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Valorization of *Cucurbita pepo* Seed Oil in Soap Production

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Abstract

The present study was conducted with the aim of extracting and characterising *Cucurbita pepo* seed oil and then producing soap with important dermatological properties. The physico-chemical results of the *Cucurbita pepo* seed oil after extraction were satisfactory and indicate that the oil obtained contains long-carbonaceous fatty acids. The production of the soap was based on the realisation of a saponification reaction by reflux heating between the extracted oil and a solution of soduim hydroxyde 7M for one hour. The soap obtained is characterised by the following physico-chemical parameters: salt content, alkali content, pH, and humidity; which meet ISO 684-1974 standards. The antibacterial parameters of the soaps S1, S2, S3, S4, S5 and S6 were determined by the Agar Diffusion test using the well method and these soaps showed an interesting antibacterial activity against the germs *pseudomonas aeruginosa* and *staphylococcus aureus*. Soaps based on *Raffia sese* oil (kolo oil) and *Elaesis guineensis* oil (palm oil) have also been synthesized for comparison with soap obtained from *Cucurbita pepo* seed oil.

Keywords

Valorization, *Cucurbita pepo* Seeds, *Cucurbita pepo* Oil, Soap, Antibacterial Activity

1. Introduction

Fats are a set of complexes of organic compounds. They have been used for many years due to their multiple properties in several fields, especially in industry, for the production of soap, paints, cosmetics, oils and margarine [1]. Although the properties of fats have been known for a long time, it was not until 1815 that Michèle Eugène Chevreul showed the exact chemical nature of a fat [2]. Over the past four decades, physical, chemical and biochemical knowledge of fats has taken a forward leap which allowed us to: analyze the composition of fats, set standards for each type of product, improve the quality, purity, conservation and stability of products, adapt their storage method and preserve their nutritional qualities [3]. Vegetable oils are fatty substances composed mainly of triglycerides of fatty acids whose chain length varies between 14 and 20 carbon atoms [4]. Each oil is characterized by its fatty acid composition, which is identified by gas chromatography. Therefore *Cucurbita pepo* seed oil is targeted by numerous studies for its richness in phenolic compounds, carotenoids, vitamins E, phytosterols and also for these different biological, antimicrobial and antioxidant properties [5]. Based on the properties of this oil, we have chosen it in order to prepare solid soaps that can have dermatological properties. Nowadays, several oils are used for the manufacture of soap due to their physico-chemical and biological characteristics, among which we can mention: olive oil, palm oil, coconut oil, peanut oil, kolo oil [6]. Fats represent in volume more or less 2/3 of the raw materials used for soap production, so it is necessary to correctly choose the fatty substance during this process, since each fats has its own characteristics or physico-chemicals properties. These characteristics in turn largely determine the soap characteristics [7]. *Cucurbita pepo* seed oil contains a wide range of bioactive elements [8] related to deep hydration and soothing of the most sensitive skin. In our previous study, we showed the importance of using *Cucurbita pepo* oil obtained by the pressure method in the fight against the UV action on the skin [9]. To our knowledge, no studies have ever been done on the production of soap from *Cucurbita pepo* seed oil. Based on these different biological virtues and physico-chemical characteristics, *Cucurbita pepo* seed oil could be saponifiable, and provide the characteristics required for a good soap. In this paper, we describe the study of the extraction of *Cucurbita pepo* seed oil obtained by the soxhlet method, its valorization in the manufacture of soap as well as the antibacterial activity of the soap obtained.

2. Materials and Methods

2.1. Oil Extraction

The plant material used in this study consists of the seeds of the *Cucurbita pepo* fruit purchased at the Total market of Bacongo in the city of Brazzaville, in the department of Bouenza in the municipality of Madingou. The soxhlet type extractor was used to extract the oil from the dried and finely ground seeds.

2.2. Oil Extraction Protocol

Introduce into the soxhelet using a cartridge 80 g of dry matter and finely ground using a VOCTORIA brand artisanal grinder. In a three-necked flask equipped with a thermometer topped with a soxhelet and a cooler, introduce 350 mL of hexane then place the flask in an oil bath. The whole is brought to the boil with stirring at 65˚C (boiling temperature of the solvent). Let the mixture to cool. The separation of the oil-solvent mixture after extraction is done through simple distillation at a temperature of 65˚C.

