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Article

Determination of the critical hydrophile-lipophile balance of licuri oil from *Syagrus coronata*: application for topical emulsions and evaluation of its hydrating function

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The aims of this study were to determine the critical hydrophile-lipophile balance **(**HLB) of licuri oil, and to perform a clinical assay to evaluate its hydrating effects. For the determination of the HLB, serial emulsions were prepared with the oil. Regarding the clinical study, 13 human subjects were recruited to evaluate the hydrating power of the emulsified preparation containing licuri oil, and comparing it with the same preparation containing sweet almond oil (SAO). The critical HLB of licuri oil was represented by the zones within the concentrations of 10% for the oil and 15% for the pair of tensoactive agents, with a value of 11.8. Both preparations showed similar hydrating power. We propose that licuri oil can be considered a new lipophilic adjuvant with hydrating characteristics, which can be used in cosmetic preparations, replacing consecrated oils, such as SAO.

Uniterms: Licuri/fixed oil/determination of hydrophile-lipophile balance. Licuri/fixed oil/hydration effects. Emulsions/preparation with licuri oil. Skin hydration.

O objetivo deste estudo foi determinar o EHL crítico do óleo licuri e realizar um ensaio clínico para avaliar os seus efeitos hidratantes. Para a determinação do EHL foram preparadas emulsões seriadas contendo esse óleo. Em relação ao estudo clínico, avaliamos o poder hidratante de preparação emulsionada com óleo de licuri, comparando-a com a mesma preparação contendo óleo de amêndoas doces (OAD), em 13 voluntários. O EHL crítico do óleo de licuri foi representado pelas zonas dentro das concentrações de 10% para o óleo e 15% para o par de tensoativos, com um valor de 11,8 e ambas as preparações mostraram poder hidratante similar. Desta forma, o óleo de licuri pode ser considerado um novo adjuvante lipofílico com função hidratante, o qual pode ser usado em preparações cosméticas, substituindo óleos de consagrado uso, tais como o OAD.

Unitermos: Licuri/óleo fixo/determinação do EHL. Licuri/óleo fixo/efeitos hidratantes. Emulsão/preparação com óleo licuri. Hidratação da pele.

INTRODUCTION

The skin is the largest organ in humans, covering nearly the entire surface of the body. The skin constitutes an efficient barrier for defense and regulation, ensuring the relationship between the interior and exterior environment. The skin determines the aspect or appearance, imprints sexual and racial characteristics, and serves as a protective barrier because of its resistance, semi-permeability, and plasticity (Oliveira, 2009). The hydration of skin is a determinant of percutaneous absorption. The moisture levels of the stratum corneum (SC), transepidermal water loss, skin elasticity, and cellular renovation of the SC represent the four main mechanisms regulating its conditioning. Thus, products that possess hydrating action upon application to the skin act by changing the water content of the corneal layer, which depends on the barrier properties and the water gradient through the SC

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(Roberts *et al*., 2008). An evaluation of the hydrating efficacy of topical agents can be obtained via non-invasive biophysical methods in human subjects. Among the methods employed, quantitation of electrical properties, such as conductance and capacitance are used most often (Corte *et al*., 2007).

Oils are typically used in emulsions. Emulsions are thermodynamically unstable systems consisting of a mixture of two immiscible liquids dispersed together, with a layer of tensoactives at the oil/water interface forming a complex interfacial film (Zanin *et al*., 2002). In order to calculate the relative concentration of tensoactives needed to produce an emulsion that is physically more stable for certain combinations of oil/water mixtures, the hydrophile-lipophile balance (HLB) method can be used (Aulton, 2005). Thus, the critical HLB obtained for a specific oil corresponds to the optimal emulsification of this oil, and the emulsion obtained is a fluid emulsion characterized by a minimal droplet size and maximum stability (Silva, 2007).

According to Corte (2006), cosmetic emulsions typically possess a vegetable oil component. Vegetable oils are characterized by low molecular weight and low viscosity, and because they are less occlusive than mineral oils, they show good cutaneous penetration, greater compatibility with the skin, and constitute a source of essential fatty acids and vitamins. Therefore, topical use increases the biosynthesis of these lipids, improving the function of the cutaneous barrier.

