Phytochemical Study and in Vitro Test of the Activity of Total Extracts of *Cissampelos Mucronata* (*Menispermaceae*) Leaves on *Plasmodia Falciparum*

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Abstract:
Objectives: The objectives of the present work were to carry out a phytochemical and pharmacological study of total extracts of *Cissampelos mucronata* (*Menispermaceae*) leaves on *Plasmodium falciparum*. Methods: To achieve this we: harvested and dried the plant's leaves, and carried out phytochemical screening to detect the chemical substances contained in the plant; performed a chromatographic analysis to study the similarity of the chemical structures of these substances with modern antimalarial drugs; tested the in vitro antimalarial activity of extracts from this plant against modern antimalarial drugs: quinine, L-artem and Doxycycline. Results: The results obtained revealed that: the leaves of the *Cissampelos mucronata* plant (*Menispermaceae*) contain all the substances we're looking for, but at different levels; some of the active ingredients found in these leaves have chemical structures similar to those of the above-mentioned modern antimalarials, while others do not. Anti-malarial test confirms plant's activity against *Plasmodium falciparum*. Conclusion: alkaloids, flavonoids, glycosides, at 50%, terpenoids, lipoids, have a high concentration of active principle; saponins, phenols, terpenoids, are at 30%; steroids and quinones are at 20%. For thin-layer chromatography (TLC): In the aqueous extract of *Cissampelos mucronata* (*Menispermaceae*), there is one spot with a retention
coefficient (Rf) equal to that of L-arrt 0.9 and contains 5 different Rf spots; this means that the plant contains a single substance with a chemical structure similar to that of strong L-arrt. In the ethanolic extract of this plant, there is no substance with the same chemical structure as the antimalarial drugs in common use in the environment where they are used. Similarly, the ethanolic extract and the aqueous extract contain certain substances with the same chemical structure. In this plant, there is no active ingredient with the same chemical structure as doxycycline and ciprofloxacin. This has led us to conclude that the leaves of Cissampelos mucronata (Menispermaceae) need to be used by people in and around South Kivu province to treat malaria, but with caution, as the pharmaco-vigilance of this plant has not yet been elucidated.

**Keywords:** Phytochemical, in vitro test, activities, total extracts, cissampelos mucronata, plasmodium falciparum.

**Introduction**

**Problem Statement**

It's true that most modern medicines are derived from medicinal plants. However, modern medicine does not claim to cure all illnesses. A large proportion of the world’s population, especially in Africa, relies on herbal remedies, either because they are expensive and the population is very poor, or because they fail to produce the expected results (Utshudi, 2004).

Much of the scientific research carried out on this subject has not ignored the therapeutic virtues of the Cissampelos mucronata plant (Menispermaceae). In fact, published works on Cissampelos mucronata (Menispermaceae) have shown numerous medicinal uses, and throughout Africa the bitter rhizome is taken as an infusion, sometimes the leaves and stems, or the fruit juice, to treat gastrointestinal affections such as diarrhea, dysentery, colic, intestinal worms and digestive problems, as well as urogenital problems such as menstrual disorders, venereal diseases, infertility, azoospermia, and to trigger uterine contractions and initiate labor or abortion, then to expel the placenta (Tankó et al., 2007). Cissampelos mucronata (Menispermaceae) also has hypoglycemic activity in rats (Tankó et al., 2007). In eastern D.R. Congo, leaf decoction is used as a vermifuge against tapeworms, gastritis, diarrhea, dysentery, colic, intestinal worms and digestive problems, as well as urogenital problems such as menstrual disorders, venereal diseases, infertility, azoospermia, and to trigger uterine contractions and initiate labor or abortion, then to expel the placenta (Tankó et al., 2007). In eastern D.R. Congo, leaf decoction is used as a vermifuge against tapeworms, gastritis, diarrhea, dysentery, colic, intestinal worms and digestive problems, as well as urogenital problems such as menstrual disorders, venereal diseases, hernia, gonorrhea, snakebite, engines, tonsillitis and malaria (Balagizi, 2021; Plant Use, n.a.). The anti-malarial aspect was of particular interest to us, as the rural population of eastern D.R. Congo uses it to treat malaria. Anti-malarial plant extracts are among the essential medicines used in this region, and it is therefore necessary to think about them in order to alleviate the suffering of malaria victims (Gilbert, A. et al., 2010; WHO, n.a.). Malaria transmission is almost permanent, with 97% of the population living in regions where transmission is permanent (WHO, 2020).

