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Evaluation of the Chemical and Biological Properties of Oil Extracted from Detoxified Rubber Tree (*Hevea Brasiliensis*) Kernels

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Abstract: The rubber kernel is rich in nutrients. But its toxicity due to hydrocyanic acid is a problem for its use in food. This bitter almond contains a high concentration of cyanide (2712.4 mg/kg). Its extracts are therefore declared not to comply with the recommendations of the food standards. However, the rubber plantations in Côte d'Ivoire provide a large quantity of seeds, and only 10% of these seeds are used to make plants. The remaining 90% is left in the plantations, which represents an annual production of about 75,000 to 100,000 tons of rubber seeds to be used in Ivorian rubber farming. It is in this context that the cyanogenetic study of the kernel was conducted. The evaluation of the total and free cyanide content of the extracts during operations such as: solar drying of the seeds and kernels in an oven, optimization of the biochemical hydrolysis of cyanoglycosides and roasting of the crushed material, made it possible to develop the present process for detoxifying the extracts from the kernel, particularly the oil and the cake. The residual hydrocyanic acid content decreased from 2712.4mg/kg to 0.38mg/kg (<35mg/Kg), as recommended by the standards for cyanogenic almonds. The rubber tree oil produced had an essential fatty acid profile of 30.9% omega 6 and 6.2% omega 3. And the study of the acute and sub-acute toxicity of the oil produced was carried out with the aim of valorising the rubber seeds in the food and cosmetic field. The absence of deaths and clinical signs observed would indicate that the oil produced by this process is not toxic at the high dose of 2000mg/kg. Pc and at the dose of 5000mg/kg. Pc. Evaluation of chronic administration of the extract on relative organ weights and determination of biochemical parameters such as urea; creatine (CREA); alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) activity reveal that the oil does not contain toxic substances that would contribute to disrupting the integrity of liver tissue. The evaluation of the chemical and biological properties reveals that, after being detoxified into cyanides, rubber tree oil can be used in food or in cosmetics.

Keywords: Detoxification, Cyanogenic Glycosides, Total Cyanides, Acute Toxicity, Subacute Toxicity, Rubber Oil

1. Introduction

The rubber tree (*Hevea basiliens*) is a plant cultivated in our country, Cote d'Ivoire, for the sole purpose of extracting LATEX to produce rubber. However, these plantations provide, on average, 500 kilograms per hectare according to data from the National Centre for Agronomic Research (CNRA). And only 10% of this production is used to make seedlings [1], which represents an annual production of about

75,000 to 100,000 tonnes of rubber seeds to be used in Côte d'Ivoire [2]. These seeds remain on the ground and pollute the environment by depositing hydrocyanic acid, a toxin that also infiltrates the soil and has a negative impact on fauna and flora [3, 4, 5]. However, the kernel of this seed provides 52% oil, and protein-rich cake (38.7%) and 6% miscellaneous waste [6]. Unfortunately, it contains about 2712.4mg/kg of hydrocyanic acid, a level that is quite high. Therefore, its extracts need to be detoxified, prior to their use in food [7]. Especially the oil is drying and rich in omega 3 and omega 6, essential fatty acids for our health [8, 9]. In this plant, this substance, which is largely derived from the secondary metabolism of the plant, plays a natural antibacterial and antifungal role in the plant, serving as a repellent against rodents, insects and predators [10, 11]. This detoxification study was conducted with the aim of valorizing this agricultural by-product through its oil. It enabled the hydrocyanic acid content of the grind to be reduced to 0.38mg/kg of HCN before the oil was extracted by soxhlets. Analysis of the extract (Figure 2a) by HPLC of the WATERS ALLIANCE EMPOWER 3 type (USA) showed an essential fatty acid profile of: 30.9% omega-6 and 6.2% omega-3, either a ratio of omega 6/approximate omega of (5/1) and a content (mg/100g) of vitamin E (78.2); vitamin A (55.2); vitamin B3 (54); vitamin B6 (30.3) and vitamin B12 (26.8). It is in view of these interesting characteristics that the study of the acute and subacute toxicity of oil extracts was carried out to reassure the application of this fat in the food field. Hydrocyanic acid is the toxin that prevents most bitter almonds from being used in food, despite their high nutrient content [12, 13]. Indeed, the simple cyanide ion CN- and the hydrocyanic acid HCN are the most toxic forms of cyanides by ingestion, inhalation and skin absorption [14, 15].

