International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

Check for updates

Formulation of anti-malaria capsules based on the fruit of *Picralima nitida* (Stapf) T. Durand and H. Durand (Apocynaceae)

NDJAFANG JATSA Kevin Mike [1], KANMOGNE Dayo Lydie Carole [1], DJOKO Ernest [1,*], Foutse Yimta Wandji [2] and Wouessidjewe Denis [3]

[1] *Galenic laboratory- «Université des Montagnes Bangangté, Cameroon.*
[2] *Phytochemestry laboratory, Université des Montagnes- Banganté, Cameroon.*
[3] *University of Grenoble Alpes-. UFR of Pharmacy Department of pharmaco-chemistry 5063 France.*

International Journal of Biological and Pharmaceutical Sciences Archive, 2023, 06(01), 059–067

Publication history: Received on 23 July 2023; revised on 08 September 2023; accepted on 11 September 2023

Article DOI: https://doi.org/10.53771/ijbpsa.2023.6.1.0086

Abstract

Malaria is a parasitic disease that is widely spread throughout the world. The annual number of cases is estimated at around 500 million; the African continent is the most affected, accounting for 95% of cases. For the treatment, WHO recommends artemisinin-based combinations; unfortunately, the cost of these combinations is relatively high, making them difficult to afford. This justifies the search for new antimalarial drugs. A large number of plant species in Cameroon have been identified as anti-malarial medicinal plants. *Picralima nitida* is one of the plants traditionally used to treat malaria in traditional medicine, and its *in vitro* and *in vivo* antimalarial activity and toxicological profile have already been scientifically demonstrated. The aim of the present study was to formulate capsules based on the aqueous extract of *Picralima nitida* fruit, in an attempt to rationalize its use in the treatment of malaria.

The aqueous extract of *Picralima nitida* fruit was prepared by maceration and then stabilized; its physicochemical characteristics were determined. The daily quantity of extract required for the treatment of malaria was determined in milligrams of total alkaloids. Finally, the extract was stabilized before being filled into capsules using European pharmacopoeia techniques. The capsules were also tested according to European Pharmacopoeia 11th edition techniques.

The extract was obtained in 6.25% yield. The extract was soft, hygroscopic, brown in color, very bitter in flavor and caramel in odor. The following phytochemical groups were present in the extract: alkaloids, phenolic compounds, flavonoid tannins, anthocyanins, saponins, terpenes, sterols. Total alkaloid content was 0.11g per gram of extract. To stabilize the extract and make it suitable for capsule filling, 25% colloidal silica and 13% microcrystalline cellulose were added.

The stabilized extract showed good flowability (flow time 6s, Carr index 11.8% and Hausner index 1.13), a moderately fine powder texture (d50 < 300um) and homogeneous distribution. The daily dose determined for a 60 kg adult was 3900mg of extract, corresponding to 313 mg of total alkaloids, which could be divided into 8 N°0 or 12 N°00 capsules. On inspection, the capsules were found to comply with the requirements of the 11th edition of the European Pharmacopoeia.

The characteristics of the formulated capsules are favorable to make them a good candidate for the various phases of clinical trials with a view to making them an improved traditional medicine (ITM)

Keywords: *Picralima nitida*; Formulation; Aqueous extract; Capsule; Antimalarial

* Corresponding author: DJOKO Ernest; Email: edjoko16@gmail,Com

1. Introduction

Today, malaria still remains a major public health problem in Cameroon as in other tropical countries. [1].The treatment recommended by the WHO consists of polytherapies combining artemisinin derivatives [1]. Unfortunately, these treatments are expensive, given the low incomes of the populations concerned, who turn to traditional practitioners, most of whom use herbal preparations [2]. *Picralima nitida* is one of the plants used in traditional medicine in Cameroon to treat malaria [3]. Its antimalarial activity [4] and toxicological profile have already been scientifically evaluated [5 ; 6]. The results of this work being highly promising, the present study set out to develop a galenic form based on a *Pricralima nitida* fruit extract to facilitate and improve the valorization of this local resource. Because of the fruit's pronounced bitterness [7] (due to its alkaloids), the capsule proved to be the most suitable dosage form.

