World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Antiplasmodial, anti-inflammatory and DPPH scavenging activities of extracts of the stem barks of *Discoglypremna caloneura* (Pax) Prain

Paul Djouonzo Toukam[1,2], Lauve Rachel Tchokouaha Yamthe[2] , Alembert Tiabou Tchinda[2], Fabrice Fekam Boyom[3] and Joseph Tanyi Mbafor[1]

[1]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaoundé, Cameroon
[2]Institute of Medical Research and Medicinal Plant Studies, P.O. Box 13033, Yaoundé, Cameroon
[3]Laboratory for Phytobiochemistry and Medicinal Plants Studies, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon

Received: 01-04-2017 / Revised: 05-05-2017 / Accepted: 20-05-2017 / Published: 27-05-2017

ABSTRACT

In this study, the stem bark of *Discoglypremna caloneura* was subjected to phytochemical study and biological activity screenings. The hexane and ethyl acetate extracts exhibited significant inhibitory activities on strain W2 of *Plasmodium falciparum* with IC50 values 4.78 and 4.6 µg/ml respectively. None of the extracts was toxic to erythrocytes at concentrations up to 10 µg/mL. In the anti-inflammatory test, negligible inhibition of serum bovine albumin denaturation was observed for all extracts. The DPPH scavenging activity, although also insignificant was limited to ethyl acetate and methanol extracts, the hexane extract showed no activity. Tricosanoic acid, 3-*O*-acetylaleuritolic acid and a mixture of β-sitosterol and stigmasterol were isolated from the hexane extract.

Keywords: *Discoglypremna caloneura*, *Plasmodium falciparum*, antiplasmodial, DPPH scavenging, denaturation, isolation.

INTRODUCTION

Malaria is still the most important tropical infectious disease. Resistance of *Plasmodium* parasites to currently used antimalarial chemotherapies is a serious threat. In Cameroon, many plants are used in folk medicine to treat malaria and related symptoms, and different types of inflammatory diseases. Many studies previously reported the antimalarial, antioxidant and anti-inflammatory effects of some of these medicinal plants [1,2]. With the growing interest in the use of medicinal plants in pharmaceuticals, a systematic investigation of plant extracts is becoming more and more important.

Discoglypremna caloneura is a large dioecious tree up to 45 m tall and belongs to the family of Euphorbiaceae. It is found in rainforest and old secondary forest, from sea-level up to 1100 m altitude. It occurs from Guinea east to Uganda and to Democratic Republic of Congo. In West Africa a decoction of the crushed leaves is taken as an expectorant in bronchial problems. The seed or seed oil is taken as an emetic and purgative against dysentery, diarrhoea and oedema, and to help in cases of difficult childbirth and as an abortifacient. The seed oil mixed with bait is used to kill unwanted animals. In Congo a bark decoction is taken to relieve coughing fits and intestinal pain caused by food poisoning, and as an emetic. A maceration of ground leaves in water is applied to the head to kill lice[3]. Bark powder is applied to sores to promote healing and its extract is used in Cameroon for the healing of liver related disorders [4]. Previous research works reveal the presence of tannins in the stems of root and bark and diterpenes in the stem bark [5]. The crude ethanol extract of leaves showed a moderate *in vitro* bacteriostatic effect against *Staphylococcus aureus* and *Enterococcus faecalis*[6]. The root and stem bark methanol extracts of *D. caloneura* did not show activity against hepatitis C virus [2]. Nyasse et al. (2006) demonstrated that 3-O-acetyl aleuritolic acid isolated from *D. caloneura* exhibited significant inhibitory activities on the vitality of

***Corresponding Author Address:** TOUKAM DJOUONZO Paul, Research Scientist, Institute of Medical Research and Medicinal Plant Studies, Ministry of Scientific Research and Innovation, C/O IMPM-CRPMT (laboratory of phytochemistry), P.O Box: 13033 Yaoundé-Cameroon; Email : touks241@yahoo.fr*

adult male worms of *Onchocerca gutturosa* [5]. To the best of our knowledge, there are no studies describing the anti-*Plasmodium*, anti-inflammatory and antioxidant activities of *D. caloneura*. In this study, we investigated different extracts from *D. caloneura* stem bark for their antiplasmodial, anti-inflammatory, DPPH scavenging properties and elucidated chemical structures of some isolated compounds.

