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Investigation of the anti-salmonellal and antiplasmodial properties of leaf extracts of *Rourea coccinea* Beninese medicinal plant

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ABSTRACT

Rourea coccinea, also called *Byrsocarpus coccineus* is a medicinal plant widely used in primary health care in West Africa and in this case in Benin. In the present study, the hydroethanolic extract of these leaves was investigated for its anti-salmonella and antiplasmodial properties. The evaluation of anti-salmonella activity was carried out by the micro dilution method associated with resazurine while that of antiplasmodial activity by method described by Syber Green. Minimum inhibitory concentrations (MICs) of the hydroethanolic extract against *Salmonella* strains were higher than 2000 µg/mL. This extract was active against multidrug-resistant *Plasmodium falciparum* strains (PfDd2) with an inhibitory concentration (IC_{50}) of 32.26 µg/mL. These works on *Rourea coccinea* justify that the plant has a clearly remarkable antiplasmodial activity rather than anti-salmonella one and its use in traditional medicine to treat malaria in Benin

Keywords: *Rourea coccinea*, leaves, hydroethanolic extract, *Salmonella* strains, *Plasmodium falciparum*, malaria.

INTRODUCTION

Plants are very useful natural resources both in terms of food and health for humanity (Horrigen et al., 2002; Altieri, 2002; Adomou et al., 2017). They represent the main inexhaustible source of bioactive molecules for the fight against microbes harmful to humans and in strengthening their immune system (Andryukov et al., 2019; Teixeira et al., 2019). Numerous ethnobotanical surveys in West Africa have shown that *Rourea coccinea* of the Connaraceae family (Yedomonhan et al., 2009; Tossou et al., 2012) is used in the treatment of several infectious diseases. Also, it is used in the treatment of male (Azonbakin et al., 2021) and female sterility (Agbodjento et al., 2021),

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in the health care of animals (Tchetan et al., 2021) in the nervous system (Kantati et al., 2016) and in the treatment of malaria (Asase et al., 2010). Similarly, several pharmacological works have shown the effectiveness of this plant in several areas. The plant has also been indicated to have antileishmanial, antitripanosomal (Bero et al., 2011), antioxidant and antibacterial properties (Parekh et al., 2007).

Previously, a study of the antioxidant, anti-shigella and anti-leishmania properties of the ethanolic extract of *Rourea coccinea* leaves was carried out. Given the ethnobotanical use of *Rourea coccinea*, the present work focused on the study of the anti-salmonella and antiplasmodial properties of the hydroethanolic extract of leaves of this plant.

MATERIALS AND METHODS

Plant material

Rourea coccinea leaves were harvested in Avogbanna, a village located in the Zou department of Benin in March 2021. The plant was identified and stored at the Abomey-Calavi National Herbarium under the number YH761/HNB. These leaves were dried away from the sun at 5°C and then powdered.

Biological material

The biological material consists of three bacterial strains *Salmonella enteritidis* (SE), ST: *Salmonella typhi* (ST), STM: *Salmonella typhimirium* and a parasitic strain which was the multi-resistant strain of *Psalmodium falciparum* (PfDd2) which were provided by the Pasteur Center of Cameroon (CPC) and provenance BEI Resources. The multidrug-resistant *Plasmodium falciparum* strain Dd2 was cultured on human red blood cells of group O rhesus positive, Rh+. These strains were stored in the laboratory of Phytobiochemistry and Medicinal Plant Study/Antimicrobial and Biocontrol Agent Unit (AmBcAU) in tubes containing Muller Hinton agar by slant culture at 4EC.

Methodology

Extraction

Extraction was alone by maceration of 50 g of *Rourea coccinea* leaf powder in ethanol-water mixture (70:30 v/v) for 72 hours. The macerate obtained was filtered using filter paper and concentrated with a rotary evaporator (Buchi, 011) at 60EC. The recovered ethanol was again introduced into the mixture and then filtered and concentrated three times in a row. The crude extract obtained was dried in an oven at 50°C then weighed and stored in a refrigerator at 4°C.

Antibacterial activity

Preparation of stock solutions of the extract, and reference antibacterial

The stock solution of hydroethanolic extract of *Rourea coccinea* was prepared at 100 mg/mL by dissolving 100 mg of extracts in 1 mL of 100% DMSO. Ciprofloxacin was prepared under the same conditions at 1 mg/mL by dissolving 1 mL of powder in sterile acidified distilled water and served as a positive control.

Preparation of Inocula salmonella

Inocula salmonella were prepared according to the standard standard 0.5 McFarland as described in our previous work. For this, a stock suspension was prepared with turbidity 0.5 Mc Farland (corresponding to an approximate concentration of $1.5x10^8$ cells/mL) from young cultures of 24 h on Muller Hinton Agar (MHA) and then diluted to $5x10^5$ CFU/mL for testing.

