DOI: 10.21776/ub.jpacr.2023.012.02.3314 *J. Pure App. Chem. Res.*, **2023**, 12 (2), 57-64
28 August 2023

Phytochemical Screening, Antioxidant, Antishigella and Antileishmanial Activities of Ethanolic Extract of *Rourea coccinea* (Schumach & Thonn.) Benth Leaves

Basile Goueti,[1,2,3] Bénédicta Kpadonou-Kpoviessi,[1,2] Raymond Fatondji,[1] Bardieu Atchade,[1] Paul Djouonzo Toukam,[3] and Salomé Kpoviessi[1,2]*

[1]Laboratory of Physic and Synthesis Organic Chemistry (LaCOPS), University of Abomey-Calavi (UAC), Faculty of Sciences and Technics (FAST), 01 BP : 4166 Cotonou, Benin
[2]Laboratory of Pharmacognosy and Essential Oils (LAPHE), University of Abomey-Calavi (UAC), Faculty of Health Sciences (FSS), Faculty of Sciences and Technics (FAST), 01 BP: 188 Cotonou, Benin
[3]Center for Studies on Medicinal Plants and Traditionnal Medicine (CRPMT) Institute of Medical Research and Medicinal plants Studies (IMPM), PO. Box 13033, yaoundé, Cameroon

*Corresponding email: kpovsalome@yahoo.fr, Salome.kpoviessi@fast.uac.bj

Received 10 June 2023; Accepted 28 August 2023

ABSTRACT

Rourea coccinea is widely used as a medicinal plant in the world, especially in West Africa. The phytochemical screening of ethanolic extract of leaves by applying the Houghton and Raman method with some modifications revealed the presence of alkaloids, phenolic compounds, terpenoids, tannins, coumarins, anthocyanins and anthraquinones. The antiradical test was performed using DPPH (2,2-diphenyl-1-picryhydrazyl), ABTS (2,2-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt) and FRAP (Ferric Reducing Antioxidant Power) with a Scavenging concentration 50 (SC_{50}) of 101.19 µg/mL, 34.73 µg/mL and 88.93 µg/mL respectively. The extract anti-shigella activity determined by the microdilution method was low. According to Jair Siquera-Neto et *al*., the antileishmanial study using the colorimetric resealing method showed activity against promastigote strains of *Leishmania donovani* with an inhibitory concentration of IC_{50}=32.26 µg/mL. The traditional use of *Rourea coccinea*, to treat several diseases, would be justified by these pharmacological property's tests.

Key word: *Rourea coccinea,* medicinal plant, Antioxidant, antileishmanial and anti-shigella activity

INTRODUCTION

An infectious disease has been an extreme problem for humanity since old days [1], [2]. One of the diseases is shigellosis and leishmaniosis which are epidemiological diseases [3], [4] that ruin the whole world especially the countries with low reviews. In the fight against these diseases, some synthetic molecules and natural substances are used [5], [6], [7]. The resistance of the microorganisms responsible for these diseases to molecules which means that the search for new bioactive molecules tolerated by the body is considered relevant [8]. The plants are an inexhaustible and natural source of bioactive compounds [9], [10].

Rourea coccinea is a plant used in the African tradition for several diseases' treatment. Indeed, in Chad, the root is used to treat a jaundice and dysentery [11]. In Nigeria, this plant's leaf is used to treat fever, sexually transmitted infections, diarrhea, etc. [12], [13]. In

The journal homepage www.jpacr.ub.ac.id
p-ISSN : 2302 – 4690 | e-ISSN : 2541 – 0733

Benin, the stem and leaf are used to treat malaria and couple infertility problems [14]. Similarly, several pharmacological properties have been studied worldwide. Indeed, the plant is known to have anti-allergic [15], anti-inflammatory [15] antimicrobial [16] and anti-trypanosomial [17] properties.

This work aims to study the phytochemical screening of the extract, the antioxidant power, the anti-leishmanial and anti-shigella properties of *Rourea coccinea* leaf.

