

# **STUDY OF THE PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF AN ORGANIC OINTMENT.**

## **Abstract**

Women have always used cosmetics to maintain their hair. To help this category of people with non-harmful ointments, an ointment made from palm kernel oil and shea butter was developed. In the presence of water, the palm kernel paste was boiled at 100°C to bring the oil to the surface of the water. The supernatant is collected in a kettle, and after cooling and filtration is collected in a bottle. The shea butter (used as an excipient) is melted in a bain-marie, then the required quantity is added to the corresponding palm kernel oil in a container. The mixture is homogenized until a single phase is obtained, then poured directly into 150mL jars.

The jars were then left to stand at a temperature below 30°C until the contents solidified. Physical, chemical, microbiological and other tests showed that the ointment was suitable use.

**Key words:** Oil, Almonds, Palm kernel, Shea, Ointment.

## **INTRODUCTION**

Hair is one of the elements of human hair found on the head. Hair is made up of keratin. As a whole, hair forms a protective barrier against external aggressions (shocks, sunrays, rain...). It also acts as a thermal insulator, with the scalp's sweat glands absorbing excess moisture to maintain the surface temperature of the area it covers.[1]. In addition to its aesthetic character, hair is a symbol of strength and virility for men, and femininity and seduction for women[2]. In these conditions, hair loss is always a source of concern for both men and

women, especially when the problem persists. Many people affected by early hair loss try to hide it by any means possible, such as wearing a wig, weaving or wearing a hat, especially as these people no longer fully benefit from its protective action, but also from psychological and social suffering. Men no longer feel virile, while women feel ugly and have lost their femininity. These people are often subject to a masked form of mockery, stigmatization and discrimination in society. Generally speaking, the consequences of hair loss are: loss of self-esteem, difficulty in withstanding the gaze of others, and isolation. [1]. However, there are several solutions for combating premature hair loss, such as taking certain pills, using hair lotions, hair transplants and hair ointments. With regard to ointments, [3] have formulated a dermal ointment based on *Mitracarpus scaber* extracts [4] worked on the formulation of an analgesic and anti-inflammatory ointment based on the same medicinal plant, [5] have formulated an antimicrobial ointment based on *Bersama abyssinica* extract to combat skin ailments. It is in this context that plant species such as *Elaeis guineensis* caught our attention, as it is commonly used by rural populations for hair maintenance and growth.,

## **I. MATERIAL AND METHODS**

### **I.1. MATERIAL**

#### **Plant material**

The plant material consisted of *Elaeis guineensis* kernels from the Arecaceae family (figure 1), obtained after removing the pulp from a few households..



**Figure1** Oil palm tree

## Technical equipment

### Laboratory equipment and materials

The main laboratory equipment and materials used in this work are:

- - Precision balance, Precisa® JB 12000C (capacity = 2100 g sensitivity = 0.1 g), (Switzerland);
- - autoclave ;
- - Memmertoven (Germany) ;
- - HANNA® HI 8010 pH meter ;
- - Glassware: sight tubes, Petri dishes, 1 ml pipettes, 100 mL graduated burette, beakers;
- - Retsch SK 100 milling machine.

- Culture media and reagent
- PCA (Plate Count Agar);
- BEA (Bile Esculin Azide) ;
- Sabouraud ;
- VRBG (Glucosée Biliée au CristalvioletandAurouge Neutre).

## **I.2.METHOD**

### **Production of palm kernel oil**

The processing stages are: reception, drying (for a week) and crushing of the pulped seeds, isolation and grouping of the kernels in a container. The kernels are then crushed (using a grinder) and finally the oil is extracted.

The oil extraction process involved boiling the palm kernel pulp in the presence of water. At the boiling temperature of the water (100°C), the oil begins to appear on the surface of the water. All the supernatant is collected in a jar.

The jar is brought to the boil until all the water has evaporated, leaving only the oil which, after cooling, is filtered and collected in a bottle.

