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PHYTOCHEMICAL ANALYSIS OF THE FRUIT EXTRACTS OF THE *Cordia collococca* SPECIES

Análisis fitoquímico de los extractos del fruto de la especie *Cordia collococca*

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ABSTRACT. There are endemic species exist in the Pinar del Rio municipality, Cuba. That in one season of the year birds and pigs take advantage of their fruits for their feeding. They are developed in different ecosystems, presenting different characteristic in the period of fructification. Among these species the *Cordia collococca* is used for the farmers to feed hens and pigs. It was carried out the gathering of their fruits, in "El avioncito" locality later pulping and watery and alcoholic extracts were prepared, used to carry out rehearsals of sifting phytochemical in function of determinate the presence of secondary metabolites. It was weighed 5 grams of the sample, they were added 50 mL of alcohol and reflowed. They were identified in abundance alkaloids, reducers' compounds, free aminoacids, flavonoids and mucilage and japonicas in smaller proportion. Also they were carried out acceptability rehearsals of the foods in substitution birds of 34 days. The period of adaptation began with the observation method during four weeks. The acceptability tests allowed evaluate in a short time the preference of the food, providing fast and sharp results. In the conclusions we realize that present active principles in the extracts show a low toxicity, what allows contining their development for their evaluation like a conventional alimentary alternative.

Key words: food, phytochemica, acceptability test, toxicology

RESUMEN. Existen especies endémicas en el municipio Pinar del Río, Cuba, que en una época del año las aves y los cerdos aprovechan sus frutos para su alimentación. Estas se desarrollan silvestres en diferentes ecosistemas, presentando características diferentes en cuanto al periodo de fructificación. Dentro de estas especies se encuentra la *Cordia collococca*, utilizada por campesinos para alimentar gallinas y cerdos. Los frutos fueron colectados en la localidad "El avioncito", posteriormente se despulparon y se llevó a cabo la preparación de los extractos acuosos y alcohólicos, utilizados para realizar ensayos de tamizaje fitoquímico en función de determinar la presencia de metabolitos secundarios. Se pesaron 5 gramos de la muestra, se le adicionaron 50 mL de alcohol y se reflujaron. Se identificaron en abundancia alcaloides, azúcares reductores, aminoácidos libres, flavonoides y en menor proporción mucilago y saponinas. También se realizaron ensayos de aceptabilidad del alimento en aves de reemplazo camperos de 34 días. Se inició el periodo de adaptación por el método de observación durante cuatro semanas. La prueba de aceptabilidad permitió evaluar a corto tiempo la preferencia del alimento, proporcionando pronósticos rápidos y certeros de los resultados. Se concluyó que los principios activos presentes en los extractos presentan una baja toxicidad, lo que permite continuar su desarrollo para su evaluación como alternativa alimentaria no convencional.

Palabras clave: alimento, fitoquímica, prueba de aceptabilidad, toxicología

INTRODUCTION

Knowledge about diversity is the basis for the conservation and efficient use of their genetic resources and it is often unknown in certain countries and regions. Empowering local development on the basis of self-knowledge processes (1), taking into account local actors and the relationships am them, among are a fundamental element in the implementation levels of strategy development (2).

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Implementing strategies for animal feeding, taking into account the natural resources that the locality has and the management by the actors knowing their potentialities is one of the offerings that are addressed in this work. Trees and shrubs contribute to the supply of food as long as some of its parts are edible (leaves, fruits, buds, shoots, flowers). The quantitative and qualitative importance of this direct source of tree food is in most cases still unknown and its potential even in terms of world economy is exploited inappropriately.

The food supplied by the trees can be used directly by man or indirectly when consumed by the animals, which will end up producing food fibers or services for man (3-6).

