Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(1):931-937

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antiplasmodial flavonoid from young twigs and leaves of *Caesalpinia bonduc* (Linn) Roxb

Olubanke O. Ogunlana[a*], Hye-Sook Kim[b], Yusuke Wataya[b], Joseph O. Olagunju[c], Afolabi A. Akindahunsi[d] and Ning H. Tan[e*]

[a]*Department of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023, Ota, Ogun State, Nigeria.*
[b]*Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama, Okayama 700-8530, Japan.*
[c]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Lagos State University, Ikeja, Lagos State, Nigeria.*
[d]*Department of Biochemistry, Federal University of Technology, Akure, Nigeria.*
[e]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China.*

ABSTRACT

Young twigs and leaves of Caesalpinia bonduc (Linn) Roxb is used in the Southwestern part of Nigeria, as an antimalarial medicinal decoction together with other medicinal plant. In order to discover new antimalaria drug or drug lead and also to verify the traditional use of C. bonduc in this area, the in vitro antiplasmodial activity of crude extract, solvent fractions and compounds isolated from C. bonduc were investigated. In vitro antiplasmodial evaluation was carried out using chloroquine sensitive strain of Plasmodium falciparum FCR-3 and selective toxicity determination was investigated using sensitivity study on Mouse mammary tumor FM3A cells as a host model. Ethyl acetate and petroleum ether soluble solvent fractions of C. bonduc showed antiplasmodial activity with EC_{50} values of 16 and 18 µg/mL and selectivity index (SI) of 0.69 and 0.29 respectively. Bioassay guided fractionation of C. bonduc led to the isolation of seven flavonoids, 7-hydroxy-4'-methoxy-3,11-dehydrohomoisoflavanone (1), 4,4'-dihydroxy-2'-methoxy-chalcone (2), 7,3'-dihydroxy-3,11-dehydrohomoisoflavanone (3), Luteolin (4), quercetin-3-methyl ether (5), Kaempferol-3-O-β-D-xylopyranoside (6) and Kaempferol-3-O-α-L-rhamnopyranosyl-(1→2)-B-D-xylopyranoside (7). Structures of compounds 1 to 7 were elucidated by spectroscopic methods. Compound 2 exhibited moderate antiplasmodial activity with EC_{50} and SI values of 33 µM and 0.33 respectively while compounds 1, 3 to 7 showed insignificant antiplasmodial activity. The investigation revealed that the moderate antiplasmodial activity exhibited by the solvent fractions of C. bonduc might be mediated by 4,4'-dihydroxy-2'-methoxy-chalcone.

Keywords: *Caesalpinia bonduc*; flavonoids; *Plasmodium falciparum* FCR-3; Mouse mammary tumor FM3A

INTRODUCTION

Malaria remains a major parasitic disease in many tropical and subtropical regions of the world [1]. It appears to be the most prevalent of human diseases, especially in these areas and as such constitutes a major public health hazard. In 2010, an approximated 3.3 billion individuals were at risk of malaria, with majority of the population from people living in sub-Saharan Africa [2]. Pregnant women and children under the age of five living in Sub-Saharan Africa are the most severely affected groups in the population [3, 2]. In 2010, it was estimated that 91 % deaths have

occurred in WHO African region, in children below 5 years of age and pregnant women with malaria cases [4, 1]. As a result of its associated high morbidity and mortality, concerted research efforts are currently being channeled into the eradication of the disease across the globe [5].

Caesalpinia bonduc, (family: *Caesalpiniaceae*, genus *Fabaceae*), commonly known as Gray Nicker nut (English) and Ayó (Yoruba), is a prickly shrub with grey, hard, globular shaped seeds with a smooth shining surface [6]. It is a medicinal plant predominantly distributed in the tropical and subtropical regions of Africa, Asia and the Caribbean [7]. It has a lot of applications in folk medicine. For instance, the pharmacological screening of the plant extracts reveals their anticancer, antimalarial, antihyperglycemic, anti-inflammatory, antirheumatic, anticonvulsant, anti-measles and antipyretic activities [8, 9, 10, 11].