The northeast region of Brazil shows a great potential for the production of vegetable oils, which needs to be explored primarily in the semiarid region. Among the most commonly found oils, licuri oil has attracted interest because of its vast applicability. However, this culture is still explored in an extractivist way (Maia *et al*., 2009). Licuri (*Syagrus coronata* (Martius) Beccari), is the fruit of a palm tree native of the semi-arid and cerrado regions of Brazil. An oil can be extracted from its nut that contains high levels of medium chain saturated fatty acids (caprylic, capric, and lauric acids), that are shorter than coconut oil, giving this oil excellent spreadability and penetration (Gomes Neto *et al*., 2009). The saturated fatty acids consist of natural fixed oils, giving them emollient properties when incorporated into dermocosmetic formulations (Pereira *et al*., 2005). Therefore,, it is of great importance to investigate the characteristics of licuri oil (*Syagrus coronate*) in order to prove its hydrating properties, for the future utilization of this oil as a new cosmetic ingredient. The aims of this work were to determine the critical HLB of licuri oil, to develop cosmetic emulsions from this oil, and perform a clinical study for preliminary evaluation of its hydrating effects on the skin of healthy volunteers.

MATERIAL AND METHODS

Determination of the critical HLB

For determination of the critical HLB of licuri oil, serial emulsions of the oil were prepared. A pair of tensoactives with known HLB were used to obtain the emulsions, which were mixed in variable proportions (5%, 10%, and 15%), defined as having scaled values of HLB between 7 and 12, which is characteristic of vegetable oils (Silva, 1997). The components are represented in Tables I and II. The tensoactives used were sorbitan monooleate (Span 80, HLB 4.3) and polysorbate 80 (Tween 80, HLB 15.0). The formula below was used to calculate the HLB of the tensoactive mixture:

$$HLBm = HLBl \,.\, (X) + HLBh \,.\, (Y) / 100$$

HLBm = HLB of the tensoactive mixture,
HLBl = HLB of the lipophilic tensoactive,
HLBh = HLB of the hydrophilic tensoactive,
X – percentage of the lipophilic tensoactive that was used,
Y – percentage of the hydrophilic tensoactive that was used

Nine serial emulsions were prepared, each with a total of five formulations, resulting in 45 emulsions.

TABLE I - Concentration of the compounds that were used in the emulsion

Conc. of Licuri´s oil	Total conc. of tensoactives	% Span 80	% Tween 80	Amount of emulsion
10, 15 e 20%	5, 10, 15%	70 %	30 %	200 g
10, 15 e 20%	5, 10, 15%	60 %	40 %	200 g
10, 15 e 20%	5, 10, 15%	50 %	50 %	200 g
10, 15 e 20%	5, 10, 15%	40 %	60 %	200 g
10, 15 e 20%	5, 10, 15%	30 %	70 %	200 g

TABLE II - HLB of the tensoactive mixture that was used

% Span 80	% Tween 80	HLB of the system
70	30	7.5
60	40	8.5
50	50	9.6
40	60	10.7
30	70	11.8

Preparation method of the emulsions for determination of the HBL

The emulsions were produced using licuri oil, butylated hydroxytoluene (BHT) as an antioxidant, Span 80 and Tween 80 as emulsifying agents, Carbopol 940 as a viscosity agent and to promote stability (only for emulsions submitted to clinical tests), and with methylparaben and propylparaben added as antimicrobial agents.

The emulsions were prepared by heating the phases separately at 70 ± 5ºC. The aqueous phase was dispersed in Carbopol 940, and pH was adjusted to 5.5 with 0.5 mL 20% NaOH (w/v). The oily phase was added to this mixture, while agitating at 900 rpm for 20 min.

Control of long-term stability

Macroscopic aspect

The macroscopic aspect of the emulsion was evaluated for possible alterations in the general appearance, including color, consistency, presence or absence of visible indicators of instability such as phase separation, sedimentation, and lump formation (Brasil, 2004).

pH Measurement

The pH of all formulations was measured using a digital pH meter equipped with a glass electrode and temperature sensor, previously calibrated with pH 4.0 and 7.0 buffer solutions at ± 25 ºC.

Phase

The emulsion (2 mL) was diluted with the same amount of water in a test tube. In cases where the water was incorporated into the emulsion, the emulsion was considered to be an O/W type. Likewise, 2 mL of the emulsion was diluted into the same volume of oil, and if the incorporation was good, it was classified as a W/O type (Silva, 1997).