EXPERIMENT

Chemicals and instrumentation

Equipment and reagents: a rotary evaporator (Buchi, 011) at 60EC. A laminar flow hood equipped with an ultraviolet lamp for sterilization, a CO_2 incubator, cell culture flasks of 25 mL/12.5 cm^2 and 250 mL/80 cm^2, polypropylene tubes with conical bottom of 15 mL and 50 mL, multi-well plates (96 wells) with flat bottom and round bottom, sterile single-use serological pipettes of 5 mL and 10 mL, manual and automatic micropipettes. A 1000 mL specimen, ethanol (Merck), methanol (Merck), Mayer's reagent, sulphuric acid, iron chloride, trichloromethane, Fehling liquor, ammonia, sodium hydroxide distilled water, volumetric flask with a capacity of 500 mL, DPPH, ABTS and FRAP. The biological material used for the anti-leishmania test consists of promastigote strains that have been grown in medium 199 (Sigma, Darmstadt, Germany). This is also added 10% SIFBS (Sigma, Darmstadt, Germany) and 100 IU/mL penicillin/streptomycin contained in suitable vials (Tsukuba, Japan) which supplied RAW 264.7 strains, *Shigella flexneri* (SFNR518), *Shigella sonnei* (SONR519) and *Shiguella dysenteriae* (SD CPC).

The *Rourea Coccinea* leaf harvested in March 2021 in central Benin at Avogbanna which is identified to the national herbarium in the botanical garden of the Abomey-Calavi National of University (UAC) under the number YH761/HNB.

Procedure reaction

Ethanolic extraction

Rourea coccinea ethanolic extract is obtained by macerating for 72 hours 50 g of plant leaf powder in 500 mL of ethanol at room temperature. The mixture result is filtered on filter paper which is then evaporated at the rotary vacuum evaporator at 50°C. The macerate obtained is concentrated at the rotary evaporator under reduced pressure at 40°C. After heating in the oven at 50°C, we obtain 4.40 (8.80) g raw extract stored in the refrigerator at a temperature of -4°C.

Phytochemical screening

The phytochemical screening of ethanol extract was carried out based on Houghton [18] method and adapted to the conditions of phytochemistry laboratory at Institut Recherches Médicinales (IMPM) of Cameroon.

Antioxidant activity

The antioxidant activity was evaluated by three methods: the trapping of DPPH radicals, the trapping of ABTS radicals and FRAP (Ferric Reducing antioxidant Power).

Trapping of radical DPPH

The DPPH radical (2,2-diphenyl-I-picryhydrazyl) is one of the most stable organic nitrogen radicals, with a purple color. It is a colorimetric method based on the loss of color at 517 nm and the radical DPPH radical reduction proof (2,2-diphenyl-1-picryhydrazyl). The

protocol for trapping the radical DPPH (2,2-diphenyl-1-picrilhydrazyl) is Bassene (2012) with some modifications [19], [20].

Trapping of ABTS radicals

Anti-radical activity was also evaluated by the $ABTS^{+\bullet}$ radical discoloration test using Khan et al. (2012) technique with some modifications [21].

FRAP (Ferric Reducing Antioxidant Power)

The Fe^{3+} reduction test was performed using the protocol described by Path Canada (1994) with some modifications by the FRAP method [19], [21].

Anti-shigella activity of raw extracts

The extract inhibition parameter was evaluated by determination of minimum inhibitory concentrations (MICs) using the liquid microdilution method described by CLSI [22].

The method principle

The principle of the micro dilution method was based on the ability of a microorganism to develop in an environment with or without antimicrobial substances. MICs were revealed by the resazurin colorimetry method which is principally based on the reduction of blue resazurin to pink resorufin by dehydrogenases of viable cells.