Determination of Minimum Inhibitory Concentrations

The inhibition parameters of the extract were evaluated by determination of Minimum Inhibitory Concentrations (MICs) by the liquid microdilution technique as described by CLSI (CLSI, 2012), protocol M07-A9) for bacteria (CLSI, 2008). Indeed, the tests were carried out in duplicate in the sterile microplates of 96 wells and described in previous work carried out.

Plasmodium falciparum strain cultivation protocol

The cultivation technique used was that of TRAGER and JENSEN (1976). The multi-resistant strain Dd2 was grown in human red blood cells, fresh group O Rhesus positive at 4% hematocrit in full RPMI medium [500 mL RPMI 1640 (Gibco, UK) supplement with 25

mM HEPES (Gibco, UK), 0.50% Albumax I (Gibco, USA), 1X hypoxanthine (Gibco, USA) and 20 µg/mL gentamicin (Gibco, China)] and incubated at 37ºC in a humidified incubator consisting of 92% N_2, 5% CO_2 and 3% O_2. The medium was replaced daily with complete RPMI medium to facilitate the growth of the parasite in cultivation. Subsequently, fine blood smears were made and stained with Giemsa and then observed under a microscope at the 100X objective with immersion oil in order to follow all stages of the cell cycle and evaluate parasitemia. Before each antiplasmodial activity test, parasitic cultures containing mostly ring stages (> 80%) were synchronized to the same evolutionary stage (ring stage) by treatment with sorbitol 5% (w/v) for 10 min according to the protocol of (Lambros and Vanderberg, 1979). The use of cultures synchronized at the same evolutionary stage compared to mixed stage cultures makes it possible to evaluate the effect on the hydroethanlic extract of *Rourea coccinea* on all three phases of evolution (rings, trophozoite, schizonte) of the 48-hours life cycle of *P. falciparum.*

Preparation of stock solutions of artemisinin, chloroquine (referenced drug)

The stock solutions were prepared in 10% DMSO at concentrations of 100 mg/mL and 1mM, respectively for the extract of each plant and artemisinin and chloroquine references. For this, 100 mg of the hydroethanolic extract of *Rourea coccinea* was dissolved in 100 µL of dimethyl sulfoxide (DMSO). Then, the stock solution prepared was homogenised. Finally, after dissolution the volume was completed to 1 mL so as to obtained a solution of 100 mg/mL. The solution obtained was filtered and the filtrate was used for anti-plasmodial activity tests. The preparation of intermediate concentrations of the hydroethanolic extract of *Rourea coccinea*, the positive control (artemisinin and chloroquine) and *in vitro* test for inhibition of the growth of *P. falciparum* based on the fluorescence of SYBR green that were used are as described by Amang à Ngnoung et al. (2023).

Statistical analysis

Half-inhibitory concentrations (IC_{50}), and minimum inhibitory concentrations were determined using concentration-response curves obtained by plotting the log concentration as a function of percent inhibition using Graphpad Prism software.

RESULTS

Results of the hydroethanolic extraction yield of *Rourea coccinea* leaves

The result of the extraction yield is shown in Table 1. The hydroethanolic extraction yield of dried *Rourea coccinea* leaves was 20.4%.

Evaluation of anti-salmonella activity

Anti-salmonella activity was performed on three salmonella strains: *Salmonella enteritidis* (SE), *Salmonella typhi* (ST), *Salmonella typhimirium* (STM) and the results are presented on three in Table 2. The reference positive control is Ciprofloxacin. These results indicated that the minimum inhibitory concentrations (MICs) of the extract on these different strains were higher than 2000 µg/mL, while those of the positive control vary between 0.87 and 1.95 µg/mL.

Evaluation of antiplasmodial activity

The antiplasmodial activity was evaluated against a multi-resistant strain of *Plasmodium falciparum* (*PfDd2)* with artemisinin and chloroquine as positive controls. The results reported in Table 3 showed that the half-inhibitory concentration (IC_{50}) of the hydroethanolic extract was 32.26 µg/mL. This extract was moderately active against *Plasmodium falciparum*. The half-inhibitory concentrations of the references were 0.03 and 0.46 µM respectively for artemisinin and chloroquine.

Table 1: Hydroethanolic extraction yield from *Rourea coccinea* leaves.

Plant	Part	Powders (g)	Mass of hydroethanolic extracts (70:30) (g) and yields in %
Rourea coccinea	Leaves	50	10.2 (20.4)

Table 2: Anti-Salmonella activity of Hydroethanolic extract of *Rourea coccinea*

Salmonella strains	SE CPC MIC (*µ*g/mL)	STM CPC MIC (*µ*g/mL)	ST CPC MIC (*µ*g/mL)
Hydroethanolic extract	>2000	>2000	>2000
Positive control ciprofloxacin	0,96	0,87	1,95

Salmonella enteritidis(SE), Salmonella typhi (ST), Salmonella typhimirium (STM).

