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Development of a cocoa-based cream (*Theobroma cacao*), to take advantage of its anti-inflammatory properties in Ecuador

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***Abstract*---**Ecuadorian cocoa is recognized worldwide for its marked characteristics of aroma and color. In addition, all derivatives are being given importance for their high health benefits. In this sense the objective of this work was: to develop a cream based on cocoa (*Theobroma cacao*), to take advantage of its anti-inflammatory properties. Therefore, bromatological analyses were made to the cocoa bean, in the same way the levels of flavonoids of the cocoa mass were analyzed. Also, different levels of cocoa butter were studied in combination with plantain infusion to obtain anti-inflammatory cream. Finally, the anti-inflammatory effect was studied by means of an analysis of the length of healing in wounds caused to young guinea pigs. After analysis, the physical and chemical characteristics of the raw material contrast with the data permitted by the NTE INEN 176 and 620 standards. In the analysis of total flavonoids such as quercetin, the 3 values obtained showed to have a great antioxidant potential. In the analysis of the anti-inflammatory effect, treatment 4 (A_2B_2) (54% cocoa butter + 27,89% Plantain infusion) proved to be the best, and even superior to traditional creams. In conclusion, this work

has determined that cocoa butter in combination with plantain infusion has anti-inflammatory activity.

***Keywords*---**Cocoa, quercetin, anti-inflammatory cream, medicinal properties.

Introduction

Cocoa, of tropical origin from the forests of Central and South America, scientifically called *Theobroma cacao*, grows in equatorial climates where there is abundant rainfall throughout the year and where its temperatures are relatively stable. A cocoa bean is the fermented and dried seed from which the cocoa solids and butter are extracted; cocoa beans are the basis of chocolate (Gómez & Cordova, 2017).

Cocoa has its origin as tropical, and is a fruit that has small flowers and long petals; the bark of the fruit is woody and has a stretched shape, the same that appear on the branches. The color of the fruit can be reddish, yellowish, greenish or whitish depending on the type of cocoa. Its pulp is rich in sugars and covers the entire grain contour (Gómez & Cordova, 2017).

Cocoa in Ecuador has a high demand due to its quality, flavor and aroma; however, the need to increase research of this plant to know its properties and benefits is observed. Among the different types of cocoa we have, CCN-51 cocoa being a cloned cocoa of Ecuadorian origin, distinguished for its high productivity and quality, in addition being the most available facilitating the harvest and export of the product (Naranjo, 2014).

The quality of cocoa beans depends fundamentally on the type of tree or variety and on the fermentation and drying processes. There are two types of cocoa on the market: l. National and 2. CCN-51. The flows constitute 93% of the grain traded in the world and are represented by the peanuts of Africa and Brazil (Arévalo et al., 2017).

In addition, of the vast number of ancestral properties known, cocoa contributes to lowering blood pressure, slowing the aging process and improving the performance of mental processes, including memory (Salas & Hernández, 2015). Similarly, studies have shown that chocolate induces the production of endorphins, which produce effects of happiness and well-being, prevents heart disease, decreases blood pressure, also has antioxidants, stimulates the brain, is anticancer and even is associated with an aphrodisiac stimulant (Gómez & Cordova, 2017).

Many epidemiological studies associate the consumption of cocoa and chocolate with a reduced risk of chronic diseases, and several health benefits of cocoa compounds have been attributed to their antioxidant and anti-inflammatory potency (Ellam & Williamson, 2013). This is based on its ability to regulate at a low level the proinflammatory cytokines and their subsequent biochemical pathways (Corti et al., 2009).

The antioxidant effects of cocoa components can influence insulin resistance and reduce the risk of diabetes (Katz et al., 2011). In recent years, there has been growing interest, supported by a large number of experimental and epidemiological studies, in the beneficial effects of some phenolic substances, contained in commonly used spices and herbs, in the prevention of various pathological conditions. Thus arises the interest of medicinal plants with anti-inflammatory properties, which are identified for cases in which an inflammatory response occurs in the body such as: blows, sprains, injuries, obesity, rheumatoid arthritis, rheumatoid arthritis, allergies or bursitis (Moya, 2018). Being the plantain (Plantago major), one of the most used for this effect (Nieto, 2018).

