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# *In vitro* validation of anti-*Plasmodium* potency of plants-based extracts from *Scoparia dulcis* Linn used traditionally to treat malaria in Niger

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**Malaria is a parasitic disease that is currently considered a major public health problem. The parasite responsible, *Plasmodium falciparum*, is increasingly resistant to current anti-malarial drugs. The objective of this study was to assess the *in vitro* anti-plasmodial potential of *Scoparia dulcis* Linn (Scrophulariaceae), a plant traditionally used to treat malaria in Niger. Organic and aqueous extracts were prepared from the leafy stems of *S. dulcis* Linn. Dichloromethane, methanol, and hydro-methanol were used for organic extraction, while an alkaloid extract was prepared through a specific extraction method. Aqueous extracts were prepared by boiling water. Chemical characterization was performed using tube assays and Thin Layer Chromatography (TLC). The *in vitro* anti-plasmodial activity of the extracts was assessed using the Lactate Dehydrogenase (p-LDH) technique on chloroquine-resistant K1 and chloroquine-sensitive 3D7 strains of *P. falciparum*. Phytochemical screening revealed the presence of various chemical groups, including flavonoids, sterols, triterpenes, sterol glycosides, saponins, coumarins, tannins, anthraquinones, carotenoids, and reducing compounds. The alkaloid extracts demonstrated significant antiplasmodial activity, with an inhibitory concentration 50 (IC50) of 1.58 µg/ml for 3D7 strains and 2.69 µg/ml for K1 strains. The dichloromethane extract showed IC50 values of 2.11 µg/ml for 3D7 and 2.23 µg/ml for K1. Methanol, aqueous, and hydro-methanol extracts were less active. *S. dulcis* shows potential as a candidate in the fight against malaria due to its rich phytochemical composition and anti-plasmodial activity.**

**Key words:** Antiplasmodial activity, *Plasmodium falciparum*, *Scoparia dulcis*, Niger.

## INTRODUCTION

Malaria remains one of the most important infectious diseases globally, with the greatest burden in sub-Saharan Africa, primarily due to *Plasmodium falciparum* infection. Today, malaria is not only a public health problem but also a significant socio-economic issue. In Niger, malaria is the leading cause of consultations at health centers. According to the WHO (2023) Niger annual report, there are 5,802,330 malaria cases and 5,678 (0.1%) deaths per year, making malaria the leading cause of mortality in the country (OMS-Niger, 2023, 2019). Treatment and control of malaria are challenging due to resistance to most available drugs and partial resistance to artemisinin (Health Organization, 2023; Uwimana et al., 2020). Combination therapies, once recommended, are increasingly facing resistance issues. Therefore, new molecules must be identified or existing ones potentiated. Currently, malaria treatment relies on quinine and artemisinin derivatives. However, resistance to these drugs poses limitations, as recent research has documented artemisinin resistance in malaria parasites (Rosenthal et al., 2024).

Traditional medicine and pharmacopoeia are crucial in this context, and there is a growing need to explore traditional remedies for developing new, effective, and affordable treatments. According to the WHO Africa Region, traditional medicine remains the primary remedy for 80% of people in developing countries. Medicinal plants are vital sources of active ingredients against infections and are central to drug research. More than 50% of current drugs have natural origins.

*Scoparia dulcis* has been traditionally used in Niger to treat a variety of conditions, including diabetes, hypertension, abdominal disorders, pain, fever, inflammation, bronchitis, hemorrhoids, hepatosis, burns, herpes, pimples, dysentery, and hair loss. The leaves are used for dermatological problems and prostate disorders, while the whole plant is used in magico-religious rituals. Biological and pharmacological studies have identified several activities of *S. dulcis*, including antiviral, antitumor, antiulcerogenic, anti-inflammatory, antidiabetic, diuretic, antimicrobial, and antioxidant properties. *In vitro* and *in vivo* studies have demonstrated that *S. dulcis*acid B and C inhibit cell proliferation, Herpes simplex 1 replication, gastric acid secretion, and PTH-stimulated bone resorption. *S. dulcis* Linn is a traditional herbal remedy with numerous therapeutic uses in Niger and globally (WAHP, 2013). The aim of this study is to validate the use of *S. dulcis* Linn in the treatment of malaria through an *in vitro* assessment of its anti-plasmodial properties using extracts from the leafy stems of the plant (Scrophulariaceae).

