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Research Article

Phytochemical Screening of Some Medicinal Plants in Côte D'ivoire and Evaluation of their Extraction Efficiency

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ABSTRACT

Malaria, the most devastating disease in the tropical region, continue to be the subject of research work by many researchers because of the increasing resistance of the malaria parasite to antimalarial drugs sold in the market . These researches aim at finding effective curative drugs and especially affordable to the population of which majority are very poor mainly in the developing nations. Our objective also is to discover new active natural compounds effective on *Plasmodium falciparum*. It consisted of screening for the phytochemical composition of eight plants traditionally used to treat malaria in the Region of Toumodi in central Côte d'Ivoire. These are *Anthocleista djalonensis* (Loganiaceae), *Cochlospermum planchonii (*Cochlopermaceae), *Harungana madagascariensis* (Hipperiacaceae), *Hoslundia opposita* (Lamiaceae), *Mangifera indica* (Anacardiaceae), *Margaritaria discoidea* (Euphorbiaceae), *Pericopsis laxiflora* (Fabaceae) *and Terminalia glaucescens* (Combretaceae). Samples consisted of leaves and bark of plant, were harvested, dried and then extracted with solvents of increasing polarity (water, ethanol, and methanol). The obtained powders were subjected to quantitative phytochemicals analysis based on reactions of coloration and precipitation. The results showed that the extraction efficiency varies between 6.3% and 12.1% for the aqueous extract, 8.8% to 20.1% ethanolic extract and 5% to 21.2 % for the methanolic extract. This extraction efficiency is not significantly different from each other whatever the solvent and the plant (P> 0.05). The phytochemical analysis showed that polyphenols are present in 23 of the 24 extracts that is a rate of 95.8%, 95.8% for the alkaloids, 79.2% for the flavonoids, 62.5% for sterols and polyterpenes and 25% saponins and tannins in a small proportion of 16.7% for gallic tannins and 8.3% for catechin tannins. The abundance of chemical compounds of which their pharmacological activities have been shown by other authors, make us to conclude that our plants are very good sources of natural molecules for the fight against malaria and other emerging diseases in our country.

Keywords: Côte d'Ivoire, Phytochemical, *Plasmodium falciparum*, extraction efficiency.

INTRODUCTION

The African flora is well endowed with large reserve of medicinal and aromatic plants. Thus, medicinal plants play an important role in primary health care. Today, many medicinal plants are used to cure diseases such as malaria, jaundice, high blood pressure and others[1]. Meanwhile about 10% of these impressive plants reserve are being studied for their pharmacological properties[2,3]. The frequent use of these plants and satisfactory results obtained[4], have led some African countries to conduct further research study for the enhancement of traditional medicine. Thus, since independence, Côte d'Ivoire has supported and adopted several international resolutions on the development of traditional medicine at World Health Organization assemblies including the Declaration of Alma-Ata in 1978 and the Bamako Initiative adopted in 1987 and the Declaration of Heads of State of the African Union in 2001 declaring year 2001-2010 the "Decade of Traditional Medicine in Africa". The current policy of the Ivorian government is to integrate traditional medicine into national health systems in other to improve health care service through quality care for the service of the population. But this cannot be achieved without a proper participation of scientific researchers in order to develop an improved traditional medicine (ITM). In this context, the phytochemical analysis study was to determine the chemical constituents in most of these plants. One of the tools of these investigations is the knowledge of phytochemical analysis capable of detecting the presence of chemical groups responsible for pharmacological activities in a given drug[5]. Phytochemical analysis plays an essential role in the characterization of chemical groups in a plant. In Cote D'Ivoire several studies have been conducted on the phytochemical constituentsof plants. The objective of those studies was to characterize the chemical groups present in those plants therefore explain the therapeutic effects of medicinal plants[6-8]. The present study focused on eight plants that are traditionally used to treat malaria mainly in central Côte D'Ivoire[9]. These are *Anthocleista djalonensis* (Loganiaceae), *Cochlospermum*

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Table 1: Data on 8 anti-malaria plants according to 9 traditional healers in Toumodi

