The in vitro antisickling effect of purified alkaloids of *Cremaspora triflora* (Thonn.) K. Schum. (Rubiaceae) and *Macaranga schweinfurthii* Pax. (Euphorbiaceae)

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World Journal of Advanced Research and Reviews, 2021, 09(03), 129–137

Publication history: Received on 04 February 2021; revised on 06 March 2021; accepted on 09 March 2021

Article DOI: https://doi.org/10.30574/wjarr.2021.9.3.0074

**Abstract**

Introduction and objective: Sickle cell disease is an inherited pathology to an abnormality of hemoglobin precisely hemoglobin S for which there is no curative therapy. It mainly affects sub-Saharan African and Caribbean populations. Thus, this study aims to make the phytochemical screening of *Cremaspora triflora* and *Macaranga schweinfurthii* as well as to evaluate the antisickling activity of their purified alkaloids.

Methodology: Chemical screening was performed using color and precipitation tests as well as the foam index method. The extraction of the alkaloids was carried out with organic solvents in a basic medium while the purification by open column chromatography. The evaluation of the antisickling activity was carried out by Emmel’s test.

Results: The chemical screening highlighted alkaloids, steroids, saponins, tannins, and terpenoids in the species studied. Flavonoids and anthocyanins were present in organs of *Macaranga schweinfurthii*, but absent in *Cremaspora triflora*. The extraction showed that *Macaranga schweinfurthii* leaves contained 0.59% alkaloids and 0.73% alkaloids in *Cremaspora triflora* leaves. The alkaloids purification allowed to obtain an alkaloidal fraction MS1 (1.24 g, 70.05%) from *Macaranga schweinfurthii* and two fractions [CT2 (0.934 g, 63.97%) and CT3 (0.006 g, 0.41%)] from *Cremaspora triflora* which tested positive with Dragendorff and Wagner reagents. The antisickling activity evaluation showed that the SIR varied between 36.00% (0.25 mg/ml) and 90.66% (1 mg/ml) for the alkaloid solutions of *Cremaspora triflora* (IC\textsubscript{50} of 0.51 mg/ml) as well as between 4.00% (0.25 mg/ml) and 33.33% (1 mg/ml) for the alkaloid solutions of *Macaranga schweinfurthii* (IC\textsubscript{50} of 1.40 mg/ml).

Conclusion: This study showed that the purified alkaloids of the studied plant species have an inhibitory power on sickling.

**Keywords:** Chemical screening; alkaloid; Antisickling; Euphorbiaceae; Rubiaceae
1 Introduction

Sickle cell disease is a hereditary pathology mainly affecting populations of Central, and West Africa, Asia, the USA, the Caribbean region, and the Mediterranean region, in particular the Greeks, and Italians [1]. It is an inherited autosomal recessive genetic disease linked to an abnormal structure of hemoglobin leading to the formation of hemoglobin S (HbS). The disease systematically affects children who inherit the same mutant gene from their parents at birth. For a child with both parents who are carriers, the probability of inheriting both characteristic genes and having the disease is 25% and the probability of being a carrier is 50%. Most carriers are in good health and lead normal lives.

The modes of treatment envisaged for this pathology to relieve the patients are in particular the medullary transplant of the marrow, the repeated blood transfusion, or the taking of hydroxyurea, a molecule which would activate the genes of fetal hemoglobin (HbF) whose presence in the erythrocyte interferes with the polymerization of hemoglobin S [2,3,4]. Local populations cannot afford such treatments. This is how the majority of the population resort to the use of medicinal plants. Indeed, herbal medicine is currently presented as an alternative that can offer relief to sickle cell disease and its action on the body depends on the composition of the plants. Several plants are reported to be used for the treatment of sickle cell disease [5,6,7]. Medicinal plants can contain a wide variety of bioactive substances such as alkaloids, flavonoids, saponins, steroids, terpenoids, anthocyanins, and quinones which are endowed with several biological activities [8,9].