## MATERIALS AND METHODS

### Plant

The plant material was obtained by harvesting the leafy stems of *S. dulcis* collected in the village of Tapoa (GPS 12° 28′ 30″ N, 2° 25′ 29″ E), located 151 km west of Niamey, Niger. This plant was selected based on previous ethnobotanical surveys, which indicated its widespread use in traditional medicine for treating malaria and its symptoms. The plant was identified at the Biology Department of the Faculty of Science and Technology of Abdou Moumouni University (FAST/UAM) based on its local name and the specimen collected for the herbarium. The collected plant was washed, dried, and pulverized into a powder. This powder was then used for the extractions.

### Extraction methods

The extraction was carried out using dichloromethane (DCM), methanol (MeOH), and hydro-methanol (MeOH/$H_2O$) as organic solvents. Alkaloid extracts were prepared using a specific extraction method. All organic extracts were obtained by macerating 10 g of each powder in 100 ml of dichloromethane, methanol, and hydro-methanol, successively, for 16 h.

The aqueous extracts were prepared by boiling 10 g of plant powder in 100 ml of water for 30 min. After cooling, the solution was filtered.

The dichloromethane extract was concentrated using a rotary evaporator (type R-215) and dried at ambient laboratory temperature. The methanol and hydro-methanol extracts were concentrated using the rotary evaporator and then freeze-dried with a freeze-dryer. The aqueous extracts were freeze-dried using a lyophilizator (type ALPHA 1-2 LD plus).

For the total alkaloid extracts, the plant powder was alkalinized with 28% ammonia to pH 9 for 10 min and then extracted with dichloromethane for 16 h. The organic phase obtained after percolation (100 ml) was concentrated and subjected to liquid-liquid extraction with 3% $H_2SO_4$ to adjust the pH to 2-3. The aqueous phase was further alkalinized with 28% ammonia to pH 9-10 and extracted again with dichloromethane. The crude alkaloid extract was obtained after concentration. All the extracts were weighed and stored for the evaluation of their antiplasmodial activity and phytochemical investigation.

### Chemical characterisation by tube reaction

The characterization of the chemical groups present in the plant extracts was carried out using tube reactions according to the techniques described by Ciulei (1982). Table 1 shows the reactions used and the phytochemical groups screened.

### Chemical characterisation by thin layer chromatography (TLC)

Fluorescent silica gel plates (60F254) were used for phytochemical screening by the TLC method, with chemical compounds migrating according to their differential polarity and affinity to the eluents. For this, 5 µl of each extract were deposited on the plates (aluminium support; silica gel adsorbent, CAMAG lot 990220). The plates were air-dried and then placed in migration tanks containing the eluent. The solvent was allowed to migrate 8 cm from the spot line. The

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**Table 1.** Phytochemical screening of extracts using the tube reaction.

| Chemical group | Test/Reaction | Observations | Reference |
|---|---|---|---|
| Alkaloids | Mayer | Whitish yellow | Wagner (2009) |
| | Dragendorff | Orange-red color | Wagner (2009) |
| Polyphenols/Tanins | $FeCl_3$ | Dark blue | Wagner (2009) |
| Flavonoids | Shibata test | Red and orange coloration | Wagner (2009) |
| Carotenoids | Carr and Price reaction | Blue or greenish blue | Wagner (2009) |
| Triterpene and steroid aglycones | Liebermann-Burchard | Greenish blue or violaceous | Wagner (2009) |
| Anthracenosides | Bornstrager reaction | Red coloration | Wagner (2009) |
| Coumarins | $NH_4OH$ 10% | Greenish blue or violaceous | Wagner (2009) |
| Carbohydrates | Fehling's test | Brick red | Wagner (2009) |
| Saponosides | Foam test | Formation of persistant foam | Wagner (2009) |

plates were then dried and examined under UV light (254/366 nm), and developed with specific reagents for each chemical group. For the identification of chemical compounds, a mixture of *n*-hexane-ethyl acetate-toluene (6:2:2 v/v/v) was used as the mobile phase for coumarins.

