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Original Article

Antiplasmodial Biflavanones from the Stem Bark of *Garcinia buchananii* Engl.

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ABSTRACT

Introduction: Plants of the genus *Garcinia* are traditionally used treat a range of infectious and non-infectious diseases. *Garcinia* species are reported to have been shown to have a range of biological activities including cytotoxicity antimicrobial, antifungal, antioxidant, antimalarial and HIV-1 protease inhibitory activity among others. **Methods:** Solvent extraction was done using CH_2Cl_2: MeOH (1:1). Isolation was done using column chromatography with silica gel as the stationery phase and ethyl acetate and n-hexane used as mobile phase in increasing polarity. Thin layer chromatography was used to monitor the isolation. Structure elucidation was done using nuclear magnetic resonance and mass spectroscopic techniques. Chloroquine resistant (W2) and chloroquine sensitive (D6) *P. falciparum* strains were used for antiplasmodial assay. **Results:** Further bioassay guided fractionation of a CH_2Cl_2: MeOH (1:1) extract of *Garcinia buchananii* led to the isolation of two already reported biflavanones, isogarcinol (1) and guttiferone (2) with promising antiplasmodial activity against a chloroquine resistant (W2) *Plasmodium falciparum* strain with an IC_{50} of 2.8 ± 0.90 µg/mL for compound 1 and IC_{50} of 3.94 ± 0.38 µg/mL for compound 2. Compounds 1 and 2 also exhibited moderate activity against the chloroquine sensitive (D6) *Plasmodium falciparum* strain with IC_{50} of 7.03±0.60 and 10.64±4.50 µg/mL, respectively. **Conclusion:** The results provide proof to support the use of *G. buchananii* by the indigenous community for antimalarial therapy.

Key words: *Garcinia buchananii*, Isogarcinol, Guttiferone F, Antiplasmodial activity.

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INTRODUCTION

Garcinia buchananii Engl, a plant found in Eastern, Central and Southern Africa is used to treat dysentery, abdominal pain, malaria and a range of infectious diseases.[1-3] The aqueous extract of the stem bark of *G. buchananii* has anti-inflammatory and anti-nociception effects[4] and it is reported to interfere with diarrhea by reducing peristalsis through inhibition of neurotransmission.[5] Studies have shown that an aqueous ethanolic extract of the stem bark exhibited strong antioxidant activity and bioassay guided isolation yielded compounds with high antioxidative power.[6] According to a literature search, no antiplasmodial activity has previously been reported for this plant. Our previous work indicated that a CH_2Cl_2: MeOH (1:1) extract inhibited the growth of malaria parasites *in vivo* by about 66 %.[7] This work reports on antiplasmodial biflavanones isolated from the CH_2Cl_2: MeOH (1:1) extract of stem bark *G. buchananii* together with antiplasmodial activity of fractions. The structures of the compounds were determined using nuclear magnetic resonance spectroscopy (1D and 2D NMR) and comparison with literature data for known compounds.

MATERIALS AND METHODS

Plant collection and solvent extraction

The stem bark of the plant was collected with the help of a botanist in Mau forest, Nakuru County Kenya in May 2015. The voucher specimen (RO2015/05) is deposited at the School of Biological Sciences (SBS) herbarium, University of Nairobi. The samples were dried under shade for a period of two weeks and then ground into powder using a miller. The crude extract was obtained by maceration using CH_2Cl_2: MeOH (1:1). The extract was concentrated under reduced pressure using a rotary evaporator and stored at - 4°C until the time of use.

Isolation

The crude extract (50g) of the stem bark of *G. buchananii* obtained using CH_2Cl_2: MeOH (1:1) was subjected to column chromatography using hexane and ethyl acetate (90:10, 0:100) in increasing polarity. This yielded fraction RAO-25F (0.1g) and RAO-25H (1.2 g) as the major fractions. Fraction RAO-25F was further subjected to column chromatography using hexane/ethyl acetate in increasing polarity followed by sephadex LH 20, CH_2Cl_2: MeOH (1:1) to obtain compound 1 and 2 (Figure 1).

