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Phytochemical screening of some medicinal plants used to treat malaria in Côte d'Ivoire (West Africa)

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Abstract

Because of the resistance of *Plasmodium falciparum* to most of the antimalarial drugs and the burden of malaria, there is an urgent need for the discovery of new antimalarial compounds. Many investigations showed that plants are often the main sources of new medicines. In this study, a phytochimical screening was performed with plants used in traditional medicine in Côte d'Ivoire to treat malaria. We identified the chemical compounds groups that could guide us towards pharmacological studies. The 20 crude extracts obtained from medicinal plants were tested for the detection of chemical groups. An abundance presence of alkaloids in all extracts and an absence of steroids in all extracts except the decoction extract of *Diospyoros monbuttensis* have been established. The other chemical groups were present in all the 5 plants. Their presence in the different extracts varied according to extraction method. The whole results of the phytochemical screening would explain in a rational manner, the enthusiasm of traditional healers for these plants as antimalarial drug.

Keywords: Antimalarial Drug, Malaria, Phytochemical Screening, Medicinal Plants

Contents

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1. Introduction

Population growth, increasing pauperization, cultural habits and expensiveness of the pharmaceutical drugs are responsible for the use of traditional medicines such as herbal medicine in rural and urban populations [1, 2]. Indeed, indigenous plants play an important role in the treatment of many diseases and an estimated of 80% of people worldwide to use herbal remedies [3, 2]. Traditional pharmacopoeia is used to treat many infections against which

plants used are as effective as the pharmaceutical drugs [4, 5, 2]**.** The secondary metabolites in these plants are involved into their medical properties. Further works on these secondary metabolites may represent a first step toward determination of active principles [6, 7]. This work was implemented to achieve the phytochemical screening of plants used against malaria in the ivorian traditional medicine. The aim of this work is to establish correlation between the active principles present in these medicinal plants and their therapeutic powers particulary their use as antimalarial drug.

2. Materials and Methods

Vegetal material

The vegetal material are leaves of *Diospyros monbuttensis, Dialium dinklagei, Newbouldia laevis, Cnestis ferruginea* and *Trema orientalis.* There are few data available on the antiplasmodial activity of these 5 plants. The leaves of *Diospyros monbuttensis* were collected during January, 2013 from Talahini-Sokoura in Department of Sandegué (North-eastern Côte d'Ivoire). The leaves of *Dialium dinklagei, Newbouldia laevis, Cnestis ferruginea* and *Trema orientalis* were collected during March, 2013 in the area of Abidjan (South of Côte d'Ivoire). Plant species were identified by Professor Ake-Assi from the "Centre National de Floristique", University of Felix Houphouet-Boigny, Abidjan, Côte d'Ivoire. Vegetal material was air-dried for 7to10 days at room temperature (25°C) and then powdered. The different extracts were prepared from the powder obtained.

Preparation of crude extract

The decoction of each plant was obtained from the fine powder as of the traditional preparation method. Then, 3 successive extractions by solvents of increasing polarity (*n*-hexane, methanol and water) were implemented described previously by Bekro *et al.* and Zirihi *et al.* [8, 9].

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 by 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 previous operations were repeated on the marc of the methanolic extract with 150mL of water for the aqueous extract.

Phytochemical screening

The phytochimical analysis was based on the staining characteristics in order to highlight the major chemical groups. All the above extract was tested. Various chemical groups were identified by reference to the methods described by Nemlin and Brunel, Bekro *et al.* and Bruneton [8, 10, 11]**.** The results were classified as follows:

-: Absence; +: Presence in low concentration; ++: Presence in moderate concentration; +++: Presence in high concentration

Steroids: *Salkowski test*

Five drops of concentrated H_2SO_4 were added to 1mL of extract, the appearance of a red color indicated the presence of steroids.

Sterols and polyterpenes

0.1g of extract is dissolved at hot in 1mL of acetic anhydride in a capsule. The resulted solution was poured in a test tube and then 0.5mL H_2SO_4 was added. A violet coloration that turned in blue, then in green revealed the presence of sterols and triterpenes.

Saponins (Foam index)

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

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

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

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.

Anthocyanins
Five milliliters of 10 % H_2SO_4 was added to 5mL of 5 % extract, and then a base (NH_4OH). 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.

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 indicates the presence of gallic tannins, the green or dark green coloration shows the presence of catechic tannins.

