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Development of *Garcinia kola* capsules

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Abstract

Garcinia kola usually referred to as "bitter cola" is a tree found in the rainforests. Many studies have revealed the properties of its seeds: antimicrobial, analgesic, anti-inflammatory, hepato-protective, antidiabetic, antioxidant. In the West Region of Cameroon these seeds are mainly used against nausea and vomiting; unfortunately, the generalization or the widening of the use of these seeds encounters some problems: the production is seasonal (June to September), the plant only grows in a small area (the departments of Moungo and Mbam and Inougou), the long storage of the seeds is not possible (they harden quickly and become unfit for consumption).

The non-toxicity of these seeds having already been demonstrated by Udenze et al in 2012, in this study we wanted to develop a galenic form that is easy to produce and likely to help better use this resource by circumventing these difficulties.

Garcinia kola seeds purchased in the city of Bafia, were cleaned, crushed and dried for 2 days at the temperature of 30°C and finally ground. The powder obtained was used to prepare capsules. The powder, calibrated through a 125 sieve, was homogeneous and had a residual humidity of 4%; its flavonoid content was 28 mg quercetin equivalent per gram. The capsules contained 400 mg of seed powder and titrated 3 mg quercetin equivalent of flavonoids per unit. They have responded favorably to the pharmacotechnical tests recommended by the European Pharmacopoeia 5th edition and appear to be able to allow the use of *Garcinia kola* seeds everywhere and in any season.

Keywords: *Garcinia kola* Heckel; Flavonoids; Capsules; Improved traditional medicine.

1. Introduction

Garcinia kola (Clusiaceae) commonly called "Bitter cola", is a medium-sized tree found in the forests of the regions of Central and East Africa. It produces reddish, yellowish or orange fruits containing 2 to 4 seeds [1]. The large consumption of these seeds has prompted studies which have shown that they contain phenolic compounds, steroids, benzophenones, flavonoids, tannins, alkaloids, anthraquinones, saponosides, anthocyanins and heterosides, as well as minerals such as phosphorus, magnesium, calcium and potassium [2, 3]. Other studies have shown their pharmacological properties; thus they are considered to be aphrodisiacs for men, but can suppress or delay fertility in women [4,5]; they are also antipyretic [6], anti-inflammatory, analgesic [7], hepato-protective [2], antidepressants, antioxidants, antidiabetics; antimicrobial, antiulcer, anti-diarrheal, antiemetics, antihypertensives, anti-asthmatic [8,9].

The wide use in traditional medicine and the work of Udenze [10] had demonstrated the non-toxicity of these seeds; in 2018 Djoko had developed garcinia lozenges effective against nausea and vomiting [11]. These pellets unfortunately presented a problem of heat stability which, associated with the seasonal aspect of the availability of seeds as well as

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the difficulty of storing these seeds, precludes the full exploitation of the seeds of *Garcinia kola*. Indeed, the seeds are mainly available in the period from June to September on the one hand and on the other hand they dry out over time and become very hard and unfit for consumption. The objective of this study is to develop capsules, a simple dosage form capable of ensuring better use and good storage of *Garcinia kola* seeds.

2. Equipment

The plant material consisted of fresh seeds of *Garcinia kola* purchased in the department of Mbam and Inoubou. The laboratory equipment included, among others: an ERWEKA ZT6-1-D disintegration apparatus, an OHAUS Adventurer balance, a sieve column: VS1000 RETSCH sieve, a standardized European Pharmacopoeia funnel: ERWEKA GT, a semi-automatic capsule filler, a spectrophotometer, a JEL Type STAV2003 volumetric meter, various reagents, packaging items.

3. Methods

4. Preparation and characterization of the powder

The fresh seeds were washed, cleaned, grated, dried, and then finely ground. The characterization of the powder consisted in establishing its organoleptic characteristics, measuring its residual moisture level, checking its particle size and its phytochemical composition. For the organoleptic control of the powder we noted the color, flavor and odor of the powder.

4.1. Residual moisture

Three 0.5 g test portions P1, P2 and P3 were introduced respectively into 3 pre-tared crystallizers and then placed in an oven at 105 ° ± 2 ° C for 3 hours. P'1, P'2 and P'3, being the respective masses of the 3 test portions after drying, the humidity level (T)

$$T = \frac{100 \times (\Sigma p - \Sigma p')}{\Sigma p}.$$

4.2. Phyto-chemical screening of *Garcinia kola* nut powder.

Usual phytochemical tests were carried out in order to highlight the chemical groups contained in the seed. [10, 12].