2. Material and methods

2.1. Material

The *Picralima nitida* fruits used were purchased at the "A" market in Bangangté, western Cameroon. The technical equipment used included: a grinder, a rotary evaporator, an oven, a semi-automatic capsule-maker, a precision balance and an autoclave. The study required the use of numerous reagents and culture media.

2.2. Methodology

2.2.1. Preparation of the extract

After purchase, the fruits were cleaned, cut and crushed using an electric mill; the crushed material was placed in distilled water for 24 hours, then underwent 3 successive macerations; the 3 macerates obtained were combined, filtered on No. 2 wattman paper, then the filtrates were concentrated in a rotary evaporator at 50°C before being dried in an oven at 50 °C for 48 hours. The extraction yield (R) was calculated according to the formula

$$R = \frac{\text{starting fruit mass} - \text{dry extract mass}}{\textit{starting fruit mass}} \times \mathbf{100}$$

2.2.2. Extract characteristics

Organoleptic characteristics (color, odor and taste) were recorded. The pH was then determined 3 times on a 10% dispersion in distilled water; the average was taken into account.

Solubility. To determine the solubility of the extract in water, 100 mg were diluted in 10 ml of osmosis water; increasing volumes of water were then added under magnetic stirring, with a visual check for the disappearance of particles.

2.2.3. Phytochemical screening

Precipitation and coloration tests using standard reagents [8] were used to determine the phytochemical groups present in the extract.

2.2.4. Alkaloid assay

Place 100 ml of a 1% w/v aqueous solution of the extract in a separatory funnel, add the amount of ammonia required to adjust the pH to 11 and 100 ml of chloroform. Shake the flask for 2 min, leave to stand and recover the chloroform phase containing the total alkaloids, mass m1, in a ROTAVAPOR flask. Mount the flask on the apparatus and remove the chloroform by rotary evaporation at 45° C; re-weigh the flask, noting its mass m2. The difference m2 - m1 is the mass of alkaloids contained in 1 g of extract. This protocol was carried out 03 times and the average value was used.

2.2.5. Capsule formulation and manufacture.

Specifications

As shown in Table I, the specifications for the formulation of *Picralima nitida* capsules include sensory, cost and formulation constraints.

Table 1 Specifications for the formulation of *Picralima nitida* capsules

Constraints	**Capsules**
Formulation constraints Size:	suitable capsule size
Sensory constraints	Medium size Masking extract odor Mask extract taste
Regulatory constraints	Compliance with the requirements specified in the "European Pharmacopoeia 11 edition".
Therapeutic function	Anti-malarial property
Cost	Lower cost

Determination of daily dose

A first method based on the results of preclinical studies was used to establish the equivalent human dose (EHD) to that obtained in animals, according to the recommendations of the Food & Drug Administration (FAD) in Table II.

Table 2 Conversion factors for determining the Equivalent Human Dose (EHD) [9]

Species	**EHD (mg/kg) = animal dose divided by**	**EHD (mg/kg) = animal dose multiplied by**
Mouse	12,3	0,081
Hamster	7,4	0,135
Rat	6,2	0,162
Rabbit	4,6	0,216
Guinea pig	3,1	0,324
Dog	1,8	0,541
Marmoset	6,2	0,162
Baboon	1,8	0,541

A second method based on the experience of traditional practitioners was used to verify the first method. Interviews with traditional healers revealed that, for the effective treatment of uncomplicated malaria, 1 kg of fruit is macerated in 2 liters of water, and the patient is asked to take 1 glass (250 ml) of the macerate twice a day for 7 days.

The alkaloids contained in the 500 ml macerate are measured on a preparation prepared by the traditional practitioner, and the daily dose of alkaloids is compared with that determined by the 1st method.