Determination of minimum inhibitory concentrations

The sterile microplates test of 96 wells consists of introducing the wells an MHB culture medium at a rate of 160µL in the first wells and 100µL in the rest of the wells. It was then sampled and introduced into corresponding wells. 40µL of a sterile solution of extract is concentrated at 10mg / mL before proceeding to a series of five dilutions of order 2 geometry. Finally, the distribution of 100µL of a bacterial suspension into the charge of 10 exposing 6 cells / mL was made for both the wells test and the negative control wells. It should be recalled that the concentration of extracts and ciprofloxacin in the wells vary respectively from 100µL / mL to 31.125µL / mL and from 1.95µL / mL to 0.0153µL / mL; the final inoculum load in each well-being $5x10^5$cells/mL. The culture medium was the sterility control, while the positive control consisted not only the culture medium but also inoculum and ciprofloxacin. After covering the microplates, they were incubated at 37 degrees Celsius for twenty-four hours. At the end of incubation, it was adding 10µL of a freshly prepared resazurin solution (0.15mg/mL) to all wells before having another incubating under the same conditions at the microplates for 30 minutes. The smallest concentration in the case of the unchanged blue coloration to pink was the MIC reflection that lack of visible bacterial growth.

Protocol of the anti-leishmanial activity test

The *in vitro* antileishmanial study has been performed on extract mentioned in this sheet paper according to the protocol described by Jair L. Siqueira-Neto et al. (2010) [23] to determine the IC_{50} of the tested samples. Briefly, 10 µL of each tested extract from intermediate plates were introduced in each plate well. Then, 90 µL of parasitic load at de 4 x 10^5 cells/mL of promastigotes of *Leishmania donovani* were introduced in the wells for a final volume of 100 µL and the plates were incubated at 28°C for 48 hours. After incubation, 10 µL of rezazurin at 1 mg/mL were introduced in each well and the plates were incubated

again for 44 hours. Additionally, the final incubation took 72 hours. After 72 hours of incubation, the fluorescence was read at spectrophotometer at $\lambda_{excitation}$ = 530nm and $\lambda_{emission}$ = 590 nm. The percentage of inhibition of each extract was calculated using Microsoft Excel Software. The extract was screened at the concentration 100 µg/mL and 50 µg/mL, respectively.

Statistical analysis

Trapping or reducing percentages of 50% were calculated using the GraphPad Prism 5.0 software.

RESULT AND DISCUSSION

Extraction efficiency

The crude ethanolic extract of 4.40 g was obtained from 50 g of *Rourea coccinea* leaf powder (Table 1). The extraction efficiency of *Rourea coccinea* leaf with ethanol is 8.80%.

Table 1. *Rourea coccinea* ethanolic extraction yield

Plant	Part	Powder (g)	Ethanolic mass extract (g)	Yield (%)
Rourea coccinea	Leaves	50	4.40	8.80

Phytochemical Screening of ethanol extract

Phytochemical screening was carried out at the IMPM Cameroon Plant Chemistry Laboratory and the results were recorded in Table 2.

Table 2. Phytochemical screening of *Rourea coccinea* leaves

Tests	Ethanolic extract from *Rourea coccinea*
Alkaloids	+
Phenolic compounds	+
Flavonoids	+
Terpenoids	+
Steroids	-
Tannins	+
Glucosides	-
Anthraquinones	+
Coumarins	+
Anthocyanins	+
Saponins	-

Unidentified presence: (-), and presence: (+)

Qualitative analysis of the secondary metabolites of the ethanolic extract revealed the presence of alkaloids, phenolic compounds, flavonoids, terpenoids, tannins, anthraquinones (quinones bound), coumarins, anthocyanins and the absence of sterols, glucosides and saponosides in the ethanolic extract of *Rourea coccinea* leaves. The same secondary metabolites were identified in Nigeria for the *Rourea coccinea* leaf [6]. Our results are

similar to those obtained by Agbodjento and his collaborator except alkaloids and anthocyanins [24]. These authors also identified the presence of carbohydrates that we had not sought as well as saponosides, steroids and glucosides that are out of our investigation [24]. These differences results can be explained by the influence of location, season, period, and vegetative stage of harvest [14], [25-26]. The presence of these large families of secondary metabolites would be responsible for biological activities noticed this plant extracts.

Antioxidant activity of the ethanol extract.

The antioxidant potency of *Rourea coccinea* leaf was evaluated by three methods and the results are recorded in Table 3.

