conocarpus erectus* (yana) (10,11).

The ethnomedicine use of *Rhizophora mangle* L. for treating diverse pathologies such as: leprosy, asthma,

hemostatic and tuberculosis has been reported in the book "The Medicinal Plant, Aromatic or Poisonous of Cuba" (15). Studies on *Rhizophora mangle* bark aqueous extract have corroborated effectiveness as antiseptic and healing of open surgical wounds (2,4,5,8,). Two products were developed from it: CIKRON, an antiseptic and accelerator in the healing of open wounds (6;9,18;) and UDERTAN, a mammary postmilking disinfectant (1). Both products constitute an important therapeutic alternative for Public Health and Veterinary Medicine in Cuba.

Previous studies about the chemical composition of the *Rhizophora mangle* L. bark aqueous extract

showed that it presents a high content of tannins (54.78%), where condensed tannins are higher with 80% of the total tannins. Other compounds like: fatty acids C10:0-C24:0 (40 mg/ g freeze dry, 4%), carbohydrates (17.5%), phyto-sterols (0.0285%) and volatile or semivolatile components (0.0205%) were also identified (17).

The chemical and physical characteristics of red mangrove barks once collected and stored at room temperature are not known yet. The knowledge of these characteristics will allow to establish the quality requirements for its use in the development of the products: CIKRON and UDERTAN. The objective of this work was to evaluate the physico-chemical characteristics of *Rhizophora mangle* L bark coming from the western area of Cuba as a source of raw material for the obtainment of phytomedicaments.

MATERIALS AND METHODS

Plant material.

Rhizophora mangle L bark was collected in six zones of the western region of the island (Bahia Honda; San Cristobal, Artemisa, San Nicolas, Nueva Paz and Batabano) between October 2003 - July 2004. In all the cases, plant authenticity was confirmed by the National Botanical Garden, keeping a sample of this.

The previously mentioned zones were separated for their geographical location in North (Bahia Honda) and South (San Cristobal, Artemisa, San Nicolas, Nueva Paz and Batabano). The material collected was dried at room temperature (25±5°C) during 72-120 hours until reaching a humidity inferior to 15%, all dried barks stayed in polyethylene sacks and stored in a fresh place at room temperature and relative humidity between 70-90%.

Preparation of the aqueous extract.

One liter of water was added to 100 g of *Rhizophora mangle* L bark previously ground (for a final concentration of 10%). The whole mixture was put in a reactor of stainless steel of 2,5 L with agitation of 200 rpm at a temperature of 90°C during 60 minutes. Subsequently, it was centrifuged at 3000 rpm to eliminate the solid residuals. The aqueous extract was stored at room temperature in polyethylene plastic flasks of high density until its analysis.

Physico-Chemical Indicators

Extraneous Organic Matter: (EOM): The extraneous organic matter was separated in the whole bark by handpicking and the percentage in weight was calculated (12).

Total Ash (TA): The percentage was determined by differences in weight in the sample incinerated (bark ground) until obtaining a constant mass (13).

Humidity (H): The percentage was determined in the bark by the gravimetric method (NRSP 309).

Total Soluble Solids (TSS): The bark ground aqueous extract (10%) was evaporated until dryness and weight was calculated until obtaining a constant mass (12).

Total Tannins (TT): A quantitative precipitation of the tannins in the bark aqueous extract was carried out using bovine serum albumin. The precipitate was dissolved with a mixture of Sodium Dodecil Sulfate and Triethanolamine and a solution of Ferric Chloride was added. After 15 min, absorbance was measured at 510 nm on an UltroSpec Spectrophotometer 2100PRO. The standard curve was carried out with known concentration of tannic acid (20)

Polymerized Polyphenolic Compounds (PPC): A quantitative precipitation of the PPC in the bark aqueous extract was carried out using Sodium Chloride (14).

Analysis by Liquid Chromatography

Samples were analyzed in a Knauer liquid chromatograph system equipped with a solvent degasser, Two Pumps (model 64), UV/VIS Filter Photometer (280 nm in a range of 0.02), Oven column, Rheodine 7125 injector valve with 20 µl loop and a column of reverse phase (Kromasil 100C18 5µm 15x0,4 cm). A methanol solution at 60% in acetic acid at 1% was used as mobile phase. Ten microliters were applied to the sample previously filtered by 0,45µm. Chromatograms were registered using an interface TIC-8EA and processed with the software BIOCROM.

