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Chemical characterization and study of the anti-plasmodial activity of the herbal tea « palustop » from medicinal plants of Côte d'Ivoire

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Abstract
This study is a contribution to the valorization of medicinal plants from Ivorian flora and the traditional medicines derived from them. The objective is to determine the chemical composition and study the anti-plasmodial activity of the herbal tea "palustop" which is used in Côte d'Ivoire to treat malaria. To achieve this, we made two extracts, aqueous and hydro-ethanolic at 70%. Colorimetric tests of the aqueous extract were performed to determine the major chemical families present in « palustop ». Then the aqueous and hydro-ethanolic extracts were tested against twelve clinical isolates of *Plasmodium falciparum*. Finally, a microbiological quality analysis of the aqueous extract of « palustop » has been conducted.
The chemical analysis of the aqueous extract revealed the presence of quinones and saponosides, as well as a significant presence of alkaloids, sterols, and polyterpenes. The aqueous and hydro-ethanolic extracts of « palustop » were effective against 9 and 8 of the tested isolates, respectively. Specifically, the inhibitory concentrations of 50% of the aqueous extract range from 0.16 µg/mL to 39.48 µg/mL. And those from the hydroalcoholic extract range between 4.17 µg/mL and 92.10 µg/mL.
The aqueous extract has a better anti-plasmodial activity with an average inhibitory concentration of 50%, IC_{50} = 11.54 µg/mL, while that of the hydroethanolic extract is 16.37 µg/mL. The microbiological analysis revealed that there are no colonies of germs in the tested sample.
This good anti-plasmodial activity and the absence of germ colonies would be justified among other things by the secondary metabolites present in the extract. The herbal tea "palustop" could be an excellent alternative in the fight against malaria in Côte d'Ivoire. Following this study, a labeled packaging has been proposed for « palustop ».

Keywords: Herbal tea, palustop, anti-plasmodial, Côte d'Ivoire

Introduction
Medicinal plants are widely used by populations for their basic health care [1]. They play an important role for the health of populations in several countries [2]. Moreover, in Côte d'Ivoire, traditional medicine has today become a component of health policy [3]. Then, given the role that traditional medicines derived from medicinal plants play in the public health program, health authorities and the population attach importance to their safety, effectiveness, and quality [4].
Plant-based medicines are used for virtually all pathologies, including malaria. It is an infection caused by *Plasmodium falciparum*, a common microbial species in the tropics, responsible for the most severe form of the disease [5]. Malaria is endemic in Côte d'Ivoire and all the population is exposed to it. This country is among the ten countries with the highest rates of malaria cases and deaths. In 2020, globally, 3.1% of cases and deaths were reported, along with 2.5% of global deaths and 6.5% of malaria cases in West Africa. The estimated number of cases increased by 10.4% between 2017 and 2020 according to USAID [6] and WHO [7]. This raises real concern. Although many medical treatments exist against this disease, resistance to the most commonly used molecules has been observed in many regions [8].
In addition, there is the economic precariousness of the populations, which promotes the regular use of medicinal plants in the treatment of malaria. Indeed, several traditional preparations or medicines are widely used by the Ivorian populations, among which is « palustop », one of the herbal teas.
The general objective of this study is to characterize chemically and evaluate *in vitro* anti-the plasmodial activity of the herbal tea « palustop » on twelve clinical isolates of *Plasmodium falciparum*.

2. Materials and Methods

2.1 Materials

2.1.1 Plant Material

The study was conducted using 500 g of the herbal tea powder "palustop" purchased from the market in Bingerville.

2.1.2 Biological Material

The biological material consists of twelve clinical isolates of *Plasmodium falciparum* from the Research and Fight Against Malaria Center (CRLP) at the National Institute of Public Health (INSP).

2.2 Method

2.2.1- Extraction of phytocompounds

1. Obtaining the aqueous crude extract by maceration

Fifty grams (50g) of powder were weighed and poured into a flask containing 1000mL of distilled water. The mixture was vigorously stirred using a magnetic stirrer for 24 hours. The filtrate was dried in an oven set at 40°C for 72 hours to obtain the aqueous crude extract.

2. Obtaining the hydroethanolic crude extract by maceration

Fifty grams (50g) of powder were weighed and poured into a flask containing 1000mL of 70% ethanol and then vigorously mixed using a magnetic stirrer for 24 hours. The resulting mixture was successively filtered through hydrophilic cotton and Whatman filter paper (grade 3). The obtained filtrate was allowed to evaporate for 48 hours in a dry oven set at 40°C. The powder collected after drying constituted the hydroethanolic extract.

3. Phytochemical sorting

The search for the main secondary metabolites in the extracts was carried out using specific chemical tests for each family of compounds [9-13]. Alkaloids were detected with Dragendorff and Büchard reagents, saponins by observing persistent foam after agitation, and sterols and polyterpenes by the Libermann reaction (appearance of a colored ring). Polyphenols were highlighted by ferric chloride, flavonoids by cyanidin, catechin tannins by Stiasny's reagent, and quinones by Bornstraëgen's reagent.