Viscosity

Viscosity was measured using a rotational viscometer (Rheology International brand) set at a rotation speed of 30 rpm.

Control of accelerated aging

Centrifugation

Sedimentation was performed with 5 mL of each emulsion at 3500 rpm for 20 min, using a Centribio centrifuge.

Evaluation of additional macroscopic characteristics

For evaluation of temperature stability 24 h after preparation, 5 mL of each emulsion was placed into glass tubes and subjected to incubation at high (45 ± 2 ºC) and low (5 ± 2 ºC) temperatures.

Size and distribution of droplets

The analysis of distribution and mean droplet size was conducted with an optical microscope, through bright field observation at ×400 and ×1000 image zoom (Souza, 2007).

Evaluation of the hydrating action of emulsions

A corneometer was used to quantitate the aqueous content of the SC. This instrument operates according to the electrical capacitance principle, to detect variations in the dielectric constant of water.

In 13 previously selected volunteers, the region chosen to assess the hydrating effects of the formulations was the forearm. Three zones were delimited using a ruler. One zone was designated for the application of emulsion containing licuri oil, another one for emulsion containing sweet almond oil, and the third zone served as a control. The sweet almond oil (SAO) was used as a reference, since it is a recognized cosmetic, possessing good emollient action to smooth and tone dry skin.

Ten measurements were performed on the forearm region of each volunteer, and mean values were calculated. The number of measurements performed was determined by the size of each region, assuring that the entire area was evaluated (DAL' BELO *et al*., 2005). The study was submitted to and approved by the Research Ethics Committee of Federal University of Pernambuco/Brazil, under registration number 167/11 (CEP/CCS/UFPE) and SISNEP FR – 4147189. All volunteers signed the Informed Consent Term (ICT) before the test began.

RESULTS AND DISCUSSION

Determination of the critical HLB of licuri oil

All emulsions were prepared in order to determine the critical HLB of licuri oil at concentrations of 10%,

15%, and 20%, together with the pair of tensoactives Span 80 and Tween 80 at concentrations of 5%, 10%, and 15%. These emulsions were submitted to the above-mentioned controls, immediately following completion of the preparation, and 1, 7, 15, 30, 90, and 180 d afterward.

Emulsions containing 5% tensoactives (partial concentrations of the hydrophilic and lipophilic tensoactives ranging from 30 to 70%) varying the concentrations of licuri oil in 10%, 15%, and 20%, presented to be white and fluid; lipophilic/hydrophilic phase behavior; pH between 5 and 6.5 and phase separation 24 h after preparation.

None of the emulsions containing 5% tensoactives remained stable 24 h after preparation, since a clear phase separation was observed. Thus, there was no change on its stability results, when the oil concentration was changed. This result may indicate that 5% tensoactives is insufficient to maintain a stable system.

To be considered stable, an emulsion must maintain the oily phase compounds in the dispersing phase, or vice versa, despite being subjected to stresses caused by variations in temperature, agitation, and gravity (Antonio, 2007). An emulsion containing 10% tensoactives remained stable for a maximum of 90 d for all parameters evaluated. Instability of these emulsions was typically observed when emulsions were treated with high temperatures and centrifugation.

High temperatures accelerate physico-chemical and chemical reactions, causing alterations in the activity, viscosity, aspect, color, and odor of compounds. Low temperatures may accelerate physical alterations such as turbidity, precipitation, and crystallization. The centrifugation test simulates an increase in gravity and induces stress in the sample, increasing the mobility of droplets and possibly promoting instability (Brasil, 2004).

Preparations containing 15% tensoactives yielded the best results. Some emulsions containing 10%, 15%, or 20% oil remained stable for more than 90 d. However, only one emulsion remained stable for more than 180 d after preparation. This preparation, composed of 10% of licuri oil and 15% tensoactives with 30% Span 80 and 70% Tween 80, had a critical HLB of 11.8 for licuri oil. Tables III and IV show the results of controls performed with emulsions containing 15% tensoactives immediately after preparation, and at 30 d and 180 d later.

Table V represents the droplet size control of emulsions containing 15% of tensoactives S80/T80.