Powder preparation

Although dry, the extract was not suitable for capsule filling because it was pasty and hygroscopic. It had to be triturated with silica and microcrystalline cellulose in various proportions to make it powdery. Flow was assessed using the standardized funnel method [8,10,11]. The European Pharmacopoeia requires 100 g to flow in less than 10 sec. Flow time was the average of 3 trials. Settling ability was determined on the volumenometer: 100 g powder was introduced into a graduated test tube; volume V_0 was measured, followed by volumes V_{10}, V_{500} and V_{1250} successively after 10, 500 and 1250 settlements [11]; then the Carr index (C.I) and Hausmer index (H.I) were determined by the formulae.

$$Carr\ Index\ = 100 \times \frac{(V_0 - V_{250})}{V_0} \qquad \text{and} \qquad Hausner\ Index = \frac{V_0}{V_{250}}$$

By comparing C.I and H.I with the flowability scale in Table III, we were able to conclude on the powder's flowability. The granulometric distribution was studied using the sieve method, which enabled us to conclude on the fineness of the powder: 100 g of powder were placed at the top of a column of pharmacopoeia granulometric analysis sieves, and after 10 minutes' agitation, the refusal masses of the different sieves were determined by weighing, then the histogram of simple and cumulative frequencies enabled us to determine the median size (d50) corresponding to the diameter of 50% of the powder [10 ;11].

Table 3 Flowability scale [8,10]

Carr index (%)	Flowability	Hausner index
1-10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair Good	1.19-1.25
21-26	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

In the light of Table IV, the powder could be designated by the appropriate descriptive term.

Table 4 Classification of powders by fineness

Descriptive term	d50 (µm)
Coarse	> 355
Moderately fine	180 – 355
Fine	125 – 180
Very fine	≤ 125

d50= Median particle size (50% of particles are smaller and 50% larger).

Capsule filling and control

Taking into account the characteristics of the powder, it was possible to proceed with capsule filling using the shaving method on a 100-count semiautomatic capsule filler.

- The batches were subjected to the usual pharmacopoeia controls [10]:
  - Organoleptic control: color, appearance, absence of cracks
  - Mass uniformity. Determination of the maximum acceptable deviation from the average mass of 20 capsules taken at random.
    - If the average mass **m** is < 300 mg, the deviation is 10% of m
    - If the average mass **m** is ≥ 300 mg, the deviation is 7.5% of m
  - Disintegration time. None of the capsules placed in the 6 tubes of the disintegration apparatus should show any solid residue after 30 min of operation of the apparatus at 37° ± 2 °C.

Microbiological quality. Microbiological quality control was carried out in accordance with the techniques recommended by the European Pharmacopoeia [10]. A stock solution corresponding to 1 g/ml was prepared by homogenizing capsule powder in water for injection; two 1/10th dilutions were then placed in 90mm PETRI dishes. As shown in Table V, the culture medium and incubation time depended on the type of microbe sought.

Table 6 Conditions for microbial testing

	Culture medium	**Incubation time**	**Incubation temperature**	**Observation at the end of incubation**
ETGA	Agar	24 H	33°C	Colonies to count
ETMY	Sabouraud	24 H	33°C	Colonies to count
Escherichia Coli	Mac Conkey	48 H	44°C	No red colony + light ring
Staphylococcus aureus	Chapman	72 H	33°C	No yellow or white colony
Enterococcus	Bile esculin	72 H	33°C	No colonies

ETGA = Enumeration of Total Aerobic Germs ETMY= Enumeration of Total Moulds and Yeasts

3. Results

Pricralima nitida fruits purchased at the Bangangté "A" market were identified at the Herbier National du Cameroun as conforming to specimen ASM6176 registered under Voucher no. 2547SRF/cam.

The aqueous extract was obtained in 6.25% yield and appeared as a dark brown paste with a caramel fragrance and a very bitter taste. It had a pH of 6.89 and a solubility of 100-50 g/l. As shown in Table VI, the extract contained mainly alkaloids, polyphenols, flavonoids, tannins, saponins, anthraquinons and steroids; glucosides and anthocyanins were absent.