Several studies on antisickling activity showed that polyphenols (anthocyanins, flavonoids) can normalize sickle cells in vitro [3,10-14]. Besides, other studies showed that several other natural substances are antisickling agents. These include phenylalanine, p-hydroxybenzoic acid and its derivatives, maslinic, oleanolic, and betulinic acids [15], limonoid [16], cepharantine [17], saponins [18], and alkaloids [19,20].

Thus, this study will proceed to the phytochemical screening of harvested organs of Cremaspora triflora (Thonn.) K.Schum. (Rubiaceae), and Macaranga schweinfurthii Pax. (Euphorbiaceae), to extract and purify their alkaloids and to evaluate the antisickling activity of the purified alkaloids of these two species.

2 Material and methods

2.1 Plant material

The plant material consisted of the leaves, stems or their bark and roots, or their bark of C. triflora [Mubaba wanika (Bemba, Lala, and Lamba)], and M. schweinfurthii [Munkala (Luba), and Kilongalong (Lunda)]. These two species were collected in the forest gallery of the Kafubu River on the Sambwa side. The identification of the studied species was carried out by comparison with the reference herbaria at INERA-Kipopo. The harvested organs were dried out of direct sunlight at the Department of Chemistry of the Faculty of Sciences of the University of Lubumbashi. They were then crushed, reduced to a coarse powder, and then stored in plastic packaging.

2.2 Biological material

Blood was collected with the informed consent of the patient, and his parents whose SS sickle cell disease was confirmed by electrophoresis. This 5-year-old patient was not transfused or treated with hydroxyurea during the six months before the blood collection. After collection, the blood was kept in the fridge (Samsung brand, made in South Korea) at 4°C. The sample was taken from the patient's elbow crease and blood was collected in the EDTA tube [21].

2.3 Phytochemical screening

The highlighting of chemical substance groups in solution is based on coloring reactions (due to complexation by charge transfer), precipitation (by phase separation), and formation of foams. These techniques concerned the search of alkaloids, flavonoids, anthocyanins, quinones, saponins, tannins, steroids, and terpenoids found in organs of the species studied [22-27].

2.4 Extraction of alkaloids and their purification

200 g of ground material was macerated in 200 ml of methanol in an Erlenmeyer flask for 24 hours. The solution obtained was filtered and then concentrated to dryness at a rotary evaporator. The crude extract obtained after evaporation was taken up in 400 ml of 1% HCl (Sigma-Aldrich, Saint-Quentin Fallavier, France) and filtered through filter paper; the filtrate was basified with 20 ml of NH₄OH (Sigma-Aldrich, Saint-Quentin Fallavier, France) to reach a pH of 9. The alkaloids were then extracted thrice with 200 ml of chloroform (Sigma-Aldrich, Saint-Quentin Fallavier, ...
France) in a separatory funnel. After complete depletion of the alkaloids, the settled organic phase was filtered through filter paper, then evaporated in a rotary evaporator (Büchi brand, manufactured in Flawil, Swisse). This solution was then taken up twice in 200 ml of 0.5 M citric acid (Sigma–Aldrich, Saint-Quentin Fallavier, France) and then basified with 10 ml of 12% NH₄OH and extracted four times with 50 ml of chloroform. Chloroformed phases were combined, and filtered through filter paper (Whatman brand) wet with chloroform. The evaporation of the solvent to dryness at reduced pressure provided the extract of total alkaloids [28, 29].

The total alkaloid extracts chromatographed on an open column of silica gel 60F₂₅₄ were successively eluted in step-gradient mode using AcOEt-MeOH as mobile phase (100: 0, 90:10, 80:20, 70:30, 60:40, 0:100 v/v). The different fractions were subjected to thin-layer chromatography (TLC) on aluminum plates using as mobile phase AcOEt-MeOH-NH₄OH (90: 9.5: 0.5, v/v/v), and TLC plates were sprayed with Dragendorff's, Wagner's, and iodine reagents. The fractions with identical profiles were combined, evaporated at 30-40 °C to dryness and then stored in the fridge at 4°C before carrying out the biological tests.

