

# Obtainment of pellets using the standardized liquid extract of *Brosimum gaudichaudii* Trécul (Moraceae)

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## ABSTRACT

**Background:** The standardized liquid extract of *Brosimum gaudichaudii* Trécul is an alternative for the treatment of vitiligo. There is a shortage of solid oral dosage forms developed from standardized extracts of this plant specie. **Objective:** This study is aimed to obtain pellets with a standardized liquid extract of *B. gaudichaudii*. **Results:** The standardized liquid extract of *B. gaudichaudii* was obtained through maceration and percolation with a 55% ethanol-water solution (v/v). Pellets were obtained through a mixture of extract of 500 g of *B. gaudichaudii* standardized extract, 500 g of microcrystalline cellulose PH101 and 10 g of hydroxypropyl methylcellulose K100. The pellets obtained presented a homogeneity yield of 92%, aspect ratio of  $1.16 \pm 0.65$ , shape factor  $e_r$  of  $0.35 \pm 0.09$  and Feret diameter of  $0.87 \pm 0.27$ . These pellets were coated with a suspension composed of titanium dioxide, aluminum red lacquer, ethyl cellulose, talc and magnesium stearate. Before the photostability test, the uncoated pellets showed psoralen content equal to  $0.13 \pm 0.01\%$  and to the 5-MOP was  $1.40 \pm 0.27\%$ . After exposure to one level ( $3 \text{ J.cm}^{-2}$ ) of UVB irradiation the uncoated pellets presented a degradation of 2.16% of psoralen and 8.1% of 5-MOP. After exposure to three levels (10, 20 and  $30 \text{ J.cm}^{-2}$ ) of UVA irradiation the uncoated pellets exhibited photodegradation of 9.78, 17.64, 24.21% of psoralen and 18.95, 23.68, 28.48% for 5-MOP. The coated pellets were unaffected after photostability test. **Conclusion:** Pellets were obtained with the standardized liquid extract of *B. gaudichaudii* and coating is a technological alternative to ensure the stability of the formula.

**Key words:** Bergapten, coating, extrusion, photostability, psoralen

## INTRODUCTION

*Brosimum gaudichaudii* Trécul. (Moraceae) roots, is traditionally used in Brazil for the treatment of patients with vitiligo, this effectiveness is attributed to the psoralens, psoralen and 5-methoxypsoralen (5-MOP).<sup>[1]</sup> Agronomic studies have been developed to enable its systematic cultivation.<sup>[2]</sup> Also, alternative technologies assures the optimal extraction of psoralen and 5-MOP from the roots of this specie.<sup>[3]</sup>

Vitiligo is an acquired depigmentation disorder that affects approximately 2% of the world's population<sup>[4]</sup> and it is characterized by the destruction of melanocytes causing a loss of skin pigmentation with the formation

of white macules.<sup>[5]</sup> The most accepted etiology is that it is related to an autoimmune disbalance, associated with a genetic predisposition. Neurohumoral imbalances and states of oxidative stress are also associated with the expression of vitiligo.<sup>[6]</sup> Therapeutic approaches are based on topical steroids (e.g. clobetasol), calcineurin inhibitors (e.g. tacrolimus), phototherapy using ultraviolet B (UVB) with restricted spectrum (311-312 nm) and photochemotherapy, which associates psoralens with UVA exposure.<sup>[7]</sup> Photochemotherapy it is the most effective treatment also it is used for the treatment of psoriasis a many dermatoses, nevertheless it is a long-term treatment and the ingestion of psoralens it is associated with pronounced adverse effects and with the possibility of skin cancer.<sup>[7,8]</sup>

Varanda *et al.*<sup>[9]</sup> identified that the hydroalcoholic extract obtained from the roots of *B. gaudichaudii* has a higher genotoxicity than the aqueous extract obtained from the same part of this specie. Verification was through *Salmonella*/

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microsome assay and CHO (Chinese Hamster Ovary) cells test. They conclude that the extract with higher levels of psoralens is more toxic. However, Cunha *et al.*<sup>[1]</sup> states that the dry extract obtained from the roots of *B. gaudichaudii* when administered orally in Wistar rats has an approximate a median lethal dose 351 times higher than the therapeutic dose. Until now the therapeutics dosage for this plant, extract has not been stated based on the contents of the psoralens.