Table 7 Phytochemical composition of *Picralima nitida* aqueous fruit extract

Secondary metabolites	**Reagents**	**Results**
Alkaloids	Dragendorf	+
Polyphenols	FeCl3	+
Flavonoïds	Magnésium turnings	+
Tanins	FeCl3 / HCl	+
Glucosides	Fehling	-
Saponins	Foaming index	+
Anthraquinones	NH4OH	+
Anthocyanins	HCl /NH4OH	-
Terpenoids /sterols	acetic anhydride / Chloroforme / H2SO4	+

(+): presence of secondary metabolite, **(-)**: absence of secondary metabolite

The total alkaloid content of the extract was 11% (0.11 g alkaloids/g extract). Table VII presents the composition and characteristics of the 8 formulas tested, showing that formula no. 8 had the best properties and was therefore selected for capsule filling.

Table 8 Formulas produced and their flow and settling properties

Composition	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Picralima nitida fruit extract	80 %	71 %	67 %	64,5%	40%	60%	60 %	67%
Colloidal silica (Aerosil 300)	20 % *(25% of extract)*	29 % *(40% of extract)*	33 % *(50 %of extract)*	35,5 % *(55% of extract)*	/	/	29% *(40% of extract)*	20 % *(25% of extract)*
Microcrystalline cellulose (Avicel PH 102)	/	/	/	/	60%	40%	11%	13%
Flow time	∞	∞	8 s	4 s	∞	∞	5 s	6 s
Hausner index	1,6	1,43	0.82	1,1	1,4	1,3	1,14	1,13
Carr index	34	30	17,92	10	30	24	16,10	11,8
Flow	Very poor	Poor	Fairly good	Bonne	Mediocre	Mediocre	Good	Good

As shown in figure 1 for formula 8, 53.90% of the powder was retained by the 300 µm mesh sieve. The average diameter (d50) is therefore less than 300 µm.

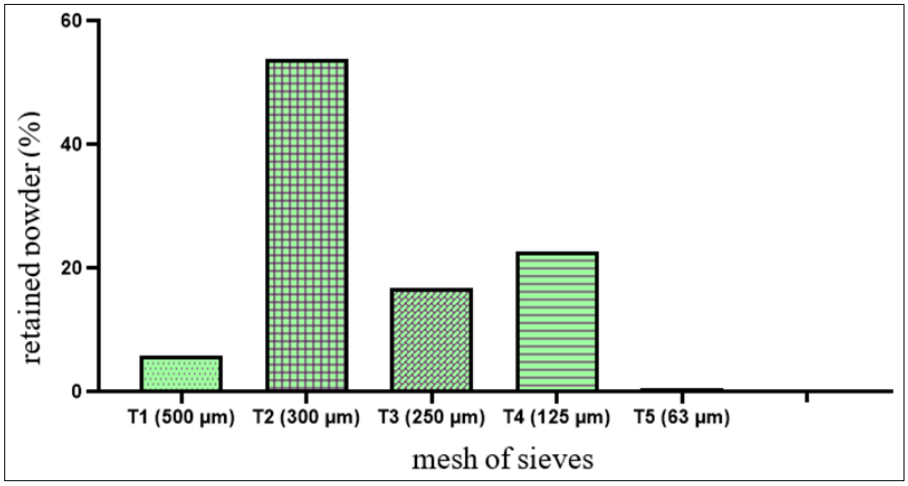

Figure 1 Powder grain size frequencies by sieves

The capsule filling table shows that 14 g of formula n° 8 powder easily fills 20 nº 00 capsules of 700 mg, or 30 nº 0 capsules of 467 mg.

- Daily dose

Pre-clinical studies [7;9] have shown that the effective dose in rats is 400 mg/Kg/day; the FAD factor in rats being 0.162, the EHD is 400 X 0.162 = 64.8 mg/Kg/day, i.e. for a 60 kg adult, 64.8 mg x 60 = 3888 mg extract per day, corresponding to 3888 x 11% = 426 mg total alkaloids per day.

The amount of alkaloids in 500 ml of the traditional practitioner's daily macerate was 430 mg alkaloids per day, corresponding to 3.909 g extract.

