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Original article

Potential use of *Leucaena leucocephala* Lam (leucaena) present in agroforestry systems of Pinar del Río

Uso potencial de *Leucaena leucocephala* Lam. (leucaena) presente en sistemas agroforestales de Pinar del Río

Uso potencial de *Leucaena leucocephala* Lam. (leucaena) presente em sistemas agroflorestais em Pinar del Río

Yusniel Dago Dueñas[1*] https://orcid.org/0000-0002-5513-0561

Jorge Candelario Milian Domínguez[1] https://orcid.org/0000-0003-4763-9779

Katiuska Calzadilla Reyes[1] https://orcid.org/0000-0001-7036-9557

María de los Ángeles Redonet Miranda[1] https://orcid.org/0000-0003-2274-4118

Yosbel López Quintana[1] https://orcid.org/0000-0002-1422-344

Lisandra Hernández Guanche[1] https://orcid.org/0000-0003-4018-4986

[1]Universidad de Pinar del Río "Hermanos Saíz Montes de Oca", Pinar del Río, Cuba.

[*]Correspondence author: yusniel.dago@upr.edu.cu

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ABSTRACT

The present work was developed at the University of Pinar del Rio "Hermanos Saíz Montes de Oca" during the months of January to March 2018, with the objective of determining the presence or not of secondary metabolites in the foliage and bark of *L. leucocephala* with potential use in agroecosystems. The plant samples were taken in an agroecosystem of Pinar del Rio and then transferred to the chemistry laboratory of the university where they were processed. The working method used for the determination of the secondary metabolites was photochemical screening where a successive extraction with solvents of increasing polarity was performed, obtaining the extracts. After the screening was done it was possible to infer that in the foliage and bark of this species there is the presence of substances such as flavonoids, alkaloids, reducing carbohydrates, tannins which proves the potential use of this plant for animal nutrition and health.

Keywords: foliage and bark of *Leucaena leucocephala*; secondary metabolite; extraction.

RESUMEN

El presente trabajo se desarrolló en la Universidad de Pinar del Río, durante los meses de enero a marzo de 2018, con el objetivo de determinar la presencia o no de metabolitos secundarios en el follaje y corteza *de Leucaena leucocephala* con potencial uso en los agroecosistemas. Las muestras de las plantas fueron tomadas en un agroecosistemas de Pinar del Río para después ser trasladadas al laboratorio de química de la universidad donde fueron procesadas. El método de trabajo utilizado para la determinación de los metabolitos secundarios fue el tamizaje fotoquímico donde se realiza una extracción sucesiva con solventes de polaridad creciente, logrando la obtención de los extractos. Después de realizado el tamizaje se pudo inferir que en el follaje y corteza de *esta especie* existe la presencia de sustancias tales como flavonoides, alcaloides, carbohidratos reductores, taninos lo cual comprueba el potencial uso de esta planta para la nutrición animal y la salud.

Palabras clave: follaje y corteza; *Leucaena leucocephala*; metabolitos secundarios; extractos.

RESUMO

O presente trabalho foi desenvolvido na Universidade de Pinar del Rio "Hermanos Saíz Montes de Oca" durante os meses de janeiro a março de 2018, com o objetivo de determinar a presença ou não de metabólitos secundários na folhagem e casca de *Leucaena leucocephala* com potencial uso nos agroecossistemas. As amostras vegetais foram coletadas em um agroecossistema de Pinar del Rio e depois transferidas para o laboratório de química da universidade onde foram processadas. O método de trabalho utilizado para a determinação dos metabólitos secundários foi a triagem fotoquímica, onde foi realizada uma extração sucessiva com solventes de polaridade crescente, obtendo-se os extratos. Após a triagem foi possível inferir que na folhagem e casca desta espécie há a presença de substâncias como flavonóides, alcaloides, carboidratos redutores, taninos que comprovam o uso potencial desta planta para a nutrição e saúde animal.

Palavras-chave: folhagem e casca; Leucaena leucocephala; metabólitos secundários; extratos.