Table 3. Antioxidant activity of *Rourea coccinea* ethanol extract

Methods	Ethanolic extract	Ascorbic acid
	Mean $SC_{50} \pm$ SD (µg/mL)	Mean $SC_{50} \pm$ SD (µg/mL)
DPPH	101.19 ± 4.62	7.36 ± 0.31
ABTS	34.73 ± 0.04	22.46 ± 2.73
FRAP	88.93 ± 2.05	33.60 ± 1.01

The antioxidant test of the ethanolic extract showed that the *Rourea coccinea* leaf exhibited a very good antiradical activity. Based on the DPPH, ABTS and FRAP methods, the scavenging concentration 50 (SC_{50}) of 101.19 µg/mL, 34.73 µg/mL and 88.93 µg/mL were obtained. Our results were similar to those obtained by Dosseh et *al*., in 2014 by applying DPPH method in evaluating the oxidizing power of ethanolic extract of *Rourea coccinea* root [27]. The same authors evaluated FRAP *in vivo* and found an increase of antioxidants at the rats' blood. The same results were obtained in the nitric oxide (NO) evaluation. It appears that the extract significantly reduced nitrate oxide (NO) ($P < 0.05$) indicating the strong antioxidant power of the plant [27]. Flavonoids and other phenolic compounds at the leaf seem to be responsible for this high antioxidant activity [28]. *Rourea coccinea* leaf is a natural source of antioxidants that helps to against the oxidative stress.

Biological activities

Anti-shigella and anti-leishmania activity *in vitro*

The results of the *in vitro* tests of the anti-leishmania and anti-shigella activities of the ethanolic extract of *Rourea coccinea* were summarized in the Table 4.

The *in vitro* anti-shigella activity of *Rourea coccinea* ethanolic extract

Table 4 suggested that the minimum inhibitory concentrations ((MICs>2000 µg/mL) of the extract were higher than 2000 µg/mL for the anti-shigella test. The ethanolic extract of *Rourea coccinea* has a non-significant activity according to the classification of criterion used. The anti-shigella study of *Rourea coccinea* has not been mentioned in the literature. Nevertheless, it has been shown that this plant methanol extract has a low antifungal activity. Indeed, the antifungal activity on isolates of Candida (C) shows inhibitions as follows: (26.6% for *C. tropicalis*, 31.6% for *C. krusei*, 12.7% for *C. parapsilosis* and 10.1% for *C. albicans*). At the end of these evaluations, these extracts have a low antifungal power

compared to fluconazol [29]. Similarly, in Nigeria, the *n*-buthanol extract has been shown to be actively against *Pseunomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* with minimum inhibitory concentrations of 1250 µg/mL to 5000 µg/mL [13]. Our results corroborate these authors' result and justify that the *Rourea coccinea* leaf has a weak anti-shigella power.

Table 4. The *in vitro* anti-shigella and anti-leishmania activities of the ethanolic extract of *Rourea coccinea*

Samples	Bacteries strains of Shigella				Stem parasites of the genus Leishmania	
	SFNR 518	SONR519	SD CPC		Promastigots of *Leishmania donovani*	
	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)		Mean IC_{50} (µg/mL)	
Extract of *Rourea coccinea*	>2000	>2000	nd	>2000	32.26 ±0.56	
Positive Controls	Amoxicillin (1µM)	0.97±0.01	0.97±0.01	0.95±0.10	nd	nd
	Amphotericin B (1µM)	nd	nd	nd	2.90±0.02	0.46±0.08

Legend: SFNR518 : *Shigella flexneri*, SONR519 : *Shigella sonnei*, SD CPC: *Shigella dysenteriae,* CPC: *Centre Pasteur of Cameroun*, nd: not determined.

The *in vitro* anti-leishmanial activity of *Rourea coccinea* ethanolic extract.

The analysis of the Table 4, *Rourea coccinea* leaf ethanolic extract shows an IC_{50} value (IC_{50}=32.26 µg/mL) against promastigous strains of *Leishmania donovani* that exhibits a moderate extract activity against these parasites. These ethanolic leaf extract results were different from Bero and colleagues in 2011. Indeed, the anti-leishmanial activity was evaluated on the extract of *Rourea coccinea* against strains with an IC_{50} > 100 µg/mL [30]. This difference can be explained firstly by the difference of leishmania strains used during their work [30]. Secondly, it can also be explained by the difference of extracts. In fact, these authors made aqueous, chloro-methanolic and methanolic extractions. Meanwhile, we have an ethanolic extraction [30]. Finally, although the plant is harvested in Benin, we also note the difference of harvest area, the vegetative state of the plant, as well as the harvest time which influences the chemical constituents of the plant [30]. This remarkable activity against *Leishmania donovani* seems caused by the secondary metabolites (terpenoids, flavonoids and phenolic compounds) present in the ethanolic extract of *Rourea coccinea* leaf which has been identified during phytochemical screening. Indeed, it has been shown that terpenoids [30], flavonoids [31] and phenolic compounds [32] have an important anti-leishmania property.

