

Short Research Articles

Phytochemical screening and polyphenolic contents of *Dialium dinklagei* and *Diospyros monbuttensis*, two Ivorian medicinal plants used to treat malaria

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 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 should be a possible alternative antimalarial reference used today because of their effectiveness in treating malaria.

Materials and methods:

From 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 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 high content of phenol and tannin *Dialium dinklagei* over *Diospyros monbuttensis*. However, the content of flavonoids *Dialium dinklagei* is relatively low 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*

1-INTRODUCTION

The high population growth, the growing impoverishment and the lack of medical facilities are the major problems faced by African populations [1]. Owing to this situation, they do not

always have access to imported pharmaceuticals. According to Sandhya [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 [4, 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* and *Dialium dinklagei* 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 yellows 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 is to investigate the correlation between the active ingredients present in these medicinal plants and their therapeutic powers, namely their use as antimalarial medicines.

2-MATERIAL AND METHODS

Plant material

The plant material consists of leaves of *Diospyros monbuttensis* and *Dialium dinklagei*. Two plants with very little been 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. They were then dried in sunlight for one week at room temperature before being reduced to a fine powder by grinding. 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]. In this 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 filtrates were gathered and evaporated in "Venticel" 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 "Venticel" oven at 55°C. This operation led to a hexane extract.

Extraction with methanol

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 is repeated on previous pomace extract with methanolic 150mL of distilled water. We obtained the aqueous extract.

Phytochemical screening

Phytochemical analysis is performed on the basis of tests for coloring characteristics to highlight the major chemical groups. It focused on 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 [17], Békro *et al.* [15] and Bruneton [18].

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

Dosage of polyphenols

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

-Dosage of total phenols

The total phenolic content of plant extracts was determined by the Folin-Ciocalteu method [19], [20]. To 0.5mL of each plant extract of concentration 100mg/mL respectively were added 5mL of Folin-ciocalteu diluted 1/10 in distilled water and 4 mL of sodium carbonate (1M). The whole is incubated at room temperature for 15 minutes. The optical densities (OD) are then read at 765nm spectrophotometer against a blank. Gallic acid is our standard which is 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. The total phenolic contents of the extracts are expressed as gallic acid (mg GAE/g extract) equivalents.

-Determination of total flavonoids

The technique used to determine the contents of total flavonoids extracted is the colorimetric method of aluminum chloride [21, 22]. And 0.5mL (100µg/mL) of each plant extract are taken, to which are successively added 1.5mL of methanol; 0.1mL of 10% aluminum chloride, 0.1mL of potassium acetate (1M) and 2.5mL of distilled water. 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 is used as a standard. The contents of flavonoids extracts are expressed as quercetin equivalents (mg QE/g extract).

-The tannins dosage

The tannin dosage is done with the modified method of the vanillin of Bainbridge *et al.* [23]. The dosage principle is based on the fact that the tannic acid (especially the flavan-3-ol) in the presence of the constituted vanillin reagent (0.1mg/mL) in an acid area (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. The results were expressed in 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 are expressed as mean $\pm$ SD. Multiple comparisons and determination of significance level are made by the univariate ANOVA followed by Tukey test. The differences are 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* (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.).

Table- 1: Selected Plants

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

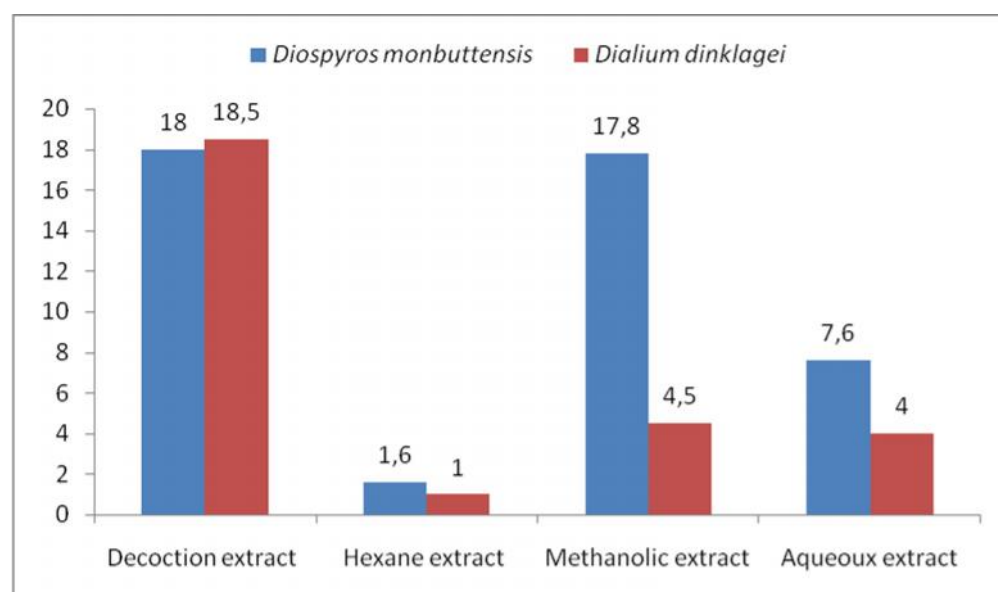


Figure 1: Yields of extractions of different extract

Table- 2: Results of phytochemical tests

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

	Aq	-	+	+++	+	-	+	+	+	++	+	+	-	+++
	Dec	-	+++	++	++	+	+	+	++	+	+++	++	+	+++
	Le													
Dialium	Hex	-	++	-	-	-	-	-	+	-	+++	+	-	-
dinklagei	Met	-	++	+	+++	++	+	+	-	-	+++	++	+	++
	Aq	-	-	+	+++	-	++	+	-	+	+++	+	-	+++

