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# *In vitro* antioxidant, antiplasmodial, antitrypanosomal, antileishmanial and cytotoxic properties of the ethanol stem bark extract of *Trichilia monadelpha* (Thonn.) JJ De Wild (Meliaceae)

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## ABSTRACT

In the course of our studies on the valorization of plants used by the Nkundo people in the Democratic Republic of the Congo, we have carried out the present work to evaluate the *in vitro* antioxidant and antiprotozoal activities of *Trichilia monadelpha*. Ethanolic stem bark extract was evaluated for the *ex vivo* antiplasmodial activity on clinical strains of *Plasmodium falciparum* as well as the *in vitro* antiprotozoal activity against *Trypanosoma brucei brucei*, *Trypanosoma cruzi*, *Leishmania infantum*, and the chloroquine and pyrimethamine-resistant K1 strain of *Plasmodium falciparum*. The test of cytotoxicity against MRC-5 cells was included to assess selectivity of activity. In addition, we carried out evaluation of the antioxidant potential using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The tested extract showed weak antioxidant potential ($IC_{50}$ > 500 µg/mL). It exhibited potent *ex vivo* activity ($IC_{50}$ < 1 µg/mL) against *P. falciparum* but low *in vitro* activity ($IC_{50}$ 20-40 µg/mL, selectivity index 2.26) against *T.b. brucei* and inactivity ($IC_{50}$ > 40 µg/mL) against the other tested protozoa. The extract was devoid of cytotoxicity ($CC_{50}$ > 64 µg/mL). These findings may support at least in part the traditional use of this plant species against malaria.



**Keywords:** Meliaceae, Ethnopharmacology, Secondary metabolites, Antiprotozoal effects, Cytotoxicity, DR Congo.

## INTRODUCTION

*Trichilia monadelpha* (Thonn.) J.J. De Wild. (syn. *Trichilia heudelotii* (Planch.) ex Oliv.) is a species of the Meliaceae family, common to the humid tropical rainforests of Africa and valued for its wood. From this family, several compounds of pharmacological interest have already been isolated and used in

 
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human therapy (Tan and Luo, 2011; Vieira et al., 2014; Happi et al., 2021). The stem barks in *Trichilia* genus are one of the most used plant parts and are reputed to have many bioactivities e.g. hepatoprotective, antimicrobial, anti-inflammatory, insecticidal or antiplasmodial activities as well as cytotoxic effects (Adeniyi et al., 2008; Tan and Luo, 2011; Ainooson et al. 2012; Tacham et al., 2015; Ben et al., 2016; Nangmo et al., 2018; da Silva et al., 2021; Passos et al., 2021).

Oxidative stress results from the disruption of the balance between the production of reactive oxygen species (ROS) and the body's antioxidant capacities. These free radicals (ROS) can be generated from both normal metabolic endogenous reactions or from exogenous sources such as air and water pollution, cigarette smoke, pathogens and drugs or exposure to radiation. Free radicals are causative agents for several pathological processes such as cancer, HIV/AIDS, neurodegenerative diseases, or even malaria (Wood et al., 2003; Favier, 2006; Akpotuzor et al., 2012). Although physiological mechanisms of antioxidant defense exist in the body, the interest of scientists for antioxidant (AOX) products from natural sources (e.g. from plants as phytomedicines and functional foods) increased these four last decades because of their appreciated capacity to early reduce or prevent the pathological processes, before irreversible harms occur (Koechlin-Ramonatxo, 2006).

Due to the low incomes of the populations and the limited access to conventional medicine in many sub-Saharan countries, as well as the lack of health insurance, it is estimated that nearly 80% of the African population use traditional medicine as first aids or even unique line of treatments in case of any health issues (WHO, 2002). For this reason, traditional medicines should be scientifically tested in order to ascertain their efficacy and safety for users (Musuyu Muganza et al., 2012; Waiganjo et al., 2020).

