

ANTIPLASMODIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *ADENIA LOBATA*, *COLA GIGANTEA* VAR. *GLABRESCENS* AND *ENTADA MANNII*, THREE TRADITIONAL PLANTS.

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ABSTRACT: Medicinal plants are currently being evaluated as source of promising antimalarial agents. Plant extracts contain phytochemical constituents for miscellaneous medicinal activities which are bioactive in nature. There is growing interest in the use of plants for the treatment and prevention of malaria. In this study, we have evaluated the claimed antimalarial properties of six extracts from three plants utilized in traditional malaria remedies and we have investigated phytochemical screening. Schizontocidal activity was measured using a standard *in vitro* assay, with NF54 and K1 *Plasmodium falciparum* strains. From the 6 extracts tested, only aqueous extract of *Entada manni* showed a promising activity ($5 \leq IC_{50} \leq 15 \mu\text{g/mL}$) on resistant strain K1. All extracts contained saponins, polyterpenes and sterols.
Key words: Traditional medicine; Medicinal plants; Malaria; Antimalarial; *Plasmodium falciparum*.

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INTRODUCTION

Malaria is currently the most deadly parasitic disease in the world, especially in developing countries (Kofi. A, *et al.*, 2012). This disease is transmitted by the female of an anophelid mosquito and caused by a protozoan of genus *Plasmodium*.

Of the five plasmodial species, only *P. falciparum*, the most widespread in Côte d'Ivoire, is responsible for the deaths of thousands of people, mostly children (Schlitzer. M, 2008).

The emergence and extension of *P. falciparum* resistant strains to the currently available antimalarials such as chloroquine, an antimalarial very reference for its cost and frequency of prescription, worsen the prognosis of this pathology (Guédé-Guina. F, *et al.*, 1995).

The impact and severity of this parasitosis on public health requires the discovery of new molecules effective on resistant strains.

In this study, we proposed to evaluate the antiplasmodial action of extracts of *Adenia lobata*, *Colagigantea* Var. *glabrescens* and *Entada manni* used traditionally against malaria access.

MATERIAL AND METHODS

Material

The plant material used consists of ethanolic and aqueous extracts of *Adenia lobata*, *Colagigantea* Var. *glabrescens* and *Entada mannii*, three plants traditionally used as a decoction in the treatment of malaria in Côte d'Ivoire.

In addition to these extracts we have also used chloroquine (CQ) for control tests.

As for the biological material, it consists of human blood parasitized by NF54 laboratory strains (strain sensitive to Chloroquine) and K1 (Chloroquine resistant strain isolated in Thailand).

The culture medium used for the *in vitro* tests was RPMI 1640 (Roswell Park Memorial Institute 1640) supplemented with 25 mM HEPES, a solution of 5% bicarbonate of sodium and 10% human serum (O⁺).

METHODS

Vegetable material and preparation of extracts

Vegetable material consisted of stem back of *Adenia lobata*, *Cola gigantea* Var. *glabrescens* and *Entada mannii*. The plants were collected from Agboville department and were identified by Floristic Center of Félix Houphouët-Boigny University. The plant samples were then dried in shade left over for 20 days and powdered with the help of grinder. Powder was extracted according to Zihiri and Kra (Zirihi. G.N, Kra. A.K.M, 2003) as follows: One hundred grams of powder were macerated in distilled water during 48 hours. The obtained homogenate was filtered successively on cotton then on Whatman paper 3 mm. The filtrate is first reduced using a rotary evaporator BÜCHI type at 60 ° C, then collected brown paste is lyophilized. We obtained the total aqueous extract (Eaq). This method was used with Ethanol to obtain ethanolic extract (Eeth).

In vitro antiplasmodial assay on *P. falciparum*

For *in vitro* culture of *P. falciparum*, we used the isotopic alternative of the microphone-test (plate of 96 wells) of Reichmann adopted by WHO (Rieckmann. K.H, *et al.*, 1978). This technique measure and quantify the capacity of drug to inhibit the growth of *P. falciparum* at the trophozoites stage.

In this technique, the strains are incubated at 37°C in an impoverished in oxygen and enriched with carbon dioxide with 95% of humidity. After 24 h, plates were removed and added tritiated hypoxanthine (0.5 µCi by well). The plates were again returned to incubator for 24 h. After the incubation, the plates were frozen and thawed. Freezing and thawing of plates free plasmodial DNA radiolabeled by hypoxanthine. The DNA is recovered after washing on a filter paper in a rectangular fiberglass tape with a cell collector. Once the collection is complete, the paper was removed and dried. The radioactivity was measured using a Wallac MicroBeta counter. All results were expressed on a listing.

Phytochemical screening

The phytochemical screening was done using the standard protocols (Uddin. G.A, *et al.*, 2012).

Test for Alkaloids: 5 mL of extract was concentrated to yield a residue. Residue was dissolved in 3 mL of 2% (v/v) HCl, few drops of Mayer's reagent was added. Appearance of the dull white precipitate indicated the presence of basic alkaloids.

Test for Saponins: 2 mL extract was shaken vigorously for 30 s in a test tube. Persistence of thick froth even after 30 mins indicated the presence of saponins.

Test for Polyphenols: In 2 mL of vegetable extract is added a drop of alcoholic solution of 2% ferric chloride. The appearance of a darker or darker blue or green color indicates the presence of polyphenolic derivatives.

