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# Antiplasmodial activity and phytochemical screening of *Landolphia heudelotii, Mitragyna ledermannii* and *Spathodea campanulata*, three traditional plants

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**Abstract**
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. Medicinal plants are currently being evaluated as source of promising antimalaria agents.
In this study, we have evaluated the claimed antimalarial properties of six extracts from three plants used in traditional medicine against malaria and we have investigated phytochemical screening.
Schizontocidal activity was measured using a standard *in vitro* assay, with NF54 and K1 *Plasmodium falciparum* strain. From the 6 extracts tested, four of them showed a promising activity ($5 \leq IC_{50} \leq 15$ µg/mL). All extracts contained Polyterpenes and sterols, Polyphenols, Flavonoids and Alkaloids.

**Keywords:** Traditional medicine; Medicinal plants; Malaria; Antimalarial; Plasmodium falciparum.

## Introduction
Malaria is currently the most deadly parasitic disease in the world, especially in developing countries [1]. This disease is transmitted by the female of an anophele 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 [2].
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 [3].
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 *Landolphia heudelotii*, *Mitragyna ledermannii* and *Spathodea campanulata* used traditionally against malaria access.

## Material and methods
### Material
The plant material used consists of ethanolic and aqueous extracts of *Landolphia heudelotii*, *Mitragyna ledermannii* and *Spathodea campanulata*, 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 leaves of *Landolphia heudelotii*, and Stem barks of *Mitragyna ledermannii* and *Spathodea campanulata*. The plants were collected from Agboville department and were identified by Floristic Center of Félix Houphouet-Boigny University.

The plant samples were then dried in shade left over for 15 days and powdered with the help of grinder. Powder was extracted according to Zihiri and Kra [4] 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 Riechman adopted by WHO [5]. This technique measure and quantify the capacity of drug to inhibit the growth of *P falciparum* at the trophozoïtes 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 Micro Beta counter. All results were expressed on a listing.

**Phytochemical screening**

The phytochemical screening was done using the standard protocols [5, 6].

**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 forth 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. $H_2SO_4$ 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 and 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 [6].

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 $IC_{50}$ values obtained. An extract showing an $IC_{50}$ value ≤ 5 µg/mL was classified as highly activity. Extracts with $IC_{50}$ values ≥ 5 µg/mL and ≤ 15 µg/mL were considered as promising activity. Extracts with $IC_{50}$ values ≥ 15 µg/mL and ≤ 50 µg/mL were considered as moderately activity and those with $IC_{50}$ values > 50 µg/mL inactive [7, 8, 9].

Aquenous extracts and ethanolic extract of *Landolphia heudelotii* and *Mitragyna ledermannii* have promising activity on NF 54 and K1 strain. These results justify the use of these plants in the treatment of malaria by traditional medicine.

The extracts of *Spathodea campanulata* have no action on *Plasmodium falciparum.* 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 [10]. Another explanation is that this plant could contain prodrugs non-active 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.

**Table 1:** *Landolphia heudelotii, Mitragyna ledermannii* and *Spathodea campanulata* antiplasmodial activity

| | Extracts | $IC_{50}$ of *P. falciparum* strains (µg/mL) | |
|---|---|---|---|
| | | NF54 | K1 |
| *Landolphia heudelotii* | Aqe | 7±0.14 | 8.11±0.65 |
| | Eeth | 5±1.77 | 18±0.51 |
| *Mitragyna ledermannii* | Aqe | 7±1.87 | 12.46±1.91 |
| | Eeth | 7±0.75 | 9.98±0.68 |
| *Spathodea campanulata* | Aqe | >50 | >50 |
| | Eeth | >50 | >50 |
| Chloroquine (nM) | | 11.03±0.23 | 124.74±4.96 |

**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 *Landolphia heudelotii* and *Mitragyna ledermannii* were shown to have same compositions.

The Alkaloids are the constituents present in all extracts. Alkaloids have been reported as one of the important groups of phytoconstituents obtained from natural sources. It plays an important role in the ecology of organisms which synthesize them. Alkaloids play an important role in the defence systems against pathogens and animals [11]. The applications of alkaloids are not limited to biological control of herbivores but also have pharmacological, veterinary and medical importance. Alkaloids belonging to beta-carboline group possess antimicrobial, anti-HIV and antiparasitic activities [12].

**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*** |
|---|---|---|---|---|---|---|---|---|
| *Landolphia heudelotii* | Eaq | + | + | + | - | - | ++ | + |
| | Eeth | ++ | ++ | ++ | - | - | + | - |
| *Mitragyna ledermannii* | Eaq | ++ | + | + | - | - | + | - |
| | Eeth | ++ | ++ | ++ | - | - | + | - |
| *Spathodea campanulata* | Eaq | + | + | ++ | + | + | ++ | - |
| | Eeth | ++ | ++ | ++ | - | - | + | - |

+: present + +: Abundantly present - : Absent

## 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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