The plates were developed using a 1N KOH solution. Under UV light (254 or 366 nm), blue or green spots indicated the presence of coumarins. For the identification of steroid and/or triterpene glycosides, a solvent mixture of n-hexane, toluene, and ethyl acetate (3:1:2 v/v/v) was used. Revelation was carried out with a solution of anisaldehyde in concentrated sulfuric acid and ethanol (0.5:5:85 v/v/v). After drying the plates in an oven at 100°C for 5 min, tetracyclic triterpenes and phytosterols produced a violet to blue coloration, while pentacyclic triterpenes were identified by a persistent violet coloration.

For saponosides, a mobile phase of chloroform, glacial acetic acid, methanol, and water (16:8:3:2 v/v/v/v) was employed. The spots were revealed with anisaldehyde in concentrated sulfuric acid and ethanol (0.5:5:85 v/v/v), showing brown or purple spots after drying the plates at 100°C for 5 min.

In the case of flavonoids, a mobile phase of toluene, acetone, and formic acid (5:5:1 v/v/v) was used. Under UV light at 366 nm, a solution of diphenylborinate in ethanol and ethylene glycol (100 mg: 50 ml: 400 mg) revealed yellow or yellow-orange spots, indicating the presence of flavonoids.

To characterize tannins, a mixture of toluene, acetone, and formic acid (5:5:1 v/v/v) served as the eluent. The plates were revealed using a 2% ferric chloride solution in 80% ethanol (1:1 v/v). Blue spots indicated the presence of hydrolysable (gallic) tannins, while greenish spots suggested catechic (condensed) tannins.

For the detection of alkaloids, a mixture of toluene, ethyl acetate, and diethylamine (7:2:1 v/v/v) was used as the eluent. The appearance of dark yellow spots upon treatment with the Draggendorf reagent confirmed the presence of alkaloids.

Finally, for emodols and anthracenosides, a mobile phase of n-hexane, ethyl acetate, and toluene (3:1:1 v/v/v) was used. The presence of emodols or anthracenosides was indicated by the appearance of red or yellow-orange spots after revealing with a 1N KOH solution.

## Biological tests

### *Plasmodium falciparum* strains

The strains of *P. falciparum* used for assessing antiplasmodial activity are responsible for the most significant cases of malaria in sub-Saharan Africa. The study utilized both chloroquine-resistant K1 strain and chloroquine-sensitive 3D7 strain of *P. falciparum*. The K1 strain was obtained from the London School of Hygiene and Tropical Medicine (LSHTM) in London, United Kingdom, while the 3D7 strain was acquired from the NMIMR Laboratory (Noguchi Memorial Institute for Medical Research) in Ghana. These two parasite strains were maintained in continuous culture in human blood at the pharmacognosy laboratory of the Centre National de Recherche et Formation sur le Paludisme (CNRFP) in Ouagadougou, Burkina Faso.

### Continuous culture of parasites *in vitro*

The strains of *P. falciparum* were maintained in culture using the technique described by Trager and Jensen in 1976. The culture medium was renewed every 24 h, and parasitaemia was monitored. Parasites were cultured in flasks containing RPMI 1640 Complete Culture Medium, 2% L-glutamine, 2% HEPES Buffer, 0.5% Gentamicin, 5% Albumax, and 9.5% hypoxanthine. The flasks were incubated in a $CO_2$ incubator at 37°C under conditions optimized for parasite growth (2% $O_2$, 5% $CO_2$, 93% $N_2$). After renewing the culture medium, parasitaemia was monitored by taking blood smears. When the parasitaemia reached 6%, a subculture was performed using fresh, non-parasitized blood of blood group A+.