Despite being promising the authors did not find reports available on studies related to the technological aspects regarding the preparation of solid dosage forms containing the extract obtained from the roots of *B. gaudichaudii*. The use of pellets obtained by extrusion-spheronization is an interesting approach to the incorporation of this plant extract into solid dosage forms. Pellets are classified as a multiparticulate drug delivery system with many related biological advantages.<sup>[10,11]</sup> Technologically, encapsulated pellets allow for dosage adjustment without formula modifications,<sup>[12]</sup> which is very useful for the development of new formulations with *B. gaudichaudii* extract.

In this study, the standardized extract of *B. gaudichaudii* was incorporated into pellets by extrusion-spheronization. The roundest pellets with a large amount of the extract were selected for the coating process. The characteristics of the pellets and photostability of psoralen and 5-MOP have been determined and compared.

## MATERIALS AND METHODS

### Materials

*B. gaudichaudii* hydroalcoholic extract (containing 1.2% w/w psoralen and 2.4% w/w 5-MOP). Analytical standard of psoralen ( $\geq 99\%$ ) and 5-MOP (99%) were acquire from Sigma-Aldrich® Brazil CO. (São Paulo, SP). Acetonitrile, Methanol HPLC grade was purchased from Scharlau® commercial representative in Brazil (LAS do Brasil, Goiânia, GO). Ultrapure water was processed via Millipore® Milli-Q system (Bedford, MA, USA). Microcrystalline cellulose PH101 were purchased form Blanver (Itapevi, SP, Brazil) and hydroxypropyl methylcellulose K100 were purchased from Dow Chemical Company (Ribeirão Preto, SP, Brazil).

### Methods

#### Extraction procedure

*B. gaudichaudii* roots were dried with an oven with air-circulation system, at 50°C during 72 h, followed by being ground in a knife mill Tecnal® (SP, Brazil). The powdered material (1 kg) was macerated with nine liters of ethanol/water solution 55/45 (v/v) for 24 h under

constant agitation. The macerated material underwent percolation with free flow of the extract. The extract was collected and re-percolated, this process was repeated five times. The obtained extract was concentrated on a rotavapor R-220 Buchi® (Essen, Germany) at 40°C which generated the concentrated extract with 10% of solids content that was stored at -17°C and protected from light.

### HPLC-PDA psoralen and bergpten

The qualitative and quantitative of the psoralen and 5-MOP analyses were performed in a HPLC Alliance e2695 (Waters®, USA) with a photodiode array (PDA) detector model 2998. Empower 2.0 chromatography data software was employed for the control of equipment and for the treatment of the data. The separation was carried out with a chromatography column zorbax Eclipse XBD-C8 (4.6 × 250 mm × 5µm) (Agilent®, USA).

The mobile phase was composed of acetonitrile and ultrapure water (45:55 v/v) at a flow rate of 0.6 mL/min. The injection volume was set to 20 µL. The detection wavelength was set at 244 nm for psoralen and 220 nm for 5-MOP. The chromatography column was maintained at 30°C, and the run time at 30 min.<sup>[8]</sup> This chromatographic method was revalidated and system suitability parameters were checked according to the international parameters.<sup>[13]</sup>

The chromatographic analysis of the extract of *B. gaudichaudii* and pellets was conducted under the same conditions used with the standard, respecting the linear dynamic range.

### Pellets formulation

The wet mass was obtained by two different ways. In the first moment the powdered material were manually shaken during 15 min and then transferred to a bowl to wet granulation process. The powder and the granulation liquid were homogenates after 15 min of hand kneading. This process was performed until the optimal formula was obtained for scale-up process. The amount of each component was determined experimentally.

In the scale-up process, the excipients were pre-mixed by a V-type mixer (LEMAQ®, Brazil) for 20 min. The liquid-solid blend was performed in a kneader mixer (LEMAQ®) for 30 min. For the extrusion process a ram extruder (Screen Extruder 20, Caleva®, England) with an extrusion plate of 1 mm perforated. The spheronization was performed using the spheronizer MBS 250 (Caleva®), with a cross-hatched spheronization plate. The pellets were dried until attaining residual moisture equal to or greater than 3%. The residual moisture was checked with a MB35 Moisture Analyzer (Ohaus®, USA) For coating process a spouted bed LM (Labmaq®, Brazil) was used.