MATERIAL AND METHODS

Plant material and extraction: *D. caloneura* stem bark was harvested in July 2013 in Mefoup, South Region of Cameroon. The plant was identify at the National Herbarium of Cameroon where a voucher specimen was deposited under the identitification number N°4207/SRFK. The stem bark was chopped into small pieces, air-dried and grounded to powder. The resulting powder was successively extracted by percolation with hexane, ethyl acetate and mixture MeOH/DCM (1:1) in increasing polarity. A small quantity of plant powder was also extracted with DCM/MeOH by percolation without prior extraction by less polar solvents (hexane and ethyl acetate) to furnish the crude methanol extract. The organic solvents were removed using a rotary evaporator and dried to completion in a ventilated oven at 50°C. The yield of extraction was calculated as weight of the given extract divided by the weight of dried plant material and multiplied by one hundred (Table 1).

Erythrocyte susceptibility to plant extracts: A preliminary toxicological assessment was carried out to determine the highest drug concentrations that could be incubated with erythrocytes without apparent toxicity. This was done according to the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide / phenazine methosulfate (MTT/PMS, Promega) colorimetric assay[7], with some modifications[1]. The extract stock solutions in 10% DMSO (1mg/mL) were serially diluted in 96 well culture plates using RPMI 1640 and tested at the highest concentration of 20 µg/mL in triplicate against erythrocytes (2% hematocrit) (at 37°C, in a 3% O_2, 5%CO_2 and 91% N_2 atmosphere, in the presence of RPMI 1640, 25Mm HEPES, pH 7.4 for 48 h). At the end of the incubation period, the cultures were transferred into polypropylene microcentrifuge tubes and centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. A total of 1.5 mL MTT solution with 250 µL g PMS was added to the pellets. Controls contained no erythrocytes. The tubes were thereafter incubated for 45 min at 37°C, and then centrifuged, and the supernatant was discarded. The pellets were re-suspended in 0.75 mL of HCl 0.04 M in isopropanol to extract and dissolve the dye (formazan) from the cells. After 5 min, the tubes were vigorously mixed and centrifuged, and the absorbance of the supernatant was determined at 570 nm, with absorbance representing healthy cells. The highest drug concentrations producing minimal damage to the cells were considered starting points for drug dilutions.

Determination of the antiplasmodial activity

Parasites culture: *Plasmodium falciparum* strain W2 was maintained in culture in sealed at 37°C, in a 3% O_2, 5% CO_2 and 91% N_2 atmosphere in RPMI 1640, 25Mm HEPES, pH 7.4, supplemented with heat inactivated 10% human serum and human erythrocytes to achieve a 2% hematocrit.

In vitro **antiplasmodial activity:** The antiplasmodial activity was tested on parasites using the FACSort flow cytometer[6]. Briefly, parasites were synchronized at the ring stage by serial treatment with 5% sorbitol (Sigma, Taufkirchen, Germany) and studied at 1% parasitemia. Plants extract were prepared as 1 mg/mL stock solutions in dimethylsulfoxide (DMSO), further diluted as needed for individual experiments, and tested in triplicate. The stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.1% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% hematocrit culture was thereafter added and gently mixed thoroughly. Negative controls contained equal concentrations of DMSO. Positive controls artemisinin (Sigma, Taufkirchen, Germany). Cultures were incubated at 37°C for 48h. Parasites at the ring stage were thereafter fixed by replacing the serum medium by an equal volume of 1% formaldehyde in PBS. Aliquots (50 µL) of each culture were then added to 5 mL round-bottom polystyrene tubes containing 0.5mL 0.1% Triton X-100 and 1nM YOYO nuclear dye (Molecular Probes) in PBS, and parasitemias of treated and control cultures were compared using a Becton-Dickinson FACSort flow cytometer to count nucleated (parasitized) erythrocytes. Data acquisition was performed using CellQuest software. These data were normalized to percent control activity and IC50s were calculated using Prism 5.0 software (GraphPad, CA, USA) with data fitted by non-linear regression to the variable slope sigmoidal dose-response formula, $Y=100/1+10^{(\log\ IC50-x)H}$, where H is the hill coefficient or slope factor.