Contamination by the Plasmodium Falciparum parasite is the most common and most serious form of malaria, and is currently fatal in 20% of cases (NMCP, 2013). Despite the measures taken to combat malaria, this endemic still persists, and malaria is the leading cause of mortality and morbidity among children aged 0 to 5, pregnant women, PLWHA and non-immune travellers (NMCP, 2013). It is therefore vital to conduct research into new antimalarial products that are accessible and less costly for the population. Since 1987, strains of parasites have been appearing that no longer react to conventional drugs. Several studies have shown the resistance of Plasmodium to synthetic antimalarial drugs in Central and West Africa (Traoré, 2019; DVDP & Dakouo, 2020; Diabaté, 2021), so it is with the aim of contributing to the enrichment of the therapeutic arsenal in the fight against malaria that the present scientific work entitled Phytochemical study and in vitro test of the antimalarial activity of cissampelos mucronata leaves (Menispermaceae) was conceived in the field of pharmaceutical organic chemistry to make our contribution to the discovery of new natural products against malaria.
This leads us to ask ourselves a few questions:

1. Are the leaves of the *Cissampelos micronata* plant (*Menispermaceae*) really anti-malarial?
2. Are there any chemical compounds behind this activity?
3. How would extracts from this plant compare with modern antimalarial drugs?

**Working Hypothesis**

- Total leaf extracts of *Cissampelos micronata* (*Menispermaceae*) contain natural substances that are as active on *Plasmodium falciparum* as modern antimalarial drugs.
- Some of these substances are similar to modern antimalarial drugs.
- The antiplasmodial activity of total extracts of *Cissampelos micronata* (*Menispermaceae*) leaves is comparable to that of quinine, L-artemforte and doxycillin.

**Objectives**

- Determine the active ingredients contained in the leaves of this plant and classify them by phytochemical screening.
- Perform thin-layer chromatography to identify their similar chemical structures to modern antimalarial control drugs.
- Carry out an in vitro test of the antimalarial activity of total extracts of *Cissampelos micronata* (*Menispermaceae*) to determine the biological activity of its extracts on *Plasmodium falciparum*.

**Interest of the Subject**

Our choice of *Cissampelos micronata* (*Menispermaceae*) leaves was also made with a view to increasing the therapeutic arsenal in the fight against malaria. This study will help us decide whether to include the *Cissampelos micronata* (*Menispermaceae*) plant in the Congolese pharmacopoeia for the treatment of malaria, and thus put it on the list of essential medicines in the D.R. Congo.

**Materials and Methods**

**Materials**

**Laboratory equipment:** hemolysis tubes, plastic pipettes, plastic gloves, incubator, pestle, mortar, chromatographic plate, hot plate, chromatographic vat, Erlenmeyer flasks, Berckel balance, funnel, test tube, water bath, micropipette, chromatographic vat, flask, absorbent cotton, TDR blade, highly malarial blood, incubator.

**Plant material:** The leaves of the *Cissampelos micronata* plant (*Menispermaceae*) were harvested in the town of Bukavu in the commune of Kadutu, along the Karhale hill on national road no. 2 opposite the SOS center, during the month of March 2022. It was dried in the shade and crushed in a sterilized mortar to obtain a fine powder. This powder was preserved according to pharmacopoeia-accepted galenic standards.

![Figure 1. Cissampelos Micronata (Menispermaceae)](image)

**Methodology**

The methodology used in this work combined several techniques, namely:

1. **Documentary techniques:** we used these to develop all the theory we needed to interpret our results.
2. **Sample preparation:** we began by harvesting and drying the leaves of *Cissampelos Micronata* (*Menispermaceae*), then pulverizing them to obtain powders which we preserved in accordance with Pharmacopoeia standards before submitting them for analysis. To do this, we proceeded as follows:
Preparation of Aqueous and Ethanolic extracts

Extracts of the plant's leaves were prepared using conventional maceration methods, the technique most frequently used by traditional practitioners. An aqueous extract obtained by macerating 30g of each plant powder in 300ml of water, to obtain a concentration of 0.1g/ml of aqueous solution, and an ethanolic extract obtained by macerating 30g of each plant powder in 70% ethanol.

After maceration for 24 hours, the extracts were filtered to obtain filtrates for phytochemical screening and antimalarial activity testing, as well as for thin-layer chromatography.

