

1 **Original Research Article**
2 **Phytochemical screening and polyphenolic**
3 **contents of *Dialium dinklagei* and *Diospyros***
4 ***monbuttensis*, two Ivorian medicinal plants**
5 **used to treat malaria**
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ABSTRACT

Introduction: Malaria is a deadly parasitic infection in its severe form. It is caused by an endoparasite protozoan transmitted by a mosquito vector. Despite considerable efforts to eradicate it, this endemic disease remains a public health problem because of its high frequency, severity and the consequences it generates. Furthermore, the existing treatments are increasingly ineffective; it is therefore urgent to find new anti-malarial drugs.

Objective: From the phytochemical screening and the determination of the polyphenolic content of *Dialium dinklagei* and *Diospyros monbuttensis* used in traditional medicine in Côte d'Ivoire to treat malaria, we identified families of chemical compounds that can guide us towards pharmacological studies. These studies allow us to identify new molecules which could be a possible alternative antimalarial reference used today because of their effectiveness in treating malaria.

Materials and methods:

From *Dialium dinklagei* and *Diospyros monbuttensis*, we obtained extracts decoction and hexane extracts, methanolic and aqueous extraction after three solvents of increasing polarity (n-hexane, methanol, water), respectively. The crude extracts obtained were tested for chemical identification of groups that may be the basis of the expected therapeutic effects. Moreover, the decoction and methanol extracts were used to determine the polyphenolic compounds.

Results:

The results vary from one plant species to another and from one sample to another. Abundant presence of alkaloids in all samples and an absence of steroids in other extract except decoction extract of *Diospyros monbuttensis* were observed. Therefore, the other chemical groups are present in all plants in varying proportions but depending on the mode of extraction. A higher content of phenols and tannins in *Dialium dinklagei* over *Diospyros monbuttensis* was observed. However, the content of flavonoids of *Dialium dinklagei* is relatively lower compared to that observed in *Diospyros monbuttensis*.

Conclusion

The overall results of the phytochemical screening and polyphenolic content would partly explain the traditional healers' infatuation to these plants as antimalarials.

Keywords: Malaria, natural substances, chemical groups, *Dialium dinklagei*, *Diospyros monbuttensis*

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10 **1-INTRODUCTION**
11

12 The high population growth, the growing impoverishment and the lack of medical facilities
13 are the major problems faced by the most of African populations [1]. Owing to this situation,
14 they do not always have access to imported pharmaceuticals. According to Sandhya et al.
15 [2] and Séguéna et al. [3], 80% of the world population relies on traditional medicine for their

primary health care that requires the use of plant extract. Indeed, the use of traditional medicine is justified by the treatment of many infections against which the plants are used as effective as drugs imported by Africa [3-5]. Secondary metabolites present in these plants are not new to the medical properties that are attributed to them. The search for these secondary metabolites could be the first step towards the determination of active ingredients [6, 7]. That is the reason why we have undertaken the phytochemical study of plants used in the Ivorian traditional medicine against malaria. In this context, *Diospyros monbuttensis* Gürk (Ebenaceae) and *Dialium dinklagei* (Caesalpinaceae) widely used by traditional healers but little studied for their antimalarial activity have been selected.

Diospyros monbuttensis leaves have been reported to possess antibacterial and antimalarial properties [8-10]. The antimicrobial activities of the plant leaves extract was due to the presence of tannins [11, 12]. Other constituents that have been reported include saponins, anthraquinones, cardiac glycosides and alkaloids [8]. *Dialium dinklagei* is a tree of Caesalpinaceae family with trunk cylindrical right. The sheets made up have an acid taste, the flowers are of color yellow and the bark is very thick [13]. Not previously reported from Ivory Coast. This species is distributed in West and Central Africa and has been reported from Angola, Cameroon, D.R. Congo, Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria and Sierra Leone [14].

The aim of this work was to investigate the correlation between the active ingredients present in these medicinal plants and their therapeutic effects, namely their use as antimalarial medicines.