The phytochemical analysis of the plant shows that it contains saponins, alkaloids, flavonoids, triterpenoids, diterpenoids, tannins and steroids [12, 13]. Detailed toxicological studies of the leaves and young twigs of the plant have been previously reported from our laboratory [14]. A survey of published works revealed the isolation of two phytochemicals with antiplasmodial activity from the seeds of *C. bonduc* [15, 16]. As a result of the antimalarial activity of the extract of *C. bonduc* [17], its dearth of information and the established usage of the plant in the southwestern part of Nigeria as an antimalarial decoction [personal communication, 2009], we attempted to screen the solvent extracts and the isolated compounds from the young twigs and leaves of *C. bonduc* for their antimalarial activity. This paper reports the *in vitro* antiplasmodial activity of tested compounds and extract against *P. falciparum* and their selectivity for further study of selection of antimalarial seeds.

EXPERIMENTAL SECTION

2.1. *Plant material*

Young twigs and leaves of *C. bonduc* (Linn) Roxb. were collected from Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria. Plant identification was done by Dr. Conrad Omonhinmi, Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun state, Nigeria. Authentication and voucher referencing were carried out at FRIN with voucher specimen SHI108408 deposited in their Herbarium.

2.2 Other experimental materials

All reagents used were of analytical grades. Antimalarial agents, quinine hydrochloride, pyrimethamine and artemisinin, were purchased from Sigma (St. Louis, MO, U.S.A.). *P. falciparum* ATCC 30932, FCR-3 strain was used as a malaria parasite in this study. It was cultivated by a modification of the method of Trager and Jensen [18, 19] using a 5 % hematocrit of type A human red blood cells suspended in RPMI 1640 medium and supplemented with heat-inactivated 10 % type A human serum. Mouse mammary tumor FM3A cells (wild-type, subclone F28-7) were maintained in a suspension culture at 37 °C in a 5 % CO_2 atmosphere in plastic bottles containing ES medium supplemented with 2 % heat-inactivated fetal bovine serum [20].

2.3. *Extraction and solvent fractionation of plant material*

The leaves and young twigs of the plant collected were air dried at room temperature and powdered. Powdered plant (8.8 kg) was extracted with 75 % v/v ethanol (50 L), at normal room temperature (25 ^{O}C), by maceration for 72 hours using three consecutive extractions. The total filtrate was concentrated to dryness with rotary evaporator at 50 ^{o}C. The dried ethanolic extract of the plant (1120 g) was re-suspended in distilled water (H_2O) and partitioned in sequence with petroleum ether, ethyl acetate and *n*-butanol. The different solvent fractions were concentrated with rotary evaporator to yield petroleum ether - soluble fraction (150 g), ethyl acetate - soluble fraction (120 g), *n*-Butanol - soluble fraction (170 g), and H_2O - soluble fraction (630 g). Crude fraction and each of the soluble solvent fractions were subjected to biological determination.

2.4. Fractionation, isolation and identification of compounds

Ethyl acetate and petroleum ether soluble fractions were combined (270 g) and separated by column chromatography (CC) (3 kg silica gel, mesh 100-200; solvent: chloroform-methanol (100:1)). Fractions were collected in gradient and this afforded 20 different fractions (C_1 to C_{20}). Successive column separations of C_6 in (Silica gel, mesh 200 – 300; solvent: petroleum ether-acetone (15:1)) and (silica gel, mesh 10 – 40; solvent: chloroform-ethyl acetate (30:1)) afforded compound 1. Successive CC separation of C_{10} on (silica gel, mesh 200 – 300; solvent: chloroform-acetone (30:1)); (Silica gel, mesh 200 – 300; solvent: chloroform-methanol (120:1)); and (silica gel, mesh 10 – 40; solvent: chloroform-methanol (60:1)) and further separation by HPLC (column: YMC-Pack ODS-A, 10 mm × 15 cm, flow rate of 2 ml/min; solvent: methanol-water (60: 40) and (58: 42) led to the separation of compounds 2 and 3 respectively. Series of CC separation of C_{12} on (silica gel, mesh 200 – 300; solvent: chloroform-methanol (200:1)); (silica gel, mesh 10 – 40; solvent: chloroform-methanol (40:1)); (sephadex

