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# Antibacterial and Antiplasmodial Activities of Tannins Extracted from *Zizyphus mauritiana* in Mali

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MW designed the study, performed the antiplasmodial activity statistical analyses while author SK performed the antibacterial assay authors SK, ND and CC collected samples and performed extractions and chemical screening. Author LK supervised antimalarial drug assay experiments at Well Cornell Medical School at NY. Author LBM managed the literature searches and proofreading of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** This study was designed to evaluate tannins extracted from *Ziziphus mauritiana* as source of potential antimalarial and antimicrobial agents in Mali.
**Place and Duration of Study:** Collection of plant materials, tannins extraction, antibacterial activity evaluation were done at University od Sciences, Techniques and Technologies of Bamako, Mali and antiplasmodial activity assessment at Department of Microbiology and Immunology, Weill Cornell Medicine, New York, United States of America between September 2013 and February 2014.

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**Methods:** We extracted tannins from leaves of *Z. mauritiana* collected around Bamako, Mali. Antiplasmodial activity was evaluated against 3D7 (chloroquine-sensitive) and Dd2 (chloroquine-resistant) strains of *Plasmodium falciparum* using the fluorescence based SYBR® green I method. Antibacterial activity of tannins was evaluated by disc diffusion method against strains of *Escherichia coli, Salmonella Typhi, Streptococcus* and *Staphylococcus aureus* donated by the National Research Institute in Public Health in Mali and collected from infected patients suffering from different diseases.
**Results:** Tannins extracts from leaves of *Z. mauritiana* showed moderate antiplasmodial activity against 3D7 *P. falciparum* (46.9±1.12 µg/mL) and against Dd2 *P. falciparum* strains (67.8±2.39 µg/mL). They showed also an antibacterial activity on different bacterial strains showing important inhibition zones.
**Conclusion:** Tannins extracted from *Z. mauritiana* demonstrated good antiplasmodial and antibacterial activities. These data confirm the potential use of tannins as a key element in antimalarial and antibacterial drug development.

*Keywords: Tannins; antimicrobial; antiplasmodial; in vitro; traditional medicine; Ziziphus mauritiana.*

## 1. INTRODUCTION

The use of medicinal plants in the treatment of diseases has a long history worldwide. Indeed, people from developing countries often do not have access to modern therapeutics such as ACT to treat malaria because of financial, geographical and/or cultural obstacles. The WHO estimates that up to 80% of the world's population relies on traditional medicinal products for some aspects of primary health care. Better knowledge of plants from traditional pharmacopoeias could lead to access to effective, standardised, available and affordable therapeutics for management of malaria by local populations [1].

Although malaria is a preventable and curable disease, it is still responsible for many deaths-mostly children and pregnant women-especially in Africa. Artemisinin Combination Therapies (ACTs) are currently the frontline treatments against *P. falciparum* malaria. Although these treatments continue to be effective in many parts of the world, the emergence of the malaria parasite resistance to ACTs is an urgent public health concern [2].

In Africa and other countries where malaria is endemic, traditional medicinal plants are frequently used to treat or cure malaria [3]. It is a fact that conventional antimalarials such as quinine and artemisinin derivatives originated from plants. It is therefore important to investigate the antimalarial activity of medicinal plants in order to determine their potential as sources of new antimalarial agents [4]. Extensive researches have been done over the last couple decades to search for natural alternatives to in-feed antibiotics and antimalarial, and plant compounds have been identified to have great potentials [5].

*Ziziphus mauritiana,* tropical fruit tree species belonging to the family *Rhamnaceae*, is a spiny, evergreen shrub or small tree with trunk 40 cm in diameter, spreading crown and many drooping branches and fruit of variable shape and size [6]. The plant is used in African traditional medicine against different symptoms and diseases [7,8,9].

Tannins are a group of water-soluble oligomeric and polymeric polyphenolic compounds with significant astringent properties. They are present in the majority of plant parts including bark, leave, fruits, and roots [10]. They are widely used in leather, food and healthcare industries as antimicrobials [11]. The mode of antimicrobial action of tannins is potentially due to inactivation of microbial adhesins and cell envelope transport proteins [11,12,13]. Besides their efficacy against bacteria, tannins have been reported to have an inhibitory action on fungi and yeasts [10,14]. Tannins isolated from several plants have been shown to possess strong activity against Gram-negative bacteria. It is worth noting that pathogenic bacteria such as *Escherichia coli*, *Salmonella, Shigella*, *Staphylococcus*, *Pseudomonas* and *Helicobacter pylori* were all sensitive to tannins [15-19].

