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DEVELOPMENT AND PHYSICAL-CHEMICAL AND MORPHOLOGICAL CHARACTERIZATION OF NANOSTRUCTURES CONTAINING ETHANOLIC EXTRACT FROM SYZYIUM MALACCENSE (L.) MERR LEAVES. & L. M. PERRY WITH POTENTIAL PHOTOPROTECTIVE PURPOSE

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Abstract: Solar radiation reaches the Earth's surface through rays, such as Ultraviolet (UV), which cause damage to health. Therefore, there is great demand for sunscreen, including products based on natural components, used in industries. The main objective of the work was to synthesize a polymeric nanoparticle with the ethanolic extract of Syzyium malaccense leaves, using poly-n-butyl-cyanocrylate (PBCA) and carry out its physicochemical and morphological characterization, carrying out the following analyses: Electronic Microscopy Scanning (SEM), Thermogavimetry (TG), Differential Thermal Analysis (ATD) and Infrared Absorption Spectroscopy (EAIV). In the morphological and physical-chemical analyzes of the nanostructure formed, it is noted that the nanostructure undergoes an endothermic reaction when subjected to high temperature, in addition to having greater mass loss when compared to the extract, with suggestions of the presence of nanoparticles with ethanolic leaf extract of Syzyium malaccense. It is recommended that further confirmatory tests be carried out to confirm the real percentage of nanostructure formation. Furthermore, the photoprotection capacity of Syzyium malaccense leaf extract was determined and, calculated using the Mansur method, a Sun Protection Factor (SPF) of 4.5 was determined, with the need for better compliance with the minimum requirements required. by the National Health Surveillance Agency (ANVISA).
Keywords: *Syzygium malaccense*. Photoprotection. Polymeric Nanoparticle.

INTRODUCTION

Solar radiation is seen by electromagnetic radiation originated by the sun and reaches the Earth's surface, carrying out biological, chemical and physical processes [1]. In contemporary society, climate change is a major problem, including the reduction of the ozone layer and the emergence of the Antarctic ozone hole, with the purpose of being the natural solar filter, absorbing ultraviolet rays[2].

Solar radiation can bring harm to the skin, such as skin cancer and other diseases, so there is a growing search for sunscreen [3]. In the production of sunscreen, nanotechnology is used, substances that have properties that protect assets, bringing greater safety and quality to consumers [4].

Consumers are looking for natural products or products based on natural components to protect against ultraviolet rays. Therefore, there is a certain concern with the protection of nature, combating tests on animals and other actions, without the use of artifices [5].

The plant *Syzygium malaccense* (L.) Merr. & LM Perry, also known as Jambo Vermelho, belongs to the *Myrtaceae family,* which has more than 300 species of trees and shrubs distributed throughout Brazil, originating in India and Malaysia, in Brazil it is found in the North, Northeast and in warm regions of the Southeast. It belongs to the genus *Syzygium,* having approximately 500 species of trees and shrubs distributed in Brazil, with properties of protection against solar radiation, through the presence of flavonoids in its constitution [6, it presents SPF equal to 6.6 with concentration of extract equal to 0.015 mg/ml [7].

Many natural compounds are unstable, making them susceptible to reactions that lead to a decrease or loss of product effectiveness. An alternative to avoid this loss of stability is the encapsulation of the natural compound, using Nanotechnology [7].

The main objective of **this** work was to synthesize a polymeric nanoparticle with the extract of the leaves of *Syzyium malaccense,* using *n- butylcyanoacrylate* (PBCA) and perform the following morphological and physical-chemical analyses: Scanning Electron Microscopy (SEM), Thermogavimetry, Differential Thermal Analysis (DTA) and Infrared Absorption Spectroscopy (IRS) [8, 9]. In addition to that, determining the photoprotection capacity of the Syzyium malaccense plant extract, through the SPF calculated by the Mansur method was also possible.

MATERIAL AND METHODS

The development of the nanoparticles was carried out in the Semi-Industrial Laboratory of the Pharmacy Course at Universidade Presbiteriana Mackenzie in Higienópolis campus, and the physical-chemical and morphological characterization of nanostructures was held in Mack Graphe laboratories.

COLLECTION OF *SYZYIUM MALACCENSE* LEAVES

The plant species *Syzygium malaccense* grows in humid tropical and subtropical environments. Generally, they can be found in areas that are located at sea level or up to 600 meters in altitude [10]. In Brazil, it is found in the northeast region and on the southeast coast, regions with a lot of heat [11].