2.3. Physico-Chemical Characteristics of Oil

2.3.1. Chemical Characteristics

The following chemical parameters were determined: humidity, pH, acid number, saponification index and esterification index.

Humidity

This is the loss mass suffered by the product after heating to 103˚C ± 2˚C expressed as a percentage. The principle is based on the heating of a test sample at 103˚C ± 2˚C until the water and volatile matter are completely removed, while successively weighing the raw material after cooling in order to obtain of constant mass.

Weigh 5 g of the oil in a beaker and heat in an oven at 103˚C for 4 hours until the water is completely removed.

Hydrogen potential: pH

The pH was determined using a HANNA type pH meter equipped with a combined electrode.

Insert 50 mL of *Cucurbita pepo* oil into a 100 mL beaker, then immerse the pH-meter electrode (previously calibrated) in the oil and read the pH value.

Acid index: IA

This is the number of milligrams of potassium hydroxide needed to neutralize the fatty acids present in a gram of fat. The acid number was determined using the method of WOLF [10].

In a 150 mL erlenmeyer flask, put 2.5 g of *Cucurbita pepo* oil, add 75 mL of 95˚ ethyl alcohol and three drops of phenolphthalein. Titrate with a 0.1M potassium hydroxide solution with stirring until a pink color is obtained.

Saponification index: IS

The saponification index corresponds to the mass of potassium hydroxide, expressed in milligrams, necessary to neutralize the free fatty acids and saponify the esterified fatty acids contained in one gram of fat. It was determinated according to IUPAC 2.202 standard [11].

In a 250 mL flask, introduce 2 g of *Cucurbita pepo* oil, add 25 mL of a 0.5 M alcoholic potash solution. Stir the reflux mixture for 1 hour. Then add 3 drops of phenolphthalein. Titrate with a 0.5 M hydrochloric acid solution with stirring until a colorless solution is obtained. Carry out a blank test under the same conditions.

Esterification index: IE

This is the amount of potassium hydroxide expressed in milligrams, needed to saponify one gram of oil stripped of fatty acids.

2.3.2. Physical Characteristics

The following physical parameters were determined: refractive index and density.

The refractive index: Ir

It was determined using a NOVEX HOLLAND brand refractometer. The method used is that described in the AFNOR T 60 - 212 standards [12].

The density

The weighing method was used at room temperature with an analytical balance to the nearest 10 - 2 using pure water at 4˚C as a reference [13].

2.4. Soap Synthesis

The complete boiling method, known as the hot process, has been demonstrated for soap making.

Carefully place 20 mL of concentrated 7M sodium hydroxide solution and 20 mL of 95˚ ethanol into a 250 mL flask using a measuring cylinder. Then add 20 mL of *Cucurbita pepo* oil. Reflux of the mixture with stirring for 1 hour at 80 ˚C. The mixture was cooled to room temperature.

Reloading

Soap is precipitated by salting. It consists in pouring the reaction mixture into a solution of 20% sodium chloride.

After cooling, slowly pour the contents of the flask into a beaker containing a 20% sodium chloride solution, then gently shake and let stand.

Filtration and washing

Perform a simple filtration (funnel and filter paper) of the resulting mixture to recover the soap in a beaker. Rinse the beaker soap with distilled water. Then filter the contents of the beaker again and rinse it a second time.

Drying and molding

Put the soap obtained in a beaker, then dry in a water bath at 80˚C until a homogeneous paste more or less solid is obtained; introduce the resulting soap into the molds to give it a shape. After three days, unmold and let dry in the open air for a week.

Physico-Chemical Characteristics of the Soap Obtained

The following characteristics have been determined to ensure the good quality of the soap: moisture, pH, alkali content, salt content and foaming power.

Alkali content: Ta

This is the number of grams of free alkali contained in 100 g of soap after refilling with sodium chloride, expressed as a percentage. It has been determined in accordance with ISO 456-1973 (F) [14].

In a 200 mL beaker, introduce 2 g of the prepared soap, add 50 mL of the previously prepared neutralized ethanol solution, then heat to dissolve the soap. After heating, titrate the mixture with an alcoholic solution of sulfuric acid to 0.1 M until the pink color disappears.

Salt content: TS

This is the amount expressed as a percentage of salt present in the soap responsible for corrosion and the reduction of the detergent power of the soap. It has been determined in accordance with ISO 457:1983 [15].