### Extrusion and spheronization procedure

The wet masses was manually fed into the extrusion chamber and compressed against the extrusion plate at a speed of 35 rpm. The extrudates were spheronized in a cross-hatched plate with 250 g of load, at 1200 rpm for 5 min or at 1500 rpm for 6 min. The drying process was performed at 40°C for 12 h or up to three percent (w/w) of residual moisture remains.

### Coating of pellets

The coating suspension was obtained stirring in 150 mL of water, 6.25 g of titanium dioxide, 5 g of magnesium stearate 5 g of talc and 5 g of aluminum lake dye dioxide. Separately, while continuously stirred until completely dispersed, 3.75 g of ethyl cellulose were solubilized in 100 mL of ethanol/water solution 80/20 (v/v). The solution was slowly mixed with the previous prepared suspension with constant stirring for 20 min and homogenized at 6000 rpm for 20 min with an Ultra-Turrax T-18 (Ika®, Germany). Upon completion of the process ethanol/water solution 80/20 (v/v) had been added until reaching the 250-mL mark, for coating 250 g pellets. For the coating process the inlet air temperature was set at 70°C and a spray volume of 1.6 mL/min for coating suspension aspersion.

### Photostability testing

Philips lamps TL40W/12 Rs were placed 20 cm above the pellets and irradiated with 3 J/cm<sup>2</sup> of ultraviolet B (UVB, 292-320 nm) or 10, 20 and 30 J/cm<sup>2</sup> of ultraviolet A (UVA, 320-400 nm) radiation. The irradiations were measured with a UVA or UVB sensor coupled to the radiometer IL-1700 (Newburyport®, USA). The pellets were placed as a monolayer. The identification and quantification of psoralen and 5-MOP were performed with chromatographic methods previously described.

The extraction procedure of psoralen and 5-MOP were performed with 15 mg of coated or uncoated pellets, crushed with mortar and pestle. The crushed material was standardized by size separation with a 100-mesh sieve. This powder was transferred to an Erlenmeyer flask with 10 mL of methanol and subjected to ultrasonic bath during 20 min. After that, the contents inside of the flask were centrifuged at 5000 rpm for 10 min. The precipitated material was separated from the supernatant, and the extraction process was repeated. From the samples obtained aliquots of 1 mL were collected, filtered through a 0.45 µm (Millex®) and the analytes were quantificated.

For the photostability test, the values of the reaction rate constant ( $k$ ) was determined by the straight linear equation, attributing the angular coefficient ( $a$ ) corresponding to the  $k$  value. All analyzes were performed in triplicate.

### Characterization of pellets

#### Shape and size distribution

Vibrating apparatus (Bertel®, Brazil) and a set of sieves (1400, 850, 710, 630, 44 µm) were used for size distribution determinations. The characterization were performed with modal size fraction (1400-72 µm).

The micrographs of pellets were obtained at 10x magnification with Axio Cam MRC5 attached to a stereomicroscope Axio Cam MRC5 Discovery V. 20 SteREO (Carl Zeiss®, Germany). The aspect ratio, Feret diameter, and shape factor  $e_R$  were determined using the freeware ImageJ 1:47 to process the images.

#### Scanning electron microscopy

The samples were sputter-coat with Au/Pd using Desk V (Denton Vacuum®, USA) and examined with a Scanning Electron Microscope JSM – 6610, EDS and ThermoScientific NSS Spectral Imaging (JEOL®, USA) at 5 kV. The SEM-EDS (Energy-dispersive spectroscopy) were used to identify cations of aluminum, titanium and magnesium in the coated pellets surface.

## RESULTS AND DISCUSSION

The chromatographic method was precise and accurate. The percentage recovered of the psoralen was equivalent to  $100.24 \pm 1.17\%$  and  $100.69 \pm 0.75\%$  for 5-methoxypsoralen. The method has a limit of detection and quantification for psoralen equal to 0.073 and 3.805 µg/mL, and for 5-methoxypsoralen were, respectively, 0.245 and 12.68 µg/mL. The values obtained with the system suitability test are in accordance with international official parameters.<sup>[13,14]</sup> The correlation coefficients ( $r$ ) for the psoralen and 5-MOP were greater than 0.999. The hydroalcoholic extract possessed a pH of  $5.84 \pm 0.01$  (mean  $\pm$  standard deviation), a relative density of  $1.04 \pm 0.02$  g/mL and a solids content of 16.03% (w/w). The content of psoralen and 5-MOP in the hydroalcoholic extract of *B. gaudichaudii* correspond to  $1.02 \pm 0.001\%$  and  $1.69 \pm 0.018\%$ , respectively.