DPPH radical – scavenging activity: The antioxidant activity of compounds was measured on the basis of the scavenging activity of the stable 1,1-diphenyl -2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-

williams et al. (1995)[8] with slight modification. 950µL of 4% DPPH solution in methanol was mixed with 50µL solution of varying concentrations (5 - 0.15 mg/mL) of each sample. Corresponding blank samples were prepared and L-Ascorbic acid was used as reference standard. The reaction was carried out in triplicate after vigorous shaking and 30 min storage in dark, and the decrease in absorbance was measured at 517 nm using UV-Vis spectrophotometer. The inhibition percentage was calculated using the following formula: % inhibition = [(absorbance of control – absorbance of sample)/absorbance of control] x 100.

The scavenging activity was classified as moderate if SC_{50}<50mg/L and high if SC_{50}>50mg/L[9].

Anti-inflammatory activity: inhibition of albumin denaturation: The determination of the *in vitro* anti-inflammatory activity was achieved by the protein denaturation method as described by Sangita *et* al. (2012)[10] and Shyam *et* al. (2013)[11] with slight modifications. The 1000 µL of reaction mixture consisted of 40 µL serum bovine albumine (0.1% weight by volume of phosphate buffered saline), 560 µL phosphate buffered saline (pH 7.4) and 400 µL of varying concentrations of test samples. The control contained all reacting species in equal volume except the test sample. The mixture was carried out in triplicate. The reaction was allowed to run by incubating at 37±2 °C in an electrothermal incubator for 30 min, then heating at 70 °C for 15 min and cooling at room temperature for 5 min. After cooling, their absorbance was measured at 660 nm using UV-Vis spectrophotometer.

Diclofenac sodium was used as reference drug and treated similarly for determination of absorbance. The % inhibition of protein denaturation was calculated by using the following formula:

% inhibition = $(V_t / V_c - 1) \times 100$

Where V_t = absorbance of test sample, V_c = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

Statistical analysis: All data are expressed as mean ± S.D. Significance differences between the means were determined by least significant difference (LSD) test at a level $P < 0.05$.

RESULTS AND DISCUSSION

Plant extraction and fractionation: Extraction: The yield of extraction of *D. caloneura* stem bark was found to increase with the polarity of the extracting solvent (Table 1) which is closed to that reported by Nyasse et al. (2006)[5].

Fractionation: Twenty grams (20g) of the hexane extract was subjected to open column chromatography over silica gel and eluted with the gradient solvent system hexane: ethyl acetate. A total of 110 fractions of 100mL each were collected and pooled based on their TLC profile into six fractions: FH1 (3.98g), FH2 (4.48g), FH3 (2.80g), FH4 (1.73g), FH5 (1.66g), FH6 (1.38g). Fractions 12-15, 18 and 30-31 crystallized in solvent system Hexane: ethyl acetate (95:5) and their filtration using a Watman filter paper led to white powders of three pure compounds (**1**, **2** and **3** respectively) according to their TLC profile revealed with diluted sulphuric acid and gentle heating. ^{1}H and ^{13}C NMR spectral analysis of these compounds together with that found in the literature enabled to identify the three compounds as tricosanoic acid (**1**), 3-*O*-acetylaleuritolic acid (**2**) and a mixture (3:1) of β-sitosterol and stigmasterol (**3**) (Figure 1)[12,13]. The percentage content of the mixture was evaluated by integrating the NMR spectrum of compound **3**. Proton H-6 belongs both to β-sitosterol and stigmasterol whereas H-20 and H-21 belong to stigmasterol alone.

Antioxidant activities of extracts: The antioxidant activity was achieved by measuring the capacity of the extract to donate a proton for the reduction of free radicals. The results showed that, except the hexane extract that did not show activity, ethyl acetate (188.25 ± 1.42 µg/L) and methanol (176.16 ± 3.45 µg/mL) extracts showed a moderate scavenging activity far lower than that of the reference compound, ascorbic acid (5.99 ± 0.05 µg/L). However, there is no significance difference between scavenging activities of both ethyl acetate and methanol extracts.

Anti-inflammatory activity: inhibition of serum bovine albumin denaturation: All extracts prevented the denaturation of serum bovine albumin induced by heat. The highest activity was observed with the hexane extract (135.41 ± 2.67 µg/mL) followed by the ethyl acetate extract (223.32 ± 4.01 µg/mL). and the less active, the methanol extract (262.82 ± 5.47 µg/mL). The activity increased with the solvent polarity thereby implying that the inhibition of denaturation might be via the non-polar site of the protein. However, the inhibition of denaturation displayed by the extracts are lower compare to that of the reference compound diclofenac (7.20 ± 0.97 µg/mL).

