Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(4): 20-23
Received: 10-04-2022
Accepted: 16-05-2022

Vincent Assamoi Adiko
Laboratoire de Constitution et Réaction de la Matière, UFR-SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Landry Claude Ahmont Kablan
a) Laboratoire de Constitution et Réaction de la Matière, UFR-SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire
b) UFR des Sciences Biologiques, Université Péléforo Gon Coulibaly Korhogo, BP 1328 Korhogo-Côte d'Ivoire

Faustin Aka Kabran
Laboratoire de Constitution et Réaction de la Matière, UFR-SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Chardin Seri Seri
Laboratoire de Constitution et Réaction de la Matière, UFR-SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Timothée Aboua Okpekon
Laboratoire de Constitution et Réaction de la Matière, UFR-SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Barthélemy Koffi Attioua
Laboratoire de Constitution et Réaction de la Matière, UFR-SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Corresponding Author:
Vincent Assamoi Adiko
Laboratoire de Constitution et Réaction de la Matière, UFR-SSMT, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Antiprotozoal activity and phenolic constituents of ethyl acetate extract from *Parinari excelsa* sabine (Chrysobalanaceae)

Vincent Assamoi Adiko, Landry Claude Ahmont Kablan, Faustin Aka Kabran, Chardin Seri Seri, Timothée Aboua Okpekon and Barthélemy Koffi Attioua

DOI: https://doi.org/10.22271/phyto.2022.v11.i4a.14439

Abstract
Parinari excelsa Sabine (Chrysobalanaceae) is a plant used in Ivorian traditional medicine to treat various diseases including malaria. Present study aims to determine the antiprotozoal activity of the ethyl acetate extract from the trunk back and its many phenolic constituents. The ethyl acetate extract was fractioned by column chromatography method using silica gel and the obtained sub fractions were evaluated *in vitro* against *Trypanosoma cruzi, Leishmania donovani* Axen and *Plasmodium falciparum K1*. The phytochemical screening of the ethyl acetate extract (EA) led to the identification by semi-preparative HPLC method of twelve known phenolic compounds: Kaempferol (1), Apigenin (2), Hydroxybenzoic acid (3), Procyanidin B1 (4), Protocatechuic acid (5), Procyanidin B2 (6), Ferulic acid (7), Xanthotoxin (8), Chlorogenic acid (9); p-Coumaric acid (10), Scopoletin (11) and Quercetin (12). Biological study revealed height antiplasmodium, antitrypanosome and antileishmaniasis activities of ethyl acetate extract and its sub-fractions.

Keywords: *Parinari excelsa,* antiprotozoal activity, Semi-preparative HPLC, polyphenols

1. Introduction

In Africa and in many regions of the world where parasitic diseases are rife, people regularly use medicinal plants to treat themselves; this is the case of *Erythrophleum suaveolens* [Konan *et al*., 2019; Ahmont *et al*., 2020] [11, 1], *Mareya micrantha* [Gnaoré *et al*., 2020] [8] and *Parinari excelsa* [kamanzi *et al*., 2004] [10]. *Parinari excelsa,* the species that interests us here, is regularly used as a remedy against malaria in Côte d'Ivoire [Gessler *et al*., 1995] [7]. Malaria is a disease which is the cause of many deaths of children in Côte d'Ivoire and in the tropical regions where it continues to be rampant [WHO, 2020] [17]; it is considered a global pandemic by the World Health Organization (WHO) [WHO, 2020] [17]. Malaria therefore remains a real public health problem in the world; mainly because of the resistance of the parasites responsible for this disease to existing drugs, and this is the case of *P. falciparum* [Wellems and Plowe, 2001] [19]. In addition to malaria, other parasitic diseases are the cause of many deaths in tropical countries; these are, among others, *Leishmaniasis* and *trypanosomiasis* [Rocha *et al*., 2005] [18]. Even if numerous researches carried out on medicinal plants have led to encouraging results [Barthelemy *et al*., 2007; 2011; Latifou *et al*., 2008] [4, 2, 12] an effective cure for these diseases has not yet been found. It is with a view to contributing to the discovery of new active ingredients against these three tropical diseases that this study was carried out on *Parinari excelsa*. This species, belonging to the Chrysobalanaceae family, is traditionally used as an antidiarrheal [Ndiaye *et al*., 2008] [14], antimalarial [Gessler *et al*., 1995; Kamanzi *et al*., 2004] [7, 10] and vermifuge [Diehl *et al*., 2004] [6]. Our previous study on this species led to the isolation of seven compounds including a new ventiloquinone presenting antileishmanial and antiplasmodial activities [Barthélemy *et al*., 2012] [3]. This encouraging result motivated this study on the ethyl acetate extract of the trunk bark of this species. This study aims to evaluate the anti-trypanosomiasis, antileishmaniasis and antiplasmodium activities of the ethyl acetate extract, and to identify its phenolic constituents.