LH-20, solvent: chloroform-methanol (1:1)) and further separation by HPLC (YMC-Pack ODS-A, 10 mm × 15 cm, flow rate of 2 ml/min; MeOH: H_2O (50: 50)) led to the separation of compounds 4 and 5. Sequential separation of C_{16} by CC on (Sephadex LH-20, solvent: chloroform-methanol (1:1)); (silica gel, mesh 10 – 40; chloroform-methanol (20:1)) and separation by HPLC (YMC-Pack ODS-A, 10 mm × 15 cm, flow rate of 2 ml/min; solvent: methanol-water (47: 53)) afforded compound 6. Sequential CC separation of C_{20} in (Sephadex LH-20; solvent: chloroform-methanol (1:1)); MPLC (RP – 18; MeOH: solvent: water (10 – 90 %)) and HPLC (YMC-Pack ODS-A, 10 mm × 15 cm, flow rate of 2 ml/min; solvent: methanol-water (60:40)) afforded compound 7. Structural identification was carried out by spectroscopic methods.

2.5. In vitro antimalarial activity determination

Various concentrations of extract, solvent fractions and isolated compounds including positive control samples (quinine hydrochloride, pyrimethamine and artemisinin) were prepared in dimethyl sulfoxide (DMSO) or water (H_2O). An aliquot of each sample solution (10 µL) was added to an individual well of a 24-well multi-dish. Erythrocytes with 0.3 % parasitemia were added to each well containing 990 µL of culture medium to give a final hematocrit level of 3 %. The plates were incubated at 37 °C for 72 hrs in a multigas incubator (5 % CO_2, 5 % O_2, 90 % N_2 atmosphere). To evaluate the antimalarial activity of samples, thin blood films from each culture were prepared and stained with Giemsa. A total of 10000 erythrocytes per one thin blood film were examined under a microscope. All of the tested samples were assayed in duplicate at each concentration. Drug-free control cultures were ran simultaneously. The level of parasitemia in control was between 4 - 5 % at 72 hrs [21]. The parasitemia activity of samples were presented as EC_{50} values, which is the concentration of sample necessary to inhibit the increase in parasite density at 72 hrs by 50 % of the control.

2.6. *In vitro selective toxicity determination*

FM3A cells grew with a doubling time of about 12 hrs. Prior to exposure to drugs, the cell density was adjusted to 5×10^4 cells/ml. A cell suspension of 990 µL was dispensed to the test plate and 10 µL of the samples at various concentrations suspended in DMSO or H_2O were added to individual wells in a 24-well multi-dish. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 hrs. All of the tested samples compounds were assayed in triplicate at each concentration. Cell numbers were measured using a cell counter CC-130 [21]. The samples' selective toxicity were presented as EC_{50} values, which refers to the concentration of the sample necessary to inhibit by 50% the increase in cell density of the control at 48 hrs. Selectivity values (the EC_{50} value for FM3A cells per the EC_{50} value for *P. falciparum*) were calculated as follows:

Selectivity: EC_{50} value of FM3A cell / EC_{50} value of *P. falciparum*

RESULTS AND DISCUSSION

From the bioactive guided fractionation of ethylacetate and petroluem ether solvent fractions of *C. bonduc*, seven flavonoids (Compounds 1 to 7) were obtained. Compound 1 was separated as a pale yellow crystalline solid with yield of 319.30 mg. Its chemical formula was ascertained to be $C_{17}H_{14}O_4$, on the ground of the molecular ion peak of positive ESI-MS *m/z* 305 $[M+Na]^+$. Compound 1 was elucidated as 7-hydroxy-4'-methoxyl-3,11-dehydrohomoisoflavanone (Figure 1). The 1D ^1H and ^{13}C NMR spectra data (Table 1) were in accord with published work [22]. Compound 2 was separated as a pale yellow powder with yield of 15.60 mg. Its chemical formula was ascertained to be $C_{16}H_{14}O_4$, on the ground of the molecular ion peaks of positive ESI-MS m/z 271 $[M+H]^+$ and 293 $[M+Na]^+$. Compound 2 was identified as 4,4'-dihydroxy-2'-methoxy-chalcone (Figure 1). Its 1D ^1H and ^{13}C NMR spectra data (Table 2) were in accord with published work [23]. Compound 3 was separated as a pale yellow powder of 10.4 mg yield. Its chemical formula was observed to be $C_{16}H_{12}O_4$, on the ground of the molecular ion peak of positive ESI-MS *m/z* 269 $[M+H]^+$. Compound 3 was identified as 7,4'-dihydroxy-3,11-dehydrohomoisoflavanone (Figure 1). The ^1H and ^{13}C NMR spectra data (Table 1) were marked with close similarity with those of compound 1. The data were in agreement with published work [24].