### **Ointment formulation**

The ointment has been formulated using palm kernel oil as the active ingredient. To prepare it, shea butter (used as an excipient) is melted in a bain-marie, then the required quantity is added to the corresponding palm kernel oil in a container. The mixture is homogenized until a single phase is obtained, then poured directly into 150mL jars. The jars are then left to stand at a temperature below 30°C until the contents solidify (figure 2).



**Figure 2 :Ointment in tins**

- **Carrying out quality control, sterility and efficacy tests on ointment**
- **Ointment quality control**
  - - Color and odor
  - The color and odor of the ointment are determined by a panel of ten (10) people.
  - - Consistency
  - Consistency is determined by touching the ointment.
  - - Stability
  - The ointment is subjected to various temperature conditions, followed by an evaluation of its melting point.
  - - Homogeneity
  - Homogeneity is checked by observation with the naked eye, after spreading the ointment thinly on a flat surface with a spatula. The even or uneven distribution of extracts in the excipient was noted.

- pH measurement

To measure pH, 50 g of the ointment was melted at 40°C in a water bath, then, when cold, pH was determined using a HANNA HI 8010 pH meter, by inserting the probe directly into the samples. Under the same conditions, the pH of shea butter and palm kernel oil was measured.

#### Ointment sterility test

- Sampling

After melting in a water bath, a 20mL sample of the ointment was taken.

- Microbiological analysis

Germ identification and enumeration were carried out using the method based on standard procedures [6]. Enumeration included total aerobic mesophilic flora, total coliforms, yeasts and molds, and Streptococcus.

- Preparation of culture media

Culture media were prepared according to manufacturers' recommendations. Table I summarizes the quantities sampled and the germs tested for each culture medium.

Table I: Germs sought according to culture medium

Prepared media	Quantities collected	Distilled water quantity	Boiling time	Sterilization time	Indication
BEA	5.6g	100mL	5 min	121°C during 20 min	Streptococci
PCA	2.05g				FMAT
Sabouraud	4.2g				Fungal germs
VRBG	3.8g			Not autoclavable	Total

					coliformbacteria
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### Inoculation of culture medium for germ detection and enumeration

- Surface seeding was carried out for each culture medium. To do this, 0.1mL of the ointment sample was removed and aseptically deposited on the surface of the agar contained in the Petri dish. Then, using a sterile rake, the inoculum is spread over the agar surface. The plates were incubated at 30°C in the oven, then observed after 24 hours for BEA, PCA and VRBG media, and after 48 hours for Sabouraud medium.

#### Colony enumeration

According to the standard [6], Petri dishes with more than 30 colonies will be retained. The number of microorganisms present in a given product sample is obtained by the following formula [7]:

$$N = \frac{\sum C}{V (n_1 + 0,1 n_2) d} \text{ (germes/mL)}$$

- N: number of germs
- $\sum C$ : colony sums
- V: inoculum volume n1: number of Petri dishes counted at 1st dilution
- n2: number of Petri dishes counted at 2nd dilution
- d: dilution rate at first dilution

## Microbiological standard

The number of germs per mL or g of sample was calculated for each germ tested, then compared to the normative reference of microbiological criteria for herbal medicinal products in the [8].

- Ointment quality control
- The following parameters were determined:
  - Color and odor (organoleptic)
  - Appreciation of the color and odor of the ointment by a panel of ten (10) people. The ointment is yellowish in color. The odor is that of attenuated shea butter.
  - Consistency
  - The ointment has a semi-solid consistency. It feels slightly hard to the touch and softens on contact with the skin when set.
  - Stability
  - The ointment is unstable at temperatures above 30°C. Above this temperature, it begins to melt.
  - The following observations have been made concerning changes over time:
    - Three batches containing the ointment were left to stand at room temperature and examined after 0 days, 1 week, 2 weeks and 1 month of storage:
      - Batch permanently open: no change;
      - Batch permanently closed until the end of the experiment: the ointment was kept intact;
      - Batch opened and tested at each control: no change observed.
    - Homogeneity