2. Material and Methods

2.1. General experimental procedure

Solid-liquid and liquid-liquid extraction methods were employed using methanol, dichloromethane, cyclohexane and ethyl acetate as solvents. Fractionation and purification were performed using column chromatography with silica gel 60 (2300-400 mesh, Merck) as

stationary phase. The analytical method of thin layer chromatography (TLC) on percolated silica gel 60 F254 plates (Merck) was used to follow the fractionation process. HPLC-UV analysis was performed in reverse phase with a LiChrospher RP-18.5 µm, LxI.D column. (250 mm × 4.6 mm) using a Dionex Ultimate 3000 device consisting of a pump (Dionex P680), an automated injector (Dionex ASI-100) (Thermo Scientific, France), a strip detector of diodes (DAD) (Hewlett Packard, France), all controlled by Chromeleon software (Thermo Scientific, France).

2.2. Plant material

The trunk bark of *Parinari excelsa* was collected from the Petit Yap Forest, Abidjan region (Southern Côte d'Ivoire). After identification, a specimen is kept in the herbarium of the Center National de Floristique (CNF) of the University Félix Houphouët Boigny under the reference number 8772. After drying in the open air in the laboratory, the dry bark was reduced to powder to give plant material.

2.3. Preparation of the ethyl acetate crude extract and fractionation

The powdered bark of *P. excelsa* (1.5 kg) was extracted a first time with the methanol-water mixture (9:1 v/v) then with methanol (100%) for 12 hours each and under permanent magnetic stirring. The two extracts were combined, evaporated to 1/3 using a rotary evaporator, defatted by liquid-liquid extraction with chloroform (3×250 mL) then extracted with ethyl acetate (5x240 mL).

After evaporation to dryness, the ethyl acetate extract (EA: 5.73 g) was fractionated by chromatography on a column of silica gel. For this, a 3.4 cm diameter glass column was filled with silica gel with a column height of 15 cm. The fractionation of EA was carried out according to the above program: methylene chloride/ethyl acetate (100:0, v/v); (80:20, v/v); (50:50, v/v); (30:70, v/v); (00:100, v/v) and ethyl acetate/methanol (95:05 v/v). The chromatographic (TLC) profiles of the fractions collected made it possible to retain three samples considered relatively pure: EA1, EA2 and EA3. These samples were then analysis by HPLC-UV method for identifying their phenolic components. The mobile phase was a gradient of acetonitrile/formic acid (0.25% in water); solvent A (acetonitrile) and solvent B (formic acid 0.25% in water). The volume of 25µl of sample is injected each time with a solvent flow rate of 1ml/min; the elution program is done below (Table 1) and peak detection was performed at 254 nm, 280 nm and 365 nm.

