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Original Article

Triterpenoids from *Grewia plagiophylla* K. Schum

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ABSTRACT

Introduction: Plants of the genus *Grewia* have been used as medicinal agents to treat several diseases. *Grewia* species are reported in use as folk medicine for the treatment of malaria, diarrhoea, dysentery, typhoid fever, small pox, cough, irritable condition of intestine and bladder among others. **Methods:** The stem back material of *Grewia plagiophylla* was extracted using CH_2Cl_2: MeOH (1:1). Fractionation and Isolation of the extract was done using column chromatography with silica gel and eluting with hexane-ethyl acetate solvent system in increasing polarity. The structures of the isolated compounds were elucidated on the basis of NMR experiments and the comparison of their spectroscopic data with literature values. **Results:** Studies on bioactive components of the stem back of *Grewia plagiophylla* led to isolation of three triterpenoids: stigmasterol (I), butelin (II) lup-20(29)-en-3-ol (III). The triterpenoids have been isolated for the first time from this species with compound (III) being reported for the first time from the genus of *Grewia*. The isolated compounds showed varying degrees of *in vitro* antimalarial activity against *Plasmodium falciparum* chloroquine-sensitive D6 and chloroquine-resistance W2 strains with significant cytotoxic activity against Vero cells.

Key words: *Grewia plagiophylla*, Compounds, Triterpenoids, Anti-plasmodial, Cytotoxicity.

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INTRODUCTION

Plant of the genus *Grewia* (Tiliaceae family) are most widely distributed in subtropical and tropical regions and consist of approximately 150 species.[1] Some of these species have been used as medicinal agents to treat several diseases. The root and stems back of these plants have been used indigenously as traditional medicine for the treatment of malaria, diarrhea, cough, smallpox cough and sore throats.[1] Previous studies indicate that they have antimalarial[2] antibacterial, antioxidant and are hepatoprotective activities.[3]

Studies on the chemical constituents on genus of *Grewia* have resulted in isolation and characterization of wide varieties of steroids glycosides, flavones, triterpenes and lignans.[4] Previous phytochemical studies of the stem bark of *Grewia optiva* have led to the isolation of three triterpenes; betulin, betulinic acid and oleanolic acid.[5] A new coumarinolignan isolated from a sample of *Grewia bilamellata* Gagnep. (Tiliaceae), grewin, has shown antimalarial activity against D6 and W2 (IC_{50} 11.2 µg/ml ≡ 28.4 µM and 5.5 µg/ml ≡ 13.9 µM) respectively without significant cytotoxicity.[6] There are no reports of the phytochemistry of *G. plagiophylla* in the literature but it has been reported to have antimalarial activity.[7]

MATERIALS AND METHODS

Stem bark of *G. plagioplylla* was collected from Kilifi County coastal region of Kenya in February, 2016. The sample was assigned Voucher number (JG701) and deposited at the East African Herbarium at National Museum of Kenya (NMK).

Sample preparation

The plant sample material was collected and transported to Kenyatta University laboratory where it was chopped into small pieces and ground in a laboratory mill.

Extraction and isolation

The powdered stem bark (3kg) was soaked in 10L dichloromethane: methanol at a ratio of 1:1 with resoaking overnight. The extract was filtered using Whatman number 1 filter paper for three consecutive days to achieve exhaustive extraction. Filtrates were then combined and concentrated using a rotary evaporator at 40°C. The crude extract (100 g) was subjected to column chromatography using hexane and ethyl acetate (100:0, 0:100) in increasing polarity (10%) and finally with methanol to obtain 100 fractions of 500 ml each. The Collected fractions were then combined based on their TLC profiles. Fraction 30-45(G 45) yielded white needle-like crystals of stigmasterol (I), fraction 60-65(G 65) yielded a white powder of butelin (II) and fractions 70-85, (G 85) (III) yielded lup-20(29)-en-3-ol.