This study aimed at evaluating the antiplasmodial and antimicrobial activities of tannins extracted from *Z. mauritiana* in Mali.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The plant *Z. mauritiana* was collected and identified at the Department of Traditional

Medicine in Bamako. The living material was composed by leaves of *Z. mauritiana*, bacterial strains of *Escherichia coli*, *Salmonella typhi*, *Streptococcus, Staphylococcus aureus* and *Plasmodium falciparum* (3D7 and Dd2).

**Fig. 1. Photographs of *Z. mauritiana* tree, leaves and fruits**

## 2.2 Reagents and Chemicals

Chemicals including RPMI (*Roswell Park Memorial Institute*) 1640, hypoxanthine, HEPES (*N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid*), SYBR® Green I-lysis buffers, DMEM-F12 (*Dulbecco's Modified Eagle Medium Mixture F-12*) streptomycin, Albumax, Trypsin, EDTA (*Ethylene Diamine Tetra Acetic Acid*) were obtained from GIBCO/Invitrogen life Technologies, USA.

For antimicrobial activity assessment the medium Muller Hinton (MH) was used with tryptic soybean medium agar.

## 2.3 Tannins Extraction

The condensed tannins were extracted from powder by 24 hours maceration in water. The solution was then filtered, concentrated by evaporation and centrifuged to obtain condensed tannins pellet.

For gallic tannins, 100 mg of powder were boiled in 20 mL of distilled water for 10 minutes. The solution was then filtered and the presence of the gallic tannins was investigated in the filtrate [20,21].

The characterisation of the gallic tannins was done with ferric chloride while for the condensed tannins it was done with the Bath Smith reagent. The content of the total tannins in the extracts was determined by spectroscopic methods at absorbance 725 nm using 1% gallic acid as standard.

## 2.4. Antiplasmodial Activity

*In vitro* susceptibility assays of tannins extracts were performed on lab culture adapted strains of *P. falciparum*, especially on chloroquine sensitive 3D7 and chloroquine resistant Dd2 [22]. They were maintained in continuous culture under microaerophilic conditions using the method described by Trager and Jensen [23,24] with the following modifications: Both parasite strains were maintained at 3% hematocrit in human red blood cells (type A+) in media comprising RPMI 1640, 25 mM HEPES buffer (pH 7.4), 100 µM hypoxanthine, 16 µM thymidine, 20 µg/mL gentamycin and 0.5% Albumax.

Cultures were grown at 37°C in 75-cm 2 flasks after gassing with a mixture of 5% CO2, 1% O2, and 94% N2. Parasites were synchronised by 0.3 M alanine-treatment at the ring-stage prior to the assays [24]. Parasite survival while exposed to different concentrations of our extracts was determined by the SYBR-Green I fluorescence based method [22]. We used for the assay extract final concentration in range of 1000 to 7.8 µg/mL. SYBR-Green I assay was carried out following the adapted procedures described elsewhere [25,26,27]. Briefly, 25 µL of lysis/SYBR Green I solution was added directly to each 50 µL culture; the plates were wrapped with aluminium foil and incubated at room temperature for 1h prior to fluorescence reading using a microtiter plate reader (Ex/Em:485 nm/530 nm). Fluorescence counts were plotted against the drug concentration and the 50% inhibitory concentration ($IC_{50}$) was determined by analysis of dose–response curves.

Each assay was done in three independent experiments and the average was used. For calculations and statistical analysis the probit method was used, to classify the activity levels of tannins the Rasoanaivo table was used [28].

Accordingly the term "*very active*" means an $IC_{50}$ less than 5 µg/mL; active for 5-50 µg/ mL; "*weakly active*" for 50-100 µg/mL and "*inactive*" more than 100 µg/mL.

Percent suppression of parasite growth of the treated and control groups were compared using one-way ANOVA and two-tailed Student's t test (*GraphPad Prism 4.0, Graph Pad Software*), with $P<0.05$ being considered significant.