For research, leaf samples from the *Syzyium malaccense* tree were collected on the coast of the state of São Paulo, more specifically on the Riviera de São Lourenço (Bertioga), in the period of June 2019. After collection, the leaves were dried in an oven at 40º C for 4 days, being crushed with the aid of a blender and stored at room temperature, with the absence of light.

PREPARATION OF *SYZYIUM MALACCENSE* LEAVES EXTRACT

Syzyium malaccense leaves extract was carried out by means of turbolysis (w/w) with 70 g of leaves dried and ground with 500 g of ethyl alcohol 96° GL. After the mixture had been stirred for 5 minutes, the mixture was filtered through gauze with the aid of a glass funnel, until complete retention of the plant material. Then, the extract was packaged and stored [6].

SYNTHESIS OF PBCA NANOPARTICLES (NANO – PBCA)

The method used for the synthesis of PBCA (n-butyl- cyanoacrylate) nanoparticles (Nano-PBCA) was emulsion polymerization, starting with the addition of 10 ml of 0.1 M HCl (in acid medium) in an erlenmeyer, together with 100 mg of Dextran ®, the formulation's emulsifying agent. Then, 100 µL of PBCA was added. Soon after, the erlenmeyer was placed on a magnetic stirrer, leaving the mouth of the erlenmeyer covered by film paper, stirring at 200 rpm, for 4 hours straight [12]. After stirring for 4 hours, the magnetic stirrer was turned off and 0.1 M NaOH (pH = 7) was added until the colloidal suspension was neutralized. After neutralization, the product was filtered using a filter paper (Figure 1) [13,14].

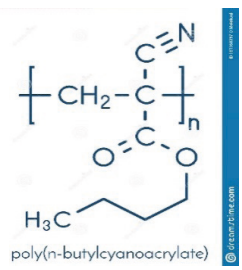


Figure 1: Representation of the structural formula of PBCA (n- butylcyanoacrylate)

Source: DREAMSTIME (2023) [14]

SYNTHESIS OF PBCA NANOPARTICLES WITH *SYZYIUM LEAF EXTRACT MALACCENSE* (NANO – EXTRACT)

For the preparation of the nanoparticle, the emulsion polymerization method was used, adding 10 ml of 0.1 M HCl (in acidic medium) to an erlenmeyer flask. Then 100 mg of Dextran ® emulsifying agent was added. Then, 100 µL of PBCA was added, initiating stirring using a magnetic stirrer at 200 rpm, with the mouth of the erlenmeyer flask covered by film paper. Then, with the help of a micropipette, 100 µl of n- butylcyanoacrylate monomer (PBCA) was added little by little, under agitation for 1 hour [13,14]. Thus, 1ml of the mixture was removed while still under agitation and conditioned; then, 1ml of *Syzyium malaccense* leaves extract was added, being shaken for 3 hours. After this waiting time, the magnetic stirrer was turned off, NaOH - 0.1 M was added, until the colloidal suspension was neutralized (pH=7), so it was filtered with the aid of filter paper [13,14].

DETERMINATION OF FPS BY THE MANSUR METHOD

The SPF was determined by spectroscopy using the height, width and location of the absorption curve within the UV spectrum. However, this method was not enough to calculate the protection factor, so the SPF analysis was performed using the Mansur method [15] (Figure **2**).

$$FPS = FC \cdot \sum_{290}^{320} EE(\lambda) \cdot i(\lambda) \cdot Abs(\lambda)$$

Figure 2 - FPS calculation model using the Mansur method.

Source: BIRTH (2009) [16]

Where FC = correction factor (equal to 10), EE = Erythematogenous effect of solar radiation at each wavelength, I =

Light intensity at each wavelength and Abs = Spectrophotometric reading of sample absorbance at each wavelength [16]. For this, the literature shows the relationship between the erythematogenous effect and the intensity of radiation at each wavelength, represented below (Table **1**):

Wavelength (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

Table 1 - Weighting used in the calculation by spectrophotometry in the Mansur method.

Source: Birth (2009) [16]

The samples were analyzed in the spectrophotometer between the wavelengths of 290 to 320 nm, with several dilutions being performed so that it was possible to perform the reading by the spectrophotometer.