In bitter kernels such as rubber, cyanides are removed as hydrogen cyanide (hydrocyanic acid) during the pre-treatment of the kernel and the sequential degradation of cyanogenic glycosides. For example: in the past, cyanogenic cassava suffered the same fate, but today, thanks to the detoxification process of cassava tubers, its by-products such as attiéké, placali, are included in the list of staple foods [16], in sub-Saharan African countries, and worldwide [17, 18]. Pre-treatment of the kernel (solar drying of the seed, oven drying of the kernel at 105°C, dehulling and grinding) removed 2077 mg/kg of total cyanides, is a detoxification yield of 35.96%. Hydrolysis of cyanogenic glycosides, mainly amygdalin, followed by steam roasting of the grindings removed 624.02mg/kg of cyanides that were locked in the heterosidic combination state in the cyanogenic glycosides.

The hydrolysis of cyanogenic glycosides is thus presented as an alternative to detoxify extracts of these bitter almonds in the food context [19]. The amygdalin, the main glycoside of this almond, by glycosidase, yields its osidic bond during emulsion [20]. This biochemical hydrolysis reaction is an enzymatic hydrolysis, using the natural enzyme found in the kernel itself (endogenous enzyme). However, it can also be carried out with an exogenous enzyme.

2. Materials and Methods

2.1. Material

2.1.1. Plant Material

The *Hevea brasiliensis* seeds were collected from the National Centre for Agronomic Research (CNRA) in Man, Cote d'Ivoire. After solar drying and hulling of the seeds, the kernels (Figure 1a) were dried in an oven at 105°C, then crushed (Figure 1b) to be stored in airtight bottles for later use.

2.1.2. Animal Material

Laboratory mice of the Wistar strain were used to study the acute and subacute toxicity of the oil samples.

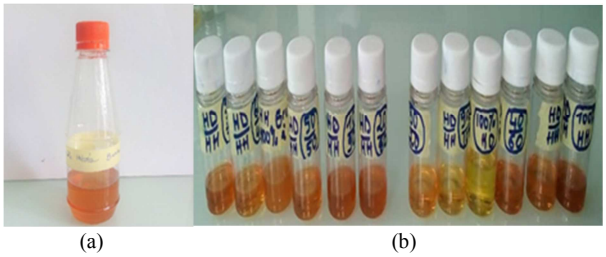
2.2. Methods

2.2.1. Development of the Extract Detoxification Flowchart

The development of the detoxification process of the rubber kernel extracts was carried out by evaluating the content of free cyanides and total cyanides of the different samples, following the following operations: solar drying of the seeds and kernels in the oven, optimization of the biochemical hydrolysis of the glycosides by maceration of the grind and roasting of the macerate by steam cooking. During the pre-treatment of the kernel (Figure 1a, 1b and 1c), the different detoxification works were carried out on the whole kernel, the kernel meal and the steam roasted meal. The elemental chemical composition of the detoxified meal, as well as its protein content, was determined by means of a Scanning Electron Microscope (SEM) associated with an X-ray Dispersive Energy (EDX) analyzer, type SH 4000 M.

(a) (b) (c)

Figure 1. *Image of kernel (a); detoxified meal (b) and detoxified cake (c).*



(a) (b)

Figure 2. *Image of oil extracts, detoxified rubber oil (a) and formulated oils (b).*

2.2.2. Optimization of the Biochemical Hydrolysis of Cyanoglycosides

Optimization was carried out by maceration, taking into account the ratio of the mass of the grind to the volume of distilled water, the speed of agitation and the duration of agitation. Three isothermal models with constant temperatures (50°C, 70°C and 90°C) were developed for this purpose, and the Y90 model (90°C) was selected after a

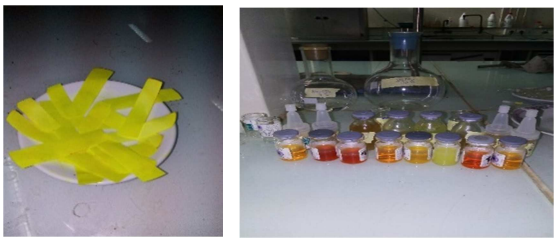
comparative study in 80 minutes. In this study, the samples were assayed following the method of Liebig Denigès, 1971 [21, 22], by a silver nitrate solution (0.02N), in the presence of ammonia solution (10%) and a KI solution (5%) until a persistent opalescent haze was reached. At this level the entrained hydrocyanic acid was first trapped by a soda solution (0.02N). The HCN content per 100 g dry matter is determined by the expression of equation (1).

2.2.3. Determination of Free Cyanides

The content of free cyanides in the various samples was determined by means of a picro-soda paper (Figure 3a), previously prepared, which provides coloured solutions of sodium cyanides (Figure 3b), determined spectrophotometrically according to the method described by Rezaul Haque M et al 2002 [23, 24]. Thus, the free cyanide content of the samples could be quantified after dilution (1/10). The spectrophotometric determination was carried out at 540nm using a JASCO type spectrophotometer (V-530).

$$T = \frac{V \text{ X } 100 \text{ X } 0{,}0054}{m} \quad (1)$$

V (volume of silver nitrate poured in ml);
m (weighed mass of grinding material in g).