Table 3: Antiplasmodial activity against *Plasmodium falciparum* multi-resistant (*PfDd2).*

Samples		IC_{50} (µg/mL)	Ecart type
Extract	Hydroethanolic	32,26	0,47
Positive controls	Artemisinin (µM)	0,03	0,00
	Chloroquine (µM)	0,46	0,01

DISCUSSION

The extraction yield of hydroethanolic leaves of *Rourea coccinea* is 20.4%. This yield is significantly better than that obtained during the ethanolic extraction of the same leaves, under the same conditions of harvesting and treatment in previous works. This difference could therefore be explained by the difference in solvent. The hydroethanolic solvent being more polar than ethanol would have extracted not only the less polar metabolites but also the most polar from the leaf powder of the plant. This extraction result is similar to that obtained by Abodjento and collaborators in 2020 from almost identical extraction conditions (Agbodjento et al., 2021). In fact, these authors obtained a yield of 17.3% from an ethanol-water solvent system (50:50, v/v). Ethanol-water (70:30, v/v) would seem to give a better extraction yield of *Rourea coccinea* leaves. The ethanol-water solvent system (70:30, v/v) seems to be best suited to optimize the extraction yield of *Rourea coccinea* leaves according this work.

The anti-salmonella activity on the three salmonella strains in the present work reveals that the minimum inhibitory concentrations of the hydroethalic extract of *Rourea coccinea* leaves are greater than 2000 µg/mL. Based on the clarification criteria used in this work, the hydroethanolic extract of *Rourea coccinea* leaves appears to be inactive against the salmonella strains used. Results obtained are similar to those of Ezeh and collaborators in Nigeria against the species *Salmonella typhi*. Indeed, it has been shown in their work that the methanolic extract of *Rourea coccinea* leaves is inactive against *Salmonella typhi* (Chukwuemeka et al., 2019). It was reported by Ahmadu et al showed that *Rourea coccinea* exhibits low anti-salmonella activity with a low Minimum inhibitory concentration of 1750 µg/mL (Ahmadu et al., 2006). In addition, Sunday and colleagues have shown that the depleted extract of the root of the plant has an inhibitory effect at a very high concentration a *Salmonella pullorum* with an inhibitory concentration of at least 3125 µg/mL

following an in vivo study on *albino Wistar* rats (Sunday et al., 2019). Results on evaluation of the anti-salmonella properties of *Rourea coccinea* reveal that the plant has an insignificant anti-salmonella activity and therefore confirm works reported in the literature. Nevertheless, it has been reported in the literature that *Rourea coccinea* exhibits significant antifungal activity (Emmanuela et al., 2019).

Evaluation of the antiplasmodial activity of the hydroethanolic extract of *Rourea coccinea* leaves showed that these leaves exhibit significant antiplasmodial activity with an inhibitory concentration (IC_{50}) equal to 32.26 µg/mL. This result is similar to those obtained by Bero et al. (2009) on *Plasmodium falciparum* (chloroquine-sensitive strain 3D7). Indeed, these authors showed that dichloromethanolic and methanolic extracts of leaves have moderate activity with inhibitory concentrations (IC_{50}) at 41.6±22.1, 54.7±21.9 µg/mL respectively (Bero et al., 2009). These authors also showed that the aqueous extract was inactive on the same strain. The work carried out by Akpan and collaborators on the study of antiplasmodial activity in vivo against *Plasmodium berghei* have shown that the ethanolic extract of *Rourea coccinea* has a curative effect of malaria (Akpan et al., 2012). It has also been reported that the leaves of this plant have good antitripanosomal activity ranging from 14.7±1.2 to 49.5±4.9 µg/mL depending on the species used and the nature of the extraction solvent (Ibrahim et al., 2014). Similarly, *Rourea coccinea* has been shown in the literature to possess significant anti-leishmania activity (Cargnin and Gnoatto, 2017). The large chemical families contained in the leaves of *Rourea coccinea* seem to be responsible for the observed antiplasmodial activity of the hydroethanolic extract of this plant. Indeed, previous work have shown that the leaf of *Rourea coccinea* contains and bioactive metabolites against plasmodium falciparum such as flavonoids, terpenoids, phenolic compounds (Klotoe et al., 2018; Bashige-Chiribagula et al., 2020).

Conclusion

At the end of this work, it should be noted that Salmonella are not very sensitive to the hydroethanolic extract of *Rourea coccinea* with a minimum inhibitory concentration higher than 2000 µg/mL. Evaluation of antiplasmodial activity revealed that this extract is active against *Plasmodium falciparum* strains with an inhibitory concentration of 32.26 µg/mL. From these results it is deduced that the leaves of *Rourea coccinea* has a much better antiplasmodial activity than an antisalmonella power. *Rourea coccinea* is therefore a good candidate for the treatment of malaria, in general for parasitic diseases.

COMPETING INTERESTS

The authors declare that there is no competing interest.

AUTHORS' CONTRIBUTIONS

All the authors contributed to the realization of this work and to the manuscript preparation.

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