Scientific Name	Family	Part used	Method of preparation	Method of administration
Anthocleista djalonensis	Loganiaceae	Bark	Kneading	Purge
Cochlospermum planchonii	Cochlopermaceae	Leaves	Decoction	Drinking
Harungana madagascariensis	Hipperiacaceae	Bark	Kneading	Purge
Hoslundia opposita	Lamiaceae	Leaves	Decoction	Drinking
Mangifera indica	Anacardiaceae	Bark	Kneading	Purge
Margaritaria discoidea	Euphorbiaceae	Bark	Kneading	Purge
Pericopsis laxiflora	Fabaceae	Bark	Kneading	Purge
Terminalia glaucescens	Combretaceae	Leaves	Decoction	Drinking

*planchonii (*Cochlopermaceae), *Harungana madagascariensis* (Hipperiacaceae), *Hoslundia opposita* (Lamiaceae), *Mangifera indica* (Anacardiaceae), *Margaritaria discoidea* (Euphorbiaceae), *Pericopsis laxiflora* (Fabaceae) *and Terminalia glaucescens* (Combretaceae). The abundance of research reports and information collected through our ethnobotanical survey make us confirm that these plants contain many chemical compounds capable of manifesting various pharmacological activities including anti-malaria[10-13]. Among the desired compounds, we are particularly interested in alkaloids, flavonoids, sterols, polyphenols and tannins, due to their therapeutic properties[14]. Thus, the objective of our study was to contribute to the phytochemical screening of the 8 plants that are mostly used in the traditional treatment of malaria in Côte d'Ivoire.

Table 2: Extraction yield based on solvent

	Yield (%)		
Plante	Aqueous extract	Ethanolic extract	Methanolic extract
B. Indima	12,4	20,1	13,6
B. Laper	8,1	12,2	10,2
L. Dismar	6,3	13,2	7,4
B. Madhar	10,4	12,4	7,2
L. Placo	7,7	19,4	14,2
L. Djantho	7,5	8,8	5
L. Glauter	12,2	9,13	21,2
B. Glauter	9,1	ND	10,4
H. opposita	11,08	ND	ND
Average yield	9,42±2,19	13,6±4,51	11,15±5,14

ND: Undeterminated ; B. Indima : Bark of *Mangifera indica*; B. Laper : Bark of *Pericopsis laxiflora*; L. Dismar : Leaves of *Margaritaria discoidea*; B. Madhar : Bark of *Harungana madagascariensis*; L. Placo : Leaves of de ; L. Djantho : Leaves of *Anthocleista djalonensis* ; L. Glauter : Leaves of *Terminalia glaucescens*; B. Glauter : Bark of *Terminalia glaucescens*; *H. opposita* : *Hoslundia opposita*.

MATERIALS AND METHODS

Presentation of the study area

Our study took place in Moronou, a town in central Côte d'Ivoire located 30 Km to Toumodi. The Department of Toumodi covers an area of approximately 2837 km^2. It is located in the southern part of the region traditionally called the V Baoulé and is the Aries region whose administrative center is Yamoussoukro. It is bounded to the north by the region of Yamoussoukro, to the east by the region of Dimbokro, south by the region of Taabo and west by the region of Oumé. The village of Moronou with an estimated population of 4000 inhabitants is a subset of the Baoulé ethnic (Akan people) called the N'gban. Courageous farmers, the people of Moronou cultivate Coffee, Cocoa (cash crops) and food crops (plantain, yams, peppers, tomatoes, okra etc.). Their climate is equatorial transition between the Sudanese and Guinean climates called baouléen climate[15].

Ethnobotanical study

The investigation on the traditional use of plants having antimalarial effect was conducted with 9 traditional healers in the village of Moronou. Ethnobotanical survey focused on the local name of the plant, method of preparation, traditional use, and mode of administration of drug. The objective of this survey was to identify plants used locally as anti-malaria and / or antipyretic in order to look for new anti-malarial molecule and to confirm, on a scientific basis, the validity of traditional use of the plants.

Plant material

Eight medicinal plants from ethnobotanical survey were used in this study. These plants were harvested on site at various locations and forwarded to the National Floristic Center of Felix Houphouet Boigny University for identification (Table 1).