(a) (b)

Figure 3. *Image of micro-soda paper (a) and samples (b) assayed by colorimetry.*

The hydrocyanic acid content is calculated using the expression in equation (2):

$$HCN\ (mg/Kg) = 420*A*100/pe \quad (2)$$

A: absorbance at 540 nm;
Pe: weight of the test (g).

The mechanism for obtaining the samples for this colorimetric assay provides for the following reaction diagram (Figure 4).

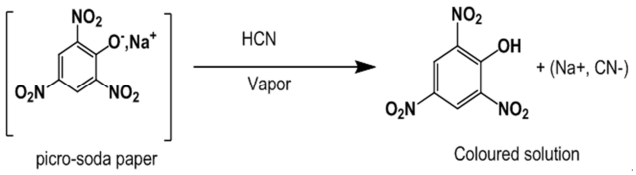


Figure 4. *Obtaining coloured sodium cyanide solutions.*

2.2.4. Determination of Total Cyanides

The total cyanides in rubber kernels were determined according to the NT ISO 2164-1975 standard for the determination of cyanogenic heterosides in legumes [25]. This step, based on the acid hydrolysis of cyanogenic glycosides, makes it possible to determine all the cyanide contained in the kernel [26]. The determination of total cyanides allowed us to assess each operating stage, thanks to the yields of HCN released. The hydrocyanic acid content per kilogram of sample (ppm) is calculated using the expression in equation (3):

$$HCN\ (mg/kg) = 0{,}54(V2-V1)\ *500*1000/250M \quad (3)$$

V1: volume in ml of ammonium thiocyanate poured in the test sample;

V2: volume in ml of ammonium thiocyanate added during the blank test;

M: mass in grams of the test sample.

2.2.5. Study of the Toxicity of Oil Extracts

1) Acute toxicity

The acute toxicity study was carried out according to the Organization for Economic Co-operation and Development (OECD, 2001) guideline No. 423 [27]. The study was performed on 12 rats per gavage. The batches consisted of three (03) rats each, namely the control batch and the batches treated with the different oils. The Controls received distilled water, while the others received the different extracts respectively. The animals were fasted for 24 hours before the experiment. After the fasting period, the animals received the different test solutions respectively. The rats of the different treated batches received the different extracts at a single dose of 2000 mg/kg CP and 5000 mg/kg CP. The animals were observed individually and regularly during the first 24 hours. After 24 hours, they were monitored daily for a period of 14 days. Observations included mobility, sensitivity to noise and pinching, feeding, spanking pattern and respiration following the method of Bürger et al. 2005 [28].

2) Subacute toxicity

This was carried out in accordance with OECD guideline 2001, No. 407 [27]. It consisted of administering the extract to rats daily by the gastric route, at doses increasing with four batches of animals, at a rate of one dose per batch for 28 days. The doses to be administered for this study were 150 mg/kg and 300 mg/kg bw. Thus 7 batches of 3 rats each were formed and the control batch received only distilled water (1ml/100g). The other batches (6) received the test solution orally (Figure 2b). The products were administered daily and at the same time throughout the experimental period. Observations were made on general condition, behaviour, respiratory movements, food intake and spanking appearance. The animals were weighed every other day.

3) Evaluation of weight gain

Weight measurements of the rats in the different batches were taken every other day for the duration of the experiment. The rats were fasted on the day before and at the end of the different experiments.

4) Collection of samples

At the end of the experiment, the animals were fasted overnight, weighed (to calculate body weight gains), and then anaesthetized as described by Badr El Said et al., 2015 [29].

Organs namely kidney, liver, heart and spleen were removed and weighed to determine relative weight. Blood was collected by decapitation technique in dry tubes for biochemical analyses. For this purpose, metabolites including urea and creatinine were measured to assess kidney integrity; enzymes assessed including liver transaminases (ASAT-ALAT) to assess liver integrity and alkaline phosphates (PAL) to assess heart integrity.

2.2.6. Statistical Analysis

For the optimization of the biochemical hydrolysis of glycosides by beta-glycosidase, the results were subjected to analyses of variance (ANOVA) using Stastica 7.0 software to compare the means. In case of significant differences, the Newman-Keuls test was used to identify the means responsible for the observed difference at the 5% threshold. The influences of the different factors in the two-level factorial design were prioritized on the basis of p-values ($p<0.05$), using the Pareto principle. The regression line used for model validation (tests in the center of the experimental domain) showed a correlation coefficient ($R=0.9779$) between the experimental and predicted responses. For the statistical analysis of the data during the toxicity study of the oil extracts, it was done with the Graph pad Prism software version 8.4.3 (686).