Several species from *Plasmodium* genus can infect vertebrates including human. The most prevalent malaria parasite is *P. falciparum*. This species can be lethal for the host and is a major public health concern worldwide, especially in tropical countries. According to the WHO (2021), there were an estimated 241 million malaria cases and 627 000 malaria deaths worldwide in 2020. This represents about 14 million more cases in 2020 compared to 2019, and 69 000 more deaths. Approximately two thirds of these additional deaths (47 000) were linked to disruptions in the provision of malaria prevention, diagnosis and treatment during the COVID-19 pandemic. Sub-Saharan Africa experiences the overwhelming share of malaria's burden, with 95 percent of cases and 96 percent of deaths, Nigeria and DR Congo representing about the half of all global malaria deaths. Among the victims, children < 5 years are the most vulnerable group affected by malaria.

Given the emergence and re-emergence of infectious diseases caused by protozoan (i.e. including neglected tropical diseases, trypanosomiasis, leishmaniasis and malaria), and insecticide resistance vectors to available molecules, it is urgent to seek new, more active and less toxic molecules to fight effectively against these scourges (Bloland and WHO, 2001; WHO, 2016; Ungogo et al., 2020).

As a contribution to these scientific efforts, the present study aimed at evaluating the antiprotozoal activities, the antioxidant potential as well as the cytotoxicity of the stem bark extract of *T. monadelpha*.

## MATERIALS AND METHODS

### Plant material collection

The choice of *T. monadelpha* was motivated by both its uses against malaria in the Nkundo traditional medicine and the consumption of its fruits by a habituated group of wild bonobos (*Pan paniscus*) in the surrounding forest of Nkundo villages

(Musuyu Muganza, *pers. comm.*). The plant material, i.e. stem bark, was collected in March 2013 at the research site Luikotale (S 02°45.610′, E 20°22.723′) previously described by Hohmann and Fruth (2003). This study site is located in the southwestern part of the southern block of the Salonga National Park, in the Mai-Ndombe province, DR Congo.

The plant was identified by Mr Boniface Nlandu of the INERA-Kinshasa (Institut National d'Etudes et de Recherches en Agronomie) and Dr Jean-Pierre Habari of the Department of Biology, Faculty of Sciences, University of Kinshasa, DRC. Voucher specimen (PROCUV-2579) of the plant has been deposited in the herbarium of this Institute. The collected plant material was air-dried in the shade and reduced to powder (IKA®A11 basic, Germany).

**Plant extraction and phytochemical screening**

Briefly, 100 g of the powdered plant material were macerated in 800 mL EtOH 80% for 48 hrs, at ambient temperature and under permanent shaking to facilitate extraction. This operation was repeated three times. The mixtures were then filtered and the filtrates pooled and concentrated using a rotary evaporator. The concentrate was then dried in an oven at 50°C until use. The yield of extract was determined.

The crude extract was submitted to a phytochemical screening using different color and precipitation reactions (Harbone, 1973).

***In vitro* antioxidant assays**

Antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical for both qualitative and quantitative analyses.

***Qualitative analysis of antioxidant properties***

This test was carried out on thin layer chromatography (TLC) as previously described by Mensah et al. (2004). About 10 µL of methanolic solution of the dry extract (20 mg/ mL) and 2 µL of the methanolic solutions (1 mg/ mL) of each reference standard (caffeic acid, chlorogenic acid, quercetin, isoquercetrin and rutin) have co-eluted on 10 x 10 cm silica gel 60 $F_{254}$ glass HPTLC plates. The mobile phase was a mixture of solvents as follows Ethyl acetate - Acetic acid - Formic acid - Water (100: 11: 11: 27; v/v/v/v). The distance of elution was 7 cm. The revelation was done by dipping the plate for 5 seconds in a tank (CAMAG chamber) filled with a methanolic solution of DPPH (0.2%). Compounds with antioxidant activity appear as yellow spots on a purple background; the Retention factor (Rf) of each spot was then calculated.