Dissolve 2.5 g of the prepared soap in a 100 mL Erlenmeyer flask containing 50 mL of boiling distilled water. Allow the solution to cool to a temperature close to 50˚C - 55˚C, add a few drops of 20% sulfuric acid. Strain and then add a few drops of phenolphthalein and neutralize with 0.25 M of sodium hydroxide. Then add a few drops of potassium chromate. Titrate the mixture with a silver nitrate solution of 0.1 M until a persistent brick red color is obtained for 30 seconds.

Humidity

The humidity of the soap was determined using the same protocol as for *Cucurbita pepo* oil.

Hydrogen potential: pH

For the determination of the pH, a soapy solution was prepared from 2 g of soap and 15 mL of distilled water, following the same protocol as for *cucurbita pepo* oil.

Statistical processing

The quantities below were determined with the R software: arithmetic means and standard deviations.

3. Results and Discussion

3.1. Extraction of *Cucurbita pepo* Oil

Table 1 presents the values of the masses and yields of the different extractions of *Cucurbita pepo* oil. Four (04) extractions were carried out with identical masses of plant matter. These experiments were carried out at different times: 6 hours, 8 hours, 10 hours and 12 hours. The results show that the oil yield of *Cucurbita pepo* seeds is proportional to the extraction time; this proves that *Cucurbita pepo* seeds have a high oil content and a good homogeneous distribution of fat throughout the raw material. In 2009, RafIdimanantso carried out the same work on *Cucurbita pepo* seeds, and found the respective yields of 24.44% and 24.24%, for test catches of 84 g and 200 g, during extraction times of 4 h and 8 h respectively [16], these results show that regardless of the mass or time used for the extraction of *Cucurbita pepo* oil, the extraction yield does not change significantly. In comparison, the results of our various extractions are almost double those results. Several causes can be at the origin of these discrepancies, such as the nature of the soil during planting, the storage period and the method of drying the seeds.

Physico-Chemical Characterisation of *Cucurbita pepo* Oil

The results reported in Table 2 illustrate the different physico-chemical parameters that allowed us to evaluate the quality of the *Cucurbita pepo* oil obtained after each extraction. These results show that the extraction time of *Cucurbita*

Table 1. Oil content of *Cucurbita pepo*.

Solvent	Hexane			
Extractions	P_1	P_2	P_3	P_4
Extraction time	6 h	8 h	10 h	12 h
Mass used (g)	80	80	80	80
Mass obtained (g)	40.12	43.67	48.40	52.32
Yield (%)	50.15	54.58	60.50	65.40

Table 2. Physico-chemical parameters of *Cucurbita pepo* oil after each extraction.

Physico-chemical parameters	Values obtained	Literature/Standard
Humidity (%)		
P_1	1.5 ± 0.09	
P_2	1.56 ± 0.09	≤1
P_3	1.5 ± 0.04	
P_4	1.5 ± 0.04	
Refractive index at 20˚C		
P_1	1.46705 ± 0.00000	
P_2	1.46707 ± 0.00000	1.474 to 1.478
P_3	1.46705 ± 0.00000	
P_4	1.46707 ± 0.00000	
Density		
P_1	0.9145 ± 0.0075	
P_2	0.9189 ± 0.0079	0.918 to 0.927
P_3	0.9180 ± 0.0080	
P_4	0.9186 ± 0.0079	
pH at 25°C		
P_1	4.56 ± 0.02	
P_2	4.55 ± 0.01	-
P_3	4.56 ± 0.02	
P_4	4.55 ± 0.01	
Acid value (mg KOH/g)		
P_1	1.4514 ± 0.0234	
P_2	1.4270 ± 0.0511	≤1
P_3	1.4664 ± 0.0322	
P_4	1.4270 ± 0.0511	
Saponification index (mg KOH/g)		
P_1	192.59 ± 0.71	
P_2	195.27 ± 0.92	174 to 197
P_3	193.58 ± 0.68	
P_4	192.59 ± 0.71	
Esterification index (mg KOH/g)		
P_1	191.11	
P_2	193.84	
P_3	192.11	-
P_4	192.88	

pepo oil does not change the physico-chemical parameters of the oil. The humidity of the different oils obtained after extraction is between 1.41% and 1.56%. A study of the literature shows that these values are slightly higher than those of the internal standard CO.G.B. LaBelle on the moisture content of fats designed for the manufacture of toilet soaps which sets a value of less than 1% [11].