3. Results and Discussion

3.1. Detoxification Results of the Extracts

The cyanogenetic of the whole rubber kernel made it possible to establish the detoxification flow chart of the extracts (Figure 7), which presents a detoxification yield in cyanides of 99.94%, is a residual content of 0.38mg/Kg. The optimization of the biochemical hydrolysis of glycosides, amygdalin in this case, provided the Y90 model (Equation 4), an isothermal model performed at 90°C that reduces the content of free cyanides from 620.4 to 0.38mg/kg under the conditions of: (2g/30ml; 80min and 60rpm). The Pareto analysis of the data (Figures 5 and 6), carried out for this purpose, revealed that, the factors stirring time and emulsion density are the factors with significant influence beyond temperature, based on p-values ($P<0.05$). Factor A (duration) had a statistical weight of 38.37% and factor C (mass/density) was in the lead with a contribution of 40.32% factor C. Recent work by Zuzana et al 2021 [20], already showed that beta-glycosidase could be optimized by activating natural enzymes of bitter almond.

The determination of total and free cyanides in this study showed that the *Hevea brasiliensis* kernel, clone PB*260 from the western part of the Ivory Coast (Man), contains a total cyanide content of up to (2712.3±1.6 mg/kg), and (620.4±2.1 mg/kg) for free cyanides, levels that are very high in the context of food. These data confirm that this almond and its extracts would expose to risks of acute and even severe toxicity in case of consumption [30, 31]. It seems necessary to warn about the risks of intoxication in case of consumption of rubber tree kernel extracts. However, the present process allows the production of extracts with a cyanide content of less than 0.38mg/kg, thus providing extracts that are consumable according to the 2019 Codex Alimentarius standards [32], and according to the European Food Safety Authority (EFSA) in its 2019 status [33]. In the regulation of this body that would be applied in January 2023, for certain foods concentrated in cyanogenic glycosides such as cassava, flax and bitter almonds the cyanide content of their flour should be: 50mg/kg for cyanogenic cassava, 150mg/kg for flax and 35mg/kg for almonds. Toxicity tests of the extracts should be able to confirm their use in food. However, the industrial production of foodstuffs should not involve too many chemical inputs in order to reduce the side effects. The alcoholic fermentation route is most often used in food production [34].

Scanning Electron Microscope (SEM) analysis associated with X-ray Dispersive Energy (EDX) analyzer type SH 4000 M of the detoxified meal (0.38mg/kg HCN) allowed to know the influence of the process on its protein content, and its chemical elemental composition (Figure 7). Its protein content was 31.563g per 100g of cake, a content that would qualify this extract for livestock feed because of the relatively low production cost. The work of Gningnini Alain Kone in 2020 [35], on this plant material already motivated its valorization in cattle feed. In addition, the hydrocyanic acid content of the experimental diets was below the EU feed standards of 2.6 to 55.4 mg/kg [33]. In any case, the irreducible content by the present process could be used to combat other pathologies in view of the interesting biological activities of certain glycosides [36].

Many studies on beta-glycosidase from sweet almond (amygdalus comminus L.) are too old, so it remains difficult, if not impossible in most cases, to use these results for comparative purposes [37]. However, they agree on the fact that almond emulsion allows the hydrolysis of its glycosides [38], in order to release hydrocyanic acid. This biochemical hydrolysis favors the synergistic action of all the endogenous enzymes of the almond [39, 40, 41].

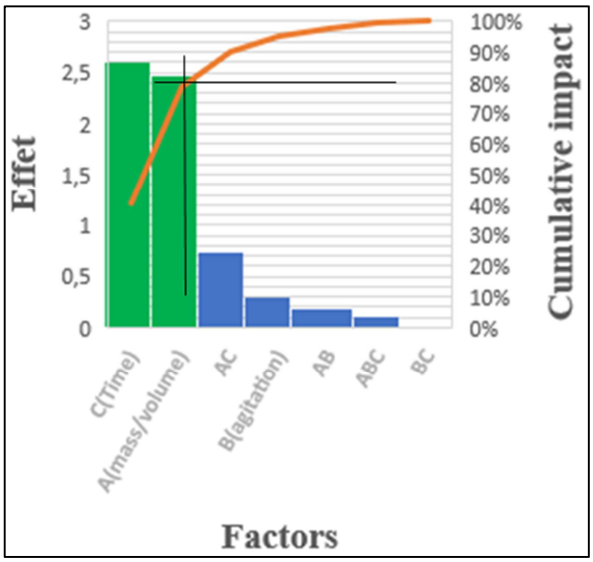


Figure 5. *Pareto chart ranking the factors according to their significant effect.*