All emulsions maintained low viscosity and showed little variation in quantifiable values during the evaluation periods. Evaluation of viscosity aids in the determination of whether a product has appropriate consistency or flow, which helps to predict the behavior of the product over time (Brasil, 2004).

The pH of a formulation must ensure the stability of the formulation ingredients, its efficacy and safety. Higher system stabilities are achieved when they are maintained with only minimal variations in pH (Garcia, Frange, 2009). Over time, the emulsions did not undergo significant changes in pH, remaining instead slightly acidic, with values between 5.5 and 6.5.

Evaluation of mean size and droplet distribution indicated that emulsions containing 10% or 15% tensoactives had values that remained stable for more than 90 d, and did not show significant variation over the evaluation period, reflecting the stability of these emulsions (Souza, 2007).

Tensoactive agents act as a barrier by modifying the coalescence ratio of the disperse phase, or by creating an interfacial film that produces repulsive electrostatic forces between the disperse phases (Garcia, Frange, 2009). This may explain why emulsions with a higher percentage of tensoactives remained stable for longer periods of time.

Hydrating function evaluation

The tables show the results of preliminary assessments of cutaneous hydration for zones 1, 2, and 3, representing control skin, the area where the preparation containing almonds oil was applied, and the area where the preparation containing licuri oil was applied, respectively.

A corneometer was used to determine skin humidity by measuring the level of humectancy of the SC based on the principle of electrical capacitance, which reflects differences between the dielectric constant of water and other substances. The measurement capacitor indicates capacitance changes according to the moisture content of samples (Courage, Khazaka, 2010).

Results were expressed in corneometric units, and in accordance with the equipment manual: Corneometer® values less than 30 represent very dry skin; values between 30 and 45, dry skin; and values greater than 45, sufficiently hydrated skin (Courage, Khazaka, 2010). In general, the volunteers' skin had hydration values of between 30 and 45, indicating dry skin.

An emulsion may have a direct hydration effect when it contributes to water retention in the SC. An emulsion may have an indirect effect when it decreases transepidermal water loss. Thus, in order to achieve successful cosmetic formulations, it is necessary to use raw materials that are biocompatible, so that the final formulation will be effective, according to the attributed benefit, without harming the skin (Maia Campos, 2002).

TABLE III - Emulsions control with 15% of tensoactives S80/T80

%	%	AFTER PREPARATION			30 DAYS							
OIL/TENS.	S80/T80	MA	pH	PB	MA	pH	PB	C	η	RT	5º	45º
10/15	70/30	W/F	6,73	L/H	W/F	6,10	L/H	(-)	30,6	(+)	(+)	(-)
10/15	60/40	W/F	6,52	L/H	W/F	6,21	L/H	(+)	30,8	(+)	(+)	(-)
10/15	50/50	W/F	6,54	L/H	W/F	5,95	L/H	(+)	32,8	(+)	(+)	(-)
10/15	40/60	W/F	5,95	L/H	W/F	5,89	L/H	(+)	33,5	(+)	(+)	(+)
10/15	30/70	W/F	5,86	L/H	W/F	5,87	L/H	(+)	31,4	(+)	(+)	(+)
15/15	70/30	W/F	6,78	L/H	W/F	6,28	L/H	(+)	32,4	(+)	(+)	(-)
15/15	60/40	W/F	6,59	L/H	W/F	6,12	L/H	(+)	31,9	(+)	(+)	(-)
15/15	50/50	W/F	5,95	L/H	W/F	5,72	L/H	(+)	32,2	(+)	(+)	(+)
15/15	40/60	W/F	5,88	L/H	W/F	5,64	L/H	(+)	30,2	(+)	(+)	(+)
15/15	30/70	W/F	5,81	L/H	W/F	5,77	L/H	(+)	31,8	(+)	(+)	(-)
20/15	70/30	W/F	6,41	L/H								
20/15	60/40	W/F	6,55	L/H	W/F	5,93	L/H	(+)	32,9	(+)	(+)	(+)
20/15	50/50	W/F	6,43	L/H	W/F	6,00	L/H	(+)	33,2	(+)	(+)	(+)
20/15	40/60	W/F	6,14	L/H								
20/15	30/70	W/F	5,82	L/H								

MA = Macroscopic Aspect; PB = Phase Behavior; C = Centrifugation; RT = Room Temperature; η = Viscosity (mPa.s); (+) = Stable; (-) = Unstable; W/F = White and Fluid; L/H = Lipophilic/Hydrophilic