Sample Preparation:

- Highly malarial blood identified by a Rapid Reaction Test and a thick drop (G.E.).
- Weigh out 1g of Quinine and dissolve in 100ml of 70% Ethanol.
- Weigh out 1g of L-Artem Forte and dissolve in 100ml of Ethanol 70%; - Take 100ml of each plant extract in 2 different test tubes.
- Evaporate all the solvent in the plant extracts and rework the solution with hexane to remove all the chlorophyll (Kaishusha et al., 2021).

Qualitative analysis of extracts (aqueous and/or organic extracts) of the Cissampelos Mucronata plant (Menispermaceae): this enabled us to detect the natural substances contained in this plant. This is the phytochemical screening. Through this screening, we have.

Search for Alkaloids (Bruneton, 2009)

Definition

Alkaloids are basic nitrogenous substances containing one or more heterocyclic nuclei. They are synthesized by plants from amino acids or their immediate derivatives, giving them physiological properties and a wide range of pharmacological activity.

Detection

It is based on the reaction of staining and/or precipitation of this, using appropriate reagents.

How it works

- Take 3 ml of each plant extract in two test tubes.
- In some tubes, add 1 ml of WAGNER reagent (2 g of diode(I )2 + 6g of potassium iodide(KI) in 75 ml of distilled water and make up to 100 ml with distilled water), others of MAYER reagent (1.36g of Hg Cl2 and 60ml of distilled water, make up to 100ml with distilled water).
- Leave the solution for 10 minutes.
- In the WAGNER reaction, the appearance of a brown, red or black precipitate indicates the presence of alkaloids, and in the MAYER reaction, the appearance of a yellowish-blue precipitate indicates the presence of alkaloids (Bruneton, 2009).

Search for Terpenoids (Bruneton, 2009)

Detection: terpenoids are detected from ethereal organic extracts in the presence of anhydrous acetic acid and concentrated sulfuric acid. Ethereal extracts containing terpenoids give purple colorations.

How it works

- 5 g powdered leaves of this plant are macerated in 50 ml petroleum ether for 24 hours.
- After extraction, filter and evaporate the solvent.
- Remove residues with 30 ml acetic anhydride.
- Take 3ml of the acidulated solution and add the Liberman-Burchard reagent (acetic anhydride solution) and concentrated H2 SO4 in one case and Hirchson (trichloroacetic acid) in the other.
- The change in color from yellow to red indicates the presence of terpenoids.

Search for Saponins (Bruneton, 2009)

Detection
It's based on their foaming power. By agitating their aqueous solution, saponins produce abundant, persistent foam.

In the presence of concentrated H₂SO₄ and K₂Cr₂O₇, they give a greenish-salty or violet to reddish-tan coloration. They are soluble in water and ethanol.

**How it works**
Macerate 5 g leaf powder in 50 ml distilled water for 24 hours and evaporate to dryness;
Recover residues with 10 ml distilled water.
Take 3 ml of this solution in 2 test tubes, shake for one minute and leave to stand for 30 minutes;
Measure the height of the persistent moss that indicates the presence of saponins.

**Search for Glucosides** (Bruneton, 2009)
Glucosides may be quinonic, chloroglucic, cyanogenic, indolic, cholanic or isosulfocyanic derivatives, and may derive from coumarins. Glycoalkaloids belong to the cardiovascular glycosides group.

**Detection**
In the presence of the appropriate reagents, the aqueous glycosidic solution gives particular colorations.

**How it works**
- Take 3 ml of each extract in test tubes and add one ml of Fehling's liqueur acidulated with 1% HCl.
- Repeat the same procedure with H₂SO₄ 84%.
- Particular colorations (brick red or reddish brown) are perceptible.

**Search for Flavonoids** (Bruneton, 2009)
Detection
In the presence of H₂SO₄, 1N, the aqueous extract containing flavonoids gives characteristic colorations to chromans (components of plant pigments), flavones, flavonols, flavonones and chalcones. In the presence of alkalis (KOH, NaOH), the aqueous extract gives deep colorations.

**Phenol Research** (Bruneton, 2009)
**Detection**
Phenols are detected using ethanoic extracts. In the presence of appropriate reagents, they give green, blue, violet or red colorations.

