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**ShortOriginal Research Articles**

**Phytochemical screening and polyphenolic contents of *Dialium dinklagei* and *Diospyros monbuttensis*, two Ivorian medicinal plants 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*, some plants 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 ~~should~~ be a possible alternative antimalarial reference used today because of their effectiveness in treating malaria.

**Materials and methods:**

From *Dialium dinklagei* and *Diospyros monbuttensis*, ~~two selected plants~~, 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. ~~The polyphenol content of the plants showed a~~ 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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**1-INTRODUCTION**

The high population growth, the growing impoverishment and the lack of medical facilities are the major problems faced by the most of African populations [1]. Owing to this situation, they do not always have access to imported pharmaceuticals. According to Sandhya et al. [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 ~~[3-54, 5, 3]~~. 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* (Caesalpiniaceae) 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, 9, 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 Caesalpiniaceae family with trunk cylindrical right. The sheets made up have an acid taste, the flowers are of color yellow~~s~~ 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 is to investigate the correlation between the active ingredients present in these medicinal plants and their therapeutic effects~~powers~~, namely their use as antimalarial medicines.

## 2-MATERIAL AND METHODS

### Plant material

The plant material consisted of leaves of *Diospyros monbuttensis* (Ebenaceae) and *Dialium dinklagei* (Caesalpiniaceae). These are two ~~Two~~ plants species, which have with very little been little studied for their antiparasmodial 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. The harvest time was chosen the morning. Plants species were identified by Professor Ake-Assi from the "Centre National de Floristique", University of Felix-Houphouët-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 grinding 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 Bekro *et al.*, [15], and Zirihi *et al.*, [16] and Beourou *et al.*, [17]. In this the following order, we used three solvents of increasing polarity: hexane, methanol and water.

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

## Extraction with hexane

50g of fine dry powder were dissolved in 150mL of hexane in a blender. The whole was vigorously agitated in 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 "VenticeI" oven at 55°C. This operation led to a hexane extract.

## Extraction with methanol

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

~~After the extraction with hexane, residue from filtrate 3 was air dried and the resulting powder was recovered in 150mL of methanol. Ten minutes of homogenization by agitation followed by a filtration led to the methanolic filtrate 1. The same operation was repeated for the two successive methanolic filtrates 2 and 3. The three filtrates obtained were also gathered and evaporated. This series of operations gave the methanolic extract.~~

## Preparation of the aqueous extract

The same operation ~~was~~ is repeated on previous ~~residue pomace~~ extract with methanolic 150mL of distilled water. We obtained the aqueous extract.

## Phytochemical screening

Phytochemical analysis ~~was~~ is performed on the basis of tests for coloring characteristics to highlight the major chemical groups. It focused on ~~decoction~~ extracts ~~decoction~~, hexane, methanol and aqueous extracts of the studied plants. Various chemical groups were identified by reference to the methods described by in the work of Nemlin and Brunel [187], Békro et al. [15] and Bruneton [198]. ~~What is asking here is that the authors mention the classes of compounds they were looking for, and the test they used to register the presence~~

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119 | of each class, for example, "the presence of flavonoids was revealed by the  $AlCl_3$  assay  
120 | described by -----"

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121 | The results have been classified according to: Highly positive: +++; Fairly positive: ++;  
122 | weakly positive: +; Negative test: -

## 125 | **Determination** Dosage of polyphenols

126 |  
127 | For the dosage of polyphenols, two extracts from each plant were selected; they are the  
128 | decoction and the methanol extracts.

## 130 | **-Determination** Dosage of total phenols

131 |  
132 | The total phenolic content of plant extracts was determined by the Folin-Ciocalteu method  
133 | [2019], 20]. To 0.5mL of each plant extract of concentration 100mg/mL respectively were  
134 | added 5mL of Folin-ciocalteu diluted 1/10 in distilled water and 4 mL of sodium carbonate  
135 | (1M). The whole ~~was~~ incubated at room temperature for 15 minutes. ~~The optical~~  
136 | ~~densities~~absorbance (OD) ~~were~~are then registered read at 765nm in a spectrophotometer  
137 | against a blank. Gallic acid ~~was~~is the our standard which standard, which ~~was~~is prepared  
138 | under the same conditions as above with a solvent mixture of methanol-/water (50: 50, V/V)  
139 | at concentrations ranging from 0 to 200mg/mL. ~~Experimentations were carried out in~~  
140 | ~~triplicate~~. The total phenolic contents of the extracts ~~were~~are expressed as gallic acid  
141 | ~~equivalents/g extract~~ (mg GAE/g extract) ~~equivalents~~.