Search for alkaloids. Leave 500 mg of powder in suspension in 5 ml of 1% aqueous HCl for 15 min, stirring at times and filter. Place 1ml of the filtrate in two test tubes and add a few drops of Dragendorff's reagent to one of these tubes and Meyer's reagent to the other. The formation of a brown or orange colored precipitate in the first or white in the second tube, indicates the presence of alkaloids.

Search for flavonoids: Heat 200 mg of powder in 2 ml of methanol. Add a metallic magnesium tablet and a few drops of concentrated HCl. The appearance of a pink or orange-red color indicates the presence of flavonoids.

Search for saponins: Bring 1g of powder to a boil in 10 ml of distilled water for 10 minutes; filter. In a graduated test tube, place 2.5ml of filtrate, make up with distilled water to 10ml and shake vigorously for 2 minutes. Leave to stand for 30 minutes, a stable foam height greater than 1 cm indicates the abundant presence of saponins.

Search for tannins: Shake 1 g of powder in 20 ml of distilled water and filter; To the filtrate, add a few drops of 10% FeCl3. A blue-black color indicates the presence of tannoids while blue-green indicates true tannins.

Testing for reducing sugars: Add 1 ml of Fehling A liquor and 1 ml of Fehling B liquor to a test tube containing 2 ml of the aqueous extract and heat in a boiling water bath for about 3 minutes. The formation of a brick red precipitate indicates the presence of reducing sugars.

4.3. Determination of flavonoids

Flavonoids have a free hydroxyl group in position 5 capable of giving, in the presence of aluminum chloride, a yellowish complex by chelation of the Al^{3+} ion. The yellow coloration produced is proportional to the amount of flavonoids present in the extract and can be evaluated by UV-visible spectrophotometry at 415nm [13]. The standard used is quercetin.

From a quercetin standard prepare a solution at 0.3 g / l then prepare a calibration range in accordance with Table 1; let stand 30 minutes in the dark and read the absorbance at 415nm with a spectrophotometer.

Using the absorbance values obtained for the different quercetin solutions, plot the calibration curve showing the absorbance as a function of the quercetin concentrations.

Table 1 Quercetin Calibration Range

Tubes	**Blanc**	**1**	**2**	**3**	**4**	**5**
Stock solution at 0.3g /l ml	0	0.2	0.4	0.6	0.8	1
Distilled water (ml)	1	0.8	0.6	0.4	0 .2	0
Quercetin concentration in g /l	0	0.06	0.12	0.18	0.24	0.3
Ethanol 95% (ml)	1.5	1.5	1.5	1.5	1.5	1.5
$AlCl_3$ 10% (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Na acetate 1M (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (ml)	2.8	2.8	2.8	2.8	2.8	2.8

For the determination of the flavonoids, leave 3.5 g of *Garcinia kola* powder in 35 ml of distilled water for 1 hour, stirring occasionally and filter; to 0.5 ml of half-diluted filtrate, subject to the treatment in Table 1 and determine the absorption at 415 nm.

Plot the absorbance on the calibration curve and determine the concentration of flavonoids expressed in quercetin equivalent (QE).

5. Capsule formulation

5.1.1. Galenic characterization of the powder

Particle size screening: Stack the sieve analysis sieves on top of each other in decreasing order of fineness, then place the sample to be analyzed on the upper sieve. After stirring the sieve column for the standard time, evaluate by exact weighing the amount of product retained on each sieve. Calculate the percentage by mass of particles retained on each sieve of known mesh size.

Once the analysis is complete, the masses obtained are added up. The total loss of material must not be greater than 5% of the mass of the initial test sample.

The analysis on a new sample was repeated, but operating at one time for a period equal to the cumulative duration of the previous stirring phases.

The end point of the analysis was reached when the mass retained on each of the sieves (refusal) became constant to within 5% or 0.1 g from the value previously measured on this sieve. A powder is considered homogeneous if a sieve retains at least 50% of the powder.

Validating the particle size analysis consists of observing and verifying whether the losses and inaccuracies associated with handling are in sufficiently low proportions so as not to distort the analysis.