Compound 4 was separated as a pale yellow powder like compounds 2 and 3 with yield of 12.80 mg. Its chemical formula was observed as $C_{15}H_{10}O_6$ from its molecular ion peak at positive ESI-MS *m/z* 287 $[M+H]^+$. Compound 4 was elucidated as Luteolin (Figure 1). The ^1H and ^{13}C NMR spectra data (Table 3) were in accord with published work [25]. Compound 5 was separated as a pale yellow crystalline solid with yield of 227.00 mg. Its chemical formula was observed to be $C_{16}H_{12}O_7$, on the ground of the chemical ion peak at positive ESIMS *m/z* 317 $[M+H]^+$. Compound 5 was elucidated as quercetin-3-methyl ether (Figure 1). The ^1H and ^{13}C NMR spectra data (Table 3) were in accord with published work [26].

Compound 6 was separated as a brown paste with yield of 24.40 mg. Its chemical formula was observed to be $C_{20}H_{18}O_{10}$, on the ground of the molecular ion peak at positive ESI-MS *m/z* 441 $[M+Na]^+$. Compound 6 was

elucidated as kaempferol-3-*O*-β-D-xylopyranoside (Figure 1). The ^{1}H and ^{13}C NMR spectra data (Table 4) were in accord with published work [27]. Compound 7 was separated as a pale yellow crystalline solid like compounds 1 and 5, with yield of 79.50 mg. Its chemical formula was observed to be $C_{26}H_{28}O_{14}$, on the ground of the molecular ion peak at positive ESIMS *m/z* 565 $[M+H]^{+}$. Compound 7 was identified to be Kaempferol-3-*O*-α-L-rhamnopyranosyl-1→2)-β-D-xylopyranoside (Figure 1). The ^{1}H and ^{13}C NMR spectra data were (Table 4) in accord with published work [28].

Figure 1 illustrates the chemical structures of compounds 1-7. Compounds 1-7 were identified and reported as follows, 7-hydroxy-4'-methoxy-3,11-dehydrohomoisoflavanone (1), 4,4'-dihydroxy-2'-methoxy-chalcone (2), 7,3'-dihydroxy-3,11-dehydrohomoisoflavanone (3), Luteolin (4), quercetin-3-methyl ether (5), Kaempferol-3-O-β-D-xylopyranoside (6) and Kaempferol-3-O-α-L-rhamnopyranosyl-(1→2)-B-D-xylopyranoside (7).

The results of the *in vitro* antimalarial activity and selectivity index determination of the ethanolic extract, solvent fractions and compounds isolated from *C. bonduc* are summarised in Table 5. Petroleum ether and ethyl acetate solvent fractions exhibited moderate antimalarial activities with EC_{50} values of 18 and 16 µg/ml and selectivity index of 0.29 and 0.69 respectively. Among the isolated compounds, only 4,4'-dihydroxy-2'-methoxy-chalcone (2) exhibited moderate antimalarial activity with EC_{50} values of 33 µM and selectivity index of 0.33 (Table 5), on the other hand, the EC_{50} values of compounds 1, 3 to 7 did not showed antimalarial activity.

The *in vitro* bioassay study of the ethanolic extract, solvent fractions and isolated compounds of *C. bonduc*, revealed its moderate antiplasmodial but poor selective activities, thus, indicating a non-selective antimalarial activity of *C. bonduc*. In spite of the less beneficiary antiplasmodial activity of flavonoids as a result of their poor selective index, the *in vitro* antiplasmodial activity of exiguaflavanone A and B, from *Artemisia indica* [29], (−)-*cis*-3-acetoxy-4′,5,7-trihydroxyflavanone, from *Siparuna andina* [30], 6-Hydroxyluteolin-7-*O*-(1″-α-rhamnoside) [31], calycosin and genistein [32] have been reported. However, this is the first report of flavonoid isolated from *C. bonduc* with moderate antiplasmodial activity. Although, the exact antiplasmodial action of flavonoids is unknown but they have been shown to inhibit the influx of L-glutamine and myo-inositol into infected erythrocytes [33]. The ability of flavonoids to form reactive oxygen species has also been linked to their antiplasmodial activity [34]. Lack of defense mechanisms against oxidative stress in *P. falciparum* makes the parasite susceptible to drugs with ability of generating reactive oxygen species [35]. Chalcones are suggested to have potential for the development of inexpensive antimalarials [36]. The antiplasmodial activity of young twigs and leaves of *C. bonduc* has been linked to the possession as 4,4'-dihydroxy-2'-methoxy-chalcone (2) has one of its bioactive constituents.