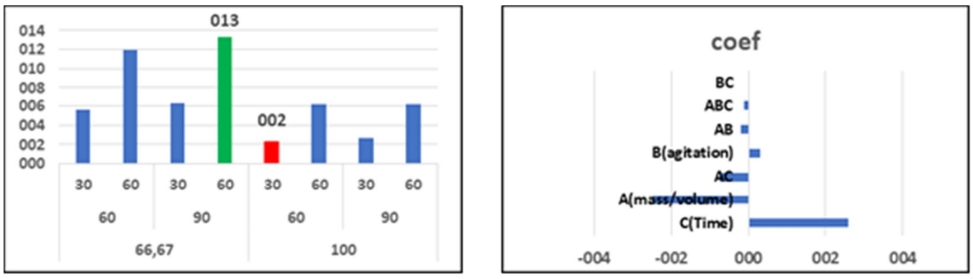


Figure 6. *The influence of factors on the experimental domain of the Y90 model.*

The mathematical expression of the model from the statistical analyses of the data is equation (4):

$$Y_{90}=6,8132875-2,4734375*XA+2,5989125*XC. \quad (4)$$

The elemental analysis of the rubber kernel from the man zone carried out by SEM spectroscopy confirms its oleaginous and proteinaceous character, as the data show that it is composed of (62.97%) carbon, (31.85%) oxygen and (5.05%) nitrogen. These elements are observed at energies lower than 1Kev thanks to the energy dispersion by X-ray photons.

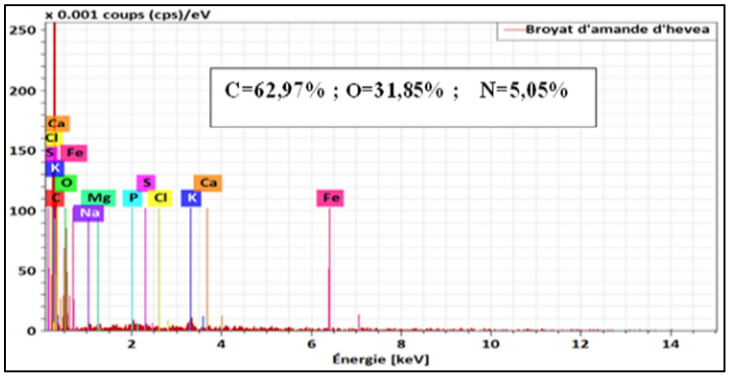
The flow chart for the production of detoxified extracts in Figure 4 is based on the process for the production of food derived from cassava, notably attiéké. It would allow any average citizen to produce food from rubber seeds, thus providing added value to this agricultural by-product. The extracted rubber tree oil was combined with refined palm oil to enrich it with omega-3. The various samples were subjected to toxicity tests for this purpose.

3.2. Results of the Study on the Toxicity of Oil Extracts

3.2.1. Assessment of the Acute Toxicity of the Extracted Rubber Tree Oil

The acute toxicity tests carried out in accordance with the OECD 423 protocol (OECD, 2001) confirmed that the oils are non-toxic by the oral route. Single administration of the oil (Figure 2a) at doses of 2000 and 5000 mg/kg/pc to the rats revealed some behavioral changes in the rats such as diarrhea and sneezing at the 5000 mg/kg/pc batch during the first 4 hours of observation. The faces observed were moist and similar to the faces of animals with diarrhea. However, 24 hours after administration of this dose to the rats, the solid faces were similar to those of the control rats. Nevertheless, the single administration of the different doses of the oil did not cause any deaths in the rats during the two weeks of observation. Table 1 summaries the different parameters on which the observations were made. The absence of deaths in the rats in the 5000 mg/kg/pc batch and the absence of clinical signs would indicate that the oil is not toxic at the high dose of 5000 mg/kg/pc. Thus, the LD50 of the oils would be greater than 5000 mg/kg (OECD, 2001). As a result, HHT oil belongs to category 5 and would be considered a non-toxic substance by the oral route, according to the Globally Harmonized System of Classification and Labelling of Chemicals (OECD, 2001).

In terms of weight gain of the animals after single administration of the solutions, there was a small weight gain of the animals treated with the different doses of the oil compared to the control rats. In addition, non-significant ($p>0.05$) variations were observed between the weight gains of rats treated with the different HHD oils and the control rats. In control rats, weight gains increased from 4.02±0.43 and 25.43±2.5 grams on day 2 and day 14 of the experiment respectively. In addition, the weight gains of the rats that received the different doses of the oil increased from 2.73±0.57 to 20.86±2.02 grams for the rats that received the HHD oil at the dose of 2000mg/kg/pc, from 0.95±0.2 to 18.42±1.5 grams for the rats that received the HHD oil at the dose of 5000mg/kg/pc. The follow-up of the weight evolution during the 14 days of observation, allowed to observe that the administration of the oil at the doses of 2000 and 5000mg/kg/pc did not influence the weight gain of the treated rats during the first days after the administration of the different solutions indeed, Kone et al. (2016; 2019) showed that detoxified rubber tree kernels incorporated in pig feed were not toxic to gilts, piglets and guinea fowl [42, 43]. These results show that detoxified rubber tree kernels are non-toxic to mammals, as well as to guinea fowl and other poultry.