**How it works**
- Take 3ml of the ethanoic extract and add 1ml FeCl₃ 1%, bright colors appear.
- Repeat the same manipulations with concentrated H₂SO₄ to develop a coloration characteristic of ether phenologies.

**Search for Steroids** (Bruneton, 2009)
**Detection**
In the presence of anhydrous acetic acid and concentrated sulfuric acid, ethereal or chloroformic organic extract containing steroids gives a mauve or green coloration.

**How it works**
- Take 10g of plant powder macerated in chloroform for 24 hours.
- After maceration, heat to approximately 96°C, filter and evaporate the solvent.
- The residue obtained is taken up in 30 ml of acetic anhydride.
- From the solution obtained, take 3ml and add the LIEBERMAN-BURCHAR reagent (acetic anhydride solution and sulfuric acid).
- The appearance of a mauve-green color indicates the presence of steroids.

**Lipid Research** (Bruneton, 2009)
**Detection** is based on ethereal or hexane organic extracts. Ether remains the best solvent for their extraction. In the presence of concentrated H₂SO₄ lipids give off violet or green stains.

**How it works**
- The ethereal extract is filtered after 10 min.
- Heat to approximately 96°C until all solvents have evaporated.
• Recover the residue with acetic anhydride and heat the solution slightly to facilitate dissolution; take 3ml from the test tube and add 1ml H₂SO₄ 1N.
• Violet or green coloration indicates the presence of lipid cells.

Search for Quinones (Bruneton, 2009)

Detection
In the presence of alkalis (NH₄OH, NaOH, KOH) quinolone gives characteristic colorations depending on the type of quinone compounds present.

How it works
Add 5ml NaOH 1 to the benzene extract. In the aqueous solution, after filtration and addition, a pinkish-red color appears, indicating the presence of quinones.

Search for Tannins (Bruneton, 2009)

Detection
In the presence of FeCl₃ (1%) or HCl (1N), aqueous tannin extracts give blue, blue-green, dark blue and green colorations.

How it works
To 3ml of aqueous extract, add 1ml FeCl₃ 1% or 1N HCl.
Take 3ml of this test solution and add 1ml of STIASNY reagent (40% formaldehyde plus 1N HCl in a 2:1 ratio), then heat in a water bath.
A blue-black precipitate appears, attesting to the presence of tannin.

Thin Layer Chroma to Graphic Analysis
It has enabled us to elucidate the similar chemical structures of the contents of this plant’s leaf extracts with modern antimalarials and antibiotics. The steps of this method are:

Sample Preparation
• Harvesting the Cissampelos mucronata plant (Menispermaceae) in the fields surrounding road no. 2 opposite the SOS/BUKAVU center.
• Dry the plant in the shade to obtain a powder.

• Maceration of 30g of powder in 300ml of pure water and 100ml of 70% ethanol for 24 hours to obtain 2 extracts: the aqueous extract and the ethanolic extract.
• After 24 hours, filter the extracts with absorbent cotton.
• Dissolve control drugs in 70% methanol and
• Heat solvents.
• Wash the residue with hexane to remove all chlorophyll.
• Recover the remainder with a methanol solution (70%).

Chromatographic Plate Preparation
• The chromatographic plate used is aluminum oxide foil;
• The 1cm lower edge area we missed with a pencil; 1cm horizontal equidistant dots depending on the number of samples to be analyzed (Horn et al., 2000; Holler et al., 1997).
• Draw the demarcation line on the top edge of the plate.

Eluent Preparation
The eluent is a solvent or mixture of solvents used to drive the constituents of a mixture through a stationary phase (Holler et al., 1997). For the present work; the eluent we used is BAW (Bitanol-Acetic-acid-water) in the proportion of 4:1:5.

Homogenize the mixture, then decant.

Comparative Thin-Layer Chromatography (c.c.m.)

How it works
• Using micro-pipettes (capillary tubes), take one drop of each sample of Cissampelos mucronata (Menispermaceae) plant extracts and place them on equidistant points previously marked with a pencil on the chromatographic plate.
• Place the eluent in the chromatographic cell up to a height of 0.5cm.
• Place the chromatographic plate in the tray so that the deposition line is 1cm from the eluent and close tightly.
• Remove the plate as soon as the eluent front is 2cm from the top edge of the plate.
• Allow the plate to dry, then proceed with development.
• The different migration speeds of active ingredients help to determine the similarity between the chemical and active ingredient structures and, by analogy, the pharmacological activity (Horn et al., 2000).