INTRODUCTION

The genus *Leucaena* has 24 species of trees and shrubs, distributed in different countries as they are: USA, Peru, Paraguay and Cuba; it is an evergreen plant, which increases the fodder in the dry season and contributes to the feeding of the animal for the families that depend economically on cattle raising, by solving problems of supply for the cattle during the dry season (Martínez, 2017).

Tree legumes are often used as a buffer to overcome interruptions in livestock feeding resulting from climate variability and in agroforestry or silvopastoral systems.

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This legume produces fodder in sufficient quantity and quality during the dry season, in order to obtain a gain in the animal's weight or for it to survive in dry conditions by reducing the contribution of fodder acquired by the producers (Lezcano, 2012).

Among the 200 species of legumes, *Leucaena leucocephala* is one of the most used in the agropastoral systems as fodder; it is a plant of great importance in livestock production, since it provides high quality food, tolerates droughts and is quickly consumed by cattle. It also has medicinal uses (stomach problems), its seeds are edible, is a fodder plant that provides animals with the necessary protein for their development (Martínez, 2017).

Plants produce a diversity of substances resulting from secondary metabolism, some responsible for the colouring and aromas of flowers and fruits, others linked to ecological interactions, such as the attraction of pollinators; some of these properties make them very attractive to animals (Milian *et al.*, 2017).

Some secondary metabolites are only present in certain species and fulfil a specific ecological function, such as attracting insects to transfer pollen to them, or animals to consume their fruits in order to disseminate their seeds; they can also act as natural pesticides in defence against herbivores or pathogenic microorganisms, or secondary metabolites can be synthesized in response to damage to some plant tissue, as well as against ultraviolet light and other aggressive physical agents, even acting as signals for communication between plants with symbionts (Milian *et al.*, 2017).

Considering the above, the objective of this work is to determine the presence or not of secondary metabolites in the foliage and bark of *L. leucocephala* with potential use in agroecosystems.

MATERIALS AND METHODS

Description of the scenarios used in the research

The place where the experiments were carried out was the Chemistry Laboratory at the University of Pinar del Río "Hermanos Saíz Montes de Oca", Cuba, where the study material was moved, as an initial and essential step; it was defibrated manually and in a rustic mill to obtain 7-8 mm particles, according to Standard 8770-58n (Ortega *et al.,* 2018). This facilitates the extraction of the active components of the sample, which was taken within the institution itself, located at 22°16'33" North latitude, 83°40'51" West longitude.

Obtaining the extracts

For the preparation of the extracts, the aerial parts of the plants (leaves, bark and fruits) were taken. The foliage, bark and fruits were processed under natural conditions for 72 hours and then in the oven for 24 hours at 60 0C, and then finely crushed to 0.8 mm particle size (Milian *et al.,* 2017). The macerations were carried out for 48 hours. The solvents of each maceration were concentrated separately under reduced pressure up to a volume of 5 ml.

Sample quality index analysis

In order to verify the quality parameters of the samples, it was necessary to carry out different studies on all the extracts for the determination of the acidity index and the humidity in the chemistry laboratory of the same institution.

Determination of the *pH* of the solution

The *pH* of the solution is determined with 0.9-1 g of the sample in 100 ml of distilled water, by direct potentiometry in a HANNA 211 pH meter.

Moisture analysis

The moisture content is determined by the gravimetric method. Between 5 and 10 g of the samples are taken, weighed on an analytical balance with an accuracy of ± 0.0001 g and dried in an oven at a temperature of 103 °C ± 2 °C until a constant mass is obtained according to Standard T-264-cm-97 (TAPPI, 1998).

A Sartorius MC 1, AC 210s, Baxun and Ziehenalemana digital analytical balance was used for this test.

The percentage of moisture: it was determined using the criteria that define it as the difference between the wet mass and the dry mass per cent (Milian *et al.,* 2017) (Equation 1).

$$m(agua) = m(h) - m(s)$$

m (h)- masa húmeda

m (s)- masa seca (1)

$$W\% = \frac{m(agua)}{m(muestra)} * 100$$

Phytochemical screening

The scheme that we propose uses the successive extraction with solvents of increasing polarity, with the purpose of achieving the biggest exhaustion of the drug, testing in each extract the metabolites that according to their solubility can be extracted in these solvents (Nogueira and Spengler, 1994). Each extract was subjected to a qualitative analysis by means of chemical reactions summarized in the guide for phytochemical screening.