With all these antecedents, the objective of this work was: to develop cocoa-based products (*Theobroma cacao*) to take advantage of their bioactive properties, in the Women's Association of "San Gerardo" of the city of Echeandía.

Materials and Methods

The present research work was carried out in the process plant of the Women's Association of San Gerardo and the laboratories of the Research Department of the State University of Bolivar.

Bromatological analysis of cocoa beans

These analyses were carried out on the basis of the following standards:
Moisture: INEN 1676; Fat: AOAC2003.06; Ash: INEN 533; Fibre: WEENDE; pH: AOAC 970.21

Determination of flavonoids in the mass of cocoa

The sample (mass of cocoa) must first be crushed, of which 30 g is weighed; the flask is completely lined with aluminum foil; 96% ethanol is placed in the flask with 4 mL of distilled water, then left to stand for 48 hours in agitation at 400 rpm at 31,9°C. Sample A is filtered and the sample is centrifuged at 15°C at 5000 rpm for 45 min. 500 µm of centrifuged sample with 500 µm of ethanol is placed in the Eppendorf tubes; subsequently, 10 mL of ethanol was prepared and dissolved with 0,2 g of Aluminum Chloride. In a 10 mL balloon, 3.00 mg of Quercetin was weighed and diluted. The tubes are coded (0, 5, 10, 15, 20, 25); in the coded tubes we add the samples according to the patterns:

$$\mathrm{V1} = \frac{C2 * V2}{C1}$$

After one hour incubation at room temperature and in a dark place the absorbance was measured at 420 nm. $A_{510}\, b_2\, m_2$

Calculations

$$\mathrm{FnT} = \frac{\mathrm{A_{510}} - \mathrm{b_2}}{\mathrm{m_2}}$$

Experimental design

An AxB factorial array block design with two Table 1 replicas was applied,

Table 1
Study Factors, Treatments

No.	Code	Factors	
		A	B
1	A_1B_1	54 % cocoa butter	18,84 % Plantain infusion
2	A_1B_2	54 % cocoa butter	27,89 % Plantain infusion
3	A_2B_1	44,95 % cocoa butter	18,84 % Plantain infusion
4	A_2B_2	44,95 % cocoa butter	27,89% Plantain infusion

The combination of factors previously detailed obeyed a process formula in relation to the calculated total weight: Mineral oil 20% (Solubilizant); Propylene glycol 5% (Cosolvent); Stearic acid 4%; Paraben 0,15% (Preservative); Chocolate perfume 0,1%; Glycerin 2%; Water (solvent): 100% difference.

Description of the process for obtaining the cream

Infusion of the plantain
It was boiled in 200mL of water, then dressed in a container with 50g of dry leaves, then covered and let rest for 10 min (Castaño, 2018).

a) Procedure for the Aqueous Phase
Weighed: the amount of cocoa butter and water was measured as described in Table 1
Heating: The cocoa butter was dissolved at a temperature of 67°C.

b) Procedure for the Oily Phase
Weighed: It was performed in weighed reagents (paraben, propylene glycol, mineral oil, glycerin, borax).
Heating: The heavy reagents were heated to bain-marie until it reached a temperature of 65°C.

c) Mixture formation of the Aqueous and Oily phase
Mixture: The aqueous phase was added over the oily phase and the mixture was carried out.
Addition of perfume: Placed 0.01 gr of chocolate perfume to the different combinations, then cooled the mixture to 25ºC.
Shake: The mixture was homogenized for 10-15 min until the emulsion formed.
Packaging and Storage, as established by Tello (2013).

Anti-inflammatory effect of the cream obtained (cutting length)

At this point, the anti-inflammatory activity of the cream obtained mainly from cocoa butter and plantain infusion was evaluated, as well as other excipients. Its purpose was to observe whether the effects of inflammation caused by the cutting of the skin of 3 young guinea pigs decrease, improving their characteristics throughout the wound. Two cm long cut was taken as a reference in the skin of

the animal and evaluations were made for 6 days to establish the reduction of the same.

Results and Discussion

Analysis of the raw material

Determination of the physical chemical characteristics in cocoa

The results presented in the table below correspond to the laboratory analyses performed on the CCN 51 cocoa beans. These are contrasted with the data of the Ecuadorian technical standard NTE INEN 176 and 620 in order to determine compliance with minimum quality parameters.