***Quantitative analysis of antioxidant properties***

The quantification of the antioxidant potential of the extract was carried out on 96-Well- microtiter plates according to the method described by Okusa et al. (2007). A fresh solution of DPPH in 80% methanol (0.004%) was prepared. The methanolic solution of the dry extract (10.5 mg/ mL) and the positive controls (ascorbic acid and quercetin at 0.75 mg/ mL) were prepared by serial half-dilution of the initial stock solution (dilution ranges 500-1.95 µg/ mL for the extract and 35.71-0.14 µg/ mL for the positive controls). The tests were carried out in triplicate. The absorbances were read at 550 nm using a spectrophotometer (ELISA Reader).

***Ex vivo* antiplasmodial assays**

All analyzes were carried out in the Parasitology Laboratory of the National Institute for Bio-medical Research (INRB) of Kinshasa (DR Congo). The patient's blood for this biological test was drawn with the consent of the parents under the advice of his attending physician. The sample was taken according to the INRB protocol on the well-being of people handling biological products of animal origin,

and according to the ethical requirements of the Declaration of Helsinki.

About 5 mL of the blood of a 4-years-old child with a high-level infection to *Plasmodium falciparum* (± 100,000 trophozoites/ µL) were taken in a heparin tube until further analysis. This test was carried out according to the method described by Rieckmann et al. (1978). Serial half-dilutions from initial stock solution (2.5 mg/mL) of both the methanolic solution of the crude extract and of the positive control (quinine) were prepared (smallest concentration 1.22 x $10^{-3}$ µg/mL). These solutions were distributed in a sterile 96-wells-microplate alongside the blank (MeOH as negative control). The plate was then placed in a desiccator until drying of the wells' contents. The mixture of the infected blood and culture medium (RPMI-1640) was added in the wells and the plate was incubated at 37°C for 48 hrs to allow the maturation of trophozoites. After the incubation, the content of each hole was placed on its corresponding microscope slide and stained with Giemsa before a microscopic examination to determine the parasitemia rate.

### *In vitro* antiprotozoal and cytotoxicity assays

Antiprotozoal and cytotoxicity (MRC-5 cells, i.e. secondary human lung fibroblasts) testing was carried out as previously described (Cos et al., 2006; Mesia et al., 2008). For the purpose of this *in vitro* screening study, the following criteria were adopted (Musuyu Muganza et al., 2012): $IC_{50} \leq 5$ µg/mL: *pronounced activity*; $5 < IC_{50} \leq 10$ µg/ mL: *good activity*; $10 < IC_{50} \leq 20$ µg/mL: *moderate activity*; $20 < IC_{50} \leq 40$ µg/mL: *low activity*; $IC_{50} > 40$ µg/mL: *inactive*. For the test of cytotoxicity, the criteria were as follows: $CC_{50} < 32$ µg/mL: *cytotoxic sample*; 32 µg/mL $\geq CC_{50} \leq 64$ µg/ mL: *sample with weak cytotoxicity*; $CC_{50} \geq 64$ µg/mL: *non-cytotoxic sample*.

### Data analysis

The DPPH radical activity was expressed as a percentage and calculated using the following equation:

$$\% \text{ DPPH scavenging effects} = (x-b) \times 100 / y$$

Where *x* is the absorbance of the product (extract or positive control) in the presence of DPPH; *y* is the mean absorbance of the negative control (blank as MeOH + DPPH); *b* is the mean absorbance of the blank (MeOH).

The *ex vivo* antiplasmodial activity was evaluated by counting the number of schizonts and trophozoites and deducing the percentage (%) of maturation, by the following formula:

Maturation (%) = Number of schizonts in the wells tested * 100/ number of trophozoites in the wells tested (1).

From equation (1), the inhibition rate (%) = 100 - Maturation rate (%).

For all the data set, from the percentages of inhibition, the measurements resulted in the calculation of the half maximal inhibitory concentration ($IC_{50}$) according to the sigmoidal curve equation under the software OriginLab® version 6.1.

## RESULTS

### Phytochemical screening

The obtained yield of the extraction of stem bark powder with EtOH 80% was 5.80 ± 0.45%.

The phytochemical analysis revealed the presence of following constituents: flavonoids, leucoanthocyanins, catechic and gallic tannins, alkaloids, coumarins, sterols and triterpenes. In contrast, anthocyanins, quinones, and cardiotonic glycosides were not found.