The antimicrobial activity as an indicator of the biological activity "in vitro" was carried out by the agar diffusion method, using strains of *Staphylococcus aureus* ATCC 29740 (3,19).

Stability Study

Indicators of total soluble solids (TSS), total tannins (TT), polymerized polyphenolic compounds (PPC) and chromatographic profiles of PPC in the *Rhizophora mangle* bark were evaluated during nine months in two areas (Pinar de Rio and Havana). The same indicators were evaluated in barks from two different areas of Havana province stored: one for nine months and the other for two years.

Statistical analysis: The method of Kruskal Wallis-Anova was applied to study the effect of the area in each indicator.

RESULTS AND DISCUSSION

A specification to fulfill in the technological process of the products CIKRON and UDERTAN is that the aqueous extract of *Rhizophora mangle L* bark presents a concentration of total soluble solids and total tannins higher than 14,5 mg/ml and 6 mg/mL respectively. This specification guarantees yields superior to 12%.

The results of different physico-chemical indicators of *Rhizophora mangle L* bark aqueous extract in each studied area are shown in Table 1.

Extraneous organic matter does not surpass 1% in any of the samples collected. Total tannins and total soluble solids vary among the areas; but in all the cases they fulfill the requirements for vegetal drug to obtain a medication with successful results.

There is a tendency of the values to be smaller in the northern area with respect to the southern one. There is a significant difference ($p < 0.05$) in the indicators of total soluble solids and concentration of PPC.

Differences between the evaluations of total tannins and total soluble solids in *Rhizophora mangle* barks by Sanchez *et al.* (16) and our results (Table 2) can be

due to the collection time and the variability of the technique where the value of the critic range is of 4.22 mg/mL when operator is changed (20).

In both studies, the specifications of *Rhizophora mangle* barks are appropriated to be used in the obtaining of veterinary drug; however it is important to consider the time and collection area of the plant since they can affect the yield of the raw material.

Besides the indicators mentioned before to evaluate the vegetable drug, the percentage and chromatographic profile of PPC are added, since they constitute the majority compound with more than 90% of the total tannins of the aqueous extract.

Figure 1 shows the chromatographic profile of PHMW obtained in a single pick from the aqueous extract of *Rhizophora mangle* bark. When there is a deterioration of the raw material due to oxidation, there is a change in the chromatographic profile and two picks appear, observing a decrease of the first pick in the area. This result indicates that the bark should not be used to obtain new lots of drugs.

The quality index required to obtain a new medication should have values superior to 14,5 mg/ml; 6 mg/ml and >85 % of the total soluble solids, total tannins and

TABLE 1. Characterization of different exploitation areas of *Rhizophora mangle L* barks./ *Caracterización de diferentes zonas de explotación de la corteza de Rhizophora mangle L.*

Area	Ash %	Humidity %	Total Soluble Solids mg/ml	Total Tannins mg/ml	PPC %	mg/ml PPC	Biological Activity (Inhibition hole) mm
North							
Bahía Honda	9.63±0.06a	10.8 ±0.2a	19.3±0.2a	11.3±1.0a	80.1±0.5a	7.04±0.4a	11.5
Suth							
San Cristobal	7.66±0.13	11.5±0.4	22.8±0.5	11.1±0.5	87.2±0.2	9.18±0.5	12.0
Artemisa	5.93±0.01	12.8±0.2	17.5±0.7	10.5±0.3	90.7±10.3	7.04±0.1	13.3
San Nicolás	7.50±0.26	11.6±0.3	39.4±10.3	15.4±0.1	91.8±0.3	13.1±0.8	15.0
Nueva Paz	8.43±0.00	12.1±0.1	35.1±0.1	13.4±0.1	91.8±0.3	10.3±0.4	16.0
Batabanó	10.72±0.03	8.57±0.02	30.9±1.2	9.2±0.1	89.6±0.2	10.2±0.1	18.0
MEDIA±SD	8,1±1,7a	11,3±1,6a	29,1±8,9b	11,9±2,5a	90,2±1,9b	9,96±2,2b	14,9±2,

TABLE 2. Comparison of *Rhizophora mangle L* bark requirements in different years./ *Comparación de las especificaciones de calidad de la cortezas de Rizophora mangle L. almacenadas a diferentes años*