The fruit of *Cordia collococca* is a wild food, used for centuries (7), as a natural sustenance for birds and other animals. For some years, developed and underdeveloped countries have been forced to investigate the use of alternative foods in order to reduce costs and obtain good results in the production and quality of meat and eggs. In this context, the country has to resort to imports in the market to meet the demand of these foods in human and animal consumption, by importing corn and soy oil at very high prices.

The characterization and safety testing of plant-derived products are important tests within the critical path of any compound that is intended to be used as a dysfunctional, drug or food. In the process of generating technologies and production systems adapted to local ecosystems (8-10) and to the specific characteristics of small producers, which are compatible with the sustainable management of natural resources, alternative poultry[A].

At present, interest has arisen in the search for food resources that can partially replace the use of costly and agroecologically distant concentrates from the environmental reality that allow to provide energy, protein and minerals in an efficient and economically viable way to herbivorous animals, A system which demonstrates that the production of the food situation is considerably improved and that the deterioration of natural resources is substantially reduced.

Many of the alternative foods have antinutritive substances that diminish their use by the animal, often altering the digestive activity of this, depressing the animal production; in addition, interfere in the total use of other nutrients like minerals and proteins, or they can produce damages to the organism of the animal that consumes them. Due to the above, the objective of the research was to carry out a phytochemical screening of the fresh fruit of *Cordia collococca* species, evaluating the acceptability of the food in 34-day field replacement birds to evaluate its possible use as an alternative non-conventional food.

MATERIALS AND METHODS

The research was carried out in November, 2014 in the research laboratory of Pinar del Río University. To perform the phytochemical analysis and the acceptability test, *Cordia collococca* fruits were harvested, using a ladder, wooden box and a sack blanket, placed under the branches that had fruits in their state of maturity. 22 kg were collected daily for a week. From the collected fruits the samples were taken for the phytochemical analysis, washed with abundant distilled water, weighed and with the aid of sterile gloves they were pulped; the extracts were prepared with the pulp.

The phytochemical screening was performed, from two extracts, aqueous and alcoholic, which were obtained by reflux, weighing to obtain each, 5 grams of fruit pulp sample of *Cordia collococca*, to which it was 50 mL of 95 % alcohol were added, they were refluxed for four hours in a reflux set consisting of a condenser, a 250 mL balloon and a warm water bath, and the hot extract was then filtered and separated into fractions, using different techniques.

For the analysis of the qualitative results, a system of crosses was used to specify the presence or absence of the families of substances with biological activity present in the fruit (11). To perform the toxicity tests 20 g of the pulp were weighed and 1 L of distilled water was added at 100 ^{0}C in a beaker precipitated for 10 min (12). The extract was dosed and applied to the birds. The toxicity by oral and dermal administration was evaluated at a single dose of the fruit extract of *Cordia collococca*.

[A]Leyva, C. *Caracterización química de harinas de frutos y hojas del árbol del pan (Artocarpus Altilis) y su empleo en la alimentación de pollos, conejos y ovinos de ceba*. Tesis de Doctorado, 2010, Ciudad de La Habana, Cuba, 100 p.

The observation method was used to evaluate the period of adaptation in 100 replacement birds of farm located in the farm "La Ceiba" of the CPA "Celso Maragoto", located at km 11 of the road of the Coloma, Pinar del Rio municipality.

It worked with unconventional food sources (fruit of *Cordia collococca*) that meets the requirements of being abundant, inexpensive and do not compete with human food, in addition to being resistant to environmental conditions.

On the other hand, the acceptance tests were started at the age of 34 days, the observation method was used for a week, with an experimental design, represented by scales with 20 farm birds, for a total of five scales, being selected a scrapbook and dividing into two groups at random. The levels of rejection or acceptance of the food were measured, which were offered early in the morning (at dawn) and in sufficient quantities to be freely consumed, accompanied by good conditions for the stay, water during the day and diet, according to the composition and nutritional contributions established by the table of consumption of technical instructions for the age. Group 1 (G1) was given industrial feed and Group 2 (G2) feed produced from farm products (maize, sorghum and salt).