For the identification of these secondary metabolites, the ferric chloride (phenols and/or tannins) extract test (ethanolic and aqueous).

The reactive agent used is a solution of 5 % ferric trichloride in saline. If the plant extract is made with ethanol, the test determines both phenols and tannins; three drops of a 5 % ferric trichloride solution in physiological saline are added to an aliquot of the ethanol extract. If the extract is aqueous, the test determines mainly tannins; to an aliquot of the extract sodium acetate is added to neutralise and three drops of the reactive solution.

A positive test can give the following general information:

1. Development of a red-wine coloration, phenolic compounds in general.
2. Development of an intense green coloration, pyrocatecholic type tannins.
3. Development of a blue coloration, tannins of the pyrogalotanic type

RESULTS AND DISCUSSION

The Table 1 shows the results of the calculations of the *pH* of alcoholic, aqueous and ethereal extracts from the different parts of the tree. The *pH* values obtained for each of the slightly acidic prepared extracts could be observed without any variation. On the other hand, Milian *et al.,* (2017) obtained higher values (*pH* 6.2 to 6.5) in the *Samanea Saman* Jacq. (carob tree).

Table 1.- *pH* values of aqueous, alcoholic and ethereal extracts of *L. leucocephala*

Sample	pH values	pH values	
	Aqueous	**Alcoholic**	**Ethereal**
Bark	6	6	6
Green foliage	6	6	6

The Table 2 shows the results of the moisture present in the bark and green foliage of the *L. leucocephala* plant, appreciating that the highest moisture contents correspond to the foliage, not being so for the green bark and fruit; these parameters indicate that the green bark and fruit can be preserved for a long period of time, which accentuates its importance from the research point of view.

Table 2.- Moisture parameters of evaluated bark and green foliage samples

Sample	**Wet Mass (g)**	**Dry Mass seca (g)**	**Wáter contents (g)**	**Moisture percent (%)**
bark	10	6,07	3,93	39,26
Green foliage	10,10	7,88	2,10	20,91

These results are higher than those obtained by Milian *et al.,* (2017), which obtained a humidity of 27 % for the bark, and lower for the humidity of the green foliage, which had 38 % for the carob tree foliage.

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Table 3. - Phytochemical screening of ethereal, alcoholic and aqueous extracts of *L. leucocephala*

Group of compounds	Essay	Extracts		
		ethereal	alcoholic	Aqueous
Saponins	Foam	-	-	-
Alkaloids	Dragendorff	+	+++	-
	Wagner	-	+++	-
Amino acids and amines	Ninhydrin	-	+++	-
Reduced Sugars	Fehling	-	+++	+++
Phenols and tannins	FeCl3	-	+++	+++
	Jelly	-	-	
Flavonoids	Shinoda	-	-	-
Mucilages	To the touch	-	-	
Essential oils and fats	Sudán	++	-	
	Non-reactive white paper			
Coumarines	Baljet	++	++	

Note: Blank spaces mean that these tests were not performed on the extract;
+ means that a positive response was obtained for that metabolite in the extract;
- means that a negative response was obtained for that metabolite in the extract

In Table 3, several metabolites were detected in the phytochemical screening carried out; in the three extracts the appearance of opalescence was evidenced in the Dragendorff tests, which showed the existence of alkaloids in small amounts. Alkaloids have a great diversity of chemical structures. They are physiologically active in animals, even in low concentrations, so they are widely used in medicine, for example: cocaine, morphine, atropine, colchicine, quinine and strychnine (Milián *et al.,*2017).

When the ferric chloride test was carried out, it was found that abundant tannins were present, especially of the pyrocatecholic type, due to the intense green colour of the sample. In the most polar extracts, alcoholic and aqueous, sugars, flavonoids, quinones and resins were detected. In addition, saponins and bitter and astringent principles were found in the aqueous extract.

On the other hand, it is interesting to point out in this Cuban species the presence of flavonoids and compound triterpenes that have also been found in other plant species such as *Ceratonia silicua* (Milian *et al.,* 2017).