The average percentage of losses (p) was determined by applying the following formula :

$$P = \frac{(\text{Test portion} - (\Sigma \text{ Test sieve refusal } 1 + \Sigma \text{ Test sieve refusal } 2)/2}{(\text{Test sample}) \times 100}$$

The sum of the rejects from each sieve must not differ by more than 2% from the test sample. Below 2%, the imprecision of the manipulations has no consequences on the analysis and the analysis is validated.

Flow through an orifice. The outlet of the dedicated funnel was sealed and then a test portion of 100 g of powder was introduced. After release from the orifice, the flow time of the entire sample was measured. This operation was carried out three times in a row. The flow time is the average of the three measurements.

The flow through a standard funnel is considered satisfactory when the mean time is less than 10 s per 100 g of substance [14].

Evaluation of the apparent density by settlement. The volume measurement as a function of settlement is carried out using a volume meter. The compressibility is evaluated from various indices: Carr index, Hausner index, V_{10} -V_{500}. The lower these indices, the better the flowability.

In the 100 ml test tube, attached to the volume meter, 100 g of the powder and lubricant mixture chosen were introduced and then the aerated volume V_0 was recorded. The apparatus was then successively adjusted for 10, 500, and 1250 settlements and the corresponding volumes V_{10}, V_{500} and V_{1250} were noted. Three tests were carried out in each case then the average was taken into account. After calculations, the indices were defined by various expressions.

V_{10} -V_{500} < 20 reflects a good reorganization of the powder

$$Carr\ Index\ = 100 \times \frac{(V_0 - V_{500})}{V_0}$$

$$Hausner\ Index\ = \frac{V_0}{V_{500}}$$

Table 2 Flowability scale [14]

Carr index (%)	Flowability	Hausner index
1-10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fairly good	1.19-1.25
21-25	Fair	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
> 38	Extremely poor	> 1.60

Choice of diluent: Usually the choice of diluent depends mainly on its solubility. For a soluble active principle, an insoluble diluent is preferable and for an insoluble AP, a soluble diluent. The main diluents used in the pharmaceutical industry for the manufacture of capsules are starch and sugars (lactose, sucrose, fructose or levulose). One of these diluents was chosen taking into account the characteristics of the plant powder.

5.1.2. Filling the capsules

Using the filling table and the apparent volume, the number of capsules to be used and the volume of inert powder to be added so that the envelopes are completely filled were determined. Then the capsules were filled homogeneously by leveling.

6. Quality control of the capsules.

Organoleptic checks: Macroscopic observation to assess the shape and the absence of dented or cracked units.

Uniformity of mass: In accordance with the European Pharmacopoeia the average mass of the contents of 20 capsules taken at random was determined; the behavior of the individual masses with respect to this average was checked. At

most 2 of the 20 units may deviate from the mean mass by e%, but no mass should deviate by more than twice the tolerable margin of error provided by the pharmacopoeia (e%).

Disaggregation test: This test was intended to determine the greater or lesser ability to disintegrate, in a liquid medium, within the prescribed time. It was carried out by standardized shaking of the capsules in water at 37 ° C. Disaggregation was considered to be achieved when there was no residue on the grid; and if a residue remained, the latter had to be constituted only by a soft mass no longer comprising a palpable nucleus and not impregnated.

Evaluation of the flavonoid content in the capsules produced: By following the dosing protocol mentioned above, the determination of the flavonoids in three capsules was carried out by spectrophotometry, and the average level was retained.

7. Results

Organoleptic and physicochemical characteristics of the powder

Organoleptic characteristics: *Garcinia kola* powder is brown in color, odor and bitter taste.

Residual moisture: Table 3 shows the amount of water not removed by drying the powder. After drying in the oven, the residual moisture level of the garcinia powder is 4%

Table 3 Moisture content of the powder of the seeds of Garcinia kola used.

	P in (g)	**P 'in (g)**	**Moisture rate in%**
Essai 1	0.50	0.48	4 %
Essai 2	0.50	0.48	4%
Essai3	0.50	0.48	4%
Average			4%

P represents the mass of powder before drying and P ' the mass of powder after drying.

7.1. Phytochemical screening

The qualitative phytochemical composition is shown in Table 4.

Table 4 Summary of the results of the phytochemical analysis

Chemical Families	**Reagents**	***Garcinia kola* Seeds**
Flavonoids	Magnesium turnings	+++++
Tannins	Foam index	+++
Saponins	$FeCl_3$	++
Reducing sugars	Fehling Reactive	++
alkaloids	Dragendorf / Meyer	+

The number of (+) is proportional to the intensity of the color or the precipitate.