2-MATERIAL AND METHODS

Plant material

The plant material consisted of leaves of *Diospyros monbuttensis* and *Dialium dinklagei*. These are two plant species, which have been little studied for their antiplasmodial activity, especially *Dialium dinklagei*. The leaves of *Diospyros monbuttensis* were collected in the month of January 2013 from Talahini-Sokoura in the Department of Sandegué (North-eastern Côte d'Ivoire). Those of *Dialium dinklagei* were collected in March 2013 in the District of Abidjan. Plants were adult and the harvest time was chosen the morning. Plants species were identified by Professor Ake-Assi from the "Centre National de Floristique", University of Felix-Houphouet-Boigny, Abidjan, Côte d'Ivoire. They were then dried in sunlight for one week at room temperature before being reduced to a fine powder by using mechanical grinder type IKAMAG. From the powder obtained after spraying the leaves, various crude extracts were prepared.

Preparation of crude extracts

First, from the fine powder obtained, decoctions of each plant were made to be as close as possible to the form of traditional use. Then we made three successive extractions with solvents of increasing polarity, according to the protocol of Beékro *et al.* [15], Zirihi *et al.* [16] and Beourou *et al.* [17]. In the following order, we used three solvents of increasing polarity: hexane, methanol and water.

Decoction

The decoction was obtained by dissolving 20g of fine dry powder in 1L of distilled water. The whole was homogenized and boiled for 30minutes. The preparation was first wrung out in a square of white cloth, and then filtered successively twice on an absorbent cotton and once on a Whatman filter paper 3mm. Filtrate 1 was thus obtained. The same operation was repeated with the residue by adding 1 liter of distilled water to obtain the filtrate 2. These two

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filtrates were gathered and evaporated in "Ventice" oven at 55°C. This series of operations led to the extract of decoction.

Extraction with hexane

A sample (50g) of fine dry powder were dissolved in 150mL of hexane, in a blender. The whole was vigorously agitated in a the blender for 10minutes. The preparation was first wrung out in a square of white cloth, and then filtered successively with an absorbent cotton and filter paper as above. The first hexane filtrate was thus obtained (hexane filtrate1). For filtrate 2, the same operation was repeated by addition of 150mL of hexane on the residue found. This activity was repeated for filtrate 3. These three filtrates were then gathered and evaporated in the "Ventice" oven at 55°C. This operation led to a hexane extract. Were the filtrates evaporated (concentrated to dryness)? If this was correct, in what solvent were they re-dissolved for the further analysis? Please, indicate this.

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Extraction with methanol

After extraction with hexane, the residual powder marc residue was dried and then extracted with . The powder obtained was recovered in 150 mL of methanol. After 10 minutes of homogenization by stirring in a blender the extract was filtered through a piece of gauze for obtaining the and filtering, methanolic filtrate 1 was obtained. The same operation was repeated twice more and allowed to obtain the methanolic filtrates 2 and the filtrate 3. All three. The 3 filtrates obtained were then pooled and evaporated in an oven "Ventice" at 55 ° C. This series of operations has led to an extract called methanol extract.

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... [2]

Preparation of the aqueous extract

The same operations were was repeated on the residual dry powder coming from the ethanolic extraction but using previous residue extract with methanolic 150mL of distilled water. We obtained the aqueous extract.

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Phytochemical screening

Phytochemical analysis was performed on the basis of tests for coloring characteristics to highlight the major chemical groups. It focused on decoction extracts, hexane, methanol and aqueous extracts of the studied plants. Various chemical groups were identified by reference to the methods described by Nemlin and Brunel [18], Békro *et al.* [15] and Bruneton [19]. The results have been classified according to: Highly positive: +++; Fairly positive: ++; weakly positive: +; Negative test: -

Saponins (Foam index)

Samples (0.1g of dry extract) were is dissolved in a test tube containing 10mL of distilled water. The samples were tube was shaken vigorously up and down for 30-45 seconds and then left for 15minutes. The height of the foam was measured. Persistent foam for more than 1cm high indicated the presence of saponins.

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Steroids: Salkowski test

Five drops of concentrated H₂SO₄ were added to 1mL of extract, the apparition appearance of a red color indicated the presence of steroids.

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Sterols and polyterpenes

Extracts (0.1g) were of extract is dissolved at hot in 1mL of hot acetic anhydride in a capsule. The resulted solutions were was poured and added with in a test tube and then 0.5mL H₂SO₄ was added. A violet coloration that turned in blue, and then in green revealed the presence of sterols and triterpenes.

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Alkaloids

Two drops of Bouchard's reagent (reagent of iodine-iodide) were added to 1mL of each extract solution. A red-brown precipitate indicated coloration meant a positive reaction.