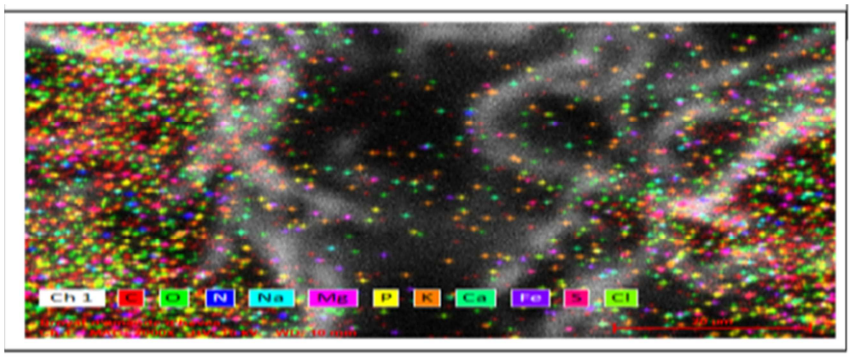


Figure 7. *Spectrum of the EDX analysis coupled with the SEM analyser type SH 4000 M.*

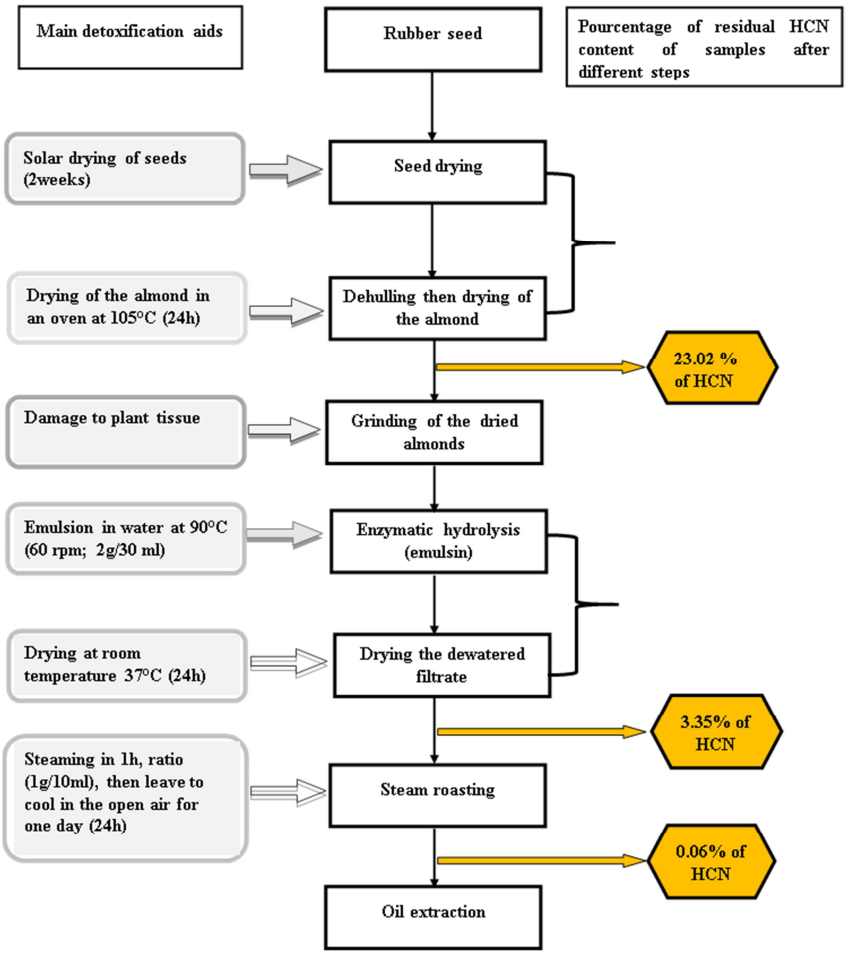


Figure 8. *Flow chart for detoxification of bitter almond extracts.*

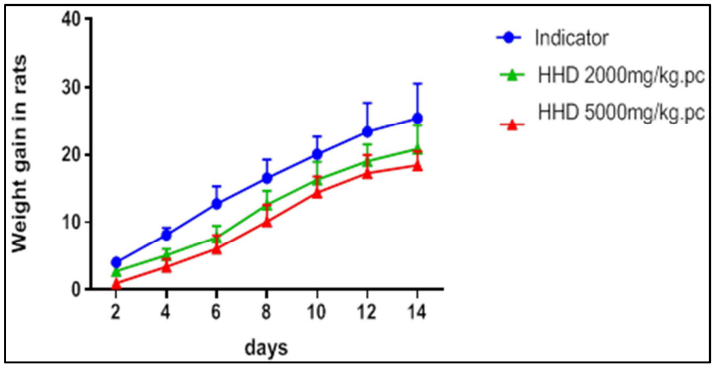


Figure 9. *Change in weight of animals during treatment.*

3.2.2. Evaluation of the Sub-Acute Toxicity of the Formulated Oils

The results of the sub-acute toxicity of the oils are broken down as follows: the behavior of the animals, the evolution of the body weight of the animals and the analysis of the biochemical constants of the blood of the sacrificed animals. During the entire treatment period, no mortality was recorded in either the control or the treated batches of rats.

Evaluation of the chronic administration of the oils on the weight of the animals

Table 1. *Parameters observed during treatment.*

Parameters	Treatment		
	Control Lots	Lots 2000	Lot 5000
Excitation	N	N	N
Stool condition (diarrhea)	N	N	P
Breathing disorder	N	N	N
Refusal of food	N	N	N
Number of deaths	0	0	0
Oral or nasal bleeding	A	A	A
Trembling	N	N	N
Coma	A	A	A
Sleepiness	N	N	N
Sneezing	N	N	P

Designations: N (No or no cases observed); P (Fair); Null (zero or no cases observed); A (No cases observed).

The results of the weight evolution of the animals in the treated groups are presented in Figure 9. Statistical analysis showed no significant difference ($p>0.5$) in the rates of change of body weight between control and treated batches at any dose level. At the end of the 28th day of the experiment, the rate of change of body mass in the animals was respectively 19.89±3.02g for the control batch, 21.65 ±1. 6 g for the HPR1 batch, 20.71±1.05 g for the HPR2 batch, 15.96±1.79 g for the HPCHD1 batch, 16.98±1.08 g for the HPCHD2 batch, 15.25±1.71 g for the HHD1 batch, 19.67±3.09 g for the HHD2 batch. The observation of weight gain in the animals treated with the oil at different doses indicates that the extract. These results show that the daily administration of the extract during the 28 days did not affect the evolution of the body mass of the animals and thus did not disturb the weight gain of the animals. This lack of disturbance in the weight gain of the rats during the treatment reflects the proper functioning of the organism [37].

Assessment of chronic oil administration on relative organ weights

Evaluation of the relative weights of the organs most targeted by the products in the case of toxicity, such as the liver, kidneys, heart and spleen, revealed no significant ($p>0.05$) difference in relative weights between the control group and the groups treated with the different oils. The relative organ weights of the control animals were 0.36±0.00 for the heart, 0.69±0.04 for the kidney, 3.97±0.29 for the liver and 0.306±0.02 for the spleen. The relative organ weights of the treated rats ranged from 0.35±0.00 to 0.4±0.19 (heart), 0.61±0.03 to 0.704±0.03 (kidney), and 3.7±0.33 to 4.07±0.19 (liver), and 0.39±0.01 to 0.52±0.05 (spleen). The lack of significant change in relative organ weights of treated animals compared to controls indicates that the oil did not affect the relative organ weights of the rats. Indeed, after exposure to a toxic substance, alterations in the relative mass of these organs reflect the toxicity of a substance [44].