Phytochemically, *Garcinia kola* seeds contain a lot of flavonoids as well as tannins, saponins, reducing sugars.

Determination of flavonoids: Determination of flavonoids in 3500 mg of powder made it possible to obtain 28 mg quercetin equivalent of flavonoids per gram of dry matter (0.8% m / m).

Capsules

Choice of dose: Based on the results of Olaleye [7], the daily dose was set at 1600 mg (4 capsules of 400 mg).

Galenic characterizations of the powder: Table 5 shows the rejects obtained on each sieve after sieving the powder from the seeds of *Garcinia kola*.

Table 5 Refusal of the sieves obtained by analytical sieving of the powder.

Sieve mesh diameter in µm		**600**	**300**	**125**	**Base**	**% losses**
Refusal of sieves in g	Test Essai 1	0.69	8.63	34.93	8.005	0.49%
	Test Essai 2	0.69	10.01	40.01	0.29	1.92%
Average						1.20%

The average rejection rate of the sieves was 1.20% which complies with the requirements of the European Pharmacopoeia; the particle size analysis by the sieve method is therefore valid.

The distribution of the powder as a function of the mesh size (FIG. 2) shows that the mesh screen 125 has retained 74.9% of powder; this powder can therefore be considered as homogeneous according to the criteria of the European Pharmacopoeia 5th edition.

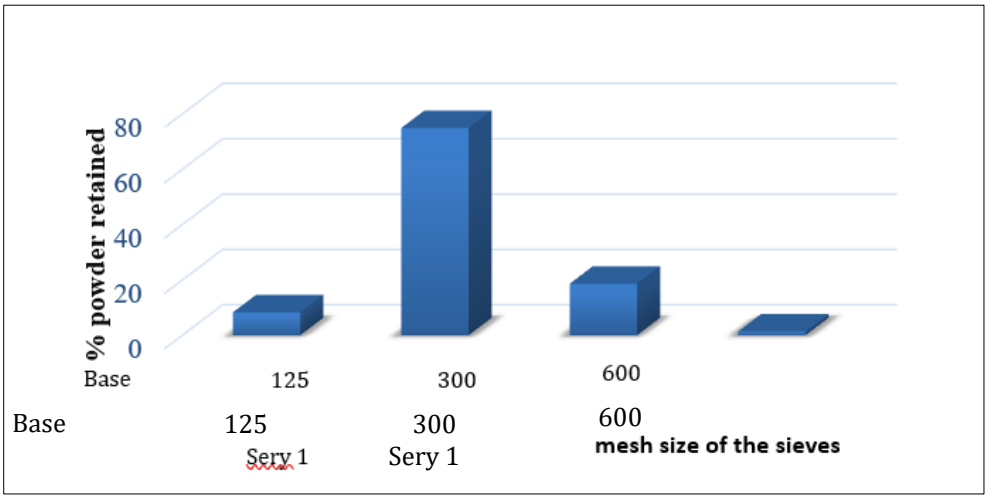

Figure 1 percentage of powder retained as a function of the diameter of the mesh of the sieve.

7.2. Choice of lubricant

Flow of the powder through an orifice: Table 6 shows the flow times of 100 g of powder as a function of some lubricants. It appears that in the absence of any lubricant, the flow of the powder is not satisfactory. Colloidal silica improves flow better than Magnesium Stearate.

Table 6 Garcinia kola powder flow time depending on the lubricant.

	Flow time in seconds
Powder without lubricant	23±1.4
Powder + 0.5% colloidal silica	11.3 ±2.4
Powder + 1% colloidal silica	6.33 ±1.4
Powder + 0.5% magnesium stearate	20±2.8
Powder + 1% Magnesium Stearate	15±1.4

It is at a rate of 1% that the colloidal silica allows flow in accordance with the requirements of the European Pharmacopoeia 5th edition.

Evaluation of the apparent density during compaction: Table 7 shows the volumes of 100 g of powder during compaction using a volume meter. These volumes were used to calculate the indices presented in Table VII.

Table 7 Volumes (in ml) of 100g powder during compaction with the volumeter

	Trial 1	**Trial 2**	**Trial 3**	**Average**
V_0	100	100	100	100
V_{10}	94	94	94	94
V_{500}	81	81	81	81
V_{1250}	81	81	81	81

Table 8 Characteristics of the powder containing 1% of colloidal silica.