4. Evaluation of the anti-plasmodial activity of the extracts

The parasitemia of the crude extracts and the anti-plasmodial controls, quinine and lumefantrine, against isolates of Plasmodium falciparum, was measured according to the SYBR Green method [14-16]. To do this, the parasites were cultured in human red blood cells (2% hematocrit, 0.5-1% parasitemia) and exposed to different concentrations of antimalarials in a 96-well plate, with controls. After 72 hours of incubation, the parasites were lysed and stained with SYBR Green, which intercalates with their DNA. The fluorescence was measured using a spectrofluorometer (λ = 535 nm) and the antiplasmodial activity, expressed as IC_{50}, was determined [17].

5. Microbiological analysis of « palustop »

The search for the microbiological quality of palustop was carried out using diffusion or dilution methods. A 1 mg/ml raw extract was prepared in sterile distilled water and then diluted in buffered peptone water to obtain a mother solution (MS). Four successive dilutions at the 10th of 10^{-1} to 10^{-4} were made from the MS according to the AFNOR standard [18]. The microbiological analysis, conducted according to AFNOR and ISO standards, focused on total mesophilic aerobic flora, total and thermotolerant coliforms, yeasts and molds, Staphylococcus aureus, and salmonellae [19-23]. Counting was performed only on plates containing between 30 and 300 colonies, and the results were expressed in the number of microorganisms per mL or g of sample [24]. The microbiological quality was then assessed by comparing these results to the reference criteria of the European Pharmacopoeia for herbal medicines [25].

3. Results and Discussion

3.1 Yield of the crude extract

The yield of the different aqueous and hydro-alcoholic extracts of the « palustop » herbal tea are respectively 3.10% and 3.58% (**Table 1**). The hydro-ethanholic extract has a slightly higher yield than that of the aqueous extract.

Table 1: mass of the samples and yield of the extractions

Extracts	maqueous (± 0.01g)	mhydro-ethanolic (± 0.01g)
Fine powder used for extraction	50	50
Extract after solvent evaporation	1.55	1.79
Extraction yield (%).	3.10	3.58

3.2 Phytochemical analysis

Chemical analysis through colorimetric tests of the aqueous extract of « palustop » revealed the presence of 4 classes of secondary metabolites (Table 2). The presence of quinones and saponins was identified, alongside a strong presence of alkaloids, sterols, and polyterpenes, while flavonoids and tannins were absent.

Indeed, following the Liebermann test, the appearance at the interphase of a purple ring that turned blue and then green indicated the presence of sterols and polyterpenes. Then, the Dragendorf test produced an orange coloration, and the addition of a few drops of Bouchard's reagent caused a brown-reddish coloration, indicating the presence of alkaloids in the extract. Furthermore, the presence of saponins was revealed by the appearance of persistent foam with a height greater than 1 cm. Finally, the Borntraeger test highlighted the presence of quinones.

Table 2: Result of the phytochemical screening of the herbal tea « palustop »

Sample / Compounds		Extrait aqueux de « palustop »
Sterols and polyterpenes		+
polyphenols		-
Flavonoïdes		-
Tannins	gallotanins	-
	Catechin	-
Quinones		+
Alkaloïds		+
Saponosides		+

(-) = negative reaction; (+) = positive reaction (presence)

Overall, secondary metabolites are recognized as biologically active compounds. Several authors have highlighted their antimicrobial activities [26-29]. They are therefore widely used in medicine. The presence of these secondary metabolites in the « palustop » herbal tea could justify its use for healthcare, especially against malaria.

3.3 Chemo-sensitivity

The values of the 50% inhibitory concentrations (IC_{50}) of the aqueous and hydroethanolic extracts of « palustop » herbal tea and those of reference molecules (quinine and lumefantrine), obtained from the chemo-sensitivity test on 12 isolates of *P. falciparum*, are presented in Table 3.

With the aqueous extract, nine isolates yielded analyzable results. These isolates were sensitive with IC_{50} values ranging from 0.16 µg/mL to 39.48 µg/mL. This extract had a better anti-plasmodial profile with an average IC_{50} of 11.54 µg/mL. It had a strong effect against six isolates: isolate 1, isolate 2, isolate 8, isolate 9, isolate 10, and isolate 12. Additionally, it had a moderate effect against three isolates: isolate 4, isolate 6, and isolate 11. According to Bero et *al.* [30] and Jansen et *al.* [31], an anti-plasmodial extract is deemed inactive if its IC_{50} > 50 µg/mL, has a moderate effect for an IC_{50} between 15 and 50 µg/mL, shows promising effects when the IC_{50} is between 5 and 15 µg/mL, and is very active, showing a powerful effect for an IC_{50} < 5 µg/mL.