CONCLUSION

The ethanolic extract of *Rourea coccinea* leaf is rich and varied in secondary metabolites. It contains alkaloids, phenolic compounds, terpenoids, flavonoids, tannins, coumarins, anthocyanins and anthraquinones. Anti-radical tests show that the *Rourea coccinea* life is rich with antioxidant that can be utilized as natural substances to against the

oxidative stress. The evaluation of anti-shigella activity reveals that the plant has very low anti-shigella activity with a minimum inhibitory concentration above 2000 µg/mL. The ethanolic leaf extract is active against promastigous strains of *Leishmania donovani* with an inhibitory concentration IC_{50} = 32.26 µg/mL. These pharmacological properties, tested for the first time in Benin, would justify the traditional use of this plant in treating several diseases.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENT

Thanks to LENTA Bruno, Full Professor, Coordinator of the YaBiNaPA project, in conducting this project which allowed us to handle in peace. Also Mrs Laure YAMTHE TCHOKOUAHA Doctor and her research team as well as all members of their laboratory without whom biological tests would not be carried out. Finally, I would like to thank Mr TCHINDA Tiabou Alembert, Doctor, Chief Laboratory of Phytochemistry of the Institute of Medical Research and Studies of Medicinal Plants (IMPM), for the collaboration at various levels of this work. Please receive here the expression of my deep gratitude.

REFERENCES

[1] Zhao, J., Yuan, Q., Wang, H., Liu, W., Liao, X., Su, Y., Wang, X., Yuan, J., Li, T., Li, J. *Clin. Infect. Dis*. **2020**, 71, 2027–2034.
[2] Tabish, S.A., Nabil, *S. Int. J. Sci.* Res. **2015**, 4, 2427–42
[3] Huigang, L., Xiaowei, X., Cui, H., Haixia, M., Zhiming, Y. *J. Biosaf. Biosecurity*. **2020**, 2, 23–26.
[4] Snacel, S. et Hadjaz, K. PhD Thesis, Université Mouloud Mammeri, **2022**.
[5] El Rhaffari, L., Hammani, K., Benlyas, M. et Zaid, A. *Biologie & Santé*. **2002**, 1(1), 45-54.
[6] Fournet, A., Hocquemiller, R., et Gantier, J. C. *Recherche*.**1995**, 26(451), 424-429.
[7] Vaissaire J., Laugier C., Baroux D., Plateau E., Laroche M., Mirial G., Coconnier M.H., Hardy J., *Bull. Acad. Vét. Fr*. **1987**, 140(3), 385-391.
[8] Andryukov, B., Mikhailov, V., Besednova, N. *J. Mar. Sci. Eng*. **2019**, 7, 176.
[9] Gori, A. PhD Thesis, Université Grenoble Alpes, **2020**.
[10] Grigoraş, C.-G. PhD Thesis, Université d'Orléans; Universitatea Vasile Alecsandri din Bacău (România), **2012**.
[11] Fowler D.G. Kirkia **2006,** 18, 35–48.
[12] Bello, A., Jamaladdeen, S., Elder, M.T., Yaradua, S.S., Kankara, S.S., Wagini, N.H., Stirton, C.H. and Muasya, M. *Bothalia-African Biodiversity & Conservation.* **2019**, *49*(1), pp.1-17.
[13] Ahmadu, A. A., Haruna, A. K., Sule, M. I., Pateh, U. U. et Akpulu, I. N. *Planta Med.*, **2006**, 72(11), 203.
[14] Bero, J., Ganfona, H., Jonville, M-C., Frédérich, M., Gbaguidi, F., DeMol, P., Moudachirou, M., Quetin-Leclercq, J. *J. Ethnopharmacol*. **2009**, 122(3), 439-444. doi: 10.1016/j.jep.2009.02.004.
[15] Dosseh, K., Kokou, I., Agbodjogbe, W. K. D., Et Agbonon, A. *W. J. Adv. Res. Rev.* **2022**, 15(3), 269-277.
[16] Dougnon, V., Legba, B. B., Gbaguidi, B., Agbodjento, E., Agbankpe, A. J., Rocha, D., Ayi, I., Azonbakin, S., Diallo, A., Bonkoungou, I.J., Klotoe, J.R., Agbangla, C. and Alitonou G.A. *Int. J. One Health*. **2022**, 8(2), 124–160