### Evaluation of anti-plasmodial activity *in vitro*

The evaluation of antiplasmodial activity involved *in vitro* exposure of plant extracts (crude) to parasites for 72 h under optimal culture conditions (Figure 1). This process measures the percentage inhibition of parasite growth. The evaluation required the presence of the parasite, culture medium, and plant extracts and was performed using the pLDH technique. The principle is based on measuring the enzymatic activity of pLDH. Lactate dehydrogenase (LDH) is a ubiquitous enzyme involved in glucose metabolism, producing the energy necessary for cells. Plasmodium LDH metabolizes 20 to 25 times more substrate than erythrocyte LDH.

The enzyme activity quantification is directly proportional to parasite development. LDH facilitates the regeneration of nicotinamide adenine dinucleotide (NAD), which is used in ATP production (the energy storage form). Both parasitic LDH (pLDH) and erythrocyte LDH use NAD to convert lactate into pyruvate. Oxidation of the NBT results in a blue color with maximum

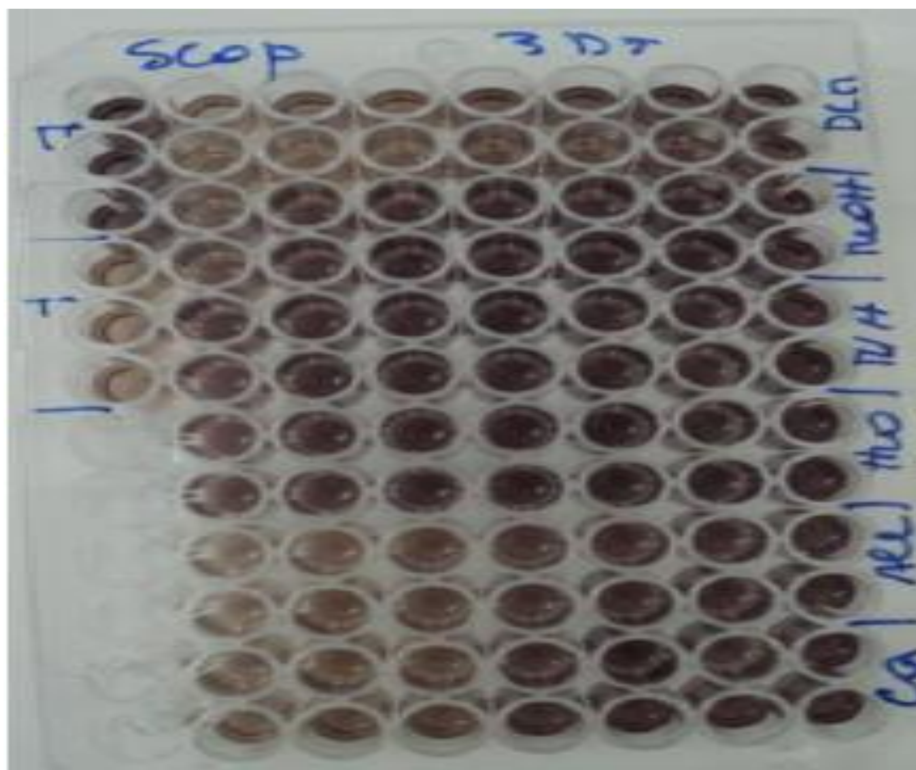


**Figure 1.** Evaluation plate test of *Scoparia dulcis* extracts against 3D7.

absorption at 650 nm. Spectrophotometric analysis at λ = 650 nm measures the absorbance, which is proportional to the parasitemia.

The plant extracts and reference drugs (Dihydroartemisinin and Chloroquine (DHA and CQ)) were dissolved in dimethyl sulfoxide (DMSO) or distilled water. Extract solutions were initially prepared at a concentration of 10 mg/ml and then diluted to obtain test concentrations of 100 µg/ml. The tests were conducted in 96-well plates. After 72 h of incubation, 100 µl of MALSTAT solution (prepared with 160 ml distilled water, 200 µl Triton X-100, 2 g L-lactate, 0.66 g Trizma base, 66 mg APAD (3-acetylpyridine adenine dinucleotide), at pH 9) was added to each well. To this, 25 µl of NBT/PES solution (prepared with 100 ml distilled water, 160 mg NBT, and 8 mg PES) and 20 µl of blood from the test plate, including positive and negative controls, were added. After 10 min of incubation, the plates were read immediately using a UV-Vis spectrophotometer at a wavelength of 650 nm. $IC_{50}$ values were determined graphically by plotting the percentage inhibition of parasite growth against the positive control. The $IC_{50}$ values of the different extracts were analyzed according to the criteria established by Deharo et al. (2001).