In Table 4 the results of the foliage are presented, where the highest content of secondary metabolites present in it was the alcohol-based extract with presence of alkaloids, amino acids, amines, reducing sugars, phenols, tannins and flavonoids; not being so for the other extracts that only had presence of oils and fats for the ethereal one, besides phenols and mucilages for the aqueous one.

Table 4. - Secondary metabolites present in the green foliage extracts of *Leucaena leucocephala*

Group of compounds	Essay	Extracts		
		ethereal	**alcoholic**	Aqueous
Saponins	Foam	-	-	-
Alkaloids	*Dragendorff*	-	-	-
	Wagner		+++	-
Amino acids and amines	Ninhidrina		+	-
Reducing Sugars	*Fehling*		+++	+
Phenols and tannins	$FeCl_3$		+++	+++
	Jelly			
Flavonoids	*Shinoda*		+++	+
Mucilages	To the touch		-	++
	Sudán	+++	-	
Essential oils and fats	Non-reactive white paper			
Coumarines	*Baljet*	++	-	

Recent studies have shown that the use of alkaloids in animal feed acts as a productive performance enhancer, through an increase in body weight, feed consumption, average daily gain and feed conversion of growing pigs (Raico-Huaccha, 2018).

Tannins are plant substances chemically characterized as polyphenols, with a high molecular weight and a high affinity for proteins. Studies have shown positive effects on animal nutrition, for example, their use to reduce ruminal protein degradation and thus increase the supply of amino acids that can be absorbed by the animal intestine (Hervás *et al*., 2001) (Table 5).

Table 5.- Main metabolites present in *Leucaena leucocephala* Lam

Group of compounds	**Bark**	**Foliage**	**Fruts**
Tannins	-	+++	+++
Carbohydrates	-	+++	+++
Flavonoids	-	+++	+++
Saponins	-	-	-
Mucilages	-	++	+++
Coumarines	++	++	++
Free amino acids	+++	+++	+++
Fenols	+++	+++	+++
Alkaloids	+++	+++	+++

From the phytochemical point of view, the tests for the determination of secondary metabolites, it was observed that the highest numbers of secondary metabolites are identified in the foliage and in the fruit, followed by the bark. The most representative metabolites are identified as alkaloids, tannins, carbohydrates, amino acids, reducing sugars and flavonoids. These results are similar to those obtained by Delgado *et al.*, (2014), which in analysis of the results of the phytochemical study of *Samanea saman* shows that this species has ample possibilities of being used to obtain natural products (bioactive substances and essential oils). In addition, the production of antioxidant compounds from chlorophyll for use in natural medicine, the production of substances used in the leather tanning process, the manufacture of beverages and the preparation of aqueous plant extracts demonstrated inhibitory activity on Escherichia coli, *Staphylococcus aureus* and *Candida albicans* (Ardonio *et al*., 2017). Flavonoids have demonstrated through numerous studies their antimicrobial activity against various agents: *Vibrio cholerae, Streptococcus mutans*, *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli, Bacillus cereus, Helicobacer pylori, Staphilococcus aureus, Lactobacillus acidophilus, Actinomyces naeslundii, Chlamydia pneumoniae* (Daglia, 2012). Good antioxidant activity and cytotoxic potential was found in extracts from the bark of *Samanea saman* (Milián *et al*., 2017). In addition, the antioxidant and organo-protective activity in the bark was attributed to the presence of polyphenolic compounds such as flavonoids and tannins.

This preliminary study describes some of the applications of the bark, foliage and fruits that constitute a natural source to enhance the development of agroforestry, as well as in animal feed with sustainability criteria. The antioxidant and organo-protective properties in the bark are attributed to the presence in it of polyphenolic compounds such as flavonoids and tannins, which can be used in the manufacture of dyes for their power to heal, as well as being astringent and insecticides (Escalante, 2019).

The foliage *L. Leucocephala* has alkaloids, tannins, carbohydrates, amino acids, coumarins, flavonoids and phenols

In the bark of *L. Leucocephala* there are mostly alkaloids, phenols, coumarins and free amino acids.

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Conflict of interests:

The authors declare not to have any interest conflicts.

Authors' contribution:

The authors have participated in the writing of the work and analysis of the documents.