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Original Research Article

ANTIBACTERIAL AND ANTIPLASMODIAL ACTIVITES OF TANNINS EXTRACTED FROM *ZIZYPHUS MAURITIANA*

ABSTRACT

Aim: This study was designed to evaluate the antiplasmodial and antibacterial activities of tannins extracts from *Ziziphus mauritiana*

Place and Duration of Study: Collection of plant materials, antibacterial activity evaluation were done in 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.

Methods: We extracted tannins from leaves of *Z. mauritiana* collected in around Bamako, Mali. Antiplasmodial activity was evaluated against the chloroquine-sensitive 3D7 *P. falciparum* and chloroquine-resistant Dd2 *P. falciparum* strains, using the fluorescencebased SYBR® green I method. Antibacterial activity of tannins was evaluated by disc diffusion method against strains obtained from infected patients suffering from vaginal, inflammatory and diarrheal urinary infections in Mali *Escherichia coli, Salmonella Typhi. Streptococcus* and *Staphylococcus aureus*

Results: Tannins extracts from leaves of *Z. mauritiana* showed moderate moderate antiplasmodial activity against 3D7 *P. falciparum* (46,9±1,12 µg/mL) and against Dd2 *P. falciparum* strains (67,8±2.39µg/mL). They showed also an antibacterial activity on different bacterial strains showing important inhibition zones.

Conclusion: Tannins extracted from leaves *of 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

Historically, plant extracts have been used as a safe, effective, and natural remedy for diseases in traditional medicine. The emergence of antibiotic resistance in pathogenic bacteria and others parasites has led to renewed interest in exploring the potential of plant-derived biological active compounds as an alternative therapeutic strategy to combat different diseases..

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 [1].

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, standardized, available and affordable therapeutics for management of malaria by local populations [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].

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Ziziphus mauritiana, tropical fruit tree species belonging to the family Rhamnaceae, is a spiny, evergreen shrub or small tree with trunk 40 cm or more in diameter; spreading crown; stipular spines and many drooping branches and fruit of variable shape and size [6]. The plant is used in African traditional medicine against different affections [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 industry, in food industry, and, as antimicrobials, in healthcare industry [11]. The mode of antimicrobial action of tannins is potentially due to inactivation of microbial adhesins and cell envelope transport proteins [11–13]. Besides their efficacy against bacteria, tannins have been reported to be inhibitory 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 tanins extracted from *Z. mauritiana* in Mali.

2. MATERIAL AND METHODS

2.1 Materials

The living material composed of leaves powdered of *Zizyphus mauritiana*, as well as the strains of *Escherichia coli*, *Salmonella typhi*, *Streptococcus, Staphylococcus aureus* and *Plasmodium falciparum* 3D7 and Dd2.

Figure1: Photographs of *Ziziphus mauritiana tree, leaves and fructs*

2.2 Reagents and Chemicals

Chemicals including RPMI (Roswell Park Memorial Institute) 1640, hypoxanthine, N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid (HEPES), glucose, albumax II, SYBR® green I-lysis buffer, DMEM-F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12), streptomycin, albumax, Trypsin, Ethylenediaminetetraacetc acid (EDTA) were obtained from GIBCO/Invitrogen life Technologies, USA.
For antimicrobial activity assessment the medium Muller Hinton (MH) was used with tryptic soybean medium and agar.

2.3 Extraction and qualitative analysis of tannins

The catechic tannins were extracted from powder by the maceration method. Brefly, we allowed to macerate 2g of powder in diluted acetone. The solution was then filtered and concentrated by evaporation. After centrifugation with sodium chloride the pellet obtained of catechic tannins was

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recovered until use. For gallic tanins, 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.

The characterization of the gallic tannins was done with ferric chloride while for the catechic 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 cultured 3D7 (CQ sensitive) and Dd2 (CQ resistant) strains of *Plasmodium falciparum* [20]. They were maintained in continuous culture under microaerophilic conditions using the method described by Trager and Jensen [21]) 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 synchronized by 0.3 M alanine-treatment at the ring-stage prior to the assays [22]. Parasite survival while exposed to different concentrations of our extracts was determined by the SYBR-Green I fluorescencebased method [20]. 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 [23,24,25]. Briefly, 25 µL of lysis/SYBRGreen I solution was added directly to each 50 µL culture; the plates were wrapped with aluminum foil and incubated at room temperature for 1 h 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 (IC50) was determined by analysis of dose–response curves.

For calculations and statistical analysis the Probit method was used, to classify the activity levels of tanins the Rasoanaivo table was used [28]. Accordingly the term "*very active*" means an IC50 less than 5 µg/mL; *active* from >5-50 µg/ mL; "*weakly active*" from >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, GraphPad Software), with $P<0.05$ being considered significant.