CHARACTERIZATION ANALYZES OF THE FORMED NANOSTRUCTURES

In order for the samples to be analyzed and for the characterization of the nanostructures formed, these were lyophilized. The characterization analyzes of the formed nanostructures were carried out in the Engineering/ MackGraphe laboratories.

SCANNING ELECTRON MICROSCOPY

The samples were submitted to the JEOL Scanning Electron Microscope equipment, model JSM of the 6510 series, where they were placed in a support, and then covered by a thin layer of gold. They were analyzed from 50X to 5,000X magnification [17], thus it was possible to detect the formed morphology of the nanostructure.

THERMOGRAVIMETRY AND DIFFERENTIAL THERMAL ANALYSIS (DTA)

The analysis was performed using the *TG/DTA* 7200 equipment, in the temperature range of 25 to 600ºC, using aluminum capsules containing 2mg of the sample [12].

INFRARED ABSORPTION SPECTROSCOPY (IRS)

The infrared light generated by the Vibrational Spectrometer suffered absorption, reflection and diffraction when incident on the sample [12]. Thus, the samples were analyzed with the presence of a Fourier transformer, using the Horizontal Attenuated Total Reflectance Accessory (ATR) of the Vibrational Spectrometer device, at room temperature, in the region of 400-500cm $^{-1}$[17].

RESULTS AND DISCUSSION

The skin is a double-layered membrane that surrounds the entire outer surface of the body. Its function is to isolate the internal structures from the external environment, divided into three layers: the epidermis, dermis and hypodermis (**Figure 3**) [3,18].

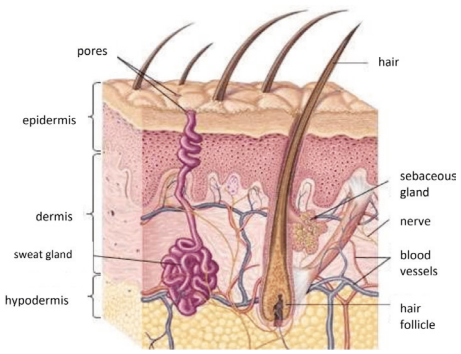


Figure 3 - Representation of human skin with skin layers

Source: BRITANNICA ESCOLA (2010) [19].

The epidermis is the outer layer of the skin, whose function is to be the protective barrier against the external environment, preventing the loss of water, electrolytes, nutrients and preventing the entry of microorganisms. It is an avascular layer; its nutrients are supplied by the dermis through the permeation of capillaries. The epidermis is subdivided into the following sublayers: *stratum corneum, stratum granulosum, stratum spinosum and stratum basale* (**Figure 4**), these represent the state of maturation of keratinocytes, according to the maturation process; thus, the cells pass from one layer to another, usually around 59 to 75 days [18]

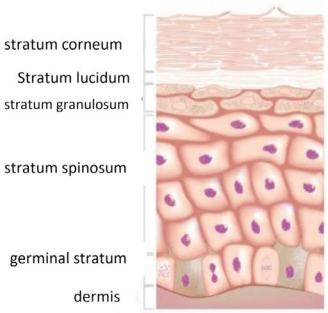


Figure 4: Representation of the epidermis with epidermis subtypes

Source: SANTOS (2023) [20].

The dermis, layer next to the epidermis, is formed by fibrous connective tissues of elastin and collagen, with the function of providing firmness and elasticity to the highly vascularized skin, providing nutrients to the epidermis. Thus, it has sublayers: the reticular dermis and capillary dermis; made up of adipose tissue, whose functions are: temperature regulation, thermal insulation, energy provider, protection and nutritional deposit [18].

Solar radiation can bring some benefits to the skin, such as having the function of stimulating the human body to produce melanin and vitamin D. It can cause harm to the skin, since solar radiation contains different types of electromagnetic waves, coming from of the sun, and each type brings different reactions on the skin. In the wavelength range between 400 and 800 nm is visible light that allows us to see. In the range between 800 and 1700 nm, there are infrared rays that cause heat, burns and, in the long term, can cause cell damage [18].