General procedures

The solvents used for column chromatography were EtOAc, n-C_6H_{14}, CH_2Cl_2 and MeOH. All the solvents used for column chromatography were double distilled. Merck silica gel (70-230 mesh) and Sephadex LH 20 were used as the stationery phase. Pre-coated aluminium silica gel plates were used in thin layer chromatography. The TLC plates were observed under UV light at 254 or 366 nm for UV active compounds, followed by placing the plate in the iodine tank. ^1H and ^{13}C NMR were recorded at 600 and 150 MHz, respectively, on a Varian–Mercury 200 MHz. Trimethylsilane (TMS) was used as internal standard, chemical shifts were recorded in ppm and coupling constants (*J*) recorded in Hz. $CDCl_3$ and CD_3OD were used as NMR solvents. Standard Bruker software was used to obtain Homo Nuclear Correlation Spectroscopy (COSY), Hetero Nuclear Single Quantum Coherence (HSQC) and Hetero Nuclear Multiple Bond Connectivity (HMBC) spectra.

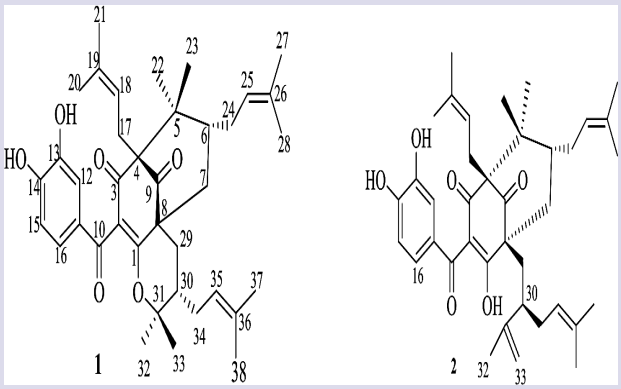

Figure 1: Structures of biflavanones, Isogarcinol (1) and guttiferone (2) isolated from the stem bark of *G. buchananii*.

Antiplasmodial activity

A semi-automated micro-dilution assay technique that measures the ability of the compounds to inhibit incorporation of [G-^{3}H] hypoxanthine into the malaria parasite was used.[8] The parasites were cultured by a method earlier described by.[9] Both D6 (chloroquine sensitive) and W2 (chloroquine resistant) *Plasmodium* strains were used. Parasites were cultured in sealed flasks at 37°C, in a 3% O_2, 5% CO_2 and 92% N_2 atmosphere in RPMI 1640, 25 mM HEPES, pH 7.4, supplemented with heat inactivated 10% human serum and human erythrocytes to achieve a 3% haematocrit. On attainment of ring stage, parasites were synchronized with 5% sorbitol and tested at 0.4% parasitemia passage into 96-well plates. Stock solutions of compounds were prepared at 1mg/ml in DMSO diluted by RPM1640 to attain 0.2% DMSO and tested in triplicate.[10] Equal concentration of DMSO was used as negative control while 1.1µm chloroquine was used as positive control. The cultures were then incubated for 48 hrs at 37°C. Thereafter, each well was pulsed with 25 µL of culture medium containing 0.5 µCi of [G-^{3}H]-hypoxanthine and the plates incubated for a further 18 hrs. The contents of each plate were harvested onto glass fibre filters, washed thoroughly with distilled water, dried and radioactivity measured using a scintillation counter.

RESULTS

Following bioassay guided fractionation we isolated two already reported compounds, isogarcinol (1) and guttiferone F (2) (Figure 1). The compounds were identified using 1D, 2D, MS spectroscopy and comparison with literature data.[6,11-15] The ^{1}H and ^{13}C NMR data for compound 1 and 2 are recorded in Table 1. Compound 1 and 2 showed 38 carbons in ^{13}C NMR. The major difference between compound 1 and 2 was observed at position C-1, C-31 and C-33. In compound 1, C-1 (δc 173.6), C-31(δc 88.3) and C-33 (δc 29.0). This indicated the presence of methyl group at C-33 and epoxidation between C-1 and C-31. In compound **2**, C-1 (δc 198.8), C-31(δc 148.1) and C-33 (δc 112.7). The carbon at δc 112.7 showed a cross peak with olefinic protons at δ_H 4.38 (*d*, 2.3) and δ_H 4.43 (*d*, 2.3).