**Revelation:**
Chromatograms of chemical species need to be revealed in order to identify the position of these species near elution. A plate containing a fluorescent substance can be used; in the presence of UV radiation; or chemical developers such as diode (Horn et al., 2000) or an acidulated solution of KMnO₄ as they interact with many chemical species to give colored products. After elution, the plate can be shaken in a closed glass vial and dipped in an acidulated solution of KMnO₄ colored spots appear with 2 developers (Horn et al., 2000). For the present work, diiodine was used as the developer.

**Calculation:**

\[ R = \frac{h}{H} \]  
(1)
Where \( h \) = height occupied by the spot; \( H \) = distance covered by the eluent; 
\( R_f \) = Retention factor.

**Malaria Testing:**
Using the necessary equipment, we were able to study the efficacy of plant extracts compared with modern antimalarial drugs (quinine and l- artem forte) and antibiotics.

This test uses two methods: the rapid reaction test (RRT) and the thick drop method.

a. **Rapid reaction test (RRT).**
• Collect the transfer sample using the micro pipette provided.
• Add 5μl of drops to well "S".

b. **Thick drop test (GE)**

**Calculating pest Density (PD)**
Determining parasite density may be required for cross-sectional surveys, epidemiological research or special studies such as evaluating the therapeutic efficacy of antimalarial drugs.

This will require two manual counters (one for the parasite and the other for the leukocytes).

The method consists of.
• Count parasites observed with one counter and leukocytes with the other, field after field, using an immersion objective.
• The number of parasites and leukocytes you count depends on their frequency and the time you have to count them. The lower the number of parasites counted, the higher the number of leukocytes counted. Once the count is complete, we calculate the number of leukocytes and prime the result in "parasites per microliter of blood" using a simple mathematical formula.

If DP is the parasite density, then:

\[ PD = \frac{\text{Nombre de parasites comptés}}{\text{Nombre de leucocytes comptés}} \times 800 \]  
(2)
In this system we have:
+=1 to 10 Parasite per 100 fields from thick drop to immersion lens.
++=11 to 100 Parasites per 100 fields from thick drop to immersion lens.
+ + + = 11 to 10 Parasites per field from thick drop to immersion lens
+ + + + = More than 10 parasites per field from the thick drop to the immersion lens (WHO, 2016).
8000 = Average number of leukocytes for a normal person recommended by the WHO.

Preparing the Thick Taste

1<sup>ère</sup> Experimentation:
- First calculate the parasitic load
- Using a plastic pasteur pipette, draw 4ml of highly malarial blood from 4 different test tubes.
- Add 4ml of aqueous extract and 4ml of ethanolic extract of the leaves of *Cissampelos mucronata* (Menispermaceae) to the 1<sup>ère</sup> test tube, 4ml of quinine sulfate solution to the 3<sup>ème</sup> test tube and 4ml of strong L-Artem solution to the 4<sup>ème</sup> test tube;
- Incubate for 24 hours in an oven at 37°C
- Microscopic examination: TDR and G.E: Parasitology examination.

Results and Interpretation

Phytochemical Screening Results

Table 1. Phytochemical Screening of *Cissampelos Mucronata* (*Menispermaceae*) leaves at the CRSN /LWIRO Malacology Laboratory in July 2022

<table>
<thead>
<tr>
<th>Chemical group and reagents used</th>
<th>Results from different reagents</th>
<th>Average concentration</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids Dragendorff reagent</td>
<td>+ + +</td>
<td>+++</td>
<td>High concentration or high content</td>
</tr>
<tr>
<td>Wagner reagent</td>
<td>+ + +</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Mayer reagent</td>
<td>+ + +</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Macreter reagent</td>
<td>+ + +</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Saponins Physical reagent K reagent CO&lt;sub&gt;2&lt;/sub&gt; + H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Flavonoids H reagent SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>+ + +</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>NaOH reagent</td>
<td>+ + +</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Glycosides Fehling + H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>+ + +</td>
<td>+++</td>
<td>Very high concentration</td>
</tr>
<tr>
<td>Fehling + HCl</td>
<td>+ + +</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Tannoids F reagent, Cl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>+ + +</td>
<td>+++</td>
<td>Very high concentration</td>
</tr>
<tr>
<td>STIASNY reagent</td>
<td>+ + +</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Phenols F reagent, Cl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>+</td>
<td>+</td>
<td>Average concentration or average content</td>
</tr>
<tr>
<td>Reactive with H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Steroids Acetic acid (Lieber Man reaction H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>+</td>
<td>+</td>
<td>Low concentration or content</td>
</tr>
<tr>
<td>Terpenoids Lieber Man reagent: H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; and acetic anhydride, Hirschson reagent (trichloroacetic acid)</td>
<td>+</td>
<td>++</td>
<td>Average concentration</td>
</tr>
<tr>
<td>Lipoids H reagent SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>+ + +</td>
<td>+++</td>
<td>High concentration or content</td>
</tr>
<tr>
<td>Quinones NaOH reagent</td>
<td>+</td>
<td>+</td>
<td>Low content or concentration</td>
</tr>
</tbody>
</table>