Quinones
An aliquot 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 $CHCl_3$ after cooling. To the organic phase was added 0.5mL of 50 % NH_4OH diluted solution. The positivity of the reaction was indicated by a red to violet indicates a positive reaction.

Coumarins
To 5mL of extract, are added 2mL of hot water, and then the solution is shared between two test tubes. In the content of one, we added 0.5 mL of NH_4OH 25%. Under the UV at 366 nm, an intense fluorescence indicated the presence of coumarins.

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% $FeCl_3$ gave a green-bluish coloration which is specific to cardiotonics glycosides.

3. Results and Discussion

Selection of plants and yields of extractions
Investigations were carried out by ethnobotanical approaches among traditional medicine actors, especially medicinal plants sellers on the markets of Abidjan area. These investigations helped in the selection of *Diospyros monbuttensis, Dialium dinklagei, Newbouldia laevis, Cnestis ferruginea* and *Trema orientalis* (Table1) considered as very used in the Ivorian traditional pharmacopoeia although paucity remains on antimalarial activity. These species have mainly been harvested both on the basis of indications from the literature and information from the healers (Table1). Of the five extracts, the decoction has produced the greatest yield (20%), and the hexane, the smallest (1%). These results suggest that the using of decoction is appropriate for preparation of plant extracts **(Fig1)**. The lowest yield was obtained with hexane. This shows that the plants analyzed contained very little of non-polar compounds. This result is similar to that obtained by Bekro *et al.* [8] with the hexane extract of *Caesalpinia benthamiana* (0.75%).

Table 1. Selected Plants

Species	Family	Local name	Part tested
Diospyros monbuttensis	*Ebenaceae*	Gnamien-Baka (Baoulé)	leaves
Dialium dinklagei	*Caesalpiniaceae*	N' séhia (Ebrie)	leaves
Newbouldia laevis	*Bignoniaceae*	Mogogbèboulou (Dioula) Tohounzué (Baoulé)	leaves
Cnestis ferruginea	*Connaraceae*	Kèrèkèssè (Baoulé)	leaves
Trema orientalis	*Ulmaceae*	Sodi goula (Dioula) N'sien (Baoulé)	leaves