Between 100 and 280 nm there is Ultraviolet C radiation, which is absorbed in the ozone layer, considered the most harmful radiation [3]. Ultraviolet B rays are found between 280 and 315 nm, reach 10% of the earth's surface, becoming more intense during the summer between 10 and 16 hours. These rays reach the epidermis, causing inhibition of the synthesis of DNA, RNA and proteins, inhibits mitosis, is mutagenic and causes late-onset erythema. In the long term, they can stimulate non-melanomic skin cancer and solar elastosis, UVA is found between 315 and 400 nm, reach the earth's surface, and the dermis, causing immediate erythema, tanning, skin aging, solar elastosis and can induce skin cancer. skin [3,18].

Sunscreens are cosmetics that seek to minimize the effects of solar radiation. They are of two types: chemical and physical. Physicists are photoprotectors that reflect solar radiation, while chemists absorb solar radiation, making it less energetic and harmful [18]. Nanotechnology deals with a technology that reduces particles and/or materials into reduced sizes in the order of nanometers (10^{-9} m) (Figure **5**).

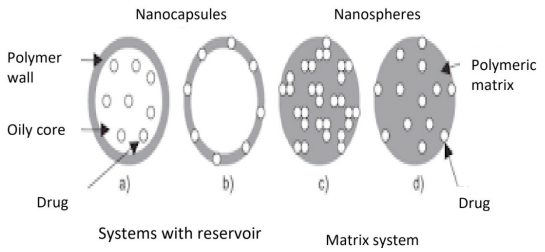


Figure 5: Schematic representation of nanocapsules and polymeric nanospheres

Source: FARIA (2009). [21]

As advantages of using this technology, it is possible to mention aid in solubility, stability, improved permeation and/or absorption, among others [3,8,6].

a) Nanocapsule with drug dissolved in its core; b) Nanocapsule with the drug adsorbed on the polymeric membrane; c) Nanosphere with drug retained in its polymeric matrix; d) Nanosphere with drug adsorbed or dispersed in the polymeric matrix

The *Syzyium malaccense*, also known as Red Jambo, is a plant of Indian origin, which is cultivated in Brazil, in the northeast, southeast and south regions, because these places are humid and have shade, which favors its cultivation [22].

This plant (**Figure 6**) is in the form of a tree, measuring about 15 meters, with large leaves and a straight trunk. The fruits are similar in size to apples, pears and peaches, are reddish, their seeds are directly related to their size, have 2 to 3 centimeters in diameter, and are brown in color [18,22,23].

Figure 6 - Representation of trees, flowers, fruits of the *Syzygium malaccense species*

Source: Moraes (2023). [24]

The leaves of the species *Syzygium malaccense* measures about 20 to 22 cm, 6 to 9 cm wide, shiny dark green on the upper part, dull green on the lower part [24] They have the following bioactives: terpenes, flavonoids, steroids, proanthocyanin reducing sugars, gallic acids and hydrolyzable tannins [18, 25, 26].

The flavonoids, present in the leaves, have in their structure two aromatic rings, joined by an oxygenated heterocyclic ring. One of its functions is to protect against the incidence of UV rays [8. In the study, it was observed that the ethanolic extract of *Syzyium malaccense* leaves was prepared and presented a greenish, fluid color with a strongly alcoholic odor.

Nano-PBCA (White PBCA Nanoparticles) have a whitish color and Nano-Extract (White PBCA Nanoparticles containing ethanolic extract of *Syzyium malaccense* leaves) has a slightly greenish white appearance, as seen in Figure 7.

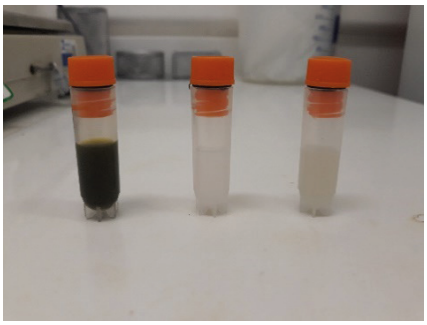


Figure 7 – Tubes containing ethanolic extract of *Syzyium malaccense* leaves

Source: authors (2022).