Preparation of extracts

In the extraction of a plant the aim is to obtain the highest possible amount of a substance as pure as possible, by acting on the plant suitable extractive solvents. For this study, three solvents have been used because of their increasing polarity: they are distilled water, ethanol and methanol. For the aqueous extraction, 100g of drug powder was extracted with 1 liter of distilled water by homogenization in a blender. After 2 cycles of homogenization, the homogenate obtained was collected in a clean square of fabric and hand-pressed by applying pressure. The collected solution was filtered twice through cotton wool and then Whatman3 mm filter paper. The resulting solution is dried in an oven type HERAEUS® at the temperature of 50 °C for 3 days. The ethanolic extract was prepared in the same method, but instead of water an alcohol-based solvent was used consisted of 30% distilled water and 70% pure ethanol[16]. For the methanolic extract, 50g of drug powder was dropped in 1.5 liter of pure methanol by homogenization in a blender. The subsequent steps are identical to those of the aqueous extraction[17]. All extracts obtained were concentrated and then weighed to know the yield (R) of each extraction. Finally, the extracts were stored away from light at 4 ° C.

Phytochemical screening

Table 3 :Phytochemical screening of 24 extracts of plant

Plants	Extracts	Sterols	Poly-phenols	Flavo-noids	Tannins		Quinones	Alkaloids		Saponins
		Polyter-penes			Gal	Cat		D	B	
B. Indima	EthOH	-	+	+	-	-	+	+	+	-
	MeOH	+	+	+	+	-	+	+	+	-
	Aq	-	+	+	+	-	-	+	+	-
B. Laper	EthOH	+	+	+	-	-	-	+	-	-
	MeOH	+	+	+	-	-	+	+	-	-
	Aq	-	+	-	+	-	-	+	-	+
L. Dismar	EthOH	+	+	+	-	-	+	+	+	-
	MeOH	+	+	+	-	+	+	+	+	-
	Aq	+	+	+	-	+	+	+	+	+
B. Madhar	EthOH	+	+	+	-	-	-	+	+	-
	MeOH	+	+	+	-	-	+	+	+	-
	Aq	-	+	+	-	-	-	+	+	-
L. Placo	EthOH	+	+	+	-	-	-	+	+	-
	MeOH	+	+	+	-	-	-	+	+	-
	Aq	-	+	+	-	-	-	+	+	-
L. Djantho	EthOH	+	+	+	-	-	-	-	-	-
	MeOH	+	+	+	-	-	+	+	+	-
	Aq	-	-	-	-	-	+	+	+	-
L. Glauter	MeOH	+	+	-	-	-	+	+	+	-
	Aq	-	+	+	+	+	-	+	+	+
B. Glauter	MeOH	+	+	-	-	-	+	+	+	-
	Aq	-	+	+	-	-	-	+	+	+
H.Opposita	EthOH	+	+	-	-	-	+	+	+	-
	Aq	-	+	+	-	-	-	+	+	+

+: Presence; -: Absence; Aq: Aqueous extract; EthOH: Ethanolic extract; MeOH: Methanolic extract; B. Indima : Bark of *Mangifera indica*; B. Laper : Bark of *Pericopsis laxiflora*; L. Dismar : Leaves of *Margaritaria discoidea*; B. Madhar : Bark of *Harungana madagascariensis*; L. Placo : Leaves of de ; L. Djantho : Leaves of *Anthocleista djalonensis* ; L. Glauter : Leaves of *Terminalia glaucescens*; B. Glauter : Bark of *Terminalia glaucescens*; *H. opposita* : *Hoslundia opposita*.

Different groups of compounds (sterol, polyterpenes, alkaloids, tannin, polyphenols, flavonoids, saponins and quinones) present in the extracts were highlighted by qualitative methods[6,18].

Sterols and polyterpenes

Table 4: Cumulative presence rate of chemical group in plant extract

Chemical Group	Extract (N)	Cumulative presence rate (%)
N	24	100
Sterols/polyterpenes	15	62.5
Polyphenols	23	95.8
Flavonoids	19	79.2
Gallic Tannins	4	16.7
Catechin tannins	2	8.3
Quinone substances	12	50.0
Alkaloids (D)*	23	95.8
Alkaloids (B)	20	83.3
Saponins	6	25.0

*D : Dragendorff ; B : Bouchardat

To highlight sterols and polyterpenes, we used the reagent LIEBERMANN. Thus, five (5) mL of each of the ten (10) plant extracts was evaporated to dryness in a capsule on sand bath or water bath. The residue was dissolved in 1 mL of hot acetic anhydride. We added 0.5 mL of sulfuric acid concentrated in triturate.

The appearance at the interphase, of a purple and purple ring, turning to blue then green, indicates a positive reaction. This test was performed with a chlorophormic solution as witness cholesterol or sitosterol.