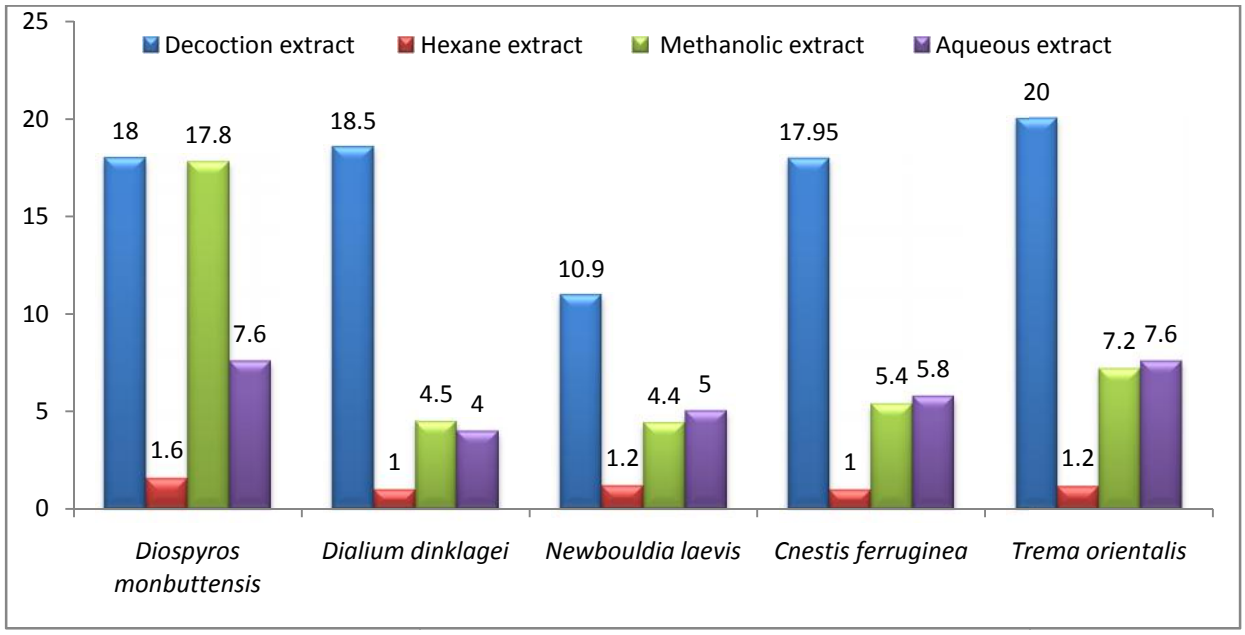

Figure 1. Yields of extractions of different extract

Table 2. Results of phytochemical tests

Natural substances			Chemical groups													
									Tannins							
Plants	**Parts**	**Extract**	**Steroids**	**Sterols and polyterpenes**	**Polyphenols**	**Flavonoids**	**Anthocyanins**	**Lucoanthocyanins**	Gallic	Catechiq	**Quinones**	**Alkaloids**	**Coumarins**	**Cardiotonics Glycosides**	**Saponins**
		Dec	+	+	+++	+	-	+	+++	-	+++	+	++	-	+++
Diospyros		Hex	-	-	-	-	-	-	-	-	-	+++	+	-	-
Monbuttensis	Le	Met	-	+	+++	+	+	+++	+++	++	++	+	+	+	++
		Aq	-	+	+++	+	-	+	+	+	++	+	+	-	+++
		Dec	-	+++	++	++	+	+	-	++	+	+++	++	+	+++
Dialium		Hex	-	++	-	-	-	-	-	-	-	+++	+	-	-
Dinklagei	Le	Met	-	++	+	+++	++	+	+	-	-	+++	++	+	++
		Aq	-	-	+	+++	-	++	+	-	+	+++	+	-	+++
		Dec	-	-	+	++	-	+	-	+	+	++	+	+	+++
Newbouldia		Hex	-	-	-	+	+	-	-	-	-	+++	+	-	-
laevis	Le	Met	-	++	++	-	+++	-	++	+++	-	++	+	++	++
		Aq	-	-	++	++	-	+	++	-	+	++	+	-	+++
		Dec	-	++	+++	+	++	++	+++	-	-	++	-	++	+++
Cnestis		Hex	-	-	-	-	+	-	-	+	+	+++	-	-	-
ferruginea	Le	Met	-	+	++	+	-	+	-	++	+	-	+	++	++
		Aq	-	-	+++	-	+	++	+++	-	+++	+	-	-	+
		Dec	-	++	+	+	-	++	+	-	++	-	++	+	+++
Trema		Hex	-	+	+	+	++	-	-	-	-	++	-	-	-
orientalis	Le	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 basis of the therapeutic use of different species of plants

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

Diospyros monbuttensis* Gürke *(Ebenaceae)

The leaves contain sterols, polyterpenes, polyphenols, flavonoids, alkaloids, saponins, leucoanthocyanins, tannins, quinones and coumarins. Anthocyanins, cardiotonics glycosides and steroids are present in very low quantity. Bouquet and Debray [12] reported similar results. They showed the presence in the leaves of some quinones, tannins, sterols and saponins and an absence of flavonoids and alkaloids. Anie *et al.* [13] noted an absence of tannins in the root and bark of stem. According to Bouquet and Debray [12]**,** this plant is considered by the Baoulé and Agni ethnic groups from Ivory Coast as a good remedy for febrile aches, stomach pains, edema and leprosy. The antimalarial therapeutic indication could be related to the presence of alkaloids since the majority of antimalarial molecules belong to this chemical family [14]. Various studies have demonstrated that the coumarins have a potential antioxidant. This antioxidant activity is due to their ability to trap the free radicals and to chelate metal ions [15]**.** It is assigned to the terpenoides and tannins some analgesics and anti-inflammatory activities. Apart from this, the tannins contribute to healing wounds [16]. The constituents present into these plants play a significant role in identifying the crude drug. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc [17]**.**

Dialium dinklagei (Caesalpiniaceae)

The leaves of *D dinklagei* contain sterols, polyterpenes, polyphenols, flavonoids, alkaloids, coumarins and saponins in large quantity. Anthocyanins, leucoanthocyanins, tannins, quinones and cardiotonics glycosides are present in low quantity. There were also no steroids. Little work has been performed on this plant, but we noted that Bouquet and Debray **[12**] have described the presence of tannins in the leaves. Existence of sterols, saponins, polyterpenes and alkaloids may explain their traditional therapeutic usages. Traditionally, the saponins are widely used as detergents, pesticides and mollucides, in addition to their industrial applications as foam and surfactants, they may also have beneficial effects on health [18]**.** These results will be helpful to phytochemists and pharmacologists for the identification of new active compounds from plants [19]**.**