Table 1: Elution program for HPLC-UV analysis of EA1-EA3 samples

Time (min)	0	30	40	50	60	70	80	90
% solvent A (MeCN)	95	90	85	80	75	70	95	95
% solvent B (AcOEt)	5	10	15	20	25	30	5	5

2.4. Biological assays

2.4.1. Antimalarial assays

The evaluation of the anti-plasmodium falciparum K1 activity *in vitro* was determined according to the method described by Desjardins *et al.* (1979) [5] and modified by Barthelemy *et al.*, (2012) [3]. This method is based on the technique of radioisotope microculture. The viability of the test is determined by the uptake of [3H] hypoxanthine by the parasites. *In vitro* cultures of asexual erythrocytic stages of Plasmodium falciparum K1 strain (multidrug pyrimethamine/chloroquine-resistant strain) are carried out then test on plant extracts. Initial concentration of plant extracts was 30 µg/mL diluted with two-fold dilutions to make seven concentrations whose lowest being at 0.47 µg/mL After 48 h incubationat 37°C of the mixture parasites and plant extracts, [3H] hypoxanthine was added and the incubation was continued for another 24 h at the same temperature. Concentrations that inhibit at least 50% of parasites (IC_{50}) are calculated according the method descript by Huber and Koella (1993) [9]. Chloroquine was used as positive reference, and the values obtained are means of two independent assays; each assay being run in duplicate.

2.4.2. Antileishmanial assays

The tests were carried out according to the method described by Barthelemy *et al.* (2011; 2012) [2, 3]; with a slight modification. The parasite used was a transgenic *Leishmania donovani* promastigote cell line showing stable expression of luciferase. Cells contained in 200 µL of growth medium (L-15 with 10% FCS) were plated at a density of 2 × 106 cells per mL in a 96-well transparent microplate. Stock solutions of the standards and extracts were prepared using DMSO as solvent. The controls and the culture medium not containing cells were incubated simultaneously, in duplicate, at 26° C, for 72 h at different concentrations of the extracts to be tested. An aliquot of 50 µL was transferred from each well to a fresh opaque/black microplate, and then 40 µL of Steady Glo reagent was added to wells. Plates were read immediately using a microplate luminometer (Polar Star galaxy). IC_{50} values were calculated from dose-response inhibition graphs; Miltefosine was used as standard.

2.4.3. Antitrypanosoma cruzi assays

The tests were carried out according to the method described by Latifou *et al.* (2008) [12]; with a slight modification. The protocol is as follows; emaciated rat myoblasts (L-6 cells) were seeded in 96-well microtiter plates containing 2000 cells per well per 100 µL in RPMI 1640 medium with 10% FBS and 2 mML-glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* were added in each well (100 µL) with or without a serial drug dilution. The plates were incubated at 37◦C in 5% CO2 for 96h, and then the minimum inhibitory concentration (MIC) was determined microscopically. The addition of CPRG/Nonidet in the wells and the color reaction which occurred during the following 2 to 4 hours made it possible to determine the IC_{50} after reading the absorbance using a spectrophotometer at 540 nm. IC_{50} values were calculated from the sigmoidal inhibition curve. The values are means of two independent assays; each assay was run in duplicate.

2.4.4. Cytotoxicity assays

Cytotoxicity activity was carried out according to the method described by Barthelemy *et al.*, (2011) [2] with a slight modification. In Costar 96-well microtiter plates at 2.2 × 105 cells/ml, 50 µL of L6 cell lines (rat skeletal muscle myoblasts) were seeded per well in MEM supplemented with 10% bovine serum heat-inactivated fetal (FBS). After this, a threefold serial dilution from 500 to 0.07 µg/mL of extract in test medium was added. Plates with a final volume of 100 µL per well were incubated at 37°C for 72 h in a humidified incubator containing 5% CO_2; The viability indicator here is the Alamar Blue. After adding the viability indicator (Alamar Blue), the mixture was incubated for another 2 hours then the plate was measured with a fluorescence scanner (SpectraMax GeminiXS, Molecular Devices). An additional incubation of 2 hours was carried out before carrying out a new reading with the same apparatus. As in the previous cases, the IC_{50} values were calculated from the sigmoidal inhibition curve.