2.5 Emmel's test

2.5.1 Preparation of solutions

Weigh 1 mg of the dry sample (Purified alkaloids); Dissolve it beforehand in a few drops of DMSO later in 1 ml of physiological water to have an initial concentration of 1 mg/ml. Diluted the main solution to get solutions of 0.5 mg/ml, and 0.25 mg/ml respectively.

The control was prepared by mixing a drop of sickle cell blood, and a drop of physiological solution (0.9% NaCl). This preparation was incubated for 24 hours and then observed on an optical microscope (Brand Nikon Eclipse E200, Melville, NY, USA) after incubation in 5 fields.

2.5.2 Assessment of antisickling activity in vitro

A drop of blood placed on a slide was mixed with a drop of the alkaloid extract. The solution obtained was protected by a coverslip and sealed with molten paraffin for a microscopic preparation. After incubation for 24 h in a water bath at 37°C, the preparations are examined under a digital optical microscope (Brand Nikon Eclipse E200, Melville, NY, USA). The images were observed in 5 different fields (on the left, on the right in the center, above, and below) by the same observer. The sickle cell inhibition rate or sickle cell preservation rate was calculated by the following formula:

\[
\text{SIR (%) = } \left( \frac{\text{NSCBT} - \text{NSCAT}}{\text{NSCBT}} \right) \times 100
\]

With NSCBT: Number of Sickle cells before treatment with the extract; NSCAT: Number of sickle cells after treatment with the extract; SIR: Sickle cell Inhibition Rate.

3 Results

3.1 Phytochemical screening

Alkaloids (Alc), flavonoids (Flav), anthocyanins (Ant), quinones (Qun), saponins (Sap), tannins (Tan), steroids (Ste) and terpenoids (Ter) were investigated in leaf powders (F), stems (T) or their bark (ET) and root bark (ER) (Table 1).

| Table 1 Chemical groups in different organs of species studied |
|---------------------------------------------------------------|
| **Plant specie**     | **PU** | **Chemical substances group** |
|                     |       | Alc | Flav | Ant | Ste | Sap | Tan | Qun | Ter |
| Cremaspora triflora | F     | +  | -   | -   | +   | +   | +   | -   | +   |
|                     | T     | +  | -   | -   | +   | +   | +   | -   | +   |
| Macaranga schweinfurthii | ER | +  | +   | +   | +   | +   | +   | -   | +   |
|                       | ET    | -  | +   | +   | +   | +   | +   | -   | -   |
|                       | F     | +  | +   | +   | +   | +   | +   | -   | +   |

Avec +: Presence; -: Absence
Alkaloids, steroids, saponins, tannins, and terpenoids were found in both species studied. Nevertheless, flavonoids and anthocyanins were present in the organs of *M. schweinfurthii* while they have did not identify in *C. triflora*. A total absence of quinones was observed in studied plant species.

### 3.2 Extraction and purification of alkaloids

The alkaloids were extracted with the organic solvent (methanol and dichloromethane) from a basified aqueous phase [9, 28] containing the extract of the leaves.

#### Table 2 Results of total alkaloids extraction.

| Plant specie              | PM (g) | CE (g) | EY (%) | Aspect | AlcT (g) | T (%) |
|---------------------------|--------|--------|--------|--------|----------|-------|
| *Cremaspora triflora*     | 200    | 29.9   | 14.95  | Greenish| 1.46     | 0.73  |
| *Macaranga schweinfurthii*| 300    | 58.8   | 19.60  | Greenish| 1.77     | 0.59  |

With **PM**: Plant material; **CE**: Crude Extract; **EY**: Extraction Yield; **AlcT**: Total alkaloids; **T**: Alkaloid content in the organ studied.