Table I ^{1}H and ^{13}C-NMR spectral data for compounds 1 and 3 (δ in ppm, *J* in Hz)

	δ_C	δ_H	δ_C	δ_H
Compounds	**1**		**3**	
2	67.53	5.35 (2H, d, *J* = 1.5)	69.05	5.35 (2H, br, s)
3	126.50	-	127.07	-
4	179.52	-	183.06	-
5	129.39	7.73 (1H, d, *J* = 8.5)	130.69	7.80 (1H, d, *J* = 8.0)
6	111.13	6.54 (1H, dd, *J* = 8.5, 2.0)	112.16	6.52 (1H, d, J = 8.0)
7	164.61	-	166.69	-
8	102.41	6.31 (1H, d, *J* = 2.0)	103.60	6.31 (1H, br, s)
9	162.47	-	164.85	-
10	114.30	-	115.90	-
11	135.21	7.63 (1H, br, s)	138.16	7.71 (1H, s)
1'	128.82	-	129.56	-
2'	132.19	7.39 (2H, d, *J* = 8.5)	133.50	7.25 (2H, d, *J* = 7.6)
3'	114.23	7.04 (2H, d, *J* = 8.5)	116.70	6.88 (2H, d, *J* = 7.6)
4'	160.26	-	160.53	-
5'	114.23	7.04 (2H, d, *J* = 8.5)	116.70	6.88 (2H, d, *J* = 7.6)
6'	132.19	7.39 (2H, d, *J* = 8.5)	133.50	7.25 (2H, d, *J* = 7.6)
4'-OCH_3	55.33	3.81 (3H, s)	-	-

^{1}H NMR and ^{13}C NMR were recorded at 500 and 125 MHz respectively in DMSO-d_6.

Table 2 ^{1}H and ^{13}C-NMR spectral data for compound 2 (δ in ppm, J in Hz)

Compounds	δ_C 2	δ_H
1	128.04	-
2	131.38	7.50 (2H, d, J = 8.4)
3	116.87	6.82 (2H, d, J = 8.4)
4	161.21	-
5	116.87	6.82 (2H, d, J = 8.4)
6	131.38	7.50 (2H, d, J = 8.4)
7	144.16	7.56 (1H, d, J = 15.6)
8	125.07	7.41 (1H, d, J = 15.6)
9	193.16	-
1'	121.76	-
2'	162.53	-
3'	100.11	6.51 (1H, br s)
4'	164.49	-
5'	108.91	6.45 (1H, d, J = 8.4)
6'	133.73	7.57 (1H, d, J = 8.4)
2'-OCH_3	56.14	3.88 (3H, s)

^{1}H NMR and ^{13}C NMR were recorded at 500 and 125 MHz respectively in DMSO-d_6.

Table 3 ^{1}H and ^{13}C-NMR spectral data for compounds 4 and 5 (δ in ppm, J in Hz)

Compounds	δ_C 4	δ_H	δ_C 5	δ_H
2	166.00	-	158.0	-
3	103.82	6.54 (1H, s)	139.52	-
4	183.85	-	180.01	-
5	163.19	-	163.08	-
6	100.09	6.20 (1H, d, J = 1.2)	99.75	6.20 (1H, d, J = 2.0)
7	166.33	-	165.94	-
8	94.97	6.44 (1H, br s)	94.70	6.39 (1H, d, J = 2.0)
9	159.40	-	158.42	-
10	105.28	-	105.83	-
1'	123.64	-	122.88	-
2'	114.11	7.38 (1H, overlap)	116.42	7.63 (1H, d, J = 2.0)
3'	147.11	-	146.50	-
4'	150.98	-	149.97	-
5'	116.75	6.90 (1H, d, J = 8.4)	116.42	7.53 (1H, dd, J = 8.4)
6'	120.28	7.38 (1H, overlap)	122.31	6.90 (1H, d, J = 8.4)
3-OCH_3	-	-	60.52	3.78 (3H, s)

^{1}H NMR and ^{13}C NMR were recorded at 500 and 125 MHz respectively in DMSO-d_6.