### *In vitro* antioxidant activities

The antioxidant activity of the tested extract of the stem bark of *T. monadelpha* was first evidenced on TLC through yellow spots

laying on a purple background (mainly at Rf = 0). The DPPH-positive substances on TLC start line were thought most likely to be heavy tannins which presence was confirmed through the phytochemical screening.

The quantitative measurement of the AOX potential of the plant extract gave the following results: the tested extract exhibited a weaker activity with $IC_{50}$ > 500 µg/mL than controls such as ascorbic acid ($IC_{50}$ = 2.13 ± 0.14 µg/mL) or quercetin ($IC_{50}$ = 0.69 ± 0.009 µg/mL).

### *Ex vivo* antiplasmodial activities

The assessment of the *ex vivo* antiplasmodial activity on the clinical strain of *P. falciparum* showed very good activity with $IC_{50}$ value of 2 x $10^{-2}$ µg/mL for the extract compared to quinine which was found to be ten times more active ($IC_{50}$ = 2 x $10^{-3}$ µg/mL).

### *In vitro* antiprotozoal and cytotoxic activities

The $IC_{50}$ values for the antiprotozoal activities and $CC_{50}$ values for the cytotoxic effects, as well as the corresponding selectivity index of the tested samples are presented in Table 1. The tested extract showed low activity ($IC_{50}$ 20-40 µg/ mL, selectivity index 2.26) against *Trypanosoma brucei brucei* and inactivity ($IC_{50}$ > 40 µg/ mL) against *Plasmodium falciparum* K-1 strain, *Trypanosoma cruzi* and *Leishmania infantum*. This extract was found to be non-cytotoxic as the obtained $CC_{50}$ value was > 64 µg/mL; the $CC_{50}$ value of the positive control (Tamoxifen) was 11.81 µg/mL.

**Table 1:** Antiprotozoal and cytotoxic activity from stem bark of *Trichilia monadelpha*

| | **Antiprotozoal activity ($IC_{50}$)** | | | | **Cytotoxicity ($CC_{50}$)** | **Selectivity Index (SI)** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test Samples** | ***T. b. brucei*** | ***T. cruzi*** | ***L. inf.*** | ***Pf-K1*** | **MRC-5** | **MRC-5/*T. b. brucei*** | **MRC-5/*T. cruzi*** | **MRC-5/*L. inf.*** | **MRC-5/*Pf-K1*** |
| Plant Extract (µg/ mL) | 28.28 | > 64 | > 64 | 41.12 | > 64 | 2.26 | nd | nd | 1.56 |
| Melarsoprol (µM) | 0.02 | nd | nd | nd | nd | nd | nd | nd | nd |
| Benznidazol (µM) | nd | 2.65 | nd | nd | nd | nd | nd | nd | nd |
| Miltefosine (µM) | nd | nd | 3.56 | nd | nd | nd | nd | nd | nd |
| Chloroquine (µM) | nd | nd | nd | 0.21 | nd | nd | nd | nd | nd |
| Tamoxifen (µM) | nd | nd | nd | nd | 11.8 | nd | nd | nd | nd |

*T. b. brucei*: *Trypanosma brucei brucei* ; *T. cruzi*: *Trypanosama cruzi*; *L. inf.*: *Leihsmania infantum*; *Pf-K1*: *Plasmodium falciparum* K-1 strain ; *MRC-5* : diploid cell culture line composed of fibroblasts, nd: not determined.

## DISCUSSION

The phytochemical screening from the stem bark extracts showed the presence of the following constituents: flavonoids, leucoanthocyanins, catechic and gallic tannins, alkaloids, coumarins, sterols and triterpenes. These findings broadly agreed with those of previous studies confirming the presence of most constituents of our findings (Adeniyi et al., 2008; Ainooson et al., 2012; Ben et al., 2013; Opawale et al., 2015; Bankole et al., 2016; Clark and Omo-Udoyo, 2021).