Note: + + + : high concentration of active ingredient; + + : average concentration of active ingredient; + : low concentration of active ingredient; - absence of an active ingredient.
Analysis of the table reveals that: the *Cissampelos mucronata* plant (*Menispermaceae*) contains all the chemical groups sought, but at different levels. Five active ingredients found in *Cissampelos mucronata* (*Menispermaceae*) plants are in high concentrations: alkaloids, glucosides, tannoids and lipoids. Of a total of 10 active ingredients found, the 5 are very abundant, accounting for 50% of the total. 3 active ingredients are of average content, namely terpenoids, phenols and saponosides, making up 30% of the total. Other active ingredients, such as quinones and steroids, are in low concentrations, representing 20% of the total.

**Chromatography result**

Comparative thin-layer chromatography carried out at the malacology laboratory of the Centre des Recherches en Sciences Naturelles (CRSN)/LWIRO yielded the following results.

![Figure 2. Chromatography Paper with Results](image)

The C. Mucronata plant has 5 spots in its aqueous extract and 5 spots in its ethanolic extract. These extracts occupy different heights, some of them at the same position.

Where CA=Cissampelos Mucronata extract; CE=Ethanolic extract of Cissampelos Mucronata; LA= Methanolic solution of strong L-artem; Q=Methanolic solution of Quinine sulfate.

Chromatogram analysis involved measuring the height (H) travelled by the solvent and the heights (h) reached by the sports (spots).

The species present in each task is characterized by its frontal ratio (RF) or retention coefficient

\[
RF = \frac{h}{H} \quad (3)
\]

Where:

- h - the distance travelled by the analyzed substances to the standard
- H - the distance covered by the eluent

Figure 2 shows the following results, from left to right: *Cissampelos mucronata* (*menispermaceae*) leaf extract, control drug solutions, such as quinine sulfate and L- artem forte.

### Table 2. Results of Chromatographic Experiments Carried Out in the Malacology Laboratory at CRSN Lwiro in July 2022

<table>
<thead>
<tr>
<th>Drug Witnesses</th>
<th>Cissampelos Mucronata plant extracts</th>
<th>Ethanol extracts (C&lt;sub&gt;Et&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous extracts (Caq)</td>
<td>Ethanol extracts (C&lt;sub&gt;Et&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Rf quinine</td>
<td>Rf L-Art</td>
<td>Rf1</td>
</tr>
<tr>
<td>0,8</td>
<td>0,92</td>
<td>0,2</td>
</tr>
</tbody>
</table>

**Note:** Caq: Aqueous extract of *Cissampelos Mucronata*, C<sub>Et</sub>: Ethanol extract of *Cissampelos Mucronata* and Rf: Frontal ratio.
The height reached by the solvent: \( H = 50 \) cm. Comparison of the frontal ratios of the various substances to be identified with the control products. The chromatogram then counts 10 spots or blobs, corresponding to front ratios (Rf); whether the sample contains different or identical chemical spaces, depending on their respective heights. For a given chromatographic plate and eluent, the frontal ratio depends only on the nature of the bodies present in the samples.

Bodies with the same Rf on the same plate are identical.

The chromatogram reveals that:

- *Cissampelos mucronata* queux extract contains 5 different Rf spots.
- This extract contains a retention coefficient (Rf) spot equal to that of L-artem at 0.9, which means that the plant contains a substance with a chemical structure similar to that of strong L-artem. These are Rf1 and Rf6, Rf7, Rf3, and Rf8.
  - This extract contains no chemical substances similar to quinine.
  - The ethanolic extract of cissampelos mucronata contains 5 spots of very different Rf; all these Rf are different from that of quinine and L-artm. So, we notice that in the ethanolic extract of this plant there is no substance with the same chemical structure as the antimalarial drugs commonly used in our environment.