Table 4 ^{1}H and ^{13}C-NMR spectral data for compounds 6 and 7 (δ in ppm, J in Hz)

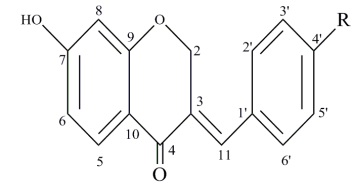
Compounds	δ_C 6	δ_H	δ_C 7	δ_H
2	158.38	-	158.12	-
3	135.31	-	134.30	-
4	179.36	-	179.12	-
5	163.00	-	162.90	-
6	99.89	6.18 (1H, br s)	99.82	6.06 (1H, br s)
7	165.96	-	165.50	-
8	94.76	6.37 (1H, br s)	94.71	6.24 (1H, br s)
9	15890	-	158.54	-
10	105.61	-	105.80	-
1'	122.58	-	122.90	-
2'	132.20	8.01 (2H, d, J = 8.4)	132.01	7.92 (2H, d, J = 8.,5)
3'	116.11	6.86 (2H, d, J = 8.4)	116.12	6.82 (2H, d, J = 8.5)
4'	161.60	-	161.23	-
5'	116.11	6.86 (2H, d, J = 8.4)	116.12	6.82 (2H, d, J = 8.5)
6'	132.20	8.01 (2H, d, J = 8.4)	132.01	7.92 (2H, d, J = 8.,5)
1"	104.64	5.16 (1H, *d*, J = 6.8)	101.22	5.53 (1H, d, J = 7.0)
2"	75.33	3.74 (1H, d, J = 4.8)	79.32	-
3"	77.52	3.48 (1H, t, J = 5.8)	77.90	-
4"	70.98	3.41 (1H, d, J = 8.2)	72.22	-
5"	67.20	3.76 (2H,s, J = 4.6)	66.90	-
1"'	-	-	102.53	5.21 (1H, br s)
2"'	-	-	71.34	-
3"'	-	-	72.33	-
4"'	-	-	74.01	-
5"'	-	-	70.01	-
6"'	-	-	17.73	-

^{1}H NMR and ^{13}C NMR were recorded at 500 and 125 MHz respectively in DMSO-d_6.

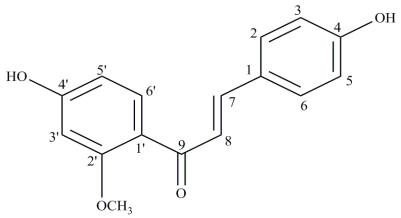
Table 5 Antimalarial activity and selectivity assay results of extracts and isolated compounds from of *C. bonduc*

Extracts/Compound	[a]EC_{50} (µg/mL, µM)	[b]EC_{50} (µg/mL, µM)	[c]Selectivity
Ethanol extract	>92.0±0.04 (51 %)	36.0±0.08	0.39
Petroleum ether extract	18.0±0.02	5.2±0.08	0.29
Ethyl acetate extract	16.0±0.01	11.0±0.08	0.69
n-Buthanol extract	>90.0±0.08 (76%)	>90.0±0.08 (80%)	1.0
Water extract	>62.0±0.06 (82%)	>62.0±0.08 (68%)	1.0
1	>27.0±0.08 (64%)	8.8±0.03	0.33
2	33.0±0.01	11.0±0.03	0.33
3	>27.0±0.03 (61%)	11.0±0.04	0.41
4	>25.0±0.02 (58%)	5.4.0±0.03	0.2
5	>9.8.0±0.03 (99%)	0.56±0.03	0.06
6	>9.7.0±0.02 (100%)	>9.7±0.03 (88 %)	1.0
7	>5.7.0±0.03 (95%)	>5.7±0.03 (97 %)	1.0
Quinine	0.2±0.02	100.0±0.01	500.0
Pyrimethamine	0.001±0.01	0.1±0.02	100.0
Artemisinin	0.01±0.04	9.0±0.02	900.0