3. Results and Discussion

3.1. Biological study of the ethyl acetate extract from *P. excelsa* Sabine

Fractionation of the ethyl acetate extract (EA: 5.73 g) of the trunk bark of *P. excelsa* led to three fractions noted EA1 (0.87 g), EA2 (0.52 g) and EA3 (1.23 g). These extract and its sub fractions were evaluated *in vitro* for their antiprotozoal activities against *Trypanosoma cruzi, Leishmania donovani axen,* and *Plasmodium falciparum* K1; all IC_{50} values are expressed in µg/mL (Table 2).

Table 2: *In vitro* antiprotozoal activities, and cytotoxicity of EA and EA1-EA3

Samples	*T. cruzi*	*L. don. axen*	*P. falc. K1*	Cytotoxicity L6
	IC_{50} (µg/L)	IC_{50} (µg/L)	IC_{50} (µg/L)	IC_{50} (µg/L)
EA	0.049	0.025	0.004	0.046
EA1	0.021	0.008	0.002	0.073
EA2	0.017	0.006	0.002	0.048
EA3	0.040	0.014	0.001	0.055
Benznidazole	0.139			
Miltefosine		0.081		
Chloroquine			0.027	
Podophyllotoxine				0.003

Concerning the Antitrypanosoma activity, it is noted that all the samples analyzed have an IC_{50} value higher than that of the reference molecule (Benznidazole, IC_{50} = 0.130 µg/L). The most active fraction was EA2; which IC_{50} value (0.017 µg/L) was eight times greater than that of Benznidazole (IC_{50} = 0.130 µg/L); comes next EA2 (IC_{50} = 0.021 µg/L) whose IC_{50} value is about seven times higher than that of Benznidazole. With the antileishmania activity, all the fractions showed higher activities (0.006<IC_{50}<0.025 µg/L) than that of the reference molecule (Miltefosine; IC_{50} = 0.081 µg/L). The best activity was obtained with EA2 (IC_{50} = 0.006 µg/L), followed by EA1 (IC_{50} = 0.008 µg/L) and EA3 (IC_{50} = 0.014 µg/L). Finally, concerning the antiplasmodium assay, the EA3 fraction showed the best activity (IC_{50} = 0.001 µg/L), followed by EA1 and EA2 (IC_{50} = 0.002 µg/L). Overall, it is noted that the activity of the AE extract improved with fractionation.

3.2. Identification of phenolic constituents of AE1, AE2 and AE3

HPLC-UV analysis of the EA1, EA2 and EA3 fractions showed that they are made up of several molecules. Also this analysis made it possible to identify by semi-preparative HPLC method their major constituents (Figure 1). This analysis showed that EA1 contained Kaempferol (1) (RT=51.11 min) in small quantities, Apigenin (2) (RT=54.37 min) in very large quantities and traces of unidentified products. The fraction EA2 was a mixture of several phenolic compounds, some of which are present in large amounts and others in small amounts. Those in large quantities, two have been identified to Hydroxybenzoic acid (3) (R_T= 15.47 min) and Procyanidin B1 (4) (R_T= 17.47 min). Five compounds are in low quantity; it is Protocatechuic acid (5) (R_T= 11.32 min), Procyanidin B2 (6) (R_T= 14.15 min); Feruilic acid (7) (R_T= 25.42 min); Xanthotoxin (8) (R_T= 51.21 min), and Apigenin (1) (R_T= 54.35 min). The EA3 fraction contains an overwhelming amount of Ferulic acid (7) (R_T= 26.35 min) and a relatively small proportion of Protocatechuic acid (5) (R_T= 11.29 min), Chlorogenic acid (9) (R_T= 15.53 min), Procyanidin B1 (4) (R_T= 17.51 min), *p*-Coumaric acid (10) (R_T= 23.97 min), Scopoletin (11) (R_T= 26.03 min) and Quercetin (12) (R_T= 50.41 min). All of the compounds identified here belong to the polyphenol family; these results confirm the reliability of the method used for the isolation of these types of compounds. Among these polyphenols identified, we have five flavonoids, five phenolic acids and two coumarin derivatives; these results confirm the richness of the *Parinari* genus in phenolic compounds [Nhukarume *et al*., 2010; Mary *et al*., 2014; Olamide *et al*., 2017] [15, 13, 16].