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## 143 | **-Determination of total flavonoids**

144 |  
145 | The technique used to determine the contents of total flavonoids in the extracts was  
146 | ~~extracted~~is the colorimetric method of aluminum chloride [224, 232]. And 0.5mL (100µg/mL)  
147 | of each plant extract ~~were~~are taken, to which ~~are successively added~~ 1.5mL of methanol;  
148 | 0.1mL of 10% aluminum chloride, 0.1mL of potassium acetate (1M) and 2.5mL of distilled  
149 | water ~~were successively added~~. After incubation at room temperature for 30 minutes, the  
150 | optical densities were measured in a spectrophotometer at 415nm. A methanolic solution of  
151 | quercetin in concentrations ranging from 0 to 100µg/mL ~~were~~is used as a standard.  
152 | ~~Experimentations were carried out in triplicate~~. The contents of flavonoids extracts are  
153 | expressed as quercetin equivalents/g extract (mg QE/g extract).

## 155 | **-Determination ofThe tannins dosage**

156 |  
157 | The tannin ~~contents were calculated by using~~ dosage is done with the modified vanillin  
158 | method of the vanillin of Bainbridge et al. [243]. The dosage principle ~~This method~~ is based  
159 | on the fact that the tannic acid (especially the flavan-3-ol) in the presence of the constituted  
160 | vanillin reagent (0.1mg/mL) in an acid ~~medium~~area (sulfuric acid 70% (v/v) gives a red color  
161 | whose maximum absorption is 500nm. The standard solution consists of tannic acid  
162 | (0.1mg/mL) prepared in 98% methanol. A range of standard was prepared with a series of 6  
163 | tubes where the first represents a blank. In the other 5 tubes are introduced respectively 0.2;  
164 | 0, 4; 0.6; 0.8; 1ml of standard solution and complete the volume of each tube with 1 ml of  
165 | distilled water. The test tube contains 1 ml of extract. To each of them are added 5 ml of  
166 | vanillin reagent. After 30 minutes of incubation in the dark, the absorbance was read at  
167 | 500nm against a blank (without extract). The tannic acid (1mg/ml) was used as the standard.  
168 | ~~Experimentations were carried out in triplicate~~. The results were expressed in mg/mL of  
169 | tannic acid equivalents.

## 171 | **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  $\pm$  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.

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### 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* (Table 1), widely used by Ivorian traditional pharmacopoeia, and very little studied for their antimalarial activity. These species were mainly collected on the basis of indications by literature and information received from healers. The decoction from 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.).

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**Table 1: Selected Plants**

| Species                       | Family                | Home name                                              | Tested part |
|-------------------------------|-----------------------|--------------------------------------------------------|-------------|
| <i>Diospyros monbuttensis</i> | <i>Ebenaceae</i>      | Gnamien-baka (Baoulé)<br>Mianômouroukalan<br>(Malinké) | Feuille     |
| <i>Dialium dinklagei</i>      | <i>Caesalpinaceae</i> | N'séhia (Ebrié)                                        | leaves      |