Polyphenols

A drop of alcoholic solution of 2% ferric chloride was added to 2mL of extracts. A blue-blackish to green more or less darkish coloration indicated a positive reaction.

Anthocyanins

Five milliliters of 10% H₂SO₄ was added to 5mL of 5 % extract, and then a base (Please give the concentration and volume of the solution of this compound NH₄OH) was added. If the coloration is accentuated by acidification and then change into blue-purplish in a basic environment, anthocyanins were present.

Leucoanthocyanins

One milliliter of extract was added to 5mL of hydrochloric alcohol. The preparation was heated in the water-bath for 15minutes. A cherry red or purplish coloration meant the presence of leucoanthocyanins.

Flavonoid

In a tube containing 3mL of the solution extract, a few drops of 10% NaOH were added. Appearance of yellow-orange color indicated the presence of flavonoids.

Quinones

An aliquot Please, give how many mL of extract was dissolved in 5mL of diluted HCl (1/5) and heated in a boiling water bath for 30minutes, and then extracted with 20mL of CHCl₃ after cooling. To the organic phase was added 0.5mL of 50% NH₄OH diluted solution. The positivity of the reaction was indicated by a red to violet color indicates a positive reaction.

Cardiotonic glycosides

This reaction is exothermic. It takes place in a glacial environment. To 2mL of aqueous solution of extract were added 1mL of glacial acetic acid and then 1mL of concentrated sulfuric acid. Adding few drops of 2% FeCl₃ gave a green-bluish coloration, which is specific to cardiotonics glycosides.

Coumarins

To 5mL of extract, are added 2mL of hot water were added, and then the solution was shared between two test tubes. In the content of one, we added 0.5mL of 25% NH₄OH 25%. Under the UV light at 366nm, an intense fluorescence indicated the presence of coumarins. And, what about the other tube containing extract and hot water?

Tannins

Two milliliters of water and few drops of 1% ferric chloride were added to 1mL of extract contained in a test tube. The appearance of a blue, blue-black or black coloration indicated the presence of gallic tannins, the green or dark green coloration showed the presence of catechic tannins.

Determination of polyphenols

For the dosage of polyphenols, two extracts from each plant species were selected; they are the decoction and the methanol extracts. The polyphenol contents were calculated for the decoction and the foliar methanol extracts of *Diospyros monbuttensis* and *Dialium dinklagei*.

Determination of total phenols

The total phenolic content of plant extracts was determined by the Folin-Ciocalteu method [20], 20]. To 0.5mL of each plant extract of concentration 100mg/mL respectively were added 5mL of Folin-Ciocalteu reagent diluted 1/10 in distilled water and 4 mL of sodium carbonate (1M). The mixture whole was incubated at room temperature for 15 minutes. Then, the absorbance was registered at 765nm in a spectrophotometer against

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a blank. Gallic acid was the standard. ~~Please explain if gallic acid was used as a standard or to construct a standard curve in order to calculate the phenol contents.~~, which was prepared under the same conditions as above with a solvent mixture of methanol/water (50: 50, V/V) at concentrations ranging from 0 to 200mg/mL. Experimentations were carried out in triplicate. The total phenolic contents of the extracts were expressed as gallic acid equivalents/g dry extract (mg GAE/g dry extract). ~~Please check if dry extract is correct.~~

-Determination of total flavonoids

The technique used to determine the contents of total flavonoids in the extracts was the colorimetric method of aluminum chloride [22, 23]. And 0.5mL (100µg/mL) of each plant extract were taken, to which 1.5mL of methanol, 0.1mL of 10% aluminum chloride, 0.1mL of potassium acetate (1M) and 2.5mL of distilled water were successively added. After incubation at room temperature for 30 minutes, the optical densities were measured in a spectrophotometer at 415nm. A methanolic solution of quercetin in concentrations ranging from 0 to 100µg/mL were is used as a standard. ~~Please explain if quercetin was used as a standard or to construct a standard curve in order to calculate the flavonoid contents.~~ Experimentations were carried out in triplicate. The contents of flavonoids extracts are were expressed as quercetin equivalents/g dry extract (mg QE/g dry extract).