**Malaria test results**

The results of in vitro testing of the antimalarial activity of extracts from the *Cissampelos mucronata* plant (*Menispermaceae*) are presented in the following table.

<table>
<thead>
<tr>
<th>Test type</th>
<th>Initial parasitic load</th>
<th>Extracts of <em>Cissampelos mucronata</em></th>
<th>Control antimalarials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Caq/20ml</td>
<td>CEt/20ml</td>
</tr>
<tr>
<td>Experience</td>
<td>TDR</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>GE</td>
<td>2400</td>
<td>1800</td>
<td>1000</td>
</tr>
<tr>
<td>Reduced parasite load</td>
<td>-</td>
<td>25%</td>
<td>58.3%</td>
</tr>
</tbody>
</table>

**Note:** Means negative: absence of parasites; + means positive test; Caq: Cissampelos Mucronata aqueous extract; CEt: Ethanol extract of Cissampelos Mucronata

The TDR revealed an initial parasitic load of 2400 rpm.

After adding 4ml of aqueous extract from the leaves of *Cissampelos mucronata* (*Menispermaceae*), the load was reduced from 2400 tr/ch to 1800 tr/ch (noted trophon per microscopic field), i.e. 25%, and after doing the same with the ethanolic extract, the initial parasite load was reduced from 2400 tr/ch to 1000, i.e. 58.3% of the reduced load. The drugs quinine and L-artem forte reduced the load to zero. These results show that the leaves of *Cissampelos mucronata* (Menispermaceae) have considerable anti-malarial activity, as the doses used are lower than those of modern anti-malarial drugs, so the efficacy is not comparable, but the effectiveness of this plant has been proven. These results are in line with the chromatographic analysis carried out, which gave a result affirming the similarity of the chemical structures of the few chemical substances contained in this antibiotic plant and then as a reference.

**Conclusion**
The aim of the present work was to determine the active principles responsible for the antimalarial action of the plant and to identify the active principles responsible for the antimalarial activity of the leaves of *Cissampelos micronata* (Menispermaceae). Experiments carried out in the Malacology laboratory of CRSN LWIRO and in the microbiology laboratory of ISTM/BUKAVU led us to the following conclusions:

For phytochemical screening:

- Alkaloids, flavonoids, glycosides, at 50%, terpenoids, lipoids, have a high concentration of active principle.
- Saponins, phenols and terpenoids account for 30%.
- Steroids and quinones account for 20%.
- For thin-layer chromatography (TLC).
  
  - In the aqueous extract of *Cissampelos micronata* (Menispermaceae) there is a spot with a retention coefficient (Rf) equal to that of L-artm 0.9 and contains 5 different Rf spots; this means that the plant contains a single substance with a chemical structure similar to that of strong L-artm.
  
  - In this extract there is no substance with a structure similar to that of quinine.
  
  - The ethanolic extract of this plant does not contain any substance with the same chemical structure as the antimalarial drugs in common use in the environment in which they are used.
  
  - Ethanolic and aqueous extracts also contain certain substances with similar chemical structures.
  
  - In this plant, there is no active ingredient with the same chemical structure as doxycycline and ciprofloxacin.

Isolating these active ingredients and studying them in depth would also contribute to the discovery of more new and costly anti-malarial products. For the anti-malarial test, the results obtained prove that the *Cissampelos Micronata* plant has anti-malarial activity, as the doses used for the tests reveal significant efficacy in relation to the initial parasite density. The aqueous and ethanolic extracts reduced the parasite load by 25% and 58.3% respectively. Our results show a moderate activity of *Cissampelos micronata* (menispermaceae) extracts compared with the control antimalarials used. However, further in vivo studies and extraction of the active ingredients and their in vitro testing are still required. So all our hypotheses are confirmed.

**Conflict of Interest**

The authors of this manuscript declare that there are no conflicts of interest.

**Authors' Contributions**

The research team was responsible for the conception of the subject, the harvesting, the drying of the plant, the spraying, the conception of the methodology, the experimental work at the various laboratories, and the writing of the manuscript. The other co-authors helped to proofread the methodology and the various versions of the manuscript to improve its scientific quality, and to raise funds to pay for publication costs.

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