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

189 Le= leaves; Dec: Decoction extract; Hex: Hexane extract; Met: Methanolic extract; Aq:

190 Aqueous extract

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192 **Phytochemical screening basis of the therapeutic use of different species of** 193 **plants**

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195 The results of the phytochemical screening carried out on the extracts of plants referred to
196 malaria are mentioned in the Table 2. These results varied from one extract to another and
197 from one plant to other.

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199 ***Diospyros monbuttensis* Gürke (Ebenaceae)**

200

201 The leaves contain sterols, polyterpenes, polyphenols, flavonoids, alkaloids, saponins, the
202 leucoanthocyanins, tannins, quinones and coumarins. Anthocyanins, cardiac glycosides and
203 steroids are present at very small quantities. These results are different from those of Anie *et*
204 *al.* [9] with the absence of tannins and roots in the stem bark. They are similar to those of
205 Bouquet and Debray [24] which showed the presence of quinones, tannins, sterols and
206 saponins in the leaves and differ from ours by the absence of flavonoids and alkaloids.
207 Previous studies [8] reported the presence of saponins, anthraquinones, cardiac glycosides
208 and alkaloids. According Bouquet and Debray [24], this plant is considered to be a good
209 remedy for stiffness, stomach ache, edema and leprosy by Baoule and Agni ethnies of Côte
210 d'Ivoire. The antimalarial therapeutic indication may be related to the presence of alkaloids
211 because we know that the majority of antimalarial drugs belong to the same chemical family
212 [25]. Various studies have shown that coumarins have a high antioxidant capacity. This
213 antioxidant activity is due to their capacity to scavenging free roots and chelating metal ions
214 [26]. Terpenoids analgesics and anti-inflammatories are attributed to tannins. Tannins also
215 contribute to wounds healing [27].

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217 ***Dialium dinklagei* (Caesalpinaceae)**

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219 The decoction of the leaves contains sterols, polyterpenes, polyphenols, flavonoids,
220 alkaloids, coumarins and saponins in large quantities. Anthocyanins, leucoanthocyanins,
221 tannins, quinones and cardiac glycosides are present in small quantities. There was also an
222 absence of steroids. These results are comparable with Akoué [13] who noted a large
223 presence of flavonoids, alkaloids, tannins and polyphenols in the ethanol extract of the
224 leaves. Very little research has been made on this plant, but we indicate that Bouquet and
225 Debray [24] confirmed the presence of tannin in its leaves. Sterols, saponins, alkaloids and
226 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 [28].

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

The contents of total phenols in plant extracts determined from the calibration curve of gallic acid ($Y=0.01810X + 0.07179$; $R^2=0.9911$) show that *Dialium dinklagei* is richer in phenol than *Diospyros monbuttensis* whatever the type of solvent used. Let's note that the highest content was obtained in the methanolic extract *Dialium dinklagei*. These levels range from 114.1mgGAE/g and 205.1mgGAE/g (Table 3). 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 different extracts of plants determined from the calibration curve of quercetin ($Y= 0.02834X + 0.01368$; $R^2= 0.9986$) show that *Diospyros monbuttensis* is richer in flavonoids than *Dialium dinklagei*. Statistically, the difference between the amounts of flavonoids in according the plant and to the solvent used and is very significant ($p < 0.0001$). These amounts vary between 6.78mgQE/g and 62.18mgQE/g (Table 3). The highest amount was obtained with the decoction from *Diospyros monbuttensis* (62.18mgQE/g).

Tannins from different plant extracts are determined from the calibration curve of tannic acid ($Y= 0.2167X - 0.004417$; $R^2= 0.9761$). Whatever the type of solvent used, they show that *Dialium dinklagei* is richer in tannins than *Diospyros monbuttensis*. Let's note that the highest amount was obtained with the aqueous extract of *Dialium dinklagei*. These contents range from 45.43mgTAE/g and 146.4mgTAE/g (Table 3). Statistically, the difference between the contents of the phenol based on the plant and solvent used is highly significant ($p < 0.0001$).

Table- 3: 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 (Dunnett's multiple comparison test) 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. On the hand, the increase in temperature favors the diffusion and solubility of substances extracted. On the other hand, it destroys certain fragile substances [29]. 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 [30]. 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 presence of other phenolic compounds such as coumarins. As to the tannin, their content in *Dialium dinklagei* is higher than that of *Diospyros monbuttensis*. 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 [31]. Thus, these compounds provide protection against cardiovascular diseases, immune / autoimmune diseases, brain disorders such as Parkinson's disease, and Alzheimer's disease [32]. 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 [33, 34]. 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.

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 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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