## RESULTS AND DISCUSSION

### Phytochemical characterisation

The yield of extracts from *S. dulcis* is shown in Table 2. The aqueous extract produced the highest yield at 12%, while the alkaloid extract yielded the least.

The results of the phytochemical screening are provided in Table 3. Sterols and triterpenes, saponins, tannins, coumarins, carotenoids, anthocyanins, flavonoids, reducing compounds, and alkaloids were detected in the leafy stems of *S. dulcis*. Thin Layer Chromatography (TLC) tests were used to confirm or refute the results of the tube tests.

In the leafy stems of *S. dulcis*, the main phytochemical compounds identified include flavonoids, sterol and triterpene glycosides, saponosides, coumarins and their derivatives, tannins, and anthracenosides.

These findings are consistent with results reported by other authors (Phan et al., 2006; Tasdemir et al., 2006). Thin layer chromatographic (TLC) analysis confirmed the presence of these chemical groups. Previous studies have shown that flavonoids act on Fab I, an enzyme involved in the synthesis of fatty acids in *P. falciparum* (Ojewole et al., 2010). The presence of flavonoids in the extracts of this plant could explain its antiplasmodial activity. The following figures show the results of the phytochemical screening using the TLC plates.

### *In vitro* anti-plasmodial activity assay

The crude extracts were tested for their antiplasmodial activity against the K1 and 3D7 strains of *P. falciparum*. Table 4 shows the results of the inhibition concentration

**Table 2.** Yield of extracts of *Scoparia dulcis* expressed in % of extraction.

| Plant | Parts used | Yield of extracts | | | | |
|---|---|---|---|---|---|---|
| | | DCM | MeOH | $H_2O$/MeOH | $H_2O$ | Alkaloid extracts |
| *Scoparia dulcis* | Leafy stems | 6.8 | 8.3 | 8.6 | 12 | 0.1 |

**Table 3.** Results of phytochemical screening.

| Solvent | Chemical group | Plant (*S. dulcis*) |
|---|---|---|
| $CH_2Cl_2$ | Sterols and Triterpenes | + |
| | Flavonic Aglicones | + |
| | Emodols | - |
| | Alkaloids bases | + |
| | Coumarins | + |
| | Carotenoïds | + |
| MeOH | Phenols (Tanins) | + |
| | Alkaloïd salts | + |
| | Reducing compounds | + |
| | Saponosids | + |
| | Glycosids sterols and triterpenes | + |
| | Flavonoids | + |
| | Anthracenosids | + |
| | Coumarin derivated | + |
| | Anthocyanosids | - |
| MeOH /$H_2O$ | Phenols (Tanins) | + |
| | Alkaloid salts | - |
| | Reducing compounds | - |
| | Saponosids | - |
| | Glycosid sterols and triterpenes | + |
| | Flavonoids | + |
| | Anthracenosids | + |
| | Coumarin derivated | + |
| | Anthocyanosids | - |
| $H_2O$ | Phenols (Tanins) | + |
| | Alkaloid salts | + |
| | Reducing compounds | - |
| | Saponosids | + |
| | Glycosid sterols and triterpenes | + |
| | Flavonoids | + |
| | Anthracenosids | + |
| | Coumarin derivated | + |
| | Anthocyanosids | - |

+: Presence; -: absence; ±: trace.

for these strains: chloroquine-sensitive 3D7 and chloroquine-resistant K1. Among the 5 extracts evaluated, alkaloid extracts demonstrated notable activity with IC50 values of 1.58 µg/ml against 3D7 and 2.69 µg/ml against K1. The dichloromethane (DCM) extracts also showed good efficacy with $IC_{50}$ values of 2.11 µg/ml against 3D7