-Determination of tannins

The tannin contents were calculated by using the modified vanillin method of Bainbridge *et al.* [24]. This method is based on the fact that the tannic acid (especially the flavan-3-ol) in the presence of the vanillin reagent (0.1mg/mL) in an acid medium (sulfuric acid 70% (v/v)) gives a red color whose maximum absorption is 500nm. The standard solution consists of tannic acid (0.1mg/mL) prepared in 98% methanol. A range of standard was prepared with a series of 6 tubes where the first represents a blank. In the other 5 tubes are introduced respectively 0.2; 0, 4; 0.6; 0.8; 1ml of standard solution and complete the volume of each tube with 1 ml of distilled water. The test tube contains 1 ml of extract. To each of them are added 5 ml of vanillin reagent. After 30 minutes of incubation in the dark, the absorbance was read at 500nm against a blank (without extract). The tannic acid (1mg/ml) was used as the standard. ~~Please explain if tannic acid was used as a standard or to construct a standard curve in order to calculate the tannin contents.~~ Experimentations were carried out in triplicate. The results were expressed ~~in as mg of tannic acid equivalents/mL mg/mL of tannic acid equivalents.~~

Statistical Analysis

Graphical representations of data were performed using the Graph Pad Prism 5.0 software (Microsoft, USA). The test results were expressed as mean ± SD. Multiple comparisons and determination of significance level are made by the univariate ANOVA followed by Tukey test. The differences were considered statistically significant at the 0.05 level.

3-RESULTS AND DISCUSSION

Plant selection and the output of the extraction

The investigations were carried out with ethnobotanical approaches among stakeholders of traditional medicine, especially herbal traders operating on the markets of the District of Abidjan. These investigations resulted in the selection of *Diospyros monbuttensis* and *Dialium dinklagei*, widely used by Ivorian traditional pharmacopoeia and very little studied

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for their antimalarial activity. These species were mainly collected on the basis of indications by literature and information received from healers. The decoction form the 5 extractions produced the highest output (20%), and hexane was the smallest (1%). These results suggest the use of the method of extraction decoction as indicated for the preparation of extracts from these plants base (Figure 1). The very low output obtained with hexane shows that the studied plants contain very little non-polar compounds. This result is similar to the one obtained by Bekro et al. [15] (0, 75%) with *Caesalpinia benthamiana* (Baill.).

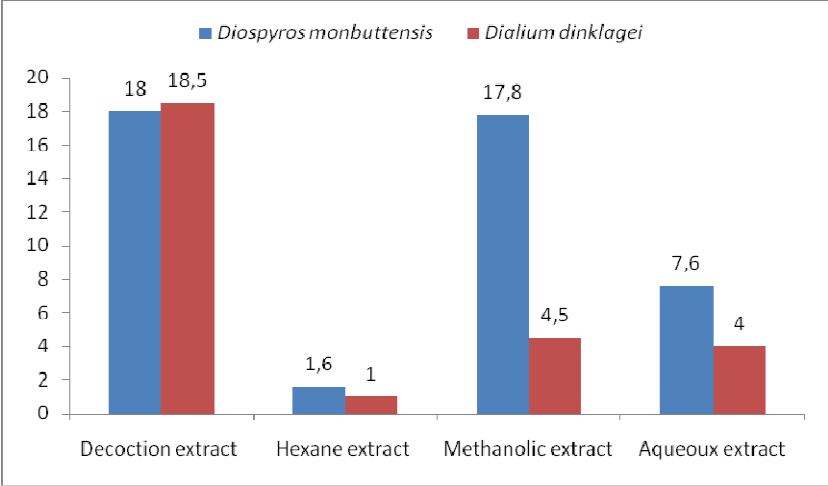


Figure 1. Yields of extracts (%)

Table 1. Results of a foliar phytochemical tests

Natural substances		Chemical groups														
Plants species																
		Parts	Extracts	Steroids	Sterols and polyterpenes	Polyphenols	Flavonoids	Anthocyanins	Leucoanthocyanins	Gallic tannins	Catechic tannins	Quinones	Alcaloids	Coumarins	Cardiotonic Glycosides	Saponins
<i>Diospyros monbuttensis</i>	Dec	+	+	+	+++	+	-	+	+++	-	+++	+	++	-	++	+
	Hex	-	-	-	-	-	-	-	-	-	-	+++	-	-	-	++
	Met	-	-	+	+++	+	+	+++	+++	++	++	+	+	+	+	++
	Aq	-	-	+	+++	+	-	+	+	+	++	+	+	-	-	+++
<i>Dialium dinklagei</i>	Dec	-	-	+++	++	++	+	+	+	++	+	+++	++	+	++	++
	Hex	-	-	++	-	-	-	-	-	+	-	+++	+	-	-	-
	Met	-	-	++	+	+++	++	+	+	-	-	+++	++	+	+	++