The fresh fruit was supplied in the early hours of the morning, at noon and in the afternoon, with a feed fasting of one hour per repetitions. These tests were the first step for the characterization of the new food.

RESULTS AND DISCUSSION

The results of the phytochemical screening performed on the fresh fruit of *Cordia collococca* are shown in Table I.

The presence of mucilages is evident, because in water they form viscous solutions that swell in them to form a pseudo gelatinous solution, not occurring in alcoholic solutions.

Coumarins are not identified in any of the extracts. This type of polyphenol appears to play a defense role in plants, as it gives off unpleasant flavors. It is very common its presence in the tender sprouts of some vegetables, what prevents the forage on the part of the animals, reason why they are rejected in the feeding.

The presence of alkaloids is abundant in the aqueous extract of the fruit; they are secondary metabolites (13), whose concentration can constitute a limitation for the consumption of the foods that contain them (14).

Table I. Qualitative results of phytochemical screening of fresh fruit of *Cordia collococca*

Chemical compounds	Fresh fruit of *Cordia collococca*	
	Aqueous	Alcoholic
Mucilages	+	-
Coumarin	-	-
Alkaloids	+++	-
Fatty compounds	-	+
Reducing compounds	+++	+++
Saponins	+	+
Tannins	+	-
Free Amino Acids	+++	+++
Carotenoids	+++	+++
Flavonoids	+++	+++

Abundant; ++ moderate; + Presence; Absence

These alkaloids could be exploited in another way, as in the phytocontrol of fungi and bacteria (15); In addition, they are derived from an amino acid, therefore, they are nitrogenated and have intense physiological action in the animals, even at low doses (16).

There was no presence of the fatty compounds in the aqueous extract; however, in the alcoholic there is presence, due to the present starches that contain small amounts of fats. Lipids associated with starch are generally polar lipids, which require polar solvents, such as methanol-water, for extraction.

Reducing compounds are abundant. The results of the analytical gait are shown in Table II.

In the trials of Molisch, Benidet and Saliwardf the presence of abundant glucose, arabinose, fructose, sucrose and starch was identified (16-20); in addition, the presence of saponins (21) was identified.

Table II. Qualitative methods for identifying reducing compounds

Tests	Results of fresh fruit
Molisch test	(+) Violet. Monosaccharides, disaccharides and polysaccharides
Benidet test	(+) reddish-yellow glucose (5 min) reducing monosaccharides (-) reddish-yellow maltosa sucrose (10-min) reducing disaccharides
Saliwardf test	(+) red fructose and glucose

Saponins are toxic and it is believed that their toxicity comes from their ability to form complexes with sterols, so they could interfere with the assimilation of these by the digestive system or break the membranes of cells after being absorbed into the bloodstream. There is a great variety of plants that contain saponins in different concentrations, such as Yucca, Ginseng, Quinoa, Quillay, among others (22).

The non-nitrogen phenolic tannins are soluble in water and not in alcohol (23) coinciding with the presence thereof in the aqueous extract and not in the alcoholic, the hydrolyzable tannins are heterogeneous polymers, formed by phenolic acids, in particular gallic acid and simple sugars identified above, the level of astringency of *Cordia collococca* fruit justifies the presence of tannins, as in apples and cashew.

This substance, from the nutritional point of view, is of great importance, since that the concentrations of the tannins are higher, increases the possibility of protein formation on bypass in the rumen (24). On the other hand, adequate tannin levels in the diet protect part of the nitrogen and favor its use in the posterior tract, modifying the routes of nitrogen excretion, decreasing the quantity eliminated in the urine and increasing the excretion in the feces, which implies having important effects on the soil. Decoctions are the most common forms of traditional preparation of the extracts, which are also used as insecticides (25).