This slightly elevated moisture content may be due to the drying of the seeds or the method used to separate the oil-solvent mixture. The high humidity of the oils is a disruptive factor during the saponification reaction; it can distort the measurement of the saponification index [11]. Therefore, these oils required drying before saponification.

The refractive index provides information about purity and oil group. The refractive index at 20˚C of *Cucurbita pepo* oil obtained after each extraction is on average 1.46705 ± 0.00000. These results are in agreement with those of the literature [17] [18], and prove that the oil is of good quality and belongs to the group of non-drying oils because it is between 1.468 and 1.470 [19]. The relative density tells us about the group to which oil belongs and also about the purity. The density of the oils obtained is between 0.9145 and 0.918. The densities of the four extracts obtained are in agreement with the data of the literature [20] and inform us that these oils belong to the group of non-drying oils [21]. The acid number defines the quality of the oil. It characterizes the purity and stability of oils at room temperature. *Cucurbita pepo* oil obtained after each extraction has low acid values, ranging from 1.3811 and 1.4664 (mg KOH/g). Our results are similar to those obtained by ADODO *et al.* in 2015 [22]. This shows that the resulting oil is rich in free fatty acids and has good stability. The pH tells us about the acidity of the oil. The pH values of the oils obtained are between 4.55 and 4.57; these values are slightly acidic, which confirms that the neutralization is complete. With regard to the saponification and esterification indices, it can be seen that the difference is not very significant. The values of the saponification indices of the oil obtained in relation to the extraction time are between 192.59 and 195.27 (mg KOH/g). These values are within the range of saponification indices of *Cucurbita pepo* seed oil found by Nicolas and Sanderson in 2003 which are 174 to 197 (mg KOH/g) [23]. These results indicate that the resulting oil contains fatty acids with a long carbon chain.

The calculated values of the esterification indices are between 191.11 and 193.94 (mg KOH/g), these values show that the oil in this study can be stored longer [10].

3.2. Soap Synthesis

3.2.1. Saponification

The soap is obtained by a saponification reaction corresponding to a basic hydrolysis of esters (triglycerides) under the action of hydroxide ions in aqueous solution in ethanol. The pH of the reaction mixture before heating is about 12. While regularly monitoring the evolution of the pH, the measurement made after one hour gives a pH value close to 11. This shows that hydroxide ions have been consumed.

3.2.2. Saponification

The results reported in Table 3 illustrate the different parameters that allowed us to evaluate the quality of the soap obtained after synthesis.

Table 3 presents the results of the physico-chemical analyses of the soaps produced from the *Cucurbita pepo* oil obtained after each extraction. The alkali content is between 0.043% and 0.049%. These values indicate the minimum amount of sodium hydroxide present in the finished product. These low values show that the removal of sodium hydroxide is carried out in our protocol. We find that by performing a single wash, we obtain very high free alkaline values that do not comply with the standard. Therefore, several washes with distilled water were necessary in order to remove excess sodium hydroxide that can have harmful effects on the cell membrane. Referring to the ISO 684-1974 [24] standard and the CO.G.B. Labelle [25] standard, our soaps can be classified in the range of soaps for the toilet, since the latter are between 0.04% and 0.08%. As regards the salt content, the values obtained are between 0.414% and 0.443%. A high salt content should be avoided in the finished product, as this is responsible for reducing the detergent and foaming power of the soap. Similarly with excess soda, several washes are important to remove the maximum amount of salt in the soap. The low salt levels show that salt removal is achieved in our protocol. The values obtained comply with ISO 684-1974 [24] and CO. Standard G.B. Labelle [25] which sets values between 0.3% and 0.5% for soaps intended for toileting. Soaps obtained after saponification have pH values between 9.01 and 9.04 to 26˚C, which are basic pH values. The pH values obtained after saponification are in accordance with the pH of Marseille soap, whose range is between 8 and 10 [26]. The resulting soaps can be used without problems for daily cleaning. On the other hand, studies have shown that a high pH value dries out and destroys the hydrolipidic film of the skin [27]. The humidity of the soaps obtained is between 11.854% and 12.815%. These humidity levels are the result of a long drying process, in order to obtain consistent soaps. Soap with a moisture content greater than 14% is not hard enough; it can have negative effects on the characteristics of the soap and its duration of use [28]. A toilet soap is very gentle on the skin, which cleanses and foams easily [29]. It should not contain more than 14% water. The soaps obtained in this study can be classified in the third range of soaps for toilets because they have humidity values between 11% and 13% according to ISO 684-1974 [24] and the co. G.B.Labelle standard [25]. Foaming power is the ability of a surfactant to form a stable and persistent foam [30]. The results of the foaming potency test carried out on the various soaps obtained after saponification show that *Cucurbita pepo* oil-based soaps have a stable and persistent foam rate for a period of up to 6 hours. This stability gives them detergent properties.