**Table 4.** antiplasmodial activity of crude extracts of *Scoparia dulcis* Linn.

| Plant | Strain | Antiplasmodial activity $IC_{50}$ in µg/mL | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | DCM | MeOH | MeOH/$H_2O$ | $H_2O$ | Alkaloid extracts | CQ (µM) | DHA (µM) |
| *Scoparia dulcis* | 3D7 | 2.11 | 21.10 | 37.30 | >50 | 1.58 | 77.24 | 1.47 |
| | K1 | 2.23 | 21.51 | >50 | >50 | 2.69 | 225.21 | 1.69 |

and 2.23 µg/ml against K1. In contrast, methanol (MeOH), methanol/water (MeOH/$H_2O$), and aqueous ($H_2O$) extracts were identified as having inactive effects with $IC_{50}$ values ≥ 10 µg/ml (Table 3), according to Deharo et al. (2001). These results indicate that the crude alkaloid and DCM extracts of the plant exhibit very good antiplasmodial activity against both K1 and 3D7 strains, with $IC_{50}$ values of less than 5 µg/ml. Conversely, the aqueous, methanol, and hydro-methanol extracts were found to be less active on both strains, with IC50 values greater than 10 µg/ml (Deharo et al., 2001).

The inhibition of parasite growth may be attributed to a possible synergistic effect of phytochemical compounds in the DCM extracts, including sterols and triterpenes, flavonoids, and anthracenosides—chemical groups previously demonstrated to have antiplasmodial properties (Laoula et al., 2021). The results can be compared to studies by Soma et al. (2017) on *Vernonia cinerea* from Burkina Faso, which reported $IC_{50}$ values of 4.25 and 2.56 µg/ml against K1 and 3D7 strains (Ouattara et al., 2014; Soma et al., 2017). The antiplasmodial properties of *S. dulcis* have also been reported by several authors (Gathirwa et al., 2008). The $IC_{50}$ values obtained with DHA ranged between 1.47 and 1.69 nM, confirming the validity of the tests conducted.

Several classes of phytochemicals contribute to the antimalarial activity of plants, including alkaloids, terpenes, steroids, and flavonoids. Alkaloids, in particular, are known for their diverse biological activities, including antimalarial effects (Jonville et al., 2013). Numerous alkaloids isolated from medicinal plants have been reported for their potent antiplasmodial activity. For instance, strychnochrysine isolated from *Strychnos nux-vomica* L. exhibits antiplasmodial activity against both chloroquine-resistant and chloroquine-sensitive strains with an $IC_{50}$ value around 10 µM (Dua et al., 2013). Conessine isolated from *Holarrhena antidysenterica* shows *in vitro* antiplasmodial activity with $IC_{50}$ values of 1.9 and 1.3 µg/ml in schizont maturation and pLDH assays, respectively. Irehline and mokluangin A, also isolated from *H. antidysenterica*, demonstrate strong antiplasmodial activity against the K1 strain with IC50 values of 1.2 and 2.0 µM, respectively (Cheenpracha et al., 2019).

The presence of alkaloids in the extracts of *S. dulcis* may explain its antiplasmodial activity, as the alkaloid extracts showed the most potent activity against both chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum*.

## Conclusion

The present study, focused on the chemical composition of dichloromethane, methanol, hydro-methanolic, and aqueous extracts of *S. dulcis* leafy stems, revealed the presence of several families of secondary metabolites. The composition is dominated by sterols and triterpenes, saponins, tannins, coumarins, carotenoids, anthocyanins, flavonoids, and alkaloids. The results of the antiplasmodial assay show that *S. dulcis* extracts exhibit *in vitro* antiplasmodial activity.

Among the extracts, dichloromethane and alkaloid extracts were the most active. These findings support the traditional use of this plant in traditional medicine and suggest it may be a source of antimalarial compounds. Further studies are needed to identify and isolate the active molecules and to confirm the safety of this plant.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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