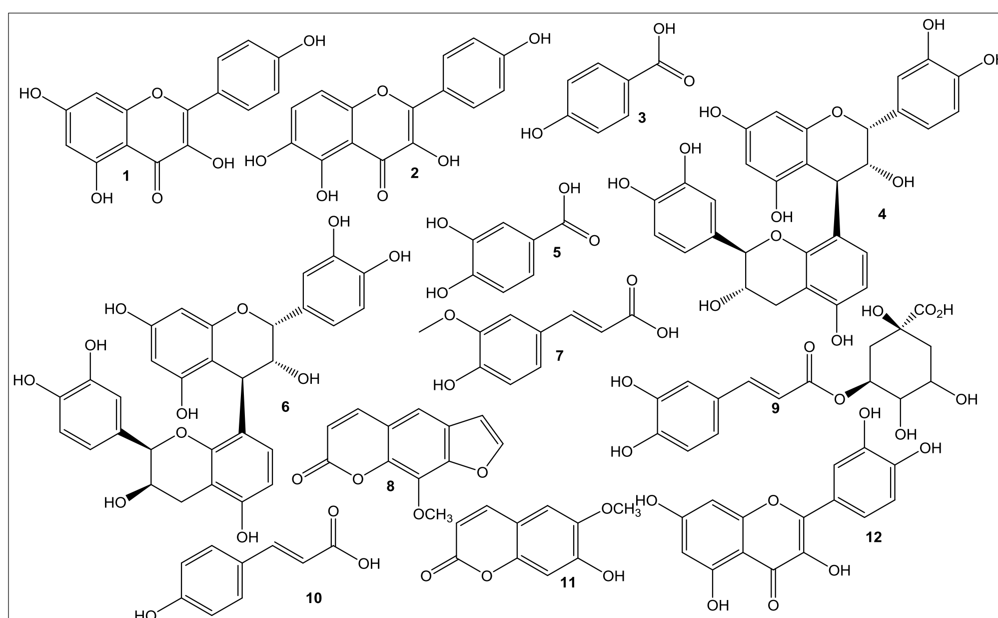

Fig 1: Phenolic compounds identified of ethyl acetate extract of *P. excelsa* : Kaempferol (1), Apigenin (2), Hydroxybenzoic acid (3), Procyanidin B1 (4), Protocatechuic acid (5), Procyanidin B2 (6), Ferulic acid (7), Xanthotoxin (8), Chlorogenic acid (9); p-Coumaric acid (10), Scopoletin (11) and Quercetin (12).

4. Conclusion
The phytochemical study of the ethyl acetate extract of *P. excelsa* led to the identification of twelve polyphenols including flavonoids, phenolic acids and coumarin. This extract and its sub-fractions have shown *in vitro* interesting antitrypanosoma, antileishmania, and antiplasmodial activities. These results confirm the traditional use of *P. excelsa* Sabine as antimalarial.

5. Acknowledgements
The authors thank PASRES (Programme d'Appui Strategique a la Recherche Scientifique en Côte d'Ivoire) for its financial support.