Table 3. Results of physico-chemical analyses of soap.

Physico-chemical parameters	Values obtained	Standars/Literature
Salt content (%)		**ISO: 684-1974**
P_1	0.442 ± 0.020	Smooth soap: 0.4 - 0.6
P_2	0.414 ± 0.002	SW Award: 0.4 - 0.6
P_3	0.414 ± 0.019	SDT: 0.3 - 0.5
P_4	0.443 ± 0.019	
Alkali content (%)		**ISO: 684-1974**
P_1	0.045 ± 0.005	Smooth soap: 0.08 - 0.18
P_2	0.048 ± 0.002	SW Award: 0.08 - 0.18
P_3	0.043 ± 0.002	SDT: 0.04 - 0.08
P_4	0.049 ± 0.013	
pH		
P_1	9.02 ± 0.05	8 - 10
P_2	9.03 ± 0.02	Marseille soap
P_3	9.01 ± 0.02	
P_4	9.04 ± 0.03	
Humidity (%)		**ISO: 684-1974**
P_1	12:815 p.m. ± 0.05	Smooth soap: 30 - 33
P_2	12.576 ± 0.02	SW: 21 - 23
P_3	12.675 ± 0.03	TDMS: 11 - 13
P_4	11.854 ± 0.03	

3.2.3. Antibacterial Activities of Soaps

1) Nature of soaps

Soap 1 - made from *Cucurbita pepo* oil obtained after 6 hours.

Soap 2 - made from *Cucurbita pepo* oil obtained after 8 hours.

Soap 3 - made with *Cucurbita pepo* oil obtained after 10 hours.

Soap 4 - made from *Cucurbita pepo* oil obtained after 12 hours.

Soap 5 - based on *Elaeis guineensis* oil (palm oil).

Soap 6 - based on *Raffia sese* oil (kolo oil).

2) Expression of results

Antibacterial activity was determined by measuring the diameter of the inhibition zone around the discs caused by the concentrations of the different soaps. Antibacterial activity is considered positive from a diameter greater than 6 mm [31] [32]. This product may have a:

- Very high activity: diameter ≥ 30 mm;
- High activity: diameter 21 - 29 mm;
- Average activity: diameter 16 - 20 mm;
- Low activity: diameter 11 - 15 mm;
- Small or no activity: diameter ≤ 10 mm.

3) Results and discussion

The antibacterial activity of our soaps has been estimated in terms of the diameter of the inhibition zone around the discs containing the soaps to be tested against the two (2) selected germs. After 24 hours of incubation at 37˚C, the results obtained are presented in Figures 1-4 below.

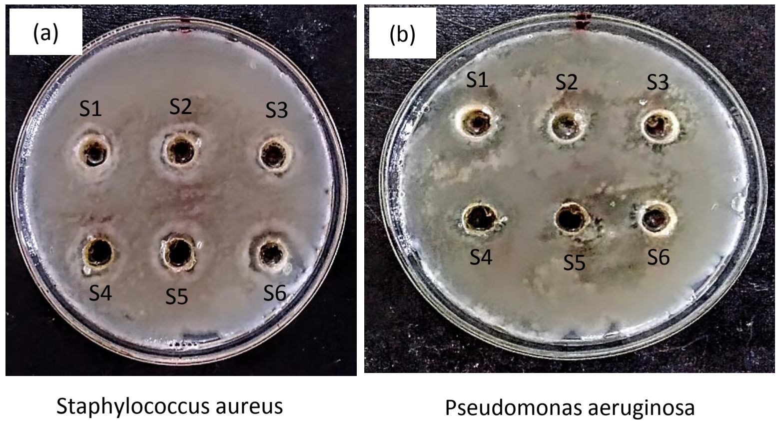

Figure 1. Antibacterial activity of different soaps at a concentration of 250 mg/ml on pathogenic bacteria.