Tests	**Results**	**Interpretation**
Hausner index	1.17	Good flowability
Carr index	14.67	Good flowability
V_{10}-V_{500}	13	Good reorganization of the Powder

The good flowability and the good ability to reorganize confirm that the colloidal silica at the rate of 1% is a good adjuvant for improving the flow of *Garcinia kola* powder.

7.3. Formulation and filling of capsules

Capsule manufacturing formula: From the filling table we obtained the following formula:

Garcinia kola powder: 400mg

Colloidal silica:4mg

Capsule number: 0

Figure 2 BTK* capsules

We therefore did not need any diluent to ensure regular, homogeneous and optimal filling of the N ° 0 capsules. By using a semi-automatic capsule filler for the filling, the regular and homogeneous capsules shown in Figure 3 were easily obtained.

Quality control.

Organoleptic controls. The capsules obtained and which we have called "BTK" are: smooth, clean, without pods, and ivory in color.

Uniformity of mass. The masses of the contents of 20 capsules selected at random are collected in Table 9

Table 9 Values of the individual masses of the contents of the capsules.

Samples numbers	Masses (mg)		Samples numbers	Masses (mg)
1	370		11	360
2	400		12	370
3	360		13	370
4	370		14	380
5	370		15	400
6	390		16	370
7	370		17	390
8	360		18	390
9	390		19	370
10	390		20	370

The average mass was M = 377mg. The maximum tolerable deviation was therefore e = 7.5% or 28.3 mg. The resulting limits were: 348.7 mg and 405.3 mg. Thus no value went outside the tolerable limits.

Disaggregation test: The technique announced above led to a disintegration time of 5 minutes, time in accordance with the standards of the European pharmacopoeia.

Flavonoid content: After manufacture, the determination of the flavonoids in the capsules of 400 mg of *Garcinia kola* powder, gave a content of 3 mg quercetin equivalent of flavonoids per capsule.

7.4. Packaging of capsules.

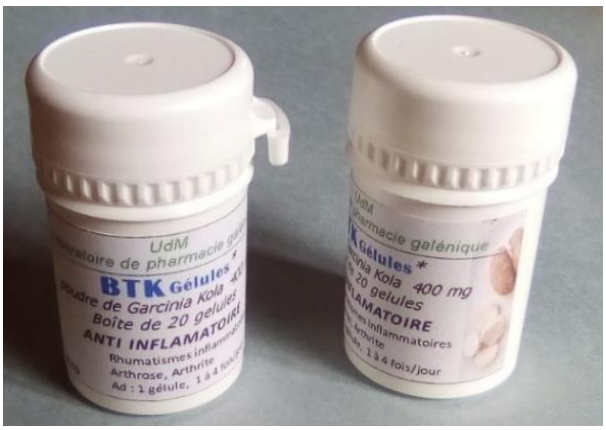

Figure 3 Presentation of BTK* capsules

The packaging was carried out in white and opaque PVC boxes, which were hermetically sealed (Figure 4). They contain 20 capsules and a desiccator capsule. The label carries information relating to the identification and traceability of the product.

8. Discussion

Garcinia kola powder turns brown slightly over time. This is probably due to the phenomenon of oxidation upon exposure to air. The residual moisture of the powder, valued at 4%, suggests that it will store well and that microorganisms will have difficulty growing in it.

The phytochemical composition of the powder revealed the presence of flavonoids, tannins, saponins and traces of alkaloids. This result corroborates that of Buba in 2016 [1] but differs slightly from that observed previously which indicated less saponin in the powder of seeds originating from Mungo [11].

The chosen galenic form (capsules) is a dry form and lends itself to good preservation of the active ingredients. These capsules may, depending on the number of units administered, be useful in the management of several pathologies, including inflammatory pathologies, disorders of the digestive tract and other claims declared for *Garcinia kola*.

9. Conclusion

At the end of this study where it was a question of developing capsules to ensure the good conservation and valorization of the seeds of *Garcinia kola*, it appears that the objective was reached because the product obtained has successfully undergone physicochemical tests and pharmacotechnics guaranteeing its quality. The initial problems (bitterness, seasonality, storage difficulties and poor spatial distribution) have been resolved. In the near future, we plan to research the effectiveness of these capsules on a number of targeted pathologies such as inflammatory diseases and infectious diarrhea.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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