The presence of flavonoids became evident in the extract with a red coloration;in the Rosenheim test, a violet color that changed to blue in 10 min. Salkowski's essay turned red. Flavonoids were identified in abundance, they have acquired public notoriety due to their biological activity in man, possess properties highly appreciated in medicine, such as antimicrobials, anticancer, reducing the risk of heart disease, among other effects.

These are responsible for the red color of the fruits that cause them to be attracted by the animals and seed dispersers (26).

Also carotenoids are abundant in the *Cordia collococca* fruit, animals are unable to synthesize carotenoids, must obtain them through their diet, these compounds being important for their biological function as pro-vitamin[A].

As an example of these compounds in nature, we can mention the best known carotenoid, carotene found in carrots, lycopene in tomato, both are identified in the fresh fruit of *Cordia collococca* in abundance, coinciding with the color that takes the egg yolk of hens that feed on *Cordia collococca* fruit (27).

Table III shows the results of the analytical gait that was performed to identify free amino acids.

Table III. Qualitative methods of amino acids and proteins

Testing	Results of fresh fruit
Ninhydrin Test	(+) Violet (proteins, polypeptides and amino acids)
Biuret test	(-) Yellow (amioacids)
Coagulation test	((-) does not coagulate (Histonas, protamines or polypeptides)
Xantoproteic Test	(+) orange (tyrosine, phenylalanine or tryptophan)
Hopkins-Cote Test	(-) colorless (Tyrosine, phenylalanine)
Test Millón -SH	(+) red (Tyrosine)
Sakaguchi test	(+) red (Arginine)

From the identified amino acids, arginine is essential and can stimulate immune function by increasing the number of leukocytes; Can stimulate the release of growth hormone (somatropin). Included in balanced diets of cats, their absence can lead to serious disorders related with excess ammonia in their tissues.

Phenylalanine was also identified, found mainly in foods rich in proteins, both animal and plant origin, in the Ninhydrin test could be observed, quickly, the presence of proteins. Tryptophan is an essential amino acid, which is only obtained through feed, in the gait it was identified with abundance.

Table IV shows the differences of groups 1 and 2, related to the acceptability of the fruit of *Cordia collococca* in 34-day campesinos, assuming the described methodology[B].

The statistical data provided by the table show the true magnitude of the acceptability effect of the fruits of *Cordia collococca* for 34-day-old farmers. Criteria of bilateral significance greater than 0,05; indicate that the data of the different observations of the samples are in correspondence with the homogeneity of the values of the population mean and the lower and upper limits, representing 95 % and finding the true magnitude of the observations of each moment of the experiment.

[B] Martínez, M. *Efectos de la suplementación con follaje de plátano (Musa paradisiaca) con indicadores morfofisiológicos, productivos y reproductivos de aves semirrústicas de reemplazos ponedoras*. Tesis de Maestría, Instituto de Ciencia Animal, 2012, Mayabeque, Cuba, 88 p.

Table IV. Testing the samples of groups 1 and 2 on the acceptability of the food

Acceptance of food by groups of experiments	Mean	Related differences				t	gl	Signification (bilateral)
		Typical deviation	from the average	95 % of interval				
				Inferior	Superior			
At the beginning G1-G2	−1,28571	1,70434	,64418	−2,86196	,29053	−1,996	6	,093
At half an hour G1-G2	−1,00000	1,41421	,53452	-,30793	2,30793	1,871	6	,111
At an hour G1-G2	,28571	,48795	,18443	-,16556	,73699	1,549	6	,172

On the other hand, the data are compatible with the observed values, in the incorporation of the chickens for the inclusion of the fruit, at the beginning, half hour and hour, concluding that the sample is sufficient, although there are not great differences to the half hour and the time of incorporation to accept the food.

In the first hours of observation, the food was difficult to peck in the rustic trough made of wild cane, for both groups (Figure 1).

After pouring the fruit into sackcloth (Figure 2), the incorporation of chickens from both groups was greater, these data being verified with the graph that appears in Figure 3.