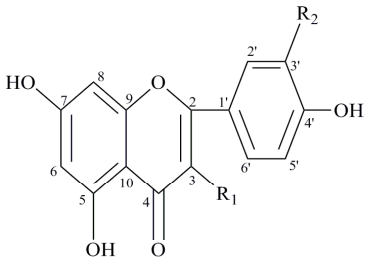
Values in parenthesis show the growth inhibition (%) of some doses. EC_{50} is effective concentration of sample necessary to inhibit the increase in parasite density at 72 hrs by 50 % of the control. EC_{50} is expressed as "µg/mL" for extract and as "µM" for isolated compounds. [a]Chloroquine sensitive strain (FCR-3) of *P. falciparum*, [b]Mouse mammary tumor FM3A cells representing a model of host, [c]Selective toxicity is calculated as EC_{50} value for FM3A cells/EC_{50} for *P. falciparum*.



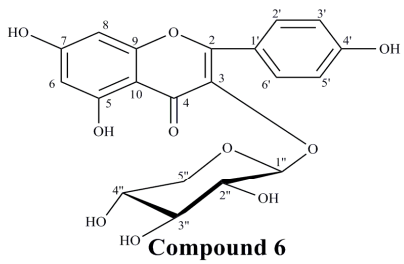
Compound 1, R = OCH_3
Compound 3, R = OH



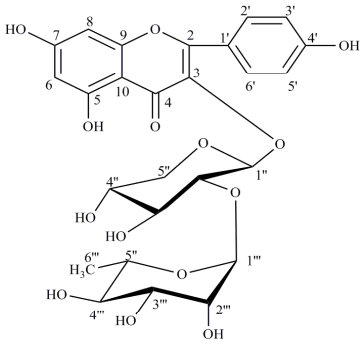
Compound 2



Compound 4, R1 = H, R2 = OH
Compound 5, R1 = OCH3, R2 = OH



Compound 6



Compound 7

Figure 1 Chemical structures of compounds 1-7 isolated from *C. bonduc*

Acknowledgements
We appreciate the Chinese Academy of Sciences (CAS) and the Academy of Sciences for the Developing World (TWAS) for their financial and material supports through the CAS-TWAS 2009 Post-Graduate Research Fellowship granted to the lead author. This work was also supported by the grant from the National Natural Science Foundation of China (30725048) given to Dr. Ning H. Tan and partially supported by the Program for Promotion of Fundamental Studies in Health Science of the National Institute of Biomedical innovation (NIBIO) (09-21, PI: Yusuke Wataya).