In Figure 7, the leaves of *Syzyium malaccense* are on the left), Nano=PBCA (in the middle) and Nano-Extract (on the right). The *Syzyium malaccense* extract sample was analyzed in a Femto 800 XI spectrophotometer. Samples with different concentrations were prepared to obtain a spectrophotometer reading of 1,000 µl/ml, 100 µl/ml and 50 µl/ml, but the spectrophotometer reading was only achieved with the 50 µl/ml sample, whose dilution factor is 201 Then, 3 aliquots of this concentration (50 µl/ml) were used to be analyzed in the following spectrum: 290 to 320 nm, obtaining the following results (Table 2):

Wave-length	1st Rate	2nd Rate	3rd Rate	Mean Absorbance
290	0.685	0.685	0.689	0.686
295	0.584	0.588	0.589	0.587
300	0.501	0.500	0.502	0.501
305	0.441	0.442	0.444	0.442
310	0.381	0.381	0.381	0.381
315	0.338	0.339	0.340	0.339
320	0.313	0.313	0.313	0.319

Table 2 - Result of the analysis of *Syzyium malaccense extract* in the spectrophotometer

Source: authors (2022).

Thus, Table 3 presents the FPS calculations found in the work:

Wave-length	Sun Protection Factor (SPF)
290	0.1029
295	0.4995
300	1.4398
305	1.4488
310	0.7101
315	0.2849
320	0.0563
ultimate fps	4.5218

Table 3 - Result of the FPS calculation through the results of the average absorbance and formula of the Mansur method

Source: authors (2022).

Thus, the SPF of the *Syzyium malaccense* extract sample at a concentration of 5 µl/ml is 4.5218. It is a relatively low SPF value, but it must be considered that the sample is at a low concentration to be able to be analyzed in the spectrophotometer and, depending on its concentration, the SPF value can be changed.

The results of the Scanning Electron Microscopy (SEM) are shown in the following images, demonstrating that there was a formation of the nanoparticle. In these images, both the Nano-PBCA **(Figure 8)** and the Nano-Extract (**Figure 9**) were found:

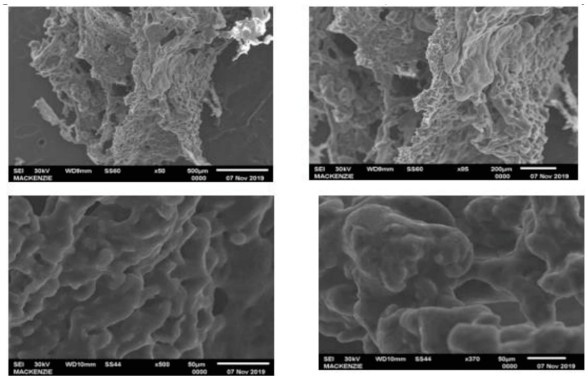


Figure 8 - Results of the Nano-PBCA samples in the SEM analysis

Source: authors (2022)

Comparing the images obtained from the Nano-Extract (Figure 9) and the Nano-PBCA (figure 8), the Nano-Extract images show the presence of defined spherical structures, grouped, with differences in size. Likewise, in the Nano-PBCA images, these structures do not appear, which suggests that there was formation of nanostructures.

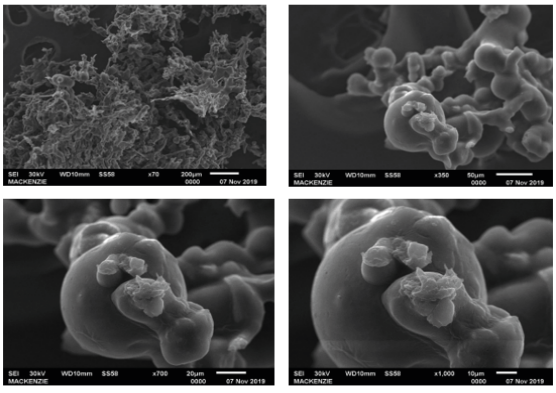
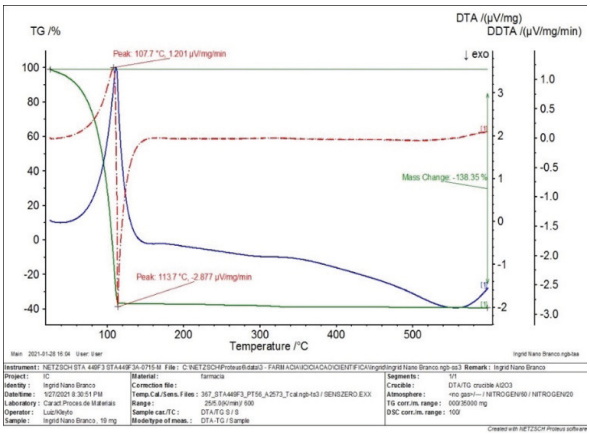