During the stages of development of new pharmaceutical product, the first trials are conducted parsimoniously to ensure that the material used is not wasted and more information about it is generated. As a result, the BG1 formula [Figure 1] was obtained only with microcrystalline cellulose and hydroalcoholic extract of *B. gaudichaudii* [Table 1]. The microcrystalline cellulose is the most popular excipient used in the production of pellets because it increases plasticity contributing for the formation of spherical shape in pellets and exhibited a weak natural adhesiveness.<sup>[15]</sup> However hydrophobic materials,

as a plant extract compromises this adhesiveness.<sup>[16]</sup> This effect was observed in BG1 formula, with the granules were powdered during spheronization. According to Shah *et al.*<sup>[15]</sup> granules should have adequate mechanical strength to avoid being pulverized in this stage.

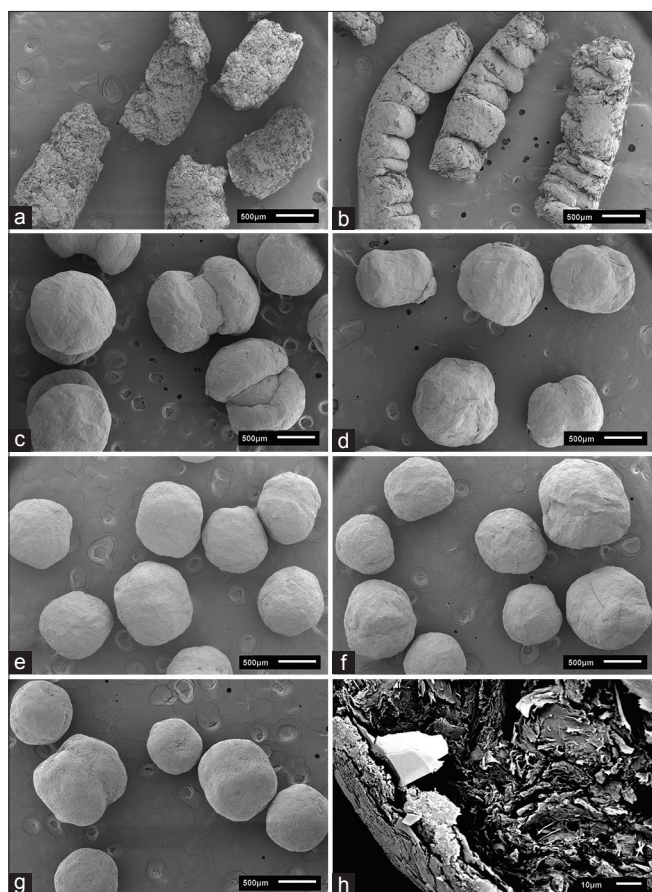
According to Beringsh *et al.*<sup>[16]</sup> this negative effect of plant extracts on pellet formation can be avoided by reducing the proportion of the extract and adding hydroxypropylmethylcellulose (HPMC). The use of HPMC is justified because this excipient provides a greater formation of hydrogen bridges.<sup>[17,18]</sup>

With BG2 formula [Figure 1] the addition of HPMC as well as the reduction in the content of *B. gaudichaudii* extract [Table 1] increased the hardness of the wet mass, making it unfit to be extruded. The addition of HPMC and the reduction in the amount of plant extract increased the mechanical strength and decreased the moisture of its wet mass. According to Lukkonen *et al.*<sup>[19]</sup> the balance between the wet and solid portion of the formula for the preparation of pellets helps in the lubrication holes in the extruder plate and allows the equipment to operate without overload.

To reduce the adhesiveness the proportion of the plant extract was increased [Table 1], obtaining BG3 formula. The modification to this formula was not enough to reduce mechanical strength, because dumbbell pellets [Figure 1] were obtained.<sup>[15]</sup> For the next formula the amount of HPMC had been reduced by half [Table 1] and thereby resulting in BG4 formula. For the first time pellets were produced, despite their low sphericity ( $e_R$ ) and elongated shape (AR) [Table 2].