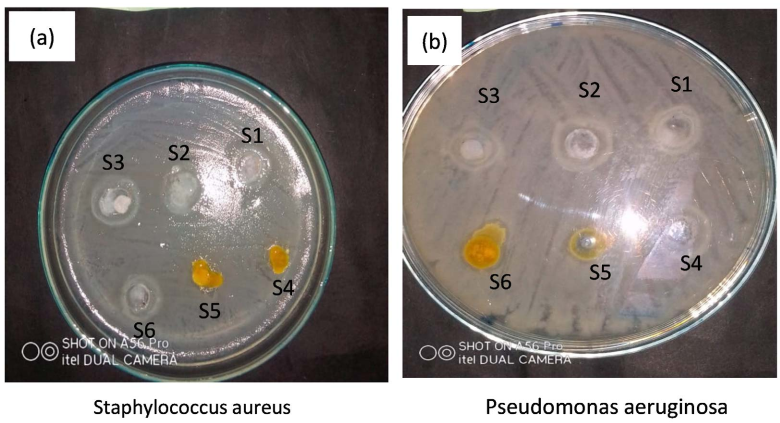

Figure 2. Antibacterial activity of different soaps at a concentration of 500 mg/ml on pathogenic bacteria.

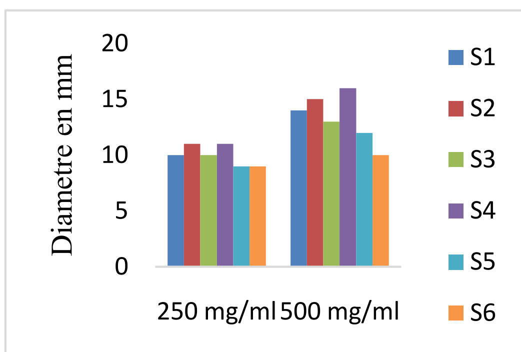

Figure 3. Staphylococcus aureus, Histograms of the main effects of diameter on the inhibition zone at 250 mg/mL and 500 mg/mL.

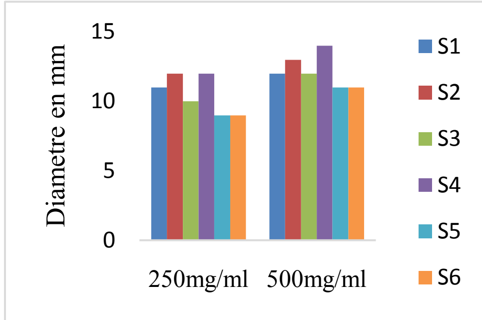

Figure 4. Pseudomonas aeruginosa, Histograms of the main effects of diameter on the inhibition zone at 250 mg/mL and 500 mg/mL.

These figures show that all soaps inhibited the growth of bacterial strains of *S. aureus* and *P. aeruginosa*. This bactericidal activity is however moderate, since almost all diameters are about 16 mm (**Figures 2-4**). S2 and S4 soaps have the best antibacterial powers against both germs, with a minimum inhibition zone diameter of about 13 mm at a concentration of 500 mg/mL. These could be used for personal hygiene.

The antibacterial activity of soaps is directly related to the composition and concentration of the active compound and the type of microorganisms targeted [33]. Oil-based soaps are rich in monoterpenes which are well known for their antimicrobial activities. According to Derwich *et al.* (2010), the antimicrobial activity of monoterpenes is explained by the presence of phenolic hydroxyl groups capable of forming hydrogen bonds with the active sites of the enzymes in the targeted cell [34]. Studies reported by Bouyahya *et al.* (2017) confirm the linear correlation between phenolic components and action against bacteria [35].

4. Conclusion

This study is a first in the context of the valorization of *cucurbita pepo* seed oil for soap production. The extraction of Soxhlet oil showed that the yield is proportional to the extraction time. We got the return of 65.40% after 12 hours. The different analyses carried out on the extracts obtained at different times showed that the extraction time does not modify the physico-chemical parameters of the oils. The saponification of *cucurbita pepo* seed oil has resulted in soaps with characteristics that meet the standards of strength. The study of antibacterial activity shows that soap based on *cucurbita pepo* oil has a more intense activity than that obtained with *Eaesis guineensis* and *Raphia sese* oils; with inhibition zones ranging from 12 to 14 mm for *Pseudomonas aeruuginosa* and from 13 to 16 mm for *Staphylococcus aureus* at a concentration of 500 mg/mL. These results showed that soap prepared with *cucurbita pepo* essential oil is effective and more sensitive to the action of these two human pathogens.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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