Figure 9 - Results of the Nano-Extract samples in the SEM analysis

Source: authors (2022)

The results of the Thermogravimetry (TG) and Differential Thermal Analysis (DTA) analyzes of the Nano-PBCA are shown in Graph 1. The green curve represents the results in Thermogravimetry, which demonstrates that there was a loss of mass (about 138.35%), in a single step, between 35

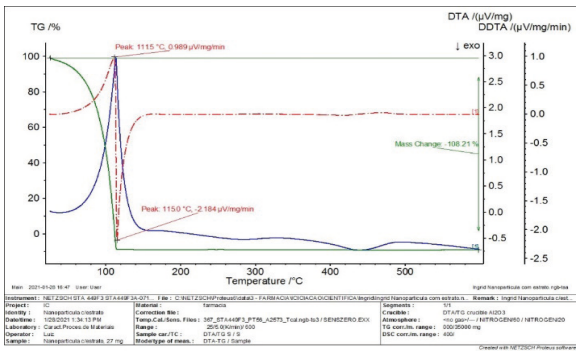
and 113.7°C. Similarly, the blue curve (DTA) shows an increasing peak, which indicates an endothermic reaction, representing the Nano-PBCA degradation process.



Graph 1 - Representation of the results of the Thermogravimetry and Differential Thermal Analysis (DTA) analyzes of the Nano-PBCA

Source: authors (2022).

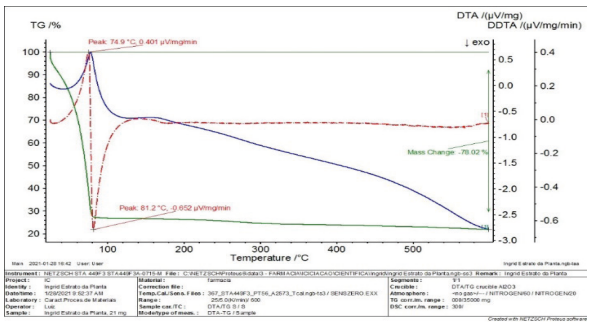
The results of the analysis of Nano - Extract (**Graph 2**) showed similar results with Nano - PBCA. The DTA results show that there is only a single rising peak, which indicates an endothermic degradation reaction, as found in the Nano-PBCA results. The thermogravimetry analysis also shows a mass loss (about 106.21%) in a single step, but this process occurs 35 between 115.0°C.



Graph 2 - Representation of the results of the Thermogravimetry and Differential Thermal Analysis (DTA) analyzes of Nano - Extract

Source: authors (2022).

The results of the DTA analyzes of the *Syzyium malaccense* leaves extract sample (Graph 3) demonstrate that an endothermic degradation process occurs. However, unlike the other samples, in the Thermogravimetry analysis of the extract, the sample suffered a mass loss of less than 100%, around 78.02%, which occurred between 35 and 81.2°C.

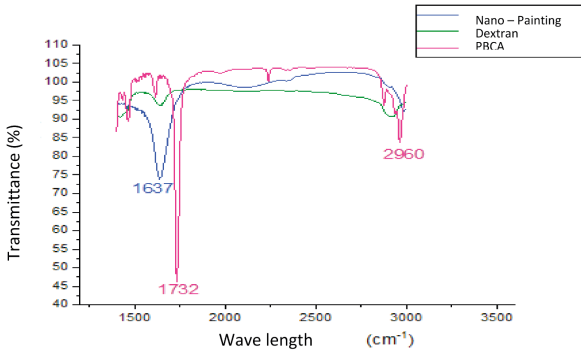


Graph 3 – Representation of the results of Thermogravimetry and Differential Thermal Analysis (DTA) of the *Syzyium malaccense* leaves extract

Source: authors (2022).

In evaluating the results of the Thermogravimetry analyses, all analyzes had a mass loss, but the nanostructures had a mass loss greater than 100%, in a temperature variation greater than 100°C. The objective of this analysis was to compare samples of the extract with the nanoparticle, to analyze if in a situation where it is in extreme situations, such as high temperature, to verify if the possible nanoparticle brought greater stability to the extract.