Influences of oils on biochemical parameters

The analyses of the biochemical parameters revealed no significant differences between the parameters of the rats in the control lot and those of the rats in the different treatment lots, Table 2. In the control rats, the values of the parameters at the end of the experiment were 0.306±0.01 g/L (urea), 4.666±0.33 mg/L (creatinine), 240±14.17 IU/L (AST), 63.666±7.75 IU/L (ALT) and 134.33±50.4 IU/L (PAL). The different values of urea, creatinine, AST, ALT and PAL of the treated rats ranged from 0.33±0.04 to 0.46±0.07 g/L, 4.66±0.33 to 5.33±0.33 mg/L, 205.33±9.27 to 261.33±12.8 IU/L, 33±6.24 to 40±12.12 IU/L and 1003±16.86 to 1053±33.19 IU/L, respectively. The different treatments did not cause a significant ($p>0.05$) change in urea and creatinine levels compared to control rats during the experiment. Transaminases are present in the liver, but also in the pancreas and other tissues. They are synthesized in the cytoplasm of cells in these organs and discharged into the circulation when these cells are damaged [45]. These enzymes are therefore important and very sensitive indicators of hepatotoxicity. A decrease or excessive release of ASAT and ALAT levels in rats indicated possible liver pathology [45]. These oils do not contain toxic substances that would contribute to the disruption of liver tissue integrity. PAL is an enzyme present in various tissues, with higher concentrations in the liver [46]. Elevated PAL levels would indicate obstruction of bile ducts, primary biliary cirrhosis; disorganization of liver architecture [47]. The concentration of PAL observed in treated rats shows that the extract did not affect these cells. In terms of renal enzymes, the oil did not induce any significant variation in urea and creatinine concentrations in the treated batches compared to the controls. Urea and creatinine are the metabolic waste products released by the kidneys [46]. Any phenomenon or substance capable of modifying its functions leads to the modification of plasma concentrations of urea and creatinine [48]. The results show that the plant has no effect on urea and creatinine metabolism. Therefore, the oils would not constitute a danger for the renal and hepatic function and would not possess any toxin such as hydrocyanic acid. Indeed, according to the work of Nathalie Allibe et al 2016, a free cyanide content of 317 µg/L in the cardiac blood would be sufficient to cause the death of the individual and that this death would occur for a level of 1100 µg/L of free cyanides in the peripheral blood [40].