As for the hydro-alcoholic extract, it was effective against eight isolates with an average inhibitory concentration of 50% of 16.37 µg/mL. It therefore has a promising effect overall. But in particular, it is very active against two isolates, isolate 1 and isolate 5; then it has a promising effect against isolate 11 and isolate 12. Finally, the hydro-alcoholic extract has a moderate effect against isolate 4, isolate 6, isolate 7, and isolate 8. This extract was inactive against isolate 3, isolate 9, and isolate 10, which could be due to handling errors considering the good results on the other isolates.

Table 3: IC_{50} values of the extracts from palustop herbal tea and reference molecules on 18 isolates of P. falciparum.

	Inhibitory concentration of 50% (IC_{50})			
Extracts / Isolates	Aqueous (µg/mL)	Hydro-ethanolic (µg/mL)	Quinine	Lum.
Isolate 1	1.74	4.48	91.50	17.48
Isolate 2	1.65	-	8.76	-
Isolate 3	-	93.3	1.58	1.98
Isolate 4	39.48	15.25	-	9.85
Isolate 5	-	4.17	-	3.30
Isolate 6	20.05	21.26	17.80	4.72
Isolate 7	-	19.28	9.29	0.66
Isolate 8	4.28	41.06	1.69	-
Isolate 9	0.91	91.50	8.76	-
Isolate 10	0.93	92.10	8.82	-
Isolate 11	34.66	12.96	25.68	91.50
Isolate 12	0.16	12.50	1.82	1.68
Average of IC_{50}	11.54	16.37	19.46	20.13

Lum.: lumefantrine

The aqueous extract of « palustop » herbal tea is effective against all isolates. The presence of secondary metabolites in palustop extracts could justify this anti-plasmodial activity. Notably, the presence of alkaloids which are a reference in terms of effectiveness against malaria. Quinine and its derivatives (dichlorohydrate of quinine) are recognized for their very strong antimalarial activity. Similarly, sesquiterpene derivatives such as artemisinin or artemether are recommended in the treatment of malaria [5].

3.4 Microbiological quality

The microbiological analysis revealed that there are no colonies of germs (Table 4), particularly of mesophilic aerobic germs, fecal coliforms, gram-negative bacteria resistant to bile salts, *Escherichia coli*, yeasts, and molds. Furthermore, Salmonella and *Staphylococcus aureus* are absent from the sample. This absence of germs could be explained by the presence of secondary metabolites in the extract. These could provide a defense against germ colonization. Additionally, there would be very good hygiene and preservation conditions for the sample.

Table 4: Microbiological quality of the aqueous extract of « palustop ».

Germs	Results	Standard (French Pharmacopoeia 10th edition)
Mesophilic aerobic germs	0	< 104 UFC/mL
Fecal coliforms	0	< 20 UFC/mL
Gram-negative bacteria resistant to bile salts	0	< 20 UFC/mL
Escherichia coli	0	< 2 UFC/mL
yeasts	0	≤ 100 UFC/mL
Molds	0	≤ 1 UFC/mL
Salmonella	Absence	Absence/ 25mL
Staphylococus aureus	absence	Absence

UFC: unit forming colony

In summary, the oral administration preparation sample complies with the microbiological standards of the French pharmacopoeia 10th edition, concerning oral preparations containing raw materials of natural origin.

4. Conclusion

This report focused on the chemical and biological study, the anti-plasmodial activity and microbiological quality, of the herbal tea « palustop » used in Côte d'Ivoire against malaria by the population. The chemical study revealed the presence of four major families of secondary metabolites, such as quinones, sterols, polyterpenes, saponins, and alkaloids. Regarding anti-malarial activity, the hydroethanolic and aqueous extracts of the herbal tea showed good activity against the growth of clinical isolates of *Plasmodium falciparum*. The aqueous extract exhibited better anti-plasmodial activity with a mean inhibitory concentration of 50% (IC_{50}) equal to 11.54 µg/mL. while that of the hydroalcoholic extract is 16.37 µg/mL. In terms of microbiological analysis, the absence of germ colonies was demonstrated. This sample would not pose a risk.

The herbal tea « palustop » could be an excellent alternative in the fight against malaria in Côte d'Ivoire. Given this effectiveness of the herbal tea, the identification of plants and/or parts of plants used to make « palustop » would be feasible, as well as the determination of minerals present in « palustop ». It would be appropriate to propose a stable galenic formulation in the development of an Improved Traditional Medicine (ITM) for malaria treatment, as well as to evaluate the toxicity of the extracts and their antiplasmodial activity in vivo in mice.

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6. Declarations

1. Conflict of interest

The authors declare no competing interests.

2. Ethical approval
This article does not contain any studies with human participants or animals performed by any of the authors.

3. Consent to participate
Not applicable. This study did not involve human participants.

4. Consent to publish
Not applicable.

7. Data Availability Statement
All data generated or analysed during this study are included in this published article.

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