Antiplasmodial activity: All the tested extracts of the stem bark from *D. caloneura* stem bark were

not toxic to erythrocytes at concentrations up to 10 µg/ml. The antiplasmodial activity has been evaluated on the resistant strain W2 of *Plasmodium falciparum*, the results are summarized in table 2.

Two of the four tested extracts, namely, hexane extract and ethyl acetate extract were active with IC_{50} ranging from 4.787 and 4.676 µg/mL respectively. These two samples were promising, with IC_{50} values below 5 µg/ml against *P. falciparum*. The fact that the crude methanol extract did not show any activity might suggest that it is enriched with secondary metabolites which are either inactive or antagonists to the inhibition of the parasite growth. Although there is no previous report on the antiplasmodial activity of *D. caloneura*, 3-O-acetyl aleuritolic acid isolated from *D. caloneura* showed a good activity against *Onchorcerca gutturosa* worms by reducing the motility and viability of the worms up to 57·1 and 64·8%, respectively[5].

Concerning the isolated compounds, the mixture of β-sitosterol and stigmasterol was reported as non-active compounds against *Plasmodium falciparum* in the literature [13]. Compound **2,** a pentacyclic triterpene belonging to the oleananes class is well known for its antiplasmodial activity. In fact, oleanane pentacyclic triterpenes showed high to low activity against *Plasmodia*[14]. Cunha et al (2003) established that the observed low or lack of *in vitro* antiplasmodial activity of oleananes is related to the absence of an acid group and/or the OH group at position 3. The derivatization or steric hindrance of these two functions also produces the same effect[15]. Compound **2** isolated from other plant species was however highly cytotoxic against human lung carcinoma A549 cells and inhibition of DNA topoisomerase II[16].

Conclusion

These results prove the efficacy of *D. caloneura* stem bark extracts in inhibiting malaria parasite, portraying anti-inflammatory and antioxidant properties. Meanwhile, further studies are ongoing to completely characterize chemical structures of all isolated compounds and carry out their *in vitro* antiplasmodial and cytotoxic activities.

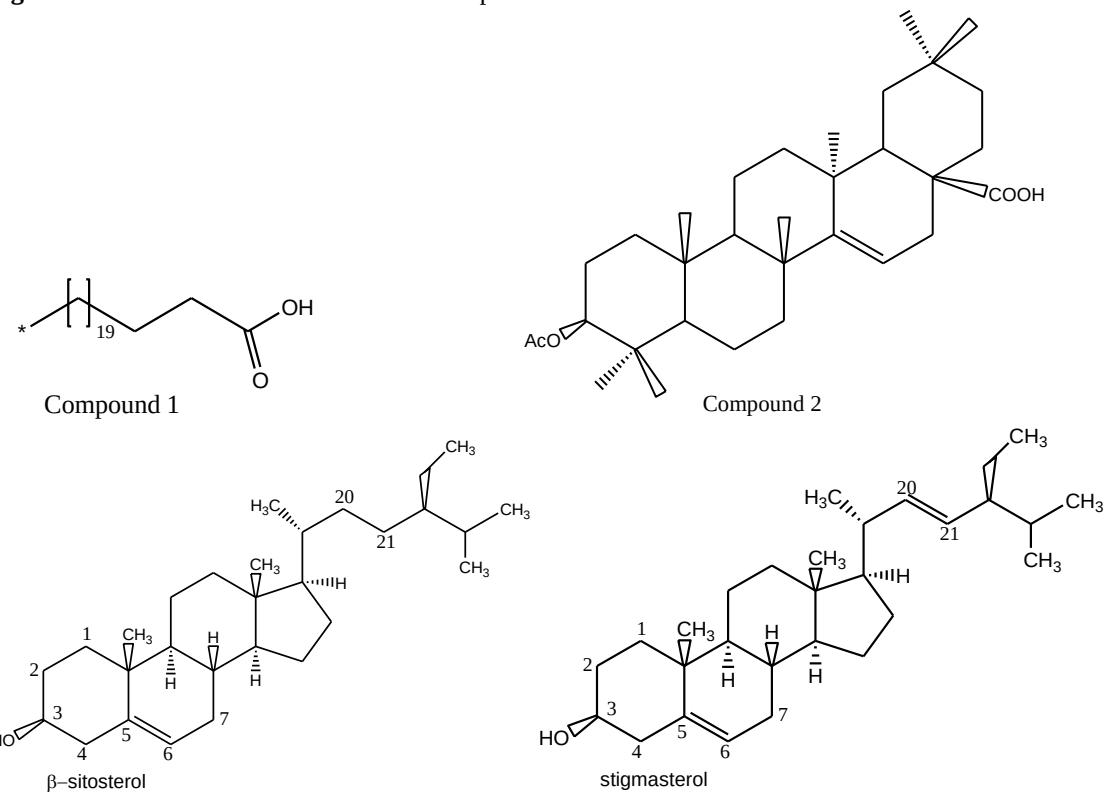
Table 1: Yield and other physical properties of extracts of *D. caloneura*