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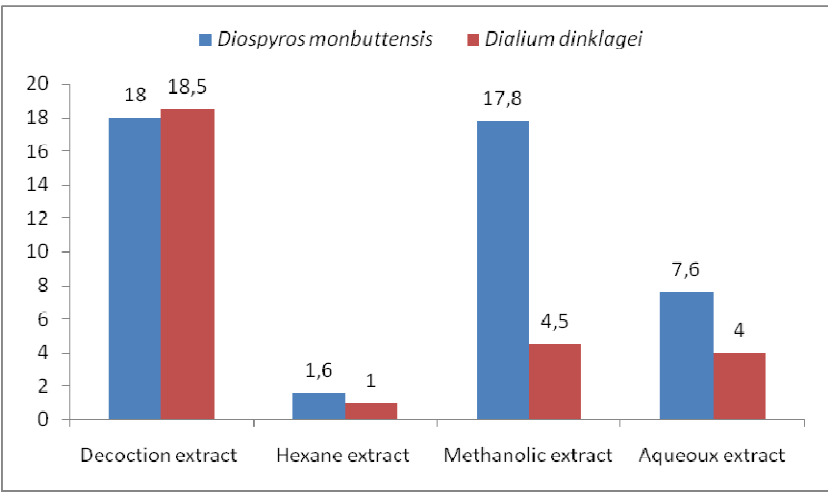


Figure 1: Yields of from of extracts Please, give the units of the yields (mg, µg, ???) and the appropriate error bars in Figure 1

ions of different extract

Table 12: Results of phytochemical tests

| Natural substances            |       | Chemical groups |          |                              |             |            |              |                     |           |          |          |           |           |                         |          |
|-------------------------------|-------|-----------------|----------|------------------------------|-------------|------------|--------------|---------------------|-----------|----------|----------|-----------|-----------|-------------------------|----------|
| Plants                        | Parts | Extracts        | Steroids | Sterols and epolyterpenes??? | Polyphenols | Flavonoids | Anthocyanins | Lucoanthocyanins??? | Gallic??? | Catechiq | Quinones | Alcaloids | Coumarins | Cardiotonics Glycosides | Saponins |
|                               |       |                 |          |                              |             |            |              |                     |           |          |          |           |           |                         |          |
| <i>Diospyros monbuttensis</i> | Le    | Dec             | +        | +                            | +++         | +          | -            | +                   | +++       | -        | +++      | +         | ++        | -                       | +++      |
|                               |       | Hex             | -        | -                            | -           | -          | -            | -                   | -         | -        | -        | +++       | +         | -                       | -        |
|                               |       | Met             | -        | +                            | +++         | +          | +            | +++                 | +++       | ++       | ++       | +         | +         | +                       | ++       |
|                               |       | Aq              | -        | +                            | +++         | +          | -            | +                   | +         | +        | ++       | +         | +         | -                       | +++      |
| <i>Dialium dinklagei</i>      | Le    | Dec             | -        | +++                          | ++          | ++         | +            | +                   | +         | ++       | +        | +++       | ++        | +                       | +++      |
|                               |       | Hex             | -        | ++                           | -           | -          | -            | -                   | -         | +        | -        | +++       | +         | -                       | -        |
|                               |       | Met             | -        | ++                           | +           | +++        | ++           | +                   | +         | -        | -        | +++       | ++        | +                       | ++       |

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

Strong positive: + + +; moderately positive: + +; Low positive: +; negative test:-  
Le= leaves; Dec: Decoction extract; Hex: Hexane extract; Met: Methanolic extract; Aq:  
Aqueous extract

### Phytochemical screening screening basis of the therapeutic use of different species of plants species

The results of the phytochemical screening carried out on the extracts of plants referred to malaria are mentioned in the Table 12. These results varied from one extract to another and from one plant to other. Chemical composition variations were found among the different extracts and between the two plant species analyzed

#### *Diospyros monbuttensis* Gürke (Ebenaceae)

The leaves contain sterols, polyterpenes, polyphenols, flavonoids, alkaloids, saponins, the leucoanthocyanins, tannins. The results of tannins are not shown in Table 11, 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] with the absence of tannins and roots in the stem bark. They are similar to those of Bouquet and Debray [254] 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 [245], 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 [265]. Various studies have shown that coumarins have a high antioxidant capacity. This antioxidant activity is due to their capacity to scavenging free roots and chelating metal ions [276]. Terpenoids analgesics and anti-inflammatories are attributed to tannins. Tannins also contribute to wounds healing [287].

#### *Dialium dinklagei* (Caesalpinaceae)

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 are comparable 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 we indicate that Bouquet and Debray [254] about confirmed the presence of tannin in the its 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 [298].