Years	Ash %	Humidity %	Total Soluble Solids mg/ml	Total Tannins mg/ml	Reference
November 1994	8.75	18	20	7.13±0.083	Sanchez <i>et al.</i> 1998
October-January 2002-2003	8,1±1,7a	11,3±1,6a	29,1±8,9b	11,9±2,5a	

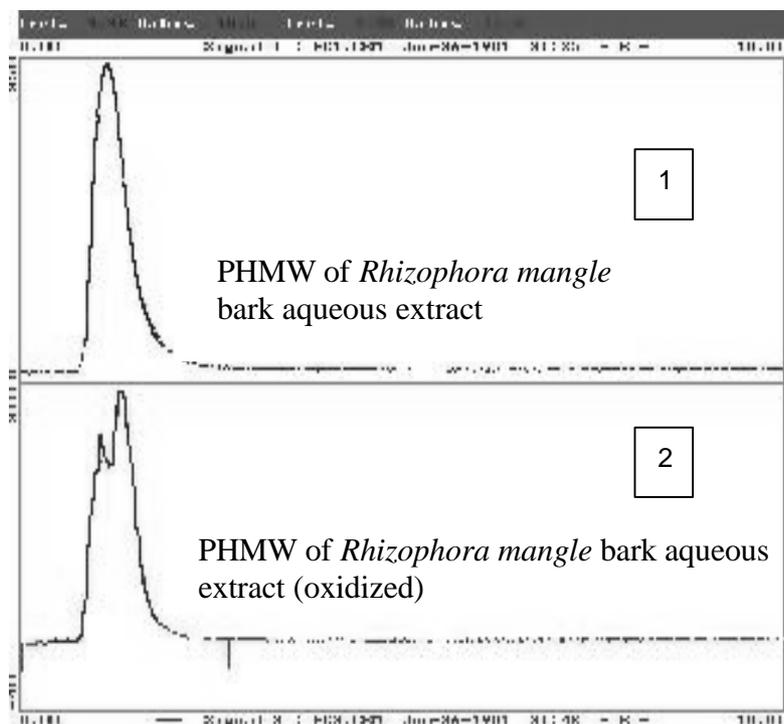


FIGURE 1. Chromatographic profile of PPC obtained from an aqueous extract of *Rhizophora mangle* bark (1 not oxidized and 2 oxidized)./ *Pefil cromatográfico de compuesto polifenólicos polimerizados a partir del extracto acuoso de la corteza de *Rizophora mangle* (1 no oxidado y 2 oxidado).*

TABLE 3. Summary of the main indicators evaluated during stability study./ *Resumen de los principales indicadores evaluado durante el estudio de estabilidad*

Months (Havana)	Total Soluble Solids mg /mL	Total Tannin mg /mL s	PHMW %	PHMW mg /mL
0	39,33±0,30	11,57±0,63	91,83±0,29	13,12±0,79
3	39,06±0,42	12,63±0,14	92,13±0,21	11,18±0,47
6	29,26±0,64	16,29±0,11	92,13±0,21	8,99±0,55
9	31,28±0,88	11,85±0,08	92,95±0,41	13,63±0,08
Pinar de Rio	Total Soluble Solids mg /mL	Total Tannin mg /mL s	PHMW %	PHMW mg /mL
0	22,83±0,50	11,00±0,54	87,24±0,23	9,19±0,49
3	19,66±0,15	13,49±0,42	88,80±0,71	13,50±0,31
6	28,26±0,29	13,13±0,16	90,00±0,03	12,62±0,31
9	30,97±0,29	12,57±0,54	86,84±1,04	10,97±0,01
Havana (zone 1)	Total Soluble Solids mg /mL	Total Tannin mg /mL s	PHMW %	
9	32,0±0,1	16,4 ±0,68	92,13 ±0,17	
24	31,8±0,9	11,84±0,16	88,95 ± 0,91	
specification	> 14,5	> 6 mg/m	>85%	

percentage and chromatographic profile of PPC respectively. Results of stability study for the two respective areas fulfill all these requirements (Table 3).

During the stability study, there was not a degradation of the active components of *Rhizophora mangle* bark (total tannins, PPC) under the conditions of storage used.

This result confirms that barks stored in polyethylene sacks, being in a fresh place at room temperature and relative humidity between 70-90%, are stable and can be used in the pharmaceutical industry.

Studies on mangroves (red mangrove) in the western region guarantee a sustainable exploitation of *Rhizophora mangle* barks for the elaboration of

drugs without affecting the ecosystem in agreement with the forest law of Cuba (7).

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