Characterization of isolated compounds

Isogarcinol (1)

Yellowish green solid; $[\alpha]^{24}_D$ -172 (c = 0.85; CH_2Cl_2); HR-ESI-MS *m/z* 603.3927 $[M+H]^+$, ($C_{38}H_{50}O_6$ requires *m/z* 603.3641), 602 $[M]^+$ (50), 575 (38), 574 (91), 465 (100), 449 (44), 341 (69), 231 (30), 137 (27), 69 (22); ^{1}H NMR ($CDCl_3$, 500 MHz) (Table 1)

Table 1: ^{1}H and ^{13}C NMR data for compound 1 and 2 (δ in ppm and *J* in Hz).

No.	1		2	
	δ_C[a]	δ_H[b], m, *J*	δ_C[a]	δ_H[b], m, *J*
1	173.6		198.8	
2	126.5		115.9	
3	196.3		194.7	
4	69.4		69.8	
5	47.0		49.6	
6	47.5	1.48 *m*	46.8	1.45 *m*
7	40.0	2.26 *d* (14.6), 2.00 *m*	42.6	2.37 *m*, 2.07 *m*
8	52.6		68.7	
9	207.9		209.8	
10	194.2		193.8	
11	131.1		128.0	
12	116.2	7.22 *d* (2.0)	124.2	6.98 *d* (2.0)
13	146.6		143.5	
14	152.6		149.6	
15	115.6	6.72 *d* (8.3)	114.4	6.63 d (8.3)
16	124.3	7.01 *dd* (8.3, 2.0)	116.5	6.97 *dd* (8.3, 2.0)
17	26.6	2.61 *m*, 2.41 *dd* (5.3, 13.4)	26.4	2.58 *m*, 2.76 *m*
18	121.2	4.90 *m*	120.2	5.09 *m*
19	135.4		135.2	
20	18.3	1.55 *s*	18.3	1.73 *s*
21	26.5	1.57 *s*	26.1	1.80 *s*
22	27.1	0.97 s	22.7	1.16 *s*
23	22.9	1.14 *s*	27.0	1.04 *s*
24	30.5	2.66 *m*, 1.81 *m*	28.9	1.94 *m*, 2.14 *m*
25	126.3	4.90 *m*	123.8	4.93 *m*
26	134.0		133.0	
27	26.1	1.67*s*	25.8	1.70 *s*
28	18.6	1.65 *s*	17.8	1.54 s
29	29.0	1.01, 0.88	36.2	2.14 *m*, 1.90 *m*
30	44.6	1.35 *m*	43.7	2.74 *m*
31	88.3		148.1	
32	21.6	1.24 *s*	18.0	1.57 *s*
33	29.0	0.88 *s*	112.7	4.38 *d* (2.3), 4.43 *d* (2.3)
34	30.5	2.10 *m*, 2.04 *m*	32.6	2.07 *m*, 1.99 *m*
35	122.9	5.19 *ddd* (6.5, 5.3, 1.2)	122.7	5.04 *m*
36	134.6		132.0	
37	26.0	1.77 *s*	25.7	1.68 *s*
38	18.1	1.62 *s*	17.9	1.60 *s*

Key: [a] recorded in 150 MHz, [b] recorded in 600 MHz; NMR solvents are $CDCl_3$ and CD_3OD, respectively.

Table 2: *In vitro* antiplasmodial activity of fractions and isolated compounds from *G. buchananii*, against D6 and W2 strains of *Plasmodium falciparum*.

Fractions/ compounds	*P. falciparum* D6 IC_{50}s (M±SD) µg/mL	*P. falciparum* W2 IC_{50}s (M±SD)
RAO-25F	7.50±0.60 µg/mL	11.98±3.0 µg/mL
RAO-25H	23.57±0.59 µg/mL	19.72±3.3 µg/mL
1	7.03±0.60	2.8±0.90
2	10.64±4.50	3.94±0.38
Chloroquine	0.019	0.057

Key: RAO-25F, RAO-25H, fractions from crude extract; 1, isogarcinol; 2, guttiferone F; D6, chloroquine susceptible; W2, chloroquine resistant.