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Aq - - + +++ - ++ + - + +++ + - +++

Strong positive: + + +; moderately positive: + +; Low positive: +; negative test:-

~~Le= leaves~~ ~~leaves were analysed in all the cases, so it does not make sense to include a column to highlight something, which was informed in the material and methods section;~~

Dec: Decoction extract; Hex: Hexane extract; Met: Methanolic extract; Aq: Aqueous extract

Phytochemical screening

The results of the phytochemical screening carried out on the extracts of plants referred to malaria are mentioned in the Table 1. Chemical composition variations were found among the different extracts and between the two plant species analyzed

Diospyros monbuttensis

The leaves contain sterols, polyterpenes, polyphenols, flavonoids, alkaloids, saponins, leucoanthocyanins, tannins, quinones and coumarins. Anthocyanins, cardiac glycosides and steroids are present at very small quantities. These results are different from those of Anie *et al.* [9], ~~who reported with the absence of tannins and in roots in and the stem bark.~~ They ~~were~~ ~~are~~ similar to those of Bouquet and Debray [25] which showed the presence of quinones, tannins, sterols and saponins in the leaves and differ from ours by the absence of flavonoids and alkaloids. Previous studies [8] reported the presence of saponins, anthraquinones, cardiac glycosides and alkaloids. According Bouquet and Debray [25], this plant is considered to be a good remedy for stiffness, stomach ache, edema and leprosy by Baoule and Agni ethnies of Côte d'Ivoire. The antimalarial therapeutic indication may be related to the presence of alkaloids because we know that the majority of antimalarial drugs belong to the same chemical family [26]. Various studies have shown that coumarins have a high antioxidant capacity. This antioxidant activity is due to their capacity to scavenging free ~~roots, radicals~~ and chelating metal ions [27]. ~~Terpenoids analgesics and anti-inflammatories are attributed to tannins~~. Tannins also contribute to wounds healing [28].

Dialium dinklagei

The decoction of the leaves contains sterols, polyterpenes, polyphenols, flavonoids, alkaloids, coumarins and saponins in large quantities. Anthocyanins, leucoanthocyanins, tannins, quinones and cardiac glycosides are present in small quantities. There was also an absence of steroids. These results ~~were~~ ~~are~~ comparable ~~to those of~~ ~~with~~ Akoué [13], who noted a large presence of flavonoids, alkaloids, tannins and polyphenols in the ethanol extract of the leaves. Very little research has been made on this plant, but our results confirm those of Bouquet and Debray [25] about the presence of tannin in the leaves of *D. dinklagei*. Sterols, saponins, alkaloids and polyterpenes help explain its traditional therapeutic uses. In addition to their industrial applications as surfactants, traditionally saponins are widely used as detergents, pesticides and mollucides and foam, and can also have advantages on health [29].

Polyphenol content of *Diospyros monbuttensis* and *Dialium dinklagei*

The contents of total phenols in the plant extracts, determined from a calibration curve of gallic acid ($Y=0.01810X + 0.07179$; $R^2=0.9911$) showed that *Dialium dinklagei* is richer in phenols than *Diospyros monbuttensis*, whatever the type of solvent used. The highest content was obtained in the methanolic extract ~~of~~ *Dialium dinklagei*. These levels ranged from 114.1mgGAE/g and 205.1mgGAE/g (Table 2). The difference between the contents of the phenol based on the plant and solvent used is highly significant ($p < 0.0001$).