[17] Ibrahim, M. A., Mohammed, A., Isah, M. B. et Aliyu, A. B. *J. ethnopharmacol*. **2014**, 154(1), 26-54.
[18] Goueti, B., Kpadonou-Kpoviessi, B., Ahoussi, L., Glinma, B., Tchinda A., Tchokouaha L., Gbenou, J, Lenta, B. and Kpoviessi, S. *J. Pharmacogn. Phytochem*. 2023, 12(3): 150-155.
[19] Bassène, E. *Presse*, Universitaire de Dakar : Dakar, **2012**, 17,94-96..
[20] Sy, A. N., Fall, A. D., Ndiaye, M., Ndiaye, K., Gueye, R.S., Bassene, E., Amadou, M. Dieye, A.M. et Sy, G.Y. *Int. J. Biol. Chem. Sci.*. **2018**, 12(4), 1816-1823.
[21] Dieng, S.I.M., Fall, A.D., Diatta-Badji, K., Sarr, A., Sene, M., Mbaye, A., William Diatta, W. et Bassene, E. *Int. J. Biol. Chem. Sci.* **2017**, 11(2), 768-776.
[22] CLSI., « Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; *Approved Standard — Ninth Edition*, **2012**, 32(2).
[23] Siqueira-Neto, J.L., Song, O-R., Oh, H., Sohn, J-H, ,Yang, G., Nam, J., Jang, J., Cechetto, J., Lee, C.B., Moon, S., Genovesio, A., Chatelain, E., Christophe, T., Freitas-Junior, L.H. *PLoS neglected tropical diseases*. **2010**, 4(5), e675.
[24] Agbodjento, E. Klotoé, J., Sacramento, I., Dougnon, V., Hounkpatin, M., Dougnon, J., Atègbo, J. *Int.l J. Biosci*. **2020**, 16(4), 230-240.
[25] Jutras, S.et Plamondon, A. P. *Écoscience*. **2021**, 28(1), 1-31.
[26] Joel, A. D., Hospice, D. G., Cossi, A. A., Aristide, H. G. H., Brice, T.et Brice, S. A. *Eur. Sci. J.*. **2017**, 13(30), 1857-7881.
[27] Dosseh, H. K., Ahozonlin, M. C. et Dossa, L. H. *Vet; Anim. Sci.*. **2021**, 14, 100-210.
[28] Said, A. A. H., El Otmani, I. S., Derfoufi, S. et Benmoussa, A. *Hegel*, **2016**, 3(3), 280-292.
[29] Emmanuela, I. N., Anthony, C. M., Ejike, E. U. et Onwumere, S. I. *Afri. J. Microbiol. Res*. **2019**,13(20), 332-340.
[30] Bero, J., Hannaert, V., Chataigné, G., Hérent, M.-F. et Quetin-Leclercq, J. *J. Ethnopharmacol*. **2011**, 137(2), 998-1002.
[31] Tasdemir, D., Kaiser, M., Brun, R., Yardley V., Thomas, J.S., Tosun, F., Rüedi, P *Antimicrob. Agents Chemother*. **2006**, 50(4), 1352-1364.
[32] Cheikh-Ali, Z., Caron, J., Cojean, S., Bories, C., Couvreur, P., Loiseau, P.M., Desmaële, D., Poupon, E., Champy, P., *Chem. Med. Chem. **2015**,* 10(2). 411-418.