For an adult weighing 60 kg, 3.9 g of extract, or 430 mg of total alkaloids, was therefore the normal daily dose.

- Dosage

The option of capsules nº. 00 containing 700 mg powder would have resulted in capsules containing 700 mg x 67% = 469 mg extract, i.e. 469 x 0.11% = 51.59 mg alkaloids, which would have required 430 mg /51.59 = 8.33 capsules per day. The option of capsules nº 0 containing 467 mg powder led to capsules containing 467 mg x 67% = 312.89 mg extract, i.e. 312.89 x 0.11% = 34.41 mg alkaloids, requiring 430 / 34.41 = 12.49 capsules per day.

So for adults, the choice was between making n° 00 capsules and giving 8 a day, or n° 0 capsules and giving 12 a day.The size of n° 00 capsules disqualified them because of their predictably poor acceptability. When choosing n° 0 capsules, they should be given in 3 doses of 4 capsules. The unit formula of the n° 0 capsules adopted is therefore that shown in Table VIII.

Table 9 Unit formula of adopted capsules (F8)

Extract	**67 %**	**313 mg (i.e. 34.4 mg alkaloids)**
Aerosil 300	20 %	93,3 mg
Avicel PH 102	13 %	60,7 mg
	100%	467 mg

3.1. Capsule tests

- Pharmacotechnical control
- Macroscopically, the capsules obtained (figure 2) showed no visible macroscopic defects.

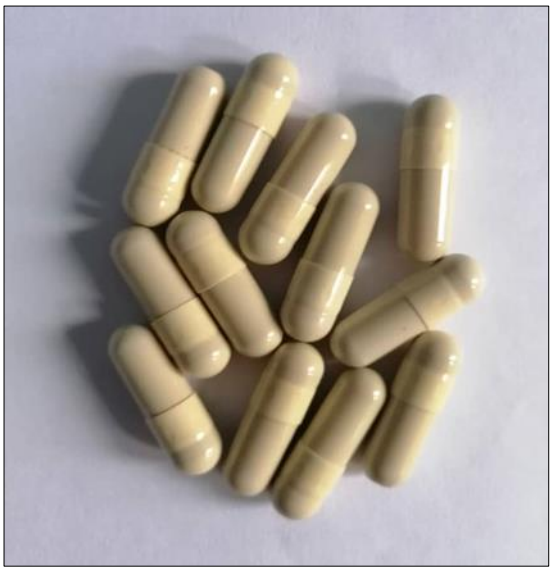

Figure 2 *Picralima nitida* fruit capsules

Figure 3 Presentation of the final product

Mass uniformity: The average mass of a capsule was 542.5 mg, giving a tolerable deviation of ± 7.5%, and acceptable limits of 501.67 mg and 583.02 mg. None of the capsules in the sample were outside these limits.

3.1.1. Disaggregation time was 5 min

From a pharmacotechnical standpoint, the capsules therefore complied with the recommendations of the 11th edition of the European Pharmacopoeia.

3.1.2. Microbiological control

The ETAG gave 6 CFU/g and the ETMY 0 CFU/g; there was no *E. Coli*, nor *staphylococcus aureus*; so microbiologically, the capsules also complied with the recommendations of the 11th edition of the European Pharmacopoeia.

3.1.3. Packaging

Finished capsules were packaged in 84-unit pill boxes (Figure 3), each corresponding to an adult treatment course. Each box bears the proposed trade name (MALARIMINE), as well as the regulatory identification and traceability informations.

4. Discussion

The bitter taste of the extract had a negative impact on acceptability and compliance [12]. The chosen galenic form (capsule) was welcome in an attempt to overcome this drawback. An alternative solution would have been to produce coated tablets, but this would require a higher level of technical expertise, which would also affect the price of the final product.

The result of the phytochemical screening confirmed that of Jiotsa in 2018 [6]. OsayemWenre [4] and Ouayogodé in 2021 [7] had noted the presence of indolomonoterpenic alkaloids in *Picralima nitida* fruit. The high alkaloid content observed in this preparation suggests good antiplasmodial activity.