Sample	Yield	Colour	Consistency
Hexane extract	0.22	yellow	oily
Ethyl acetate extract	0.70	Dark greenish	pasty
Methanol extract	3.00	Dark brownish	pasty
Crude methanol extract	3.50	Dark brownish	pasty

Table 2: Antiplasmodial activities of extracts of *D. caloneura.*

Nature of tested sample	**IC_{50} (µg/ml) ±S.D**
Hexane extract	4.787±0.211
Ethyl acetate extract	4.676±0.463
Methanol extract	>10
Crude Methanol extract	>10
Positive control: Artemisinin	0.005 ± 0.0008

Figure 1: Chemical structures of isolated compounds.



REFERENCE

1. Boyom FF et al. Antiplasmodial activity of extracts from seven medicinal plants used in malaria treatment in Cameroon. J Ethnopharm 2009; 123: 483-488.
2. Zofou D et al. In vitro antiplasmodial activity and cytotoxicity of extracts of selected medicinal plants used by traditional healers of Western Cameroon. Mal Res Treat 2011; 6 pages.
3. Schmelzer G.H.; Discoglypremna caloneura (Pax) Prain. In: Schmelzer G.H, Gurib-Fakim A, eds. PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale). Wageningen, Netherlands, 2008: 258-260.
4. Galani BRT et al. Plant extracts from Cameroonian medicinal plants strongly inhibit hepatitis C virus infection *in vitro*. Fron Microbiol 2015; 6, 488.
5. Nyasse B et al. Antifilarial activity in vitro of polycarpol and 3-O-acetyl aleuritolic acid from cameroonian medicinal plants against *Onchocerca gutturosa*. Nat Prod Res 2006; 20(4): 391-7.
6. Atindehou KK et al. Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. Phytother Res 2002; 16(5): 497–502.
7. Cedillo-Rivera R et al. A rapid colorimetric assay with the Tetrazolium salt MTT and Phenazine Methosulfate (PMS) for viability of *Entamoeba histolytica*. Arch Med Res 1992; 23: 59-61.
8. Brand-williams W et al. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol 1995; 28: 25-30.
9. Omisore NOA et al. Antitrichomonal and Antioxidant Activities of *Dorsteniabarteri* and *Dorsteniaconvexa*. Braz J Med Biol Res 2005; 38: 1087-94.
10. Sangita C et al. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacif J Tropic Biomed 2012; S178 – S180.
11. Shyam BP et al. *In vitro* anti-inflammatory activity of Raupya (Silver) Bhasma. J Chem Pharm Res 2013; 5(9):194-197.
12. Viswanadh GS et al. Chemical constituents of the heartwood and bark of *Homonoia riparia*. J Trop Med Plants 2006; Vol:7, N°2.
13. Nethengwe MF. Antiplasmodial/Antipyretic activity of some Zulu medicinal plants. Master thesis, University of Zululand: South Africa, 2012.
14. Murtala BI et al. A systematic review of pentacyclic triterpenes and their derivatives as chemotherapeutic agents against tropical parasitic diseases. Parasitology 2016; 143: 1219–1231.
15. Cunha WR et al. In vitro trypanocidal activity of triterpenes from Minocia species. Planta Med 2003; 69: 470-471.
16. Wada S, Tanaka R. Isolation, DNA topoisomerase-II inhibition, and cytotoxicity of three new terpenoids from the bark of *Macaranga tanarius.* Chem biod 2006; 3: 473 - 479.