Polyphenols

To highlight the polyphenols, reaction with ferric chloride ($FeCl_3$) was used. Thus, to 2 mL of each extract solution is added a drop of alcohol solution of 2% ferric chloride. Ferric chloride causes in the presence of polyphenol derivatives the appearance of blackishblue or green more or less dark color. The control is performed with the alcoholic solution of gallic acid.

Flavonoids

Tohighlight flavonoids, "cyanidin" reaction was used. (2) mL of each extract was evaporated and the residue was taken up in 5 mL of alcoholic hydrochloric diluted 2 times. By adding 2-3 magnesium chips, there is an exothermal

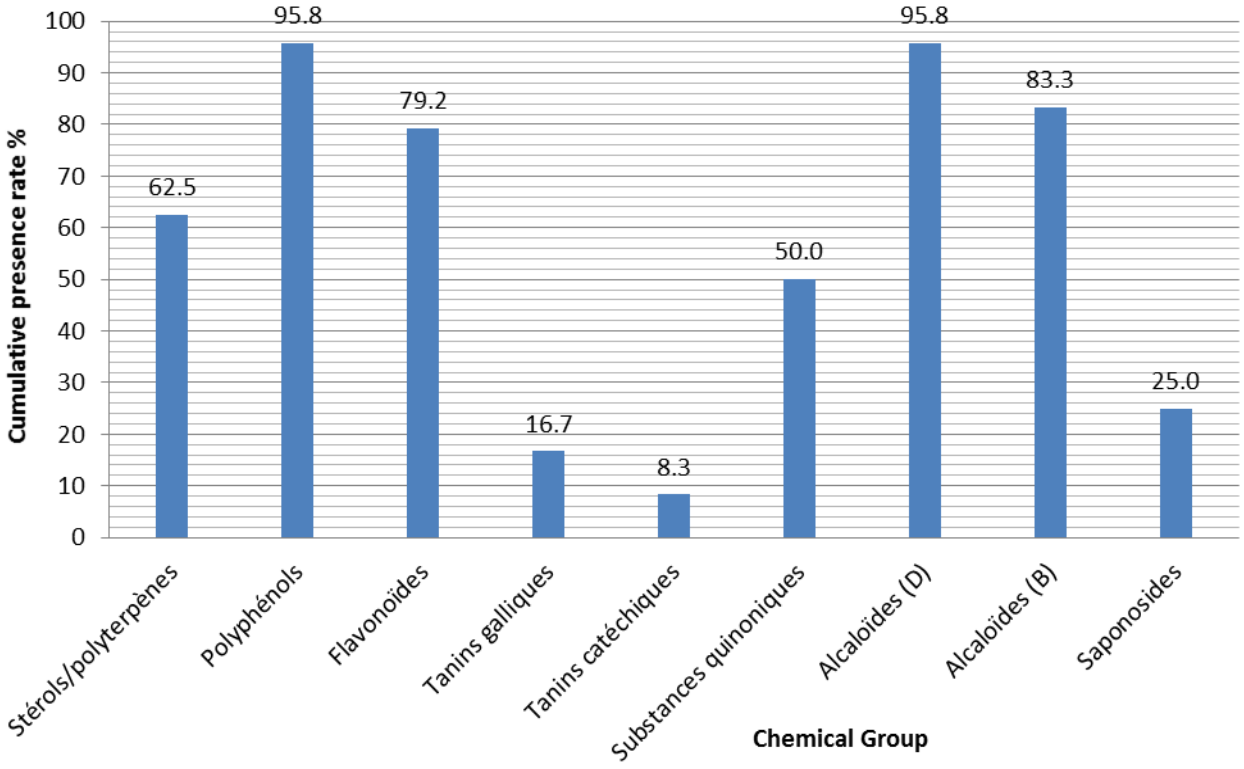

Figure 1 : Cumulative presence rate of chemical group in plant extract

heating and then a pink-orange or violet. The addition of 3 drops of isoamyl alcohol has intensified this coloration which confirmed the presence of flavonoids. An alcoholic solution of quercetin was used as control.