To reduce the formation of elongated pellets the spheronization process was changed according to the the studies proposed by Hellen, Yliruusi and Kristofferson.<sup>[20]</sup> Thus the amount of each component from BG4 remained the same to obtain BG5 formula. In this new formula the time and the speed of revolutions of the spheronizer plate had been increased [Table 1]. Although, compared with the BG4 the pellets obtained with BG5 formula were 15.38% more uniform and  $\cong$  22,22% more spherical ( $e_R$ ).

One last attempt to increase the proportion of hydroalcoholic extract of *B. gaudichaudii* was performed with the BG6 formula [Figure 1]. Therefore the ideal composition was established [Table 1] for pellets with good sphericity and homogeneity [Table 2]. Thus, we proceeded with the scale-up of BG6 for the BG7 formula, the pilot



**Figure 1:** SEM micrographs corresponding to the granules obtained with the BG1 (a) or BG2 formula(b) and pellets obtained with the respective formulas: BG3 (c) BG4 (d) BG5 (e) BG6 (f) coated BG7 (g) Coating thickness of a BG7 coated pellet (h) cut in half

**Table 1: Composition of the tested formulations obtained with the hydroalcoholic extract of *Brosimum gaudichaudii***

Formula	MCC <sup>a</sup> (g)	HPMC <sup>b</sup> (g)	Standardized extract <sup>c</sup> (g)	Spheronization profile	
				Time (min)/ Yield speed (rpm)	Yield (%)
BG1	150	0	155	5/1200	-
BG2	35.5	7	35,5	5/1200	-
BG3	150	7.5	150	5/1200	-
BG4	150	3.75	150	5/1200	76
BG5	150	3.75	150	6/1500	91
BG6	150	3	150	6/1500	92
BG7	500	10	500	6/1500	84

<sup>a</sup>Microcrystalline Cellulose; <sup>b</sup>Hydroxypropil methylcellulose; <sup>c</sup>Hydroalcoholic extract of *Brosimum gaudichaudii* with solid contentes of 16.03%, standadized in psoralen (1.2%) and 5-methoxyypsoralen (2.4%)

**Table 2: Results of the shape parameters from the pellets obtained with with the hydroalcoholic extract of *Brosimum gaudichaudii***

Formula	AR <sup>a</sup>	$e_R$ <sup>b</sup>	Feret diameter
BG4	1.31±0.16	0.04±0.16	1.07±0.13
BG5	1.18±0.09	0.33±0.09	0.98±0.09
BG6	1.14±0.07	0.36±0.10	0.88±0.07
BG7	1.12±0.07	0.37±0.10	0.85±0.05
Coated pellets	1.15±0.07	0.38±0.07	0.94±0.08

<sup>a</sup>Aspect ratio; <sup>b</sup>Shape factor  $e_R$

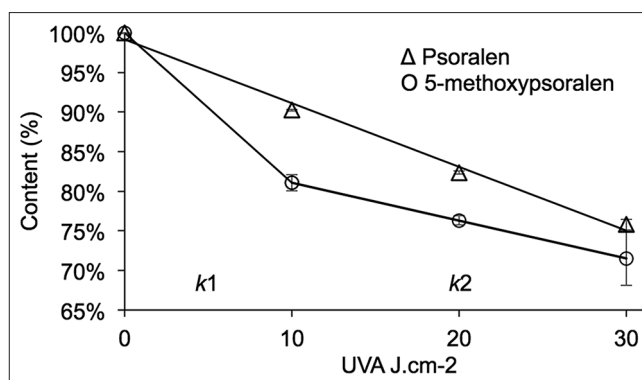
batch. The pilot batch (BG7) showed an increase in  $r$  equal to 1.09% ( $P > 0.05$ ). Despite the results achieved further studies are needed to evaluate the influence of production parameters or composition of the formula in the sphericity of pellets, prepared with the standardized extract of *B. gaudichaudii*.

Half the pellets obtained from the formula BG7 were coated [Figure 1] to determine the importance of the coating in the photostability of psoralen and 5-methoxypsoralen. The spraying of the coating suspension lasted 2h48' and generated an increase of 39.64% (w/w) on the average weight of pellets. The layer formed by coating the suspension around the pellets had approximately 5  $\mu\text{m}$  in thickness. Both pellets coated and uncoated were subjected to photostability test.