After extractions of the alkaloids, it was observed that *M. schweinfurthii* contained 1.77 g of alkaloids per 300 g of plant material, i.e. 0.59% of alkaloids in the leaves used while *C. triflora* contained 1.46 g of alkaloids per 200 g of plant material (0.73% alkaloids).

The extracted alkaloids were subjected to open column chromatography on a silica gel 60F254, with AcOEt-MeOH as mobile phase (100: 0, 90:10, 80:20, 70:30, 60:40, 0: 100, v/v) coupled to TLC analyses using as mobile phase AcOEt-MeOH-NH4OH (90: 9.5: 0.5, v/v/v), and plates were revealed with the reagents of Dragendorff, Wagner, and iodine.

#### Table 3 Purification of total alkaloids.

| Plant specie               | AlcT (g) | Alkaloid fractions characteristics |
|----------------------------|----------|-----------------------------------|
| *Cremaspora triflora*      | 1.46     | CT1 0.520 35.61 Greenish Greenish Dark - |
|                            |          | CT2 0.934 63.97 Mauve Red Orange + |
|                            |          | CT3 0.006 0.41 Mauve Red Orange + |
| *Macaranga schweinfurthii* | 1.77     | MS1 1.24 70.05 Yellow Red Orange + |
|                            |          | MS2 0.53 29.94 Greenish Greenish Dark - |

Spray reagent (I: Iodine; W: Wagner; D: Dragendorff); + : Alkaloid; - : Other substance; Obs: observation

The fractionation of the total alkaloids yielded 3 fractions from 1.46g of total alkaloids of *C. triflora*. These are the CT1 (0.520 g, 35.61%), CT2 (0.934 g, 63.97%), and CT3 (0.006 g, 0.41%). From 1.77 g of total alkaloids of *M. schweinfurthii*, two fractions MS1 (1.24 g, 70.05%), and MS2 (0.53 g, 29.94%) were obtained. CT2, CT3, and MS1 are alkaloid fractions because they were positive in contact with Dragendorff, and Wagner, two of the six reagents used for alkaloid detection.

### 3.3 Evaluation of the antisickling activity in vitro

Several tests showed that crude plant extracts or solutions of anthocyanins can normalize sickle cells in the blood of a person with sickle cell anemia. To verify this hypothesis, the alkaloid fractions (CT2, and MS1) were subjected to the evaluation of the antisickling activity by Emmel's test (Figure 1a-d, and Table 4).
Figure 1 Morphology of sickle cells before (a), and after treatment with C. triflora alkaloidal solution (b-d)

The action of the solutions of the alkaloids showed that these substances had inhibitory activity on sickle cell disease. The sickle cell count before treatment (NSCBT) and after treatment (NSCAT) with the alkaloid solutions was used to calculate the sickle cell inhibition rate (SIR) (Table 4).

Table 4 Results of the biological test.

| Plant species          | Purified alkaloid | C (mg/ml) | NSCBT | NSCAT | SIR (%) |
|------------------------|-------------------|-----------|-------|-------|---------|
| Control                | -                 | -         | 75    | 75    | 0       |
| Cremaspora triflora    | CT2               | 1         | 75    | 7     | 90.66   |
|                        |                   | 0.5       | 75    | 46    | 38.66   |
|                        |                   | 0.25      | 75    | 48    | 36.00   |
| Macaranga schweinfurthii| MS1               | 1         | 75    | 50    | 33.33   |
|                        |                   | 0.5       | 75    | 58    | 22.66   |
|                        |                   | 0.25      | 75    | 72    | 4.00    |

It appeared that only the alkaloid solution at 1 mg/ml of Cremaspora triflora gave the sickle cell inhibition rate (SIR) of 90.66%. Regarding the other solutions, the results showed that at 0.25 mg/ml the SIR was 36.00%, and at 0.5 mg/ml the SIR was 38.66%. An inhibition rate of sickling of less than 40% has been observed. Besides, the alkaloid solutions of Macaranga schweinfurthii showed an inhibition rate of less than 35% with an almost nil SIR for the 0.25 mg/ml solution.