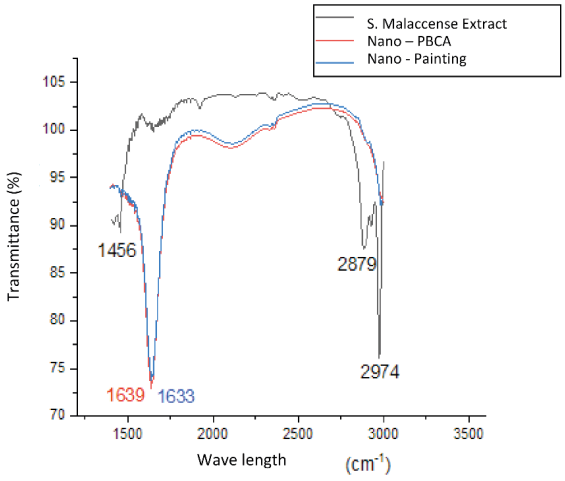
The results of the Differential Thermal Analysis (DTA) demonstrate that all samples undergo an endothermic process in their degradation, that is, in the degradation reaction, heat absorption occurs, for example: melting, vaporization, etc. In the Infrared Absorption Spectroscopy analysis, it is possible to make 2 comparisons. The first comparison deals with the following substances: PBCA, Dextran® and the complete Nanoparticle **Graph 4**).



Graph 4 - Analysis of PBCA, Dextran® and Nano - Infrared Extract

Source: authors (2022).

As can be seen (Graph 4), the characteristic bands of the reagents are present in the results of the Nano – Extract, in an approximate value, confirming the occurrence of synthesis of the nanosystems (Nanos – PBCA and Nano-Extract) and that there was no excess of reagents. In **Graph 5** it is possible to observe the following samples: *Syzyium malaccense* leaves extr*act,* Nano-PBCA and Nano-Extract.



Graph 5 - Analysis of Nano-Extract, Nano-PBCA and *Syzyium malaccense* leaves extract by infrared

Source: authors (2022).

As it can be seen, there are no significant differences in the Nano - PBCA and Nano - Extract bands, suggesting that there may have been a formation of the nanoparticle. Both Nano – PBCA and Nano – Extract show bands with approximate values (1639 cm $^{-1}$ and 1633 cm $^{-1}$), revealing the presence of a carbon linked to an oxygen [27]. As found in the 2 substances, it is suggested that the origin of this bond originated from the formed nanoparticle, and not from the extract. Evaluating the extract analysis results, it is also possible to observe the presence of a value band of 2879 and 2974 cm $^{-1}$, revealing that there is the presence of carboxylic acid in the extract, but it is not present in the nanoparticle samples. Furthermore, it is possible to observe the presence of a band in the extract with a value of 1456 cm $^{-1}$, whose presence indicates the presence of alkane carbonic bonds [27].

CONCLUSION

The results found in the morphological and physical-chemical analyses, as well as in the Scanning Electron Microscopy analysis, show that there was formation of the nanoparticle with *Syzyium malaccense* leaves extract. It is possible to determine some characteristics of the formed nanostructure, revealing that in the process of its degradation, the developed nanostructure undergoes an endothermic reaction.

It was possible to analyze that only when the nanostructure is submitted to a temperature variation greater than 100ºC, there is mass loss. When compared with the results of the extract, the extract suffered less loss of mass in temperature variations lower than 100ºC, since the greatest loss occurred at temperatures lower than 100ºC, indicating greater stability of the formed nanostructures.

The results of the IRS analysis demonstrate that the Nano – Extract formed presents the presence of carbon linked to oxygen, and this connection comes from the nanoparticle. In the extract, it is possible to identify the presence of carboxylic acid and carbons that have alkane bonds. After analyzing the results,

it can be suggested that there is the formation of polymeric nanoparticles. Therefore, it is still recommended that further tests be carried out to confirm the formation of the nanoparticle.

Thus, with the work it was possible to prepare an ethanolic extract containing leaves of *Syzyium malaccense*, in a concentration of 5 µl/ml with an SPF of 4.5. According to Anvisa's RDC number: 30 of 2012, SPF is not in the minimum requirement of sunscreens, this can be explained by the low concentration of the analyzed extract. This low concentration can be explained by the time when the leaves of *Syzyium malaccense* were collected, the time of day or the storage of the material. It may also be related to the turbolysis time performed for the extraction of *Syzygium malaccense* leaves. Therefore, it is suggested to carry out several collections at different times of the year, at different extraction times, in addition to analyzing the extract's SPF using a more sensitive method, such as the *Labsphere equipment.*

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