Prior to quantify the amount of psoralen and 5-MOP in pellets subjected to the test of photostability, the extraction process by ultrasound was conducted as described above. Thus, after the second extraction the supernatant obtained showed only traces of the presence of psoralen and 5-MOP, which were below the limits of detection and quantification. Therefore, for the extraction of psoralen and 5-MOP of coated or uncoated pellets was necessary realize only a first extraction.

Before the photostability test the uncoated pellets showed psoralen content equal to  $0.13 \pm 0.01\%$  and to the 5-MOP was  $1.40 \pm 0.27\%$ . After exposure to one level (3  $\text{J}/\text{cm}^2$ ) of UVB irradiation the uncoated pellets presented a degradation of 2.16% of psoralen and 8.1% of 5-MOP. After exposure to three levels (10, 20 and 30  $\text{J}/\text{cm}^2$ ) of UVA irradiation the uncoated pellets exhibited photodegradation of 9.78, 17.64, 24.21% of psoralen and 18.95, 23.68, 28.48% for 5-MOP. The photodegradation of psoralen and 5-MOP happened in two phases. The first phase corresponds to the irradiation of 0-10  $\text{J}/\text{cm}^2$  of UVA radiation, with  $k_1$  corresponding to -9.0978 for psoralen and -0.0019 for 5-MOP. The second phase corresponds to the irradiation of 10-30  $\text{J}/\text{cm}^2$  of UVA radiation, with  $k_2$  equal to -0.0072 for psoralen and -0.0048 for 5-MOP. The linear correlation coefficient ( $r$ ) in  $k_1$  or  $k_2$ , for both analytes, were equal to 1 [Figure 2].

Thus, the test result shows that the photodegradation for UVB irradiation or UVA radiation 5-MOP degrades more than psoralen. The conditions in which the photodegradation of these furocoumarins occurs is not yet clear.<sup>[22,23]</sup> Serrano-Pérez *et al.*<sup>[23]</sup> determined through the CASPT2 method that 5-MOP is more accessible to singlet/triplet state than psoralen. In other words, 5-MOP assumes reactive states more easily than the psoralen.



**Figure 2:** Photostability of psoralen and 5-methoxypsoralen from uncoated pellets obtained with *Brosimum gaudichaudii* hydroalcoholic extract. Standard deviation is indicated by bars. The photodegradation of psoralen and 5-MOP happened in two phases. The first phase ( $k_1$ ) corresponds to the irradiation of 0-10  $\text{J}/\text{cm}^2$  of UVA radiation and the second phase ( $k_2$ ) corresponds to the irradiation of 10-30  $\text{J}/\text{cm}^2$  of UVA

The results obtained with UVA irradiation in the uncoated pellets shows that the photodegradation of 5-MOP in  $k_1$  occurred faster than the psoralen [Figure 2]. Probably because 5-MOP it is more abundant on the surface of the pellets and hence was more exposed to UVA radiation. Based on this argument we can infer that the degradation rate decreases in  $k_2$  because of this effect would become observed in the 5-MOP molecules that are in the deeper layers of the pellets.

According to Balasaraswathy *et al.*<sup>[24]</sup> in some regions of the globe only 2 hours of sun, exposure would be sufficient to irradiate 30  $\text{J}/\text{cm}^2$  of UVA radiation. Based on our previous results this sun exposure would be sufficient for the degradation of 24.1% of psoralen and 28.48% of 5-MOP.

The pellets coated with photoprotective suspension after being exposed to UVB or to the three levels of UVA presented reduction lower than 3% in the content of psoralen or 5-MOP. The application of the photoprotective suspension creates a film that protects the pellets individually.

## CONCLUSIONS

Pellets were successfully obtained using 49.5% of *B. gaudichaudii* standardized hydroalcoholic extract, 49.5% of microcrystalline cellulose and 1% of hydroxypropyl methylcellulose. The coating of pellets prevented psoralen and 5-MOP were degraded during the photostability assay. For the first time a formulation is proposed for obtaining pellets from this plant extract is also identified a technological alternative for maintaining the stability of its chemical markers.

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