Guttiferone F (2)

Yellowish solid; $[\alpha]^{24}_{D}$ -45 (c = 0.5; CH_3OH); HR-ESI-MS *m/z* 603.3882 $[M+H]^+$, ($C_{38}H_{50}O_6$ requires *m/z* 603.3641), 602 $[M]^+$ (20), 465 (36), 279 (24), 231 (18), 167 (48), 149 (100), 69 (21); ^1H NMR (CD_3OD, 500 MHz) (Table 1)

Antiplasmodial activity

The results in Table 2 show that fractions RAO-25F and RAO-25H exhibited antiplasmodial activity against chloroquine resistant (W2) and chloroquine susceptible (D6) *P. falciparum* strains. Fraction RAO-25F showed higher activity compared to RAO-25H with IC_{50} values 7.50±0.60 vs 23.57±0.59 µg/mL against the D6 strain and 11.98±3.0 vs 19.72±3.3 µg/mL against the W2 strain, respectively. Fraction RAO-25F yielded compounds 1 and 2 which exhibited good antiplasmodial activity. Compound 1 gave IC_{50} of 7.03±0.60 and 2.8±0.90 µg/mL against the D6 and W2 strains, respectively. Compound 2 gave IC_{50} of 10.64±4.50 and 3.94±0.38 µg/mL against D6 and W2 strains, respectively.

DISCUSSION

Garcinia buchananii belongs to the family Clusiaceae and has been known to have a wide range of biological activities.[16] According to our literature search, antiplasmodial activity of *G. buchananii* is being reported for the first time. In this work, we report antiplasmodial activity of fractions/compounds from *G. buchananii* and isolation of two biflavanoids, isogarcinol (1) and Guttiferone (2). These compounds have been isolated from *Moronobea coccinea* and *Allanblackia stuhlmanni.*[12,13] Compounds 1 and 2 exhibited a very similar profile of antiplasmodial activity. These results provide evidence supporting claims for use of this plant by the indigenous Ogiek community, of Kenya, for treatment of malaria. The observed activity is similar to other related compounds previously reported. The benzophenones with a tetrahydropyrane ring have been reported to have strong antiplasmodial activity.[13] These biflavanones have also shown antioxidant, anti-HIV and anti-carcinogenic activity.[6,12,14,16]

CONCLUSION

Bioassay guided isolation of *G. buchananii* led to isolation of two biflavanones, isogarcinol (1) and guttiferone F (2) with moderate antiplasmodial activity against a chloroquine sensitive (D6) and chloroquine resistant (W2) *Plasmodium falciparum* strains. The results provide proof to support the use of *G. buchananii* by the indigenous community for anti-malarial therapy.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS

HIV: Human Immunodeficiency virus; **WHO:** World Health Organization; **NMR:** Nuclear Magnetic Resonance; **TLC:** Thin Layer Chromatography; **DMSO:** Dimethyl sulfoxide.

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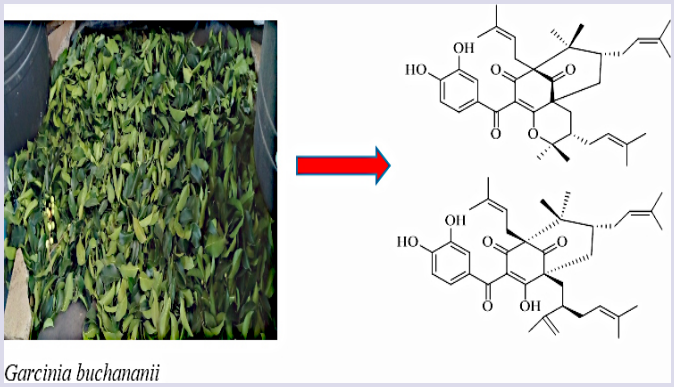
PICTORIAL ABSTRACT



Garcinia buchananii

SUMMARY

- Isogarcinol and guttiferone F was isolated from stem bark of G. buchananii.
- The compounds were elucidated using ID, 2D NMR and Mass spectroscopy.
- Fractions and the biflavanones were tested for antiplasmodial activity.

ABOUT AUTHORS

Ruth Anyango Omole is an assistant lecturer in the department of chemical science and Technology at Technical university of Kenya. She has Master of Science in medicinal Chemistry. She is in the final stage of finishing her PhD in Traditional Medicine. Her research interest are mainly phytochemistry, drug discovery and design and organic synthesis.