- The homogeneity of the ointment was checked by spreading it out in a thin layer on a flat surface using a spatula. In addition, as the oils are miscible with each other, very good homogeneity was achieved (even distribution of the extract).
- pH measurement

The pH measurement yielded:

Palm kernel oil (pH=4.16), Shea butter (pH=3.85) and formulated ointment (pH=3.93),

### **Cutaneous tolerability test for ointment**

Application of the ointment to the flank of an albino rabbit showed no skin reaction on different days. The Primary Irritation Index (PII) was zero after 24 and 72 hours of ointment application to the rabbits' flanks.

### **Ointment sterility test**

This test is a microbiological analysis carried out in agar plates, in which the ointment was surface-seeded on the respective media (PCA, BEA, Sabouraud and VRBG). The test showed that the ointment was free from contamination after 24 hours incubation at 30°C. The results of the microbiological analysis are reported in Table II.

Table II: Results of microbiological tests on ointment

Samples	Number of germs / mL sample (CFU/mL or CFU/g)			
	Yeasts and molds	Streptococci	Germs mesophilic	Total coliform bacteria
Ointment	Absence	Absence	Absence	Absence

## **II. RESULTS & DISCUSSION**

The aim of this study was to formulate a cosmetic ointment using palm kernel oil as the active ingredient and shea butter as the excipient.

The use of the traditional extraction method described by [9] enabled palm kernel oil to be extracted with a yield of 26%, providing sufficient palm kernel oil.

Quality control results obtained with the ointment show good macroscopic parameters with very good homogeneity. The attenuated shea butter smell of the ointment is a very pleasant fragrance, insofar as shea butter itself is a preferred excipient over others because of its smell [10], and it also releases the active ingredient better [11].

The pH values were determined using a HANNA HI 8010 pH meter, an electronic device providing precise pH values. The pH values obtained for palm kernel oil (pH=4.16), shea butter (pH=3.85) and formulated ointment (pH=3.93) show that palm kernel oil, shea butter and formulated ointment are acidic.

Furthermore, these different values indicate that the ointment has a pH value closer to that of the skin, as defined by [11].

At temperatures above 30°C, ointments begin to melt, so we recommend keeping them in a cool place (temperature below 30°C).

The results of the skin tolerability test indicated skin tolerance in rabbits. Researchers have always been more interested in using these animals for their work. In fact, these animals are relatively well suited to the task, as they are phylogenetically closer to humans and are easy to handle. For this study, rabbits were chosen because of the sensitivity of their skin. Furthermore, for ethical reasons, *in vivo* tests cannot be carried out directly on humans. They have to be carried out on rodents such as mice or rats, or on other animals [12] such as rabbits in this case, whose metabolism is far from resembling that of humans. The test consisted in investigating possible skin irritation reactions due to the ointment by determining the primary irritation index according to the Draize scoring system. No skin irritation reactions were observed.

So, according to the Draize scale, the ointment was considered non-irritating to the skin. This is explained by the fact that the shea butter (excipient) is not aggressive to the skin, since it has been applied for ages.

The result of the microbiological analysis of the ointments showed an absence of germs, which can be explained by the respect of hygiene measures adopted during the formulation of the ointment; This allows us to say that the ointment was produced according to Good Manufacturing Practice (GMP) standards. In addition, as the ointment consists entirely of fats, it limits contamination and the development of microorganisms.

In terms of efficacy tests, the performance of the ointments was better in male rabbits than in females. In fact, in males, the performance of the ointment can be seen from D16, with hair growth of 21 mm at the end of 28 days, whereas it was lower than in the control. We can appreciate the effectiveness of palm kernel oil, used for ages by rural populations for hair care ([9],[14],[13]). In females, on the other hand, at the end of 28 days of experimentation, hair grew faster without the ointments (in the control), reflecting a slowdown in hair growth in rabbits in the presence of the ointments.