These data made it possible to plot the curve of the sickling inhibition rate as a function of the concentration of the solutions of the alkaloids studied (Figure 2).
Figure 2 Evolution of the SIR and trend curve for alkaloid solutions of C. triflora (a-b) and M. schweinfurthii (c-d).

It appears that the evolution of the inhibition rate as a function of the concentration of alkaloid solutions of C. triflora follows an exponential trend while that of M. schweinfurthii, a logarithmic trend. The concentration that inhibits the sickling of drepanocytes at 50% was 0.51 mg/ml for the alkaloids of C. triflora, and 1.40 mg/ml for the alkaloids of M. schweinfurthii.

4 Discussion

Alkaloids, steroids, saponins, tannins, terpenoids, flavonoids, and anthocyanins were found in the species studied. These are groups of chemical substances with various biological activities including antibacterial, antifungal, anticancer, antioxidant, antimalarial, antiviral, antidiabetic, and hepatoprotective activities [8, 9, 29]. The presence of polyphenols such as anthocyanins and flavonoids is proof that the species studied contain molecules with the capacity to inhibit sickling in vitro and/or in vivo [9-11, 13, 14].

The total alkaloids content in M. schweinfurthii leaves (0.59% alkaloids), and C. triflora leaves (0.73% alkaloids) indicates that these plant species are alkaloidal because a plant is considered alkaloidal when its alkaloid content is greater than or equal to 0.01% [29, 30]. Alkaloids were always scarce in plants in general [29, 31].

In another study, Mbayo (2019) [29] showed that the alkaloid content of M. schweinfurthii was 0.1126% in leaves and 0.1084% in stem bark. These contents are less than 0.59% obtained during the extraction of alkaloids in the leaves of the same species. This discrepancy could be justified by the fact that the content and chemical composition of a plant species depends on the harvest period, the plant age at harvest, the nature of the soil, and other physical and biological characteristics of the plant ecosystem [25].

The evaluation of the antisickling activity by the Emmel method showed that the C. triflora alkaloids solutions exhibited the power to normalize the shape of sickle cells with SIR varying between 36.00% (0.25 mg/ml), and 90.66% (1 mg/ml) with an IC$_{50}$ of 0.51 mg/ml. Besides, the alkaloid solutions of M. schweinfurthii showed an inhibition rate of less than
35% with an almost zero SIR for the 0.25 mg/ml solution, and an IC50 of 1.40 mg/ml. This shows that the *C. triflora* purified alkaloid reacted with a lower IC50 is more active than that of *M. schweinfurthii* [19,20].

This study has just shown that the inhibition of sickling decreases with the decrease in the alkaloid concentration of the solution. In other words, less the solution is concentrated; the weak will be its inhibitory power. The results of inhibiting sickle cell disease at high concentrations could be interesting, but the toxicity of alkaloids may limit their use. Thus, toxicity studies and elucidation of the alkaloid structure of these two plants would allow the lethal dose to be determined, although it was found that they have low antisickling activity.

## 5 Conclusion

The objective of this study was to evaluate the antisickling activity of the total alkaloids of two plants, namely *M. schweinfurthii* and *C. triflora*. Solutions of the total alkaloid fractions showed an antisickling property which varied with increasing alkaloid concentration. These preliminary results, which corroborate existing data in the literature, would validate the traditional use of these plants in the symptomatic treatment of sickle cell disease. In perspective, the evaluation of the in vivo toxicity of the purified alkaloids and their characterization as well as the evaluation of the antisickling activity of all the purified and characterized alkaloids will be considered.

### Compliance with ethical standards

**Acknowledgments**

The authors sincerely thank the Sendwe general reference hospital in Lubumbashi for providing sickle cell blood.

**Disclosure of conflict of interest**

The authors declare no competing interests.

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