In Figure 2, voluntary consumption can be observed, however as good as this food is, its first quality is that its level of consumption is accepted by the animal. Despite the results of the phytochemical analysis, the birds serve as an instrument for evaluating a raw material and do not require sophisticated equipment, providing a fast and accurate forecast, a result similar to what could be had in a classic test of animal behavior.

In Figure 3 we can observe the matrix of the distances evaluated in the incorporation of the farmy chickens of groups 1 and 2, for the inclusion of the fruit of *Cordia collococca*.

The evaluated distances shown in the graph coincide with the experimental data (Table V), with a marked difference; the first day, at the start of Group 1 (industrial feed diet), 20 % of the chickens were incorporated, a marked difference with Group 2 (feed produced from farm products), which was incorporated 60 % the second day. At first the difference is lower, for Group 1, 50 % and for Group 2, 60 % is maintained, coinciding for both groups, 100 % of incorporation on the third day.

In the adaptation period the birds recognize the *Cordia collococca* fruit in the first hours of the food offering, achieving a familiarity with it. No food rejection was observed, the next day no rest food was collected, it was fed with 5,5 kg per group daily.

Figure 1. 34-day field chicks pecking at the feeder

Figure 2. 34-day-old farm chickens pecking ateje (*Cordia collococca*.) in blankets

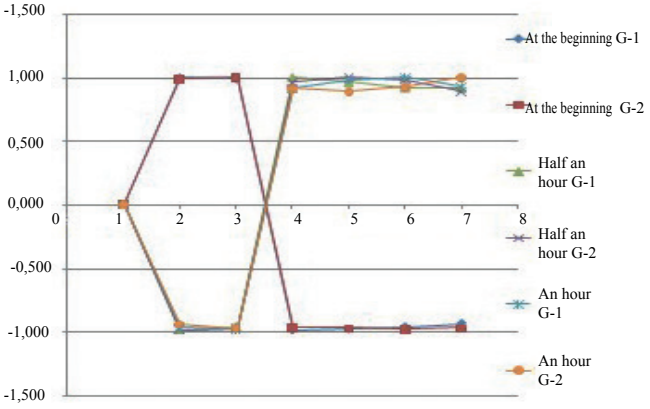

Figure 3. Matrix of the distances evaluated

On the other hand, the toxicity was evaluated with the application of the prepared extract, to a sample of 20 field chickens at the age of 34 days (Figure 4). The solution was placed in the rustic feeders and drank for three days, with no alterations observed.

In the sensitization test that was applied in the field chickens, the presentation of edemas or erythema was not found during the observation period of 72 h, and therefore it was classified as non-sensitizing dermal, and the product was classified as non-dermal irritant and ophthalmic.

CONCLUSIONES

- Phytochemical screening allowed the qualitative identification of the abundant presence of alkaloids, reducing compounds, free amino acids, flavonoids and the presence of mucilage and saponins, which makes it possible to use the species in the production of different natural products.
- The acceptability test allowed us to evaluate, in a short time, through the observational method, the food preference, in grazing, providing fast and accurate forecasts of the results.
- The active ingredients present in the extracts present a low toxicity, which allows to continue their study for evaluation as an alternative non-conventional food.

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Table V. Acceptability of the fresh fruit of *Cordia collococca*

Nu. of birds for		Observation of food acceptability of the groups by days and hours						
G-1	G-2	Hours of the 1st. day			Hours of the 2nd. day		Hours of 3rd day	
		At the beginning	Half an hour	Un hour	At the beginning	Half an hour	One hour	At the beginning
1	1			1		1	1	
2	2	2						
3	3		2					
4	4		4	4		5		
5	5				5			
6	6	6						
7	7							
8	8				8			
9	9							
10	10							20
Total of birds		8	6	5	13	6	1	20

Figure 4. Dermal and oral administration at a single dose of the fruit extract of *Cordia collococca*

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