Test for Polyterpenes and sterols: 5 mL of plant extract are evaporated to dryness. The residue is dissolved hot in 1 mL of acetic anhydride and collected in a test tube. Along the tube, 0.5 mL of concentrated sulfuric acid is added. The appearance at the interphase of a purple or purple ring, turning blue and then green, indicates a positive reaction.

Test for Quinone: 1 mL of extract was taken. 1 mL of conc. H₂SO₄ was added. Formation of red color indicated the presence of quinone.

Test for Tannins: About 0.5 g of the dried powdered samples was boiled in 20 mL of water in a test tube and then filtered.

Test for Flavonoids: A portion of the powdered plant sample was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute Ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

RESULTS

Antiplasmodial activity

The results of the *in vitro* antiplasmodial activity of extracts are presented in table I.

The antimalarial activity of extracts was defined according to the IC₅₀ values obtained. An extract showing an IC₅₀ value ≤ 5 µg/mL was classified as highly activity. Extracts with IC₅₀ values ≥ 5 µg/mL and ≤ 15 µg/mL were considered as promising activity. Extracts with IC₅₀ values ≥ 15 µg/mL and ≤ 50 µg/mL were considered as moderately activity and those with IC₅₀ values > 50 µg/mL inactive (Usman. R, *et al.*, 2012; Kipré. G.R, *et al.*, 2017 ; Bero. J, *et al.*, 2009).

Phytochemical screening

Phytochemical screening of 6 extracts showed the presence of several secondary metabolites which are summarized in Table 2. In the phytochemical screening, Aqueous extracts and ethanolic extracts of *Adenia lobata* and *Cola gigantea* Var. *glabrescens* were shown to have same compositions. Similarly ethanolic extracts and aqueous extracts of *Adenia lobata* and *Entada mannii* had same compositions

Table 1: *Adenia lobata*, *Cola gigantea* Var. *glabrescens* and *Entada mannii* antiplasmodial activity

Plants	Extracts	IC ₅₀ of <i>P. falciparum</i> strains (µg/mL)	
		NF54	K1
<i>Adenia lobata</i>	Aqe	14.57±1.16	15.88±1.41
	Eeth	9.69±0,38	25.1±0.71
<i>Cola gigantea</i> Var. <i>glabrescens</i>	Aqe	18.02±1.92	21±0.67
	Eeth	28.54±1.85	20±1.22
<i>Entada mannii</i>	Aqe	1.54±0.38	10.07±1.26
	Eeth	25.69±0.39	30±1.29
Chloroquine (nM)		11.03±0.23	124.74±4.96

Table 2: Phytochemical screening of aqueous extracts and ethanolic extracts of three antimalaria plants

Plants	Extracts	Polyterpenes and sterols	Polyphenols	Flavonoids	Tannins	Quinone	Alkaloids	Saponins
<i>Adenia lobata</i>	Eaq	++	+	+	+	-	+	++
	Eeth	+	-	-	-	-	+	++
<i>Cola gigantea</i> Var. <i>glabrescens</i>	Eaq	+	++	++	-	-	-	++
	Eeth	++	+	+	+	-	+	++
<i>Entada mannii</i>	Eaq	+	-	-	-	-	+	++
	Eeth	+	++	++	-	-	-	++

+ : present ++: Abundantly present - : Absent

DISCUSSION

Antiplasmodial activity

The main goal of this work was to investigate the potential antimalarial properties of some plants used in traditional medicine, against malaria and/or fever, and providing scientific validation for their use. Therefore, selection of plants was carried out based mainly on an ethnobotanical approach (Uddin. G.A, *et al.*, 2012).

Aqueous extracts and ethanolic extract of *Entada mannii* have promising activity on NF54 and K1 strains. These results justify the use of this plant in the treatment of malaria in traditional medicine. Aqueous extracts and ethanolic extract of *Adenia lobata* and *Cola gigantea* Var. *glabrescens* have moderately activity resistant strain K1.

However, it is important to note that this plant is frequently used to treat fever, generally associated to malaria. Therefore, an explanation for their lack of *in vitro* antimalarial inactivity could be that these plants may act as antipyretics or may enhance the immune system, rather than having direct antiparasitic activity (Bero. J, Quetin-Leclercq. J, 2011). Another explanation is that this plant could contain prodrugs inactive by themselves. In this case, these precursors of the active compounds have to be metabolized *in vivo* into active antimalarials, a major limitation in this study.

Phytochemical screening

The saponins, polyterpenes and sterols are the constituents present in all extracts. The saponins and sterols have been reported as one of the important groups of phytoconstituents obtained from natural sources. The steroid, ergosterol-5,8-endoperoxide, isolated from the aerial parts of *Ajuga remota*, showed antiplasmodial activity against FCA 20/GHA strain of *P. falciparum*, with an IC₅₀ value of 8.2 µM (Kuria. K.A.M, et al., 2002). Steroidal saponins with anti-malarial activity have also been isolated from the leaves of *Vernonia amygdalina* (Ohigashi. H, et al., 1994). Ohigashi et al., reported the isolation of vernonioside A1, this saponins had weak antiplasmodial activity against the multidrug-resistant K1 strain of *P. falciparum* (Ohigashi. H, et al., 1994).

CONCLUSION

The search for new antimalarial drugs has become increasingly urgent due to plasmodial resistance to existing drugs. As part of this global effort, the present study aimed at doing the phytochemical screening and antimalaria evaluation of three medicinal plants used traditionally.

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