The results of the flavonoids ~~contents of the different extracts of both plants species analyzed, determined from a calibration curve of quercetin ($Y= 0.02834X + 0.01368$; $R^2= 0.9986$) showed that *Diospyros monbuttensis* is richer in flavonoids than *Dialium dinklagei*.~~

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289 The differences between the amounts of flavonoids in according to the plant species and the
 290 solvent used were significant ($p < 0.0001$). These amounts vary between 6.78mgQE/g and
 291 62.18mgQE/g (Table 2). The highest amount was obtained in the decoction extract of
 292 *Diospyros monbuttensis* (62.18mgQE/g).
 293 Tannins from different plant extracts were determined from a calibration curve of tannic acid
 294 ($Y = 0.2167X - 0.004417$; $R^2 = 0.9761$). Whatever the type of solvent used, the results
 295 showed that *Dialium dinklagei* is richer in tannins than *Diospyros monbuttensis*; the highest
 296 amount was obtained in the decoction extract of *Dialium dinklagei*. These contents ranged
 297 from 46.50mgTAE/g and 146.4mgTAE/g (Table 2). Statistically, the difference between the
 298 contents of the phenols concerning the plant species analyzed and the type of solvent used
 299 were highly significant ($p < 0.0001$).
 300

301 **Table 2. Total phenols, flavonoids and tannins contents of extracts**

	Phenol content (mgGAE/g dry extract)		Flavonoid content (mgQE/g dry extract)		Tannin content (mgTAE/g dry extract)	
	Decoction extract	Methanolic extract	Decoction extract	Methanolic extract	Decoction extract	Methanolic extract
<i>Diospyros monbuttensis</i>	136,54± 12,63	114,10± 2,43	62,18± 2,03	15,25± 1,13	46,50±1,00	85,70±3,25
<i>Dialium dinklagei</i>	185,59± 5,89	205,07± 9,09	6,780± 0,61	10,90± 1,52	146,4±5,46	108,7±6,85

302
 303 The analysis of variance (ANOVA) and the post-ANOVA test (Tukey's multiple comparison
 304 test) have shown that the means of different contents of phenol, tannins and flavonoids of
 305 the two plants species were significantly different. When compared to those obtained by
 306 Akoue et al., [13] (4.42±0.45mgGAE/-g for the aqueous extract and 2.27±0.0038mgGAE/g
 307 ethanolic extract), the contents of phenol *Dialium dinklagei* for methanolic and aqueous
 308 extracts were very high. This difference could be due to the extraction solvent and the
 309 method used.
 310 In general, the decoction gave the highest contents of phenols, flavonoids and tannins. One
 311 the hand, the increase in temperature favors the diffusion and solubility of substances
 312 extracted. On the other hand, it destroys certain fragile substances [30]. This increase of the
 313 contents of flavonoids in the decocted extract of *Diospyros monbuttensis* can be explained
 314 by the destruction by the heat of polyphenol oxidases (PPO) that lowers the polyphenol
 315 content; thus the breaking of the connection between polyphenols and other substances
 316 leading to the accessibility of these active principles may explain the abundance of its
 317 hand [31]. The flavonoid content of *Dialium dinklagei*, relatively low in relation with that of
 318 *Diospyros monbuttensis*, which when it is high in total phenol, could be explained by the
 319 presence of other phenolic compounds such as coumarins. As to the tannin, their The tannin
 320 content in *Dialium dinklagei* was higher than that of *Diospyros monbuttensis*. This
 321 difference may be related to the species of the studied plants, which were different. The
 322 Tannins are usually defined as natural polyphenolic compounds with high molecular weight,
 323 capable of forming complexes with proteins. And their effects on animals are various [32].
 324 Thus, these compounds provide protection against cardiovascular diseases, immune-/
 325 autoimmune diseases, brain disorders such as Parkinson's disease, and Alzheimer's
 326 disease [33]. Traditional medicinal use of these plants is well justified because it is now
 327 recognized that phenolic compounds have a significant therapeutic interest: fight against
 328 atherosclerosis, anticarcinogenic actions, a major source of natural antioxidants to fight
 329 against cellular aging [34, 35]. Furthermore the phytochemical sorting revealed that in

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addition to phenols, flavonoids and tannins, these plants also contain polyterpenes, saponins and alkaloids which contribute to their various therapeutic activities.

4-CONCLUSION

The overall results of the phytochemical screening and polyphenolic content partly and rationally explain the interest of traditional healers for these plants as antimalarial plants. The therapeutic effects can be induced by various chemicals (alkaloid, flavonoids, polyphenols, polyterpenes, saponins, sterols and catechic tannins) which form the scientific basis of traditional therapeutic use of the studied plants. The *in vivo* and *in vitro* studies and the determination of the structure of active molecules will deepen the study of the antimalarial activity of these plants.

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Competing interests

The authors declare that they have no competing interests

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