REFERENCES

[1] M Frederic; MP Hayette; M Tits; P De Mol; L Angenot. *Antimicro. Agent. Chem.*, **1999**, 43(9). 2328-2331.
[2] World Health Organization WHO. World global malaria programme. World malaria report, **2011**.
[3] JW Tracy; LT Webster Jr. Drugs used in the chemotherapy of protozoal infections. In: *The pharmacological basics of therapeutics*, Editors. JG Hardman; LE Limbird. McGraw-Hill New York, **2001**.
[4] J Sachs; P Malaney. *Nat.*, **2002**, 415(6872), 680-685.
[5] MF Good. *Nat. Rev. Immun.*, **2001**, 1(2), 117-125.
[6] AK Nadkarni. *Indian Materia Medica*. 13th Edition, Dhootapapeshwar Prakashan Ltd, Bombay, **1954**, 1, 229-235.
[7] M Gupta; UK Mazumder; RS Kumar; TS Kumar. *Iran. J. Pharmacol. Therap.*, **2003**, 2, 30-34.
[8] SK Adesina. *Fitoterapia*., **1982**, 53, 147-162.
[9] S Chakrabarti; TK Biswas; B Rokeya; L Ali; M Mosihuzzaman; N Nahar; AK Khan-Azad; BJ Mukherjee. *J. Ethnopharma*., **2003**, 84(1), 41-46.
[10] M Gupta; UK Mazumder; RS Kumar; T Sivakumar; ML Vamsi. *J. Pharmacol. Sci.*, **2004**, 94(2), 177-184.
[11] MA Sonibare; JO Moody; EO Adesanya. *J. Ethnopharma.*, **2009**, 122, 268–272.
[12] RS Kumar; M Gupta; UK Mazumdar; Y Rajeshwar; TS Kumar; P Gomath; R Roy. *J. Toxicol. Sci.*, **2005**, 30**(**4), 265-274.
[13] OO Ogunlana; OE Ogunlana; CA Ntube; JA Olagunju; AA Akindahunsi. *Glob. Res. J. Pharmaceu.Sci.*, **2012**, 1(1), 1-4.
[14] OO Ogunlana; OE Ogunlana; AA Adeneye; OAC Udo- Chijioke; TI Dare-Olipede; JA Olagunju; AA Akindahunsi. *Afri. J. Trad. Complem. Alternat. Med.*, **2013**, 10(6), 504-512.
[15] K Pudhom; D Sommit; N Suwankitti; A Petsom. *J. Nat. Prod.*, **2007**, 70(9), 1542-1544.
[16] TZ Linn; S Awale; Y Tezuka; AH Banskota; SK Kalauni; F Attamimi; J Ueda; PB Asih; D Syafruddin; K Tanaka; S Kadota. *J. Nat. Prod*., **2005**, 68(5), 706-710.
[17] E Innocent; JM Moshi; PJ Masimba; ZH Mbwambo; MC Kapingu; A Kamuhabwa. *Afri. J. Trad. Complem. Alternat. Med.*, **2009**, 6(2), 163-167.
[18] W Trager; JB Jenson. *Sci.*, **1976**, 193, 673-675.
[19] JB Jensen; W Trager. *J. Parasitol.*, **1977**, 63(5), 883-886.
[20] A Yoshioka; S Tanaka; O Hiraoka; Y Koyama; Y Hirota; D Ayusawa; T Seno; C Garrett; Y Wataya. *J. Bio. Chem.*, **1987**, 262(17), 8235 - 8241.
[21] HS Kim; Y Shibata; Y Wataya; K Tsuchiya; A Masuyama; M Nojima. *J. Med. Chem.*, **1999**, 42(14), 2604-2609.
[22] KK Purushothaman; K Kalyani; K Subramaniam; SP Shanmughanathan. *Indian J. Chem., Section B, Organic Chemistry Including Medicinal Chemistry*, **1982**, 21B(4), 383-386.
[23] M Namikoshi; H Nakata; M Nuno; T Ozawa; T Saitoh. *Chem. Pharm. Bul.*, **1987a**, 35 (9), 3568-3575.
[24] M Namikoshi; H Nakata; T Saitoh. *Phytochem.*, **1987b**, 26(6), 1831-1833.
[25] H Wagner; VM Chari. T*etrahedron Let.*, **1976**, 21, 1799 - 1802.
[26] L Jurd; RM Horowitz. *J. Organic Chem.*, **1957**, 2, 1618-1622.
[27] LA Kruglii; VI Glyzin. *Khimiya Prirodnykh Soedinenii*, **1968**, 4(6), 379-380.
[28] SS Moon; A Abdur-Rahman; M Manir; VS Jamal-Ahamed. *Arch. Pharmacol. Res.*, **2010**, 33(8), 1169-1173.
[29] R Chanphen; Y Thebtaranonth; S Wanauppathamkul; Y Yuthavong. *J. Nat. Prod*., **1998**, 61(9), 1146-1147.
[30] K Jenett-Siems; K Siems; J Jakupovic; PN Solis; MP Gupta; FP Mockenhaupt; U Bienzle; E Eich. *Planta. Medica.*, **2000**, 66(4), 384-385.
[31] G Bringmann; M Ochse; G Zotz; K Peters; EM Peters; R Brun; J Schlauer. *Phytochem*., **2000**, 53(8), 965-969.
[32] K Kaur; M Jain; T Kaur; R Jain. *Bioorg. Med. Chem.*, **2009**, 17(9), 3229-3256.
[33] BC Elford. *Parasitol. Tod.*, 1986, 2(11), 309-312.
[34] MM Iwu; O Obidoa; M Anazodo. *Pharmacol. Res. Communicat*., **1986**, 18(1), 81-91.
[35] A Ribeiro; D Pilo-Veloso; AJ Romanha; CL Zani. *J. Nat. Prod.*, **1997**, 60(8), 836-841.
[36] SK Awasthi; N Mishra; B Kumar; M Sharma; A Bhattacharya; LC Mishra; VK Bhasin. *Med. Chem. Res.*, **2009**, 18, 407-420.