Declaration of interest
None

6. References
1. Ahmont CLK, Jacques DK, Gustav K, Faustin AK, Ballo D, Amon DN, Tapé K, *et al*. Five new cassane diterpenes from the seeds and bark of *Erythrophleum suaveolens*. Fitoterapia. 2020;146:104700. ISSN: 0367-326X.
2. Barthélemy A, Latifou L, Dodehe Y, Cyril A, Marcel K, Bernard W, *et al*. *In vitro* antiplasmodial and antileishmanial activities of flavonoids from *Anogeissus leiocarpus* (Combretaceae). Int. J. Pharm. Sci. Rev. Res. 2011;11(2):1-4, ISSN 0976 – 044X.
3. Barthélemy A, Dodehe Y, Latifou L, Ramiarantsoa H, Cyril A, Bernard W, Annelise L, *et al*. *In vitro* antileishmanial, antiplasmodial and cytotoxic activities of a new ventiloquinone and five known triterpenes from *Parinari excelsa.* Pharm. Biol. 2012;50(7):801-806. ISSN: 1388-0209.
4. Barthélemy A, Philippe C, Bernard W. Anti-plasmodial Activity of Constituents Isolated from *Croton lobatus* (Euphorbiaceae). Pharm. Biol. 2007;45(4):1-4; ISSN: 1744-5116.
5. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother,* 1979;16:710-718.
6. Diehl MS, Atindehou KK, Téré H, Betschart B. Prospect for anthelminthic plants in the Ivory Coast using ethnobotanical criteria. J. Ethnopharmacol, 2004;95:277-84.
7. Gessler MC, Msuya DE, Nkunya MH, Mwasumbi LB, Schär A, Heinrich M, *et al*. Traditional healers in Tanzania: The treatment of malaria with plant remedies. J. Ethnopharmacol. 1995;48:131-144.
8. Gnaoré YTD, Koffi BA, Yaya S, Faustin AK, Landry CAK, Marc V, *et al*. Nor-cucurbitacins from the leaves of *Mareya micrantha* (Benth.) Müll. Arg. (Euphorbiaceae). Fitoterapia. 2020;143:104538. ISSN: 0367-326X
9. Huber W, Koella JC. A comparison of the three methods of estimating EC50 in studies of drug resistance of malaria parasite. Acta Trop. 1993;55:257–261.
10. Kamanzi AK, Schmid C, Brun R, Koné MW, Traore D. Antitrypanosomal and antiplasmodial activity of medicinal plants from Côte d'Ivoire. J. Ethnopharmacol. 2004;90:221-227.
11. Konan DJ, Attioua KB, Kablan ALC, Kabran AF, Koua KBD, Okpekon AT, *et al*. Evaluation of antioxidant activities of methanol extracts and cassane diterpenoids from *Erythrophleum suaveolens* [(Guill. et Perr.) Brenan] (Fabaceae). Journal of Pharmacognosy and Phytochemistry. 2019;8(3):1051-1055. ISSN: 2278-4136
12. Latifou L, Barthélemy A, Bernard W, Marcel K, Sanni A, Catherine VS. Phytochemical Study and Antiprotozoal Activity of Compound Isolated from Thalia geniculata. Pham. Biol. 2008;46(3):162-165. ISSN: 1744-5116.
13. Mary TO, Ayodeji EA, Kayode K, Afolabi CA. Protective effects of *Parinari curatellifolia* flavonoids against acetaminophen-induced hepatic necrosis in rats. Saudi J. Biol. Sci. 2014;21(5):486-492.
14. Ndiaye M, Diatta W, Sy AN, Dièye AM, Faye B, Bassène E. Antidiabetic properties of aqueous barks extract of *Parinari excelsa* in alloxan-induced diabetic rats. Fitoterapia. 2008;79:267-270.
15. Nhukarume L, Chikwambi Z, Muchuweti M, Chipurura B. Phenolic content and antioxidant capacities of Parinari curatelifolia, Strychnos spinose and Adansonia digitata. J. Food Biochem. 2010;34(1):207-221.
16. Olamide O, Crown TR, Komolafe AC, Akinmoladun MT, Olaleye AA, Akindahunsi AAB. *Parinari curatellifolia* seed flavonoids protect against Triton-induced dyslipidemia and atherogenicity in rats. Wiley Online Library, 2017, https://doi.org/10.1002/tkm2.1082
17. OMS. World malaria report 2020, ISBN 978-92-4-001579-1.
18. Rocha LG, Almeida JR, Macêdo RO, Barbosa-Filho JM. A review of natural products with antileishmanial activity. Phytomedicine, 2005, 12, 514–535.
19. Wellems T, Plowe C. Chloroquine-resistant malaria. J. Infect. Dis. 2001;184:770-776.