Colloidal silica (Aerosil 300) and microcrystalline cellulose (Avicel pH 102), used as excipients to stabilize the extract, offer the additional advantages of a lubricating role and the absence of any noticeable effect [13].

The convergence of the 2 methods for determining the daily dose of total alkaloids is such as to inspire confidence in the value of the said dose.

The very low level of microbiological contamination testifies to the seriousness with which the formulation was carried out and the observance of good hygiene practices [14].

The advantage of the pillboxes chosen for packaging is the presence of a drawing substance in the cap.

5. Conclusion

The aim of the present study was to formulate anti-malarial capsules based on *Picralima nitida* fruits. At the end of the study, size 0 capsules containing 35 mg of total alkaloids were developed. They passed the pharmacotechnical and microbiological control tests recommended by the 11th edition of the European Pharmacopoeia. They could be improved with a view to entering the MTA visa procedure.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Yasri S, Wiwanitkit V. Artemisinin resistance: an important emerging clinical problem in tropical medicine. International Journal of Physiology, Pathophysiology and Pharmacology.2021, 13(6):152-7.

[2] Pierre S, Vroumsiatoua, Tchoibalsan, Fohouo T, Nloga A, Michel A, et al. Medicinal plants used in traditional treatment of malaria in Cameroon. Journal of Ecology and National Environmental.2011. 3: 104-17.

[3] Bikii J, Tchouya GRF, Tchouankeu JC, Tsambo E Antimalarial activity in crude extracts of some Cameroonian medicinal plants. African Journal Traditional Complement. 2007., 4(1): 107-111.

[4] Osayemwenre E, Abiodun F, Langer P. Medicinal uses, phytochemistry and pharmacology of Picralima nitida (Apocynaceae) in tropical diseases. Asian Pacific Journal of Tropical Medicine.7: 1 - 8.

[5] Awodele O, Coulidiaty AGV, Afolayan GO, Agagu S, Omoseyindemi B, Busia K Toxicological evaluation of Picralima nitida in rodents. Journal Ethno-pharmacology 2019. 236: 205-19.

[6] Jiotsa B Study of the effects of the aqueous extract of the fruits of Picralima nitida (Apocynaceae) on malaria infection induced in rats. Doctoral thesis. Pharmacy. Université des Montagnes, Bangangté 2018.

[7] Ouayogodé A, Kouakou L, Ahmont L, Adiko M, Djadji T, Kouakou SG, Bamba D, Champy P. Micrographic study and evaluation of the in vivo antiplasmodial activity of the fruit of Picralima nitida (Stapf) T.Durand & H. Durand (Apocynaceae). African Journal of Traditional Medicine. 2021; 20(2): 01-08.

[8] Development of Garcinia kola capsules. E. Djoko, W. Lanty, B. Ngantchouko and J M. Tekam . International Journal of Biological and Pharmaceutical Sciences Archive. 2021. 02(01), 184–193

[9] Anroop B, Shery J Simple practice guide for dose conversion between animals and human Journal of Basic and Clinical Pharmacy. 2016. 7; 27-30.

[10] Ph. Eur. European pharmacopoeia .2022 - 11th edition

[11] Chougouo-Nkuitchou R, N'Guessan1 A, Nkamenjo C, Djoko E, Kouamouo J, Tane P, Koffi A, Wouessidjewe D . Formulation of capsules of based on the powder of leaves and stems of Artemisia annua, Health Science and Disease. 2011. Vol 21 (4)

[12] Dibong SD, Mpondo Mpondo E, Ngoye A, Kwin MF, Betti JL. Ethnobotany and phytomedicine of medicinal plants from Douala, Cameroon. Journal of Applied Biosciences 2011. 37, 2496 – 2507

[13] Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 2006 6th edition. Pharmaceutical Press; 888p

[14] Antonio-Nkondjio C, Ndo C, Njiokou F, Bigoga JD, Awono-Ambene P, Etang J, et al. Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination. Parasite Vectors.2019. 12 (1): 501.