#### Polyphenol content of *Diospyros monbuttensis* Gürke (Ebenaceae) and *Dialium dinklagei* (Caesalpinaceae)

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The contents of total phenols in the plant extracts, determined from the 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. Let's note that the The highest content was obtained in the methanolic extract *Dialium dinklagei*. These levels ranged from 114.1mgGAE/g and 205.1mgGAE/g (Table 23). 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 the 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*. Statistically, the The differences between the amounts of flavonoids in according to the plant species and to the solvent used were and is very significant ( $p < 0.0001$ ). These amounts vary between 6.78mgQE/g and 62.18mgQE/g (Table 23). The highest amount was obtained in with the decoction extract of from *Diospyros monbuttensis* (62.18mgQE/g).

Tannins from different plant extracts were are determined from the a calibration curve of tannic acid ( $Y= 0.2167X - 0.004417$ ;  $R^2= 0.9761$ ). Whatever the type of solvent used, the results they showed that *Dialium dinklagei* is richer in tannins than *Diospyros monbuttensis*. Let's note that the highest amount was obtained in with the decoction aqueous extract of *Dialium dinklagei*. These contents ranged from 45.43mgTAE/g???? and 146.4mgTAE/g (Table 23). Statistically, the difference between the contents of the phenols concerning based on the plant species analyzed and the type of solvent used were is highly significant ( $p < 0.0001$ ).

**Table- 23. Total phenols, flavonoids and tannins contents of extracts**

|                               | Phenols content (mgGAE/g) |                    | Flavonoids content (mgQE/g) |                    | Tannins content (mgTAE/g) |                    |
|-------------------------------|---------------------------|--------------------|-----------------------------|--------------------|---------------------------|--------------------|
|                               | 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         |

The analysis of variance (ANOVA) and the post-ANOVA test (Dunnnett's multiple comparison test) (Was this test or Tukey test as indicated in the section of Material and methods, used to analyze the data) have showed that the means of different contents of phenol, tannins and flavonoids of the two plants are significantly different. When compared to those obtained by Akoue et al., [2013] (4.42±0.45mgGAE / g for the aqueous extract and 2.27±0.0038mgGAE/g ethanolic extract), the contents of phenol *Dialium dinklagei* for methanolic and aqueous extracts are very high. This difference could be due to the extraction solvent and the method used.

In general, the decoction gave the highest contents of phenols, flavonoids and tannins. One the hand, the increase in temperature favors the diffusion and solubility of substances extracted. On the other hand, it destroys certain fragile substances [3029]. This increase of the contents of flavonoids in the decocted extract of *Diospyros monbuttensis* can be explained by the destruction by the heat of polyphenol oxidases (PPO) that lowers the polyphenol content; thus the breaking of the connection between polyphenols and other substances leading to the accessibility of these active principles may explain the abundance of its hand [310]. The flavonoid content of *Dialium dinklagei*, relatively low in relation with that of *Diospyros monbuttensis* when it is high in total phenol, could be explained by the

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presence of other phenolic compounds such as coumarins. As to the tannin, their content in *Dialium dinklagei* is higher than that of *Diospyros monbutensis*. This difference may be related to the species of the studied plants which are different. The tannins are usually defined as natural polyphenolic compounds with high molecular weight, capable of forming complexes with proteins. Tannins are an important source of protein??? in animals and their effects on animals are various [321]. Thus, these compounds provide protection against cardiovascular diseases, immune / autoimmune diseases, brain disorders such as Parkinson's disease, and Alzheimer's disease [332]. Traditional medicinal use of these plants is well justified because it is now recognized that phenolic compounds have a significant therapeutic interest: fight against atherosclerosis, anticarcinogenic actions, a major source of natural antioxidants to fight against cellular aging [343, 354]. Furthermore the phytochemical sorting revealed that in addition to phenols, flavonoids and tannins, these plants also contain polyterpenes, saponins and alkaloids which contribute to their various therapeutic activities.

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#### 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 are induced by various chemicals (alkaloid, flavonoids, polyphenols, polyterpenes, saponins, sterols and catechin 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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