The antioxidant activity ($IC_{50}$ > 500 µg/mL) of the tested extract was found to be very weak. This result is in line with the study of Ben et al. (2013) who have reported a similar tendency with the EtOH stem bark extract of *T. monadelpha*, exhibiting a weak antioxidant potential with $IC_{50}$ value of $4.10^{-2}$ mg/mL. Besides, Clark and Omo-Udoyo (2021) found a concentration-dependent AOX effect from MeOH leaf extracts of *T. monadelpha* reaching up to 46% of inhibition of DPPH free radical at 2 mg/ L. These results suggested that $IC_{50}$ value is > 2 mg/L. It appears that tannins are preponderant in our extract as evidenced by the important DPPH positive spot on start line of the TLC. The weak antioxidant potential found in the quantitative test may be attributed to the poor solubility of the heavy tannins (González et al., 2022).

The antiplasmodial activity of *T. monadelpha* stem bark extract revealed a potent *ex vivo* activity ($IC_{50}$ $2.10^{-2}$ µg/mL) when using the *P. falciparum* clinic strain. But, this extract was found inactive *in vitro* ($IC_{50}$ 41.12 µg/ mL) against *P. falciparum* K-1 strain. Kamanzi Atindehou et al. (2004) found on their side, remarkable *in vitro* activity ($IC_{50}$ 3.61 µg/mL) against *P. falciparum*-K1 strain (resistant to chloroquine and pyrimethamine) from EtOH extract of *T. monadelpha* originated from Ivory Coast. The found activity could be attributed to phytochemical groups such as alkaloids, limonoids, as well as other compounds. Bankole et al. (2016) carryied out their *in vivo* antiplasmodial studies on aqueous stem bark extracts of *T. monadelpha* against *P. berghei* ANKA strain (resistant to chloroquine); they showed a poor suppression (40%) of parasite multiplication at the highest dose (800 mg/kg) in treated mice. These discrepancies in results can be attributed to many factors such as the soil composition, climate changes, or extracting solvents, leading to the different availabilities of secondary metabolites in the different tested extracts.

The 80% EtOH stem bark extract of *T. monadelpha* possessed a moderate antitrypanosomal activity ($IC_{50}$ 28.28 µg/mL, SI 2.26) against *Trypanosma brucei brucei*. On their side, Kamanzi Atindehou et al. (2004) reported with the same plant part a potent activity ($IC_{50}$ 5 µg/mL) against *T. b. rhodesiense*.

The stem bark extract of *T. monadelpha* was inactive *in vitro* against *Leishmania infantum*, with $IC_{50}$ > 64 µg/mL. There are no previous reports on investigation of this plant species on this protozoan. Nevertheless, Kowa et al. (2019) who worked on the stem bark of *T. gilgiana* a plant species from Cameroon, showed a potent activity ($IC_{50}$ < 7 µg/mL) against *L. donovani* promastigotes due to limonoid compounds, with low cytotoxicity ($CC_{50}$ values from 47.47 to > 200 µg/mL).

In this study, the stem bark extracts of *T. monadelpha* was found the non-cytotoxic with a $CC_{50}$ > 64 µg/mL. It should be noted that Opawale et al. (2015) who worked as well on the EtOH stem bark extract found quite different results with $CC_{50}$ value of 8.82 mg/mL. As Olorunniyi (2020) showed *in vivo* dose-dependent toxic effects from crude aqueous stem bark extract of *T. monadelpha*, with $LD_{50}$ value > 500 mg/kg of the body weight of mice, it would be therefore advisable to avoid the use of large doses of the *T. monadelpha* stem bark extracts.

## Conclusion

The species *Trichilia monadelpha* is a plant much cited in African traditional medicine. We wanted to test it for its antioxidant and antiprotozoal properties. Based on the obtained results in the present study, *T. monadelpha* stem bark would not be a good reducing agent to control human diseases caused by oxidative stress. Our findings may support at least in part the traditional use of this

plant species against malaria, more likely against susceptible strains of *Plasmodium falciparum*. We look forward to clarifying whether the *in vivo* test will confirm the interesting *ex vivo* results.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

PKK and DMM designed and conducted the experiments in the laboratory. UM, JYL and DMM analyzed the data. PKK, UM and DMM drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

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