2.5 Antibacterial activty

Bacterial strains obtained from infected patients suffering from vaginal, inflammatory and diarrheal urinary infections in Mali *Escherichia coli, Salmonella Typhi. Streptococcus* and *Staphylococcus aureus* were used and provided by the National Reseach Institute in Public Health. These strains were mostly resistant to commonly used antibiotics. They were then subcultured overnight at 35 C in Mueller-Hilton agar slants.

Antibacterial activities were evaluated as described elsewhere [26] and overall results of the third repetition are presented here. Brefly 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: Ciprofloxazone or ceftriaxone for *Escherichia coli* and *Salmonella typhi,* Erythromycin for *Streptococcus* and Penicillin for *Staphylococcus aureus.* The plates were kept in the fridge at 5 C for 2 h. then incubated at 35C for 24 h. The inhibition zones were measured.
Different concentrations of the tannins extract were prepared separately and loaded their requisite amount over sterilized filter paper discs in order to determine the minimum inhibitory concentrations

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(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 h. 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). [27]

3. RESULTS AND DISCUSSION

3.1 Characterization and determination of the tannins from *Z. mauritiana*

Gallic tannins and catechic tannins

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

Qualitative analysis of tannins by TLC

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 coloration with ferric chloride at Rf1 = 0.7; of catechic tannins revealed by ferric chloride which gives a greenish-brown coloration at three spots corresponding to Rf = 0.6; Rf = 0.56; and R f = 0.38. Only one spot was colorless with FeCl3.

Table I: TLC of the tannins extracts of *Z. mauritiana*

Numéros des Spots	Rf	Observation at U.V.	Nature of tannins	Coloration 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	Colorless

Evaluation of tannins concentration

The dosage of tannins by spectrophotometry revealed an average percentage of 2.4% in the leaves *Z. mauritiana* (data not shown).

The CCM of the extracts showed the presence of two types of tannins: Condensed tannins and gallic tannins. Another non-identifying substance would be present on the TLC plate.

The qualitative analysis of our extracts by the CCM showed that they are made up mainly of tannins (Catechic and Gallic tannins). Therefore, the biocidal activity of these extracts on the plasmodium would be linked to the phenolic compounds (Tannins).

3.3 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.

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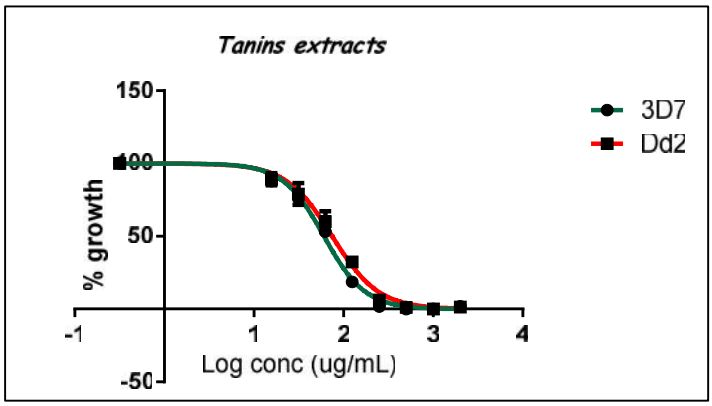

Figure2: Dose dependent curbe of parasite growth inhibition

Table II: Antiplasmodial activity of tannins extracts from *Z. mauritiana*

Antiplasmodial activity on *P. falciparum*	IC $_{50}$ *µg/mL*	
	3D7	**Dd2**
Extracts of *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;
IC_{50} is expressed in µg/mL ± SD.
**Data was obtained from three independent experiments*

Methanol extracts of *Amona senegalensis* were used as positive control and extracts from ginger powder as negative control. *A. senegalensis* has been shown to have a good antiplamodial activity in previous report [29]. In other study in India, the leaf extract of *Z. spectabilis, S. wallichiana* and *Amomum* sp showed good to weak antimalarial activity [30].

The mechanism of action might be due to the inhibition of hemozoin biocrystallization, inhibition of protein synthesis, decreased mitochondrial membrane potential and DNA fragmentation [31, 32].

3.4 Antibacterial activity of tannins extracts

Tannins extracts from leaves of *Z. mauritiana* demonstrated more antibacterial activity on *streptococcus* than *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus.* This activity was not very marked as that of standard antibiotics commonly used.

Table III: 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

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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. [33]. In other study 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* [34].

Reddy and coll 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* [35].

In fact discovery and development of antibiotics in the last century have provided assurances in clinical management all over. In the odyssey of drug development, antibiotics are introduced continually and a few of them are modified suiting to the need to overcome bacterial resistance. Today the demand for newer antibiotics for multidrug resistant bacteria in colossal scale has arisen and the tannins may play an important role in this need.

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 antiplasmodial

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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