### 2.5 Antibacterial Activity

Bacterial strains used *Escherichia coli, Salmonella typhi Streptococcus* and *Staphylococcus aureus* were a donation from the National Research Institute in Public Health in Mali and were obtained from infected patients suffering from different diseases. These strains were mostly resistant to commonly used antibiotics.

Antibacterial activities were evaluated as described elsewhere [29,30] and overall results of the third repetition are presented here. Mueller-Hilton agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. The loaded filter paper discs with different concentrations of the tannins extract were placed on the top of the Mueller-Hilton agar plates. Standard antibiotics were used as positive controls for each bacterial strain: Ciprofloxazine or ceftriaxone for *Escherichia coli* and *Salmonella typhi,* Erythromycin for *Streptococcus* and Penicillin for *Staphylococcus aureus.*

The plates were evaluated after incubation at 37°C for 18 h, after which the zones of inhibition around each disc were measured. Antibacterial activity was measured as the inhibition zone of bacterial growth (mm) produced by the tannins extracts.

Different concentrations of the tannins extract were prepared separately and loaded their requisite amount over sterilised filter paper discs in order to determine the minimum inhibitory concentrations (MIC's) of the tannins extract. The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 hours of incubation.

The Kruskal–Wallis H test for data of zone of inhibition as antibacterial activities in agar-disc diffusion method of tannins extracts against bacterial stains was used with the Statistical Package for Medical Science version 17.0 (SPSS Inc., IL, USA) [29].

## 3. RESULTS AND DISCUSSION

### 3.1 Characterisation and Determination of the Tannins from *Z. mauritiana*

Gallic tannins gave a blackish blue colour with ferric chloride while the condensed tannins gave a red colour with Bath Smith reagent.

The qualitative analysis of the tannins extracts of *Z. mauritiana* revealed five (5) spots respectively at Rf = 2.8 Rf = 3.1, RF = 3.6; Rf = 4.2 and Rf = 4.7

TLC revealed the presence of the blue highlights with a black blue colouration with ferric chloride at Rf1 = 0.7; of condensed tannins revealed by ferric chloride which gives a greenish-brown colouration at three spots corresponding to Rf = 0.6; Rf = 0.56; and R f = 0.38. Only one spot was colourless with FeCl3.

### 3.2 Antiplasmodial Activity of Tannins Extracts

Plant extracts were evaluated at concentrations up to 1000 µg / mL against 3D7 and Dd2 strains of *P. falciparum*. The tannins extracts showed moderate antiplasmodial activity on both *P. falciparum* strains.

**Table 1. TLC of the tannins extracts of *Z. mauritiana***

| Numéros des spots | Rf | Observation at U.V. | Nature of tannins | Colouration with FeCl3 |
|---|---|---|---|---|
| Spot1 | 0,7 | Visible spot | Gallic tannins | Black blue |
| Spot2 | 0,6 | Visible spot | Tanins catechic monomeric | Greenish brown |
| Spot3 | 0,56 | Visible spot | Tanins catechic dimeric | Greenish brown |
| Spo4 | 0,38 | Visible spot | Polymeric catechic tannins | Greenish brown |
| Spot5 | 0,3 | Visible spot | Not identified | Colourless |