TABLE IV - Emulsions control with 15% of tensoactives S80/T80

%	%	180 DAYS							
OIL/TENS.	S80/T80	MA	pH	PB	C	η	RT	5º	45º
10/15	70/30								
10/15	60/40								
10/15	50/50								
10/15	40/60								
10/15	30/70	W/F	5,84	L/H	(+)	34,3	(+)	(+)	(+)
15/15	70/30								
15/15	60/40								
15/15	50/50								
15/15	40/60	W/F	5,55	L/H	(+)	33,9	(+)	(+)	(-)
15/15	30/70								
20/15	70/30								
20/15	60/40								
20/15	50/50	W/F	6,01	L/H	(-)	33,0	(+)	(+)	(-)
20/15	40/60								
20/15	30/70								

MA = Macroscopic Aspect; PB = Phase Behavior; C = Centrifugation; RT = Room Temperature; η = Viscosity (mPa.s); (+) = Stable; (-) = Unstable; W/F = White and Fluid; L/H = Lipophilic/Hydrophilic

TABLE V - Droplet size control for emulsions with 15% of tensoactives S80/T80

%	%	24 HOURS	30 DAYS	90 DAYS	180 DAYS
OIL/TENS.	S80/T80	MDS ± D	MDS ± D	MDS ± D	MDS ± D
10/15	70/30	2,4 ± 0,2	2,5 ± 0,8		
10/15	60/40	2,1 ± 0,5	2,3 ± 1,5		
10/15	50/50	2,7 ± 0,8	2,8 ± 1,3		
10/15	40/60	1,8 ± 0,6	2,0 ± 1,2	2,1 ± 1,7	
10/15	30/70	1,4 ± 0,3	1,6 ± 0,7	1,7 ± 0,9	1,8 ± 1,1
15/15	70/30	2,2 ± 0,5	2,5 ± 1,6		
15/15	60/40	1,9 ± 1,6	2,3 ± 1,7		
15/15	50/50	1,7 ± 0,4	2,1 ± 0,6	2,2 ± 1,5	
15/15	40/60	1,6 ± 0,5	1,8 ± 0,9	1,9 ± 1,3	2,0 ± 1,8
15/15	30/70	2,5 ± 0,8	2,4 ± 0,7		
20/15	70/30	2,1 ± 1,5			
20/15	60/40	1,8 ± 0,8	1,9 ± 1,4	1,8 ± 0,8	
20/15	50/50	1,6 ± 0,3	1,7 ± 1,2	1,6 ± 0,3	1,7 ± 0,7
20/15	40/60	2,9 ± 0,5			
20/15	30/70	2,7 ± 0,4	2,9 ± 1,2		

MDS = Mean droplet size; D = standard deviation (μm)

TABLE VI - Mean hydrating values for zones 1, 2 e 3

VOLUNTEERS	BEFORE	AFTER	VARIATION %
ZONE 1	35	35.3	0.94
ZONE 2	35.92	46.75	25.48
ZONE 3	36.55	48.65	23.06

TABLE VII - Statistical comparison of hydrating mean values for zones 2 and 3

Parameter	Values
α (significance level)	5 %
Degrees of freedom	12
$T_{critical}$	2,.180
$T_{observed}$	0.96

Values obtained in zone 1, used as control skin, showed that the variation in the initial measure and two h later were less than 1%, indicating that small alterations in the humidity of normal skin will not significantly influence the hydrating characteristics of the emulsion being tested.

By comparing the hydration values obtained in zones 2 and 3, it became apparent that the results for preparations containing sweet almond oil and licuri oil were similar. In addition, according to the statistical test carried out, ($t_{observed} < t_{\alpha,n-1)}$, the difference between oils was not statistically significant, when the significance level was set at 5%.

It is important to emphasize that none of the volunteers who participated in the study reported adverse reactions, including redness or itching, after applying emulsions containing licuri or sweet almond oil.

CONCLUSION

Based upon the obtained results, it may be concluded that the determination of the critical HLB of licuri oil will provide the ideal emulsification containing the oil. According to this preliminary evaluation of cutaneous hydration, licuri oil may be considered a new lipophilic adjuvant possessing significant hydrating functions.

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