Moreover, according to empirical knowledge and the literature, palm kernel oil, the active ingredient in the ointment, is a product used mainly by women for hair maintenance and growth. This contradiction between test results in rabbits and information from empirical knowledge and the literature could be explained by the fact that rabbits and humans do not have the same metabolism [5].

Also, the low density of hair observed in males in the treated area may be due to the mechanical action exerted during ointment application.

## **CONCLUSION**

The galenic formulation enabled us to develop a cosmetic ointment based on palm kernel oil and shea butter as an excipient.

The results of tests carried out on rabbit hair show that these ointments stimulate hair growth, especially in males.

The ointment contains appreciable physical, chemical and microbiological properties that encourage its use.

## **REFERENCES**

[1]. **Mélanie LR, (2016)** The consequences of alopecia in women treated with chemotherapy for cancer. End-of-study work for the state nursing diploma. Nursing training institute, Caen - France. p 1-26.

- [2]. **Thomas D, (2015)**Alopecia in young people. Doctoral thesis, Université de Picardie Jules Verne, France ; 96p.
- [3]. **Sanogo R, Konipo A, Maiga A, Arama AE, Diall D, Bougoudogo, F, (2006)** Formulation of a dermal ointment based on *Mitracarpus zizanioides* (Zucc) extracts. Pharm. Med, Vol. XIV. pp. 159-177. \
- [4]. **Dembélé DL, (2011)**Formulation of an analgesic and anti-inflammatory ointment based on *Securidaca longepedunculata* Fresen (Polygalaceae). Doctoral thesis from the Faculty of Medicine, Pharmacy and Odontostomatology. University of Bamako, Mali. 143 p.
- [5]. **Bene K, Camara D, Soumahoro I.A, Kanga Y, Zirihi GN, (2017)**Galenical formulation of an antimicrobial ointment based on a hydroalcoholic extract of *Bersama abyssinica* Fresen. *Ethnopharmacologia*, n°58, p 61-69
- [6]. **AFNOR (1996)**Food microbiology. Enumeration of thermo-tolerant coliforms by counting colonies obtained at 44°C, NF V 08 60, Paris, AFNOR, 2p.
- [7]. **AFNOR (2001)**Food microbiology. General rule for microbiological examinations, NF ISO 7218 / A1, Paris, AFNOR, 12p.
- [8]. **European Pharmacopoeia (2014)**Microbiological quality of pharmaceutical preparations, p 567 – 569.
- [9]. **Ouattara H, Meite A, Dally T, Kati CS, (2016)**Study of the properties of the fruits of three Arecaceae: *Elaeis guineensis* Jacq., *Cocos nucifera* L., *Borassus flabellifer* Var. *J. Appl. Biosci.* p 10157-10169.
- [10]. **Mboui O SE, (2003)**Traditional palm kernel oil. Doctor of Pharmacy thesis from the University of Cheikh Anta Diop. 93p.

[11]. **Eyang EM,(2007)**Study of the phytochemistry and antibacterial and antifungal activities of five medicinal plants used in the traditional treatment of dermatoses in Mali,Doctoral Thesis from the Faculty of Medicine, Pharmacy and Odontostomatology, University of Bamako, Mali, 175 p.

[12]. **Kamanzi AK, (2002)**Medicinal plants from Côte d'Ivoire: phytochemical investigations guided by bioassays,State Doctorate in Science, Plant Biology, Cocody University, Abidjan, Ivory Coast, 176 p

[13]. **Sijelmassi A, (1991)**Medicinal plants of Morocco. Ricin, Casablanca: The Fennec. Pp 217.

[14]. **Sadok D, Bentounes FZ, (2016)**Study of the insecticidal activity of *Ricinus communis* and *Mentha piperita* leaf extracts against *Aphis spiraecola* green citrus aphid (Hemiptera: Aphididae). Master's thesis from Abdelhamid Ibn Badis University-Mostaganem - Algeria; 100p.