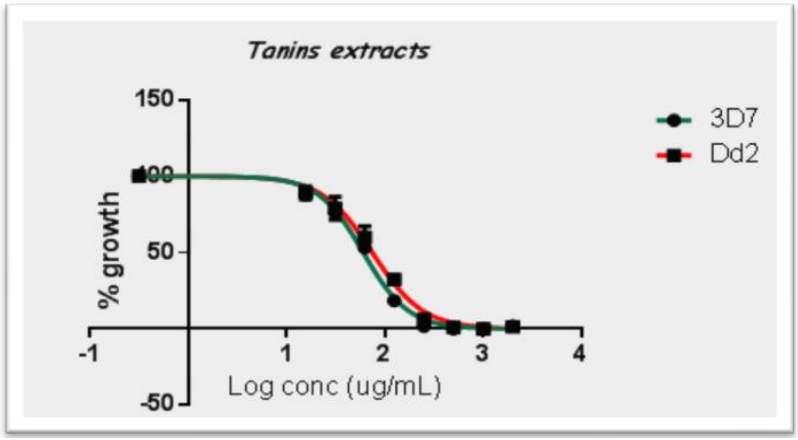


**Fig. 2. Parasite growth inhibition by Tannins extracts**

**Table 2. Antiplasmodial activity of tannins extracts from *Z. mauritiana***

| Antiplasmodial activity on *P. falciparum* | $IC_{50}$ µg/mL | |
|---|---|---|
| | 3D7 | Dd2 |
| Taninins Extracts from *Z. mauritiana* | 46.9±1.12 | 67.8±2.39 |
| Negative control | 237.4±2.6 | 371.7±4.5 |
| Positive control: *Amona senegalensis* | 23.93±0.79 | 29.47± 2.42 |

*Keys 3D7: Choloroquine-sensitve P. falciparum strain;*
*Dd2: choloroquine-resistant P. falciparum strain;*
*IC50 is expressed in µg/mL ± SD.*
**Data were obtained from three independent experiments*

The tannins extracts from *Z. mauritiana* demonstrated moderate activity on chloroquine sensitive 3D7 (46.9±1.12) and weak activity on chloroquine resistant Dd2 (67.8±2.39) of *P. falciparum* strains in our settings. Methanol extracts of *Amona senegalensis* were used as positive control and extracts from ginger powder as negative control.

In previous study on antimalarial plants from Mali [31] crude extracts of *A. senegalensis* have shown good antiplamodial activity. These extracts were more active than our tannins extracts.

Bagavan and collaborators have reported a promising antiplasmodial activity of extracts from *Phyllanthus emblica, Syzygium aromaticum*, *Abrus precatorius* and *Annona squamosa* on 3D7 and Dd2 [32]. In other study in India, the leaf extract of *Z. spectabilis, S. wallichiana* and *Amomum* sp showed good to weak antimalarial activity [33] compared to our data.

Our data suggest the use of tannins extracts as antimalarial source, however the mechanism of their action is not clear by they may interfere with different pathways such as hemozoin crystallisation, protein synthesis or DNA fragmentation [34,35].

### 3.4 Antibacterial Activity of Tannins Extracts

In our experiments standard antibiotics have produced inhibition zone around the discs showing the antibacterial activity. The tannins extracts from leaves of *Z. mauritiana* demonstrated also moderate antibacterial activity (average 7-9 mm diameter of inhibition zone) on tested strains. Especially they were more active on *streptococcus* than *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus.* The measured inhibition zones were less than those observed with standard antibiotics commonly used.

Monali and collaborators have shown that methanol extracts of *A. acuminata, P. granatum and S. febrifuga* demonstrated antibacterial activity on 11 bacterial strains with at least 25 to 29 mm diameter-sizes of zone of inhibition [36].

Ethanol extracts of *Punica granatum, Syzygium aromaticum, Zingiber officinales* and *Thymus vulgaris* were potentially effective with variable efficiency against *S. aureus, P. granatum* and *S. aromaticum* causing food poisoning diseases [37].

**Table 3. Antibacterial activity of tannins extracts from *Z. mauritiana***

| Bacterial strains | Inhibition diameter, mm |
|---|---|
| *Escherichia coli* | 6±1.4 |
| *Salmonella typhi* | 7±0.9 |
| *Streptocoque* | 9±2.1 |
| *Staphylocoque aureus* | 6±1.5 |

In other study in Nigeria Chukwujekwu and collaborators reported *in vitro* antibacterial, anti-inflammatory and antimalarial activities of 15 plant species. Most antibacterial activity was observed against *Staphylococcus aureus* with petroleum ether and dichloro methane extracts of *Mallotus oppositifolius* leaves [38].

Reddy and collaborators have reported that the mixture of tannins, gallagic acid and punicalagins exhibited antiplasmodial activity against *Plasmodium falciparum* D6 and W2 clones and different fractions revealed antimicrobial activity when assayed against *Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Cryptococcus neoformans, methicillin-resistant Staphylococcus* [39].

## 4. CONCLUSION

In the present study we have shown the antiplasmodial and antibacterial activity of tannins extracts from leaves of *Zizyphus mauritiana* in Mali. Thus, the presently used tannins could be regarded as effective and studied for further consideration for complementary medicine sources of antimicrobials against most multidrug resistant bacteria and others parasites.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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