Tannins

The catechin tannins are identified by the STIASNY reagent (formalin 30%, HCl concentration: 1 / 0.5). Five (5) mL of each extract was evaporated to dryness. After adding 15 mL of Stiasny reagent to the residue, the mixture was kept in a water bath at 80 ° C for 30 min. The observation of a precipitate in large flakes characterizes catechin tannins. Gallic tannins are identified by adding $FeCl_3$. Indeed, we have filtered the previous solution. The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of 2% $FeCl_3$ causes the appearance of a deep blue-black color indicating the presence of gallic tannins. An alcoholic gallic acid or catechin solution is used as a control.

Quinone substances

To highlight the free or combined quinone substances, we used the reagent BORNTRAEGEN. Indeed, two (2) mL of each of the extracts were evaporated to dryness. The residue was triturated in 5 ml of hydrochloric acid 1/5. The triturate is then carriedin a water bath for 30 min. After cooling, it is extracted with 20 mL of chloroform. Ammonia diluted 2-times (0.5 mL) was added to the chloroform solution. A red or purple color appeared the sign of the presence of quinones.

Alkaloids

To confirm alkaloids, Dragendorff reagent (reagent for iodobismuthate) and Bouchardat (iodoioduré reagent) were used. Thus, six (6) mL of each solution were evaporated to dryness. The residue is taken up in 6 mL of alcohol at 60 ° C. The addition of 2 drops of Dragendorff reagent to the alcoholic solution causes a precipitate or an orange color. The addition of 2 drops of reagent Bouchardat to the alcoholic solution causes a precipitate of reddish-brown color which indicates a positive reaction.

Saponins

To highlight the saponins, we introduced 10 mL of each of the aqueous extracts in a test tube. The tube is stirred for 15 seconds and allowed to stand for 15 minutes. Persistent foam, height greater than 1 cm indicates the presence of saponins.

The total phenols

The contents of total phenols in plants were determined by the Folin-Ciocalteu method. A volume of 0.5 mL of each plant extract (0.1 g / mL) or gallic acid (0.1 mg / mL) was mixed with 5 mL of diluted Folin-Ciocalteu reagent diluted by 1 / 10th of distilled water and 4 mL of sodium carbonate (1 M). Gallic acid is the reference antioxidant. After 15 minutes of incubation at room temperature (25 °C), the absorbance is read in a spectrophotometer at 765 nm. The standard curve is obtained under the same conditions as above using a range of concentrations (0- 250 mg / L) of gallic acid solution prepared in methanol. The total phenols content in plant extracts are determined graphically and expressed in terms of equivalents of gallic acid (mg / g dry matter).

Calculating the extraction yield

The extraction yield (Y) is the ratio between the amounts of extract (E) obtained on the amount of initial plant fine powder (FP). Or Y (%) = E / FPx100.

Statistical analysis

The data obtained were processed using SPSS 20.0 software. The non-parametric Kruskal-Wallis test was

used to analyze the bond between the solvent and the presence or absence of chemical groups in the various extracts.

RESULTS

Extraction efficiency (Yield)

Our work has focused on nine (9) plants powder from either bark, or leaves of eight (8) antimalarial plants. For better typographic use, these extracts were coded. Each plant fine powder was subjected to three (3) extractions, aqueous, ethanol and metanolic extraction. With three data, we have obtained 24 extracts finally, in which we have codedtheir names for a better utilization with 9aqueous (37.5%), 7 ethanolic (29.16%) and 8 methanolic (33.33%). Extractions gave an average yield of 9.42% (± 2.19) for the aqueous extract, 13.60% (± 4.51) for the ethanolic extract and 11.15% (± 5.14) for the methanolic extract (Table 2)

Phytochemical Screening

The results of the phytochemical screening are summarized in Table 3. The relative abundance of different chemical compounds in extracts was expressed in Table 4 and Figure 1.