To our knowledge, this rubber oil has not yet been the subject of a toxicity study for food purposes. However, some work carried out in this field, even though it is not too recent, justifies the fact that the oils tested are not toxic for consumption on the basis of the toxicity indexes derived from the research work of: Debora et al 2013; Sacher et al 1991; James et al 2006; Abdel Fattach et al 2020 [46, 47, 49, 50].

Table 2. *Relative weight of organs (%).*

	INDICATOR	HPR1	HPR2	HPCHD1	HPCHD2	HHD1	HHD2
HEART	0.36±0.00	0.40±0.01	0.37±0.01	0.35 ±0.00	0.38 ±0.03	0.4±0.19	0.39±0.02
RENAL	0.69±0.04	0.67±0.01	0.66±0.03	0.704 ±0.03	0.61±0.03	0.69±0.04	0.70 ±0.02
FOIE	3.97±0.29	3.85±0.26	4.07 ±0.19	3.750±0.12	3.7±0.33	3.67 ±0.38	3.93 ±0.17
RATE	0.306 ±0.02	0.48±0.04	0.39 ±0.03	0.52 ±0.05	0.41 ±0.16	0.48 ±0.03	0.39 ±0.01

Table 3. *Biochemical parameters assessed.*

	INDICATOR	HPR1	HPR2	HPCHD1	HPCHD2	HHD1	HHD2
UREA (g/L)	0.306±0.01	0.413±0.05	0.46±0.07	0.37 ±0.04	0.34 ±0.04	0.33±0.04	0.37±0.45
CREA (mg/L)	4.666±0.33	5.33±0.33	5.33±0.33	5.33±0.33	5 ±0	4.66±0.33	5±0
ASAT (UI/L)	240±14.17	213.66±30.12	211±12.16	261.33±12.86	205.33±9.27	265.66±46.67	207.33±11.46
ALAT (UI/L)	63.666±7.75	40±12.12	37±4.07	39.66 ±4.05	36±3.51	35.33±10	33±624
ALP (UI/L)	134.33±504	131±60.06	129.66±41.79	133±38.19	133±38.99	132 ±32.5	133 ±16.86

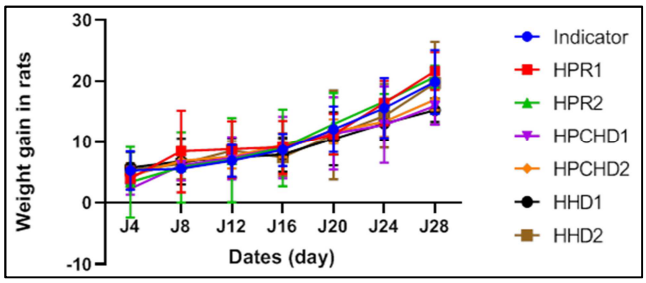


Figure 10. *Change in weight of animals during treatment.*

4. Conclusion

Detoxification of bitter almond extracts for food purposes is possible. It can be carried out by following the above-mentioned operating steps which take into account factors such as: temperature, granulometry of the grind, the ratio of grind mass to volume of water. The hydrocyanic acid, which is blocked in the combined state, is released under these conditions, which optimizes the hydrolysis of cyanoglycosides. In our study, the extract we were aiming for was rubber tree oil. This detoxification process reduced the residual HCN content of the grind to 0.38mg/kg. Also, the study of the toxicity of the rubber oil resulting from the present detoxification process reveals that this oil can be used in the cosmetic and food fields, thus providing a high added value to the rubber seeds (agricultural waste), which degrade the environment, when not removed from our plantations.

In perspective, the determination of the nutritional and therapeutic characteristics of this oil are planned, to evaluate the energy contribution of this fat, as well as its effects on the most frequent metabolic diseases in sub-Saharan Africa, notably diabetes and hypertension.

Conflict of Interest

The authors declare that they have no conflict of interest in relation to this article.

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