Table 2
Comparison of experimental and bibliographic data

Parameter	Parameter INEN 176 -620	Result
Moisture	Maximum 7%	7,35
Fat	≥ 20%	43,68
Ash	Maximum 10%	4,001
Fiber	Minimum 30%	28,87
pH	Range 5,2 – 6,5	5,7

The data obtained show that in relation to the humidity of the cocoa evaluated it has a slight increase over the parameter established in the INEN standard, exceeding this value by 0,35%, which was a very important factor because it can lead to an increase in the rate of multiplication of microorganisms causing the loss of quality of food.

Regarding the fat content, cocoa exceeds the reference value, which is positive because being a saturated fat will provide beneficial effects on consumers such as the prevention of vascular diseases due to the phenolic components it has (Gutiérrez, 2015).

The ash content is framed within the permitted range (max. 10%) defining the degree of purity of cocoa. The dietary fiber of cocoa is linked to the ability to improve intestinal transit, as well as the reduction of cholesterol levels, and controls blood sugar levels (Perea, 2019). The pH value obtained is within the parameters required by the INEN standard, which is a factor that establishes the quality of cocoa.

Determination of total flavonoids using quercetin as a standard

Table 3
Quercetin values obtained in cocoa paste

Analysis (samples)	Concentration	mg quercetin/100 g sample
1	29,03	5,13
2	28,83	5,09
3	29,24	5,17

Based on the research "Quantification of Total Polyphenols and Antioxidant Capacity in Cocoa Peel and Seed" carried out by Ordoñez et al., (2019), a value of 3,97 mg/100 g of quercetin sample is obtained in cocoa paste analysis. This value compared with the 3 values obtained in the analysis, establishes that the cocoa paste used is rich in flavonoids (quercetin), which demonstrates its great antioxidant potential.

Anti-inflammatory effect of the cream obtained (cutting length)

At this point, the anti-inflammatory activity of the cream obtained was evaluated; the cut-off reference value was a mean value of 2 cm long in the guinea pig skin. Evaluations were made for 6 days to establish the reduction of the same (Tables 4).

Table 4
Average values of cut length after applying the cream, factors A and B

Factor A	Mean	Homogeneous groups
2	1,78042	X
1	1,86458	X
Factor B	Mean	Homogeneous groups
2	1,81917	X
1	1,82583	X

After a comparison analysis of means, 2 homogeneous groups were identified, therefore, there are statistically significant differences between the 2 levels of factor A, obtaining better results with level 2 corresponding to 44,95% of cocoa butter. With respect to factor B, homogeneous groups were not identified, therefore, there are no statistically significant differences between the 2 levels of factor B: numerically, the best results were level 2 corresponding to 27,89% of plantain infusion. Thus, based on the results obtained, it is concluded that the T4 treatment with the A_2B_2 coding (Figure 1).

Figure 1. Anti-inflammatory and healing effect before and after application of the cream

Anti-inflammatory effect of the cream obtained (width of inflammation)

The purpose of this analysis was to observe whether the effect of inflammation caused by the cut of the guinea pig skin related to the improvement of its characteristics along the width of the wound decreases (Table 5).

Table 5
Average swelling width values after applying the cream, factors A and B

Factor A	Mean	Homogeneous groups
2	0,267	X
1	0,403	X
Factor B	Media	Homogeneous groups
2	0,333	X
1	0,337	X

In this analysis it was identified that there are statistically significant differences between the 2 levels of factor A: the best result was obtained in level 2 corresponding to 44,95% of cocoa butter. Similarly, in factor B no statistically significant differences were identified between the 2 levels, within which the best results are obtained with the level 2 corresponding to 27,89% of rim infusion. Therefore, T4 treatment (A_2B_2) was the best.

In short, with treatment 4, the reduction in the size of the wound up to a length of 1,64 cm was observed. Likewise, the decrease in the width of the inflammation was evaluated considering a width of 0,5 cm, in which, the inflammation decreased up to 0,03 cm. These values were compared with a control (commercial cream) in which the values of length of the final wound were 1,70 cm and the width of the wound 0,10 cm; clearly denoting the effectiveness of the developed anti-inflammatory cream.

Conclusion

This work has made it possible to determine that cocoa butter in combination with plantain infusion presents anti-inflammatory activity, denoting the importance of this new production line in the use of cocoa derivatives.

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