DISCUSSION

In the analysis of the extraction yields, we found that the aqueous extract has a minimum yield of 6.3% while the maximum yield was 12.4%. The average yield was 9.42%. The ethanol extract has a minimum yield of 8.8% and a maximum of 20.1% for an average yield of 13.6%. The methanol extract has a minimum yield of 5% and a maximum yield of 21.2% with an average yield of 11.5%. The Kruskal-Wallis test allows us to say that the distribution of the extraction yield is the same for each plant extract. The null hypothesis (H0), which states that there is equality of extraction yield between different extract, cannot be dismissed. There is no significant difference between the extraction yields by the solvent ($p > 0.05$). However, we noted a highest yield with ethanolic extracts with an average yield of 13.6%. The methanolic and aqueous extracts are relatively less abundant with respective average yield of 11.15% and 9.42%. The choice of plant organs used is justified by the fact that the leaves, bark and roots are the site of choice for the biosynthesis and even storage of secondary metabolites responsible for the biological properties of the plant[19]. For phytochemical analysis test carried out gave the following result, a strong presence of polyphenols and alkaloids in extracts with a cumulative rate of 95.5% for each chemical group. Flavonoids and sterols/polyterpenes followed with rate of 79.2% and 62.5%, respectively. Quinone substances present in a rate of 50%. Tannins and saponins are the most poorly represented with 8.3% presence for catechin tannins, 16.7% for gallic tannins and 25% for saponins. These data showed that, in general, our extracts are rich in various chemical constituents responsible for pharmacological properties. This results show that the chemical groups are concentrated in the extracts according to the type of solvent used for extraction. Thus, there is a significant difference in the concentration of chemical compounds according to the solvent ($P < 0.05$). Our findings are supported by previous work on the phytochemical composition of some plants including the bark and leaves of *Terminalia glaucescens*, the presence of tannins, saponins and flavonoids in the leaves of *Mangifera indic*a also quinone substances in the bark of *Harungana madagascariensis*[20,21]. The strong presence of alkaloids in all extracts, mainly in the leaves of *Cochlospermum planchonii*, *Anthocleista djalonensis*, suggests interesting therapeutic perspectives. Because the alkaloids have several pharmaceutical applications in humans. These applications have been clinically proven[22,23]. The complete study of phytochemical screening among 8 plants showed the presence of other chemical compounds that also possess interesting biological activities. These are among other polyphenolic substances (tannin, flavonoids), sterols, polyterpenes and saponins. These results are in accordance with the works of other authors[24]. Flavonoids are vein actifs, that is reduce the permeability of blood capillaries and increase their resistance[14]. It also gives them some antiallergic properties, hepatoprotective and antiplasmodial[25]. Flavonoids have antioxidant, anti-inflammatory and play a vital role in the treatment of cardiovascular and neurodegenerativediseases[26]. The presence of tannins, alkaloids, saponins, steroids, flavonoids and anthraquinones in the bark extracts[27] and leaves of[28] *Terminalia glaucescens* could explain the antibacterial properties of the plant. Flavonoids include chalcones have, among others, a very interesting activity on *Plasmodium falciparum*[29-31]. In addition, it was shown that the aqueous bark extract of *Terminalia glaucescens* be active on the *in vitro* growth of Enterobacteriaceae producing beta-lactamases (ESBLs)[32]. Also, the aqueous extract of roots bark of *Terminalia macroptera* would have a very significant effect on *Plasmodium falciparum* (IC_{50} = 1µg / mL); this activity is due to the presence of saponins[33]. The extract of the leaves have activity against all strains of *Neisseria gonorrhea*[34]. According to Conrad et al. 1998[35], the isolated triterpene compounds stem bark would show biological activities (antibacterial, antifungal, anthelmintic) and hemolytic properties. Hydro-ethanol extracts of the leaves, stem bark and roots manifest remarkable antifungal activities[36]. The genus *Terminalia* would contain hydrosoluble tannins responsible for antidiarrheal activity[37,38]. Several biological activities have been attributed to polyphenols especially anticancer[39] and anti-ulcer[40,41]. *Hoslundia opposita* contain essential oils effective in the treatment of stomach pain[42]. A bioguided scanning will allow us to identify the chemical compounds responsible for the anti-malaria activity of the tested plants as reported by other authors[10-13].

CONCLUSION

In sum, extraction yields were not significantly different. However, the distribution of yield is much more homogeneous in the aqueous extract; unlikewith ethanol and methanol extracts where we noticed a dispersion of yield. The phytochemical screening performed with our 8 plants we found the presence of alkaloids, phenolic

compounds, flavonoids, tannins etc. These results corroborate to those reported by other authors. Going by the presence of many chemical group and many scientific results available, we confirm that these plants showed interesting pharmacological activities. They are therefore prime targets for expanding the range of natural products used to fight against malaria and certain diseases. This study was conducted in the context of implementation of innovative initiatives that can lead in the future to the manufacture of improved traditional medicines (ITM) for the treatment of malaria in Côte d'Ivoire. The use of more sophisticated methods of phytochemical screening would be crucial to characterize the molecules and the evaluation of anti-malaria activity of the different fractions.

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