Newbouldia laevis* (P. Beauv.) Seemann ex-Bureau *(Begoniaceae)

The leaves of *Newbouldia laevis* contain plenty of saponins and alkaloids, moderate flavonoids, polyphenols, anthocyanins, tannins, and in low quantity sterols, polyterpenes, coumarins, quinones and leucoanthocyanins. There were no steroids. These results are in agreement with those of Bouquet et Debray [12] who noted the presence of sterols and saponin. In the contrary, Yemoa *et al.* [20] reported the absence of alkaloids, terpenes, steroids, saponosides and the presence of flavonoids in the leaves. According to Usman *et al*. [21]**,** there are flavonoids, tannins, terpenes, steroids and cardiotonics glycosides in the extracts. The amount of secondary metabolites in this plant could explain his permanent usages by traditional healers. In fact, the roots of *Newbouldia laevis* are used in Benin in the treatment of Buruli ulcer [20]**.** This plant is also used in cases of constipation, gastrointestinal pain and bronchipneumonia. Use externally, treats the intercostal pains, rheumatism, neuralgia and the toothache. It is also used against venereal diseases [12, 22, 23]**.** It was too deemed to facilitate childbirth [12, 24]**.** Several works confirmed the antimicrobial activity of *N. laevis* [25,21]**.**

***Cnestis ferruginea* Vahl exDC (Connaraceae)**

The leaves of *Cnestis ferruginea* contain saponins, quinones, polyphenols, gallic tannins and alkaloids in large quantity. The quantity of cardiotonics glycosides, sterols, polyterpenes, catechic tannins, leucoanthocyanins, and anthocyanins are present in average quantity. Some trace of coumarins and flavonoids and a lack of steroids were reported. The traditional use of this plant as antimalarial drug could be explained by the alkaloids. Several other usges were described. According to Bouquet and Debray [12]**,** the leaves are deworming, very active against the ascaris. These leaves are also used to treat scabies, asthenia, to calm madness and have purgative properties. Another therapeutic indication that traditional pharmacopoeia recognizes to this plant is its use as an aphrodisiac and the treatment of eye disorders. Prior works have proven the anti-inflammatory analgesic activities [26, 28], anti-depressive and anxiolytic activities [27] of *Cnestis ferruginea.* The anthocyanins help the immune system to protect and fight effectively against viral infections [29]**.**

***Trema orientalis* (L.) Blume (Ulmaceae)**

Leaves of *T. orientalis* do not steroids, but contain sterols, polyterpenes, polyphenols, flavonoids, tannins and alkaloids in varying proportions depending on the solvent. This result differs from that of Bouquet and Debray [12] who did not report alkaloids. It also differs from those of Bekro *et al.* [8] who mentionned a lack of tannin gallic and saponins. Kerharo and Adam [30] showed that the leaves have diuretics effects because of the flavonoids and deworming affects due to polyterpenes. The hypotensive effect of the plant described by the traditional healers would be due to polyphenols and flavonoids (flavonones). Dimo *et al.* [31] indicated that *Trema orientalis* has some antidiabetic properties (hypoglycaemic activity) and could be beneficial for diabetics with cardiovascular diseases. Dijoux-Franca *et al.,* [32]**,** showed the presence of dihydrophenanthrene and phenyl

dihydroisocoumarine in *Trema orientalis.* The importance of secondary metabolites in this plant could therefore explain its various therapeutical virtues.

4. Conclusion

In our opinion, the whole results of the phytochemical screening could explain the enthusiasm of traditional healers for these plants as antimalarial drug. The therapeutic effects are induced by various chemical compounds (alkaloid, flavonoids, polyphenols, polyterpenes, saponins, sterols and tannins catechics) which constitute the scientific basis of the traditional therapeutic use of plants studied. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. These results will be helpful to phytochemists and pharmacologists for identification of new active compounds from plants. We wish to deepen this phytochemical study on one hand by the studies *in vitro* and *in vivo* of antimalarial activity and on the other hand in establishing with precision the structures of active molecules.

5. Competing Interests

The authors declare that they have no competing interests.

6. Acknowledgement

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