Cytotoxicity studies

The pure compounds were tested for cytotoxicity using Vero cell growth –inhibition assay as described by Kurokawa *et al.* (1995).[8] Inhibition data were plotted as dose-response curves, from which CC_{50} was estimated. Selectivity index (SI) was used as parameter of clinical significance of the test samples by comparing general toxins and selective inhibitory effect on *P. falciparum* calculated as described by Wright and Phillipson, (1990).[9] Leishmaniasis, malaria and trypanosomiasis. In this review the potential of natural plant products as a source of antiprotozoal drugs is discussed with respect to biochemical differences between protozoa and hosts. Some of the ways in which pathogenic protozoa differ biochemically from their human hosts are described, and the modes of action of some antiprotozoal drugs which exploit these differences are mentioned. A selection of natural products of plant origin (alkaloids, terpenes, quinones and miscellaneous compounds

Antiplasmodial assay

Antimalarial assays were conducted using a semi - micro –dilution assay that determine the ability of the compounds to inhibit incorporation of [G-3H] hypoxanthine into a malaria parasite as described by Desjardins (1979).[10] Two strains of *P. falciparum*: D6 (CQ-sensitive) and W2 (CQ-resistant) were used in the study. Drug concentration proficient of inhibiting 50% of the *P. falciparum* (IC_{50}) was determined as described by Sixsmith *et al.* (1984).[11] The pure compounds with IC_{50} < 10 μg/mL were considered to have antimalarial activity.

RESULTS AND DISCUSSION

Extraction of the air dried stem bark of *G. plagiophylla* with CH_2Cl_2: MeOH (1:1) at room temperature, followed by a combination of chromatographic separations, yielded three compounds (I-III).

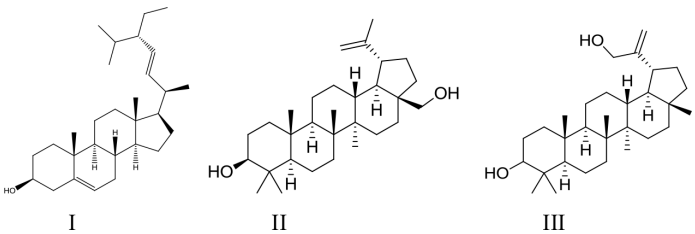
Compound I was obtained as a needle like crystals. Mass spectrum of isolated compound (I) showed parent molecular ion [M^+] peak at mlz 412 which corresponds to the molecular formula $C_{29}H_{48}O$.^{1}H-NMR spectrum of compound I showed Six methyl protons appearing at δ_H 1.23, δ 1.19, δ 1.06, δ 1.00, δ 0.98 (Table 1). The proton corresponding to the H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.20. Three olefenic protons appeared downfield at δ 5.24, 4.57 and 4.14. The ^{13}C-NMR showed recognizable signals at 140.8 and 121 ppm, which corresponds to double bond at C-22 and C-6 double bonds respectively. One more double bond in between C-5 and C-23 showed signals at 130.1 and 129.1 ppm. The above spectral data supported the presence of sterol skeleton having a hydroxyl group at C-3 position with two double bonds at C-5/C-6 and C-20/C-21 with six methyl groups which was supported by the key COSY and HMBC correlations as shown in Figure 1. Finally its identity was confirmed by comparison of its spectral data with those reported in literature.[12]

Compound (II) was obtained as white crystals. The molecular formula, $C_{30}H_{50}O_2$was determined on the basis of EI-MS. (calcd. 442 for $C_{30}H_{50}O_2$). The ^{1}H NMR spectrum (Table 2) showed six methyl signals at δ 1.68, 0.97, 0.90, 0.85, 0.76 and 0.65 ppm. A doublet of doublets was present at 3.08 ppm which is a characteristic of a α-oriented proton at C-3 at δ H 3.18 ppm. Doublets for geminal protons at δ 4.70 and 4.59 ppm, along with the methyl signal at δ 1.68 ppm, suggested that compound II was a lupane-type triterpenoid. A pair of oxymethylene doublets at δ 3.70 and 3.20 ppm, indicated the presence of a second hydroxyl group in the molecule.^{13}C NMR spectra revealed 30 carbon atoms (Table 2). This further confirmed a pentacyclic structure. The chemical shift at δ 150.8 and 109.3 were characteristic peaks of sp^2 carbons comprising the double bond of lupeol type skeleton assigned to C-20, to C-29 respectively. The Oxygenated C-3 and C-8 shifts were observed at δ 78.7 and 60.2 respectively corresponding to reported literature.[13]Adenium obesum. Methods: The stem-bark, after air-drying and powdering, was subjected to sequential hot-continuous extraction using petroleum spirit (60 - 80ºC Based on the above spectroscopic result compound (II) was considered to be betulin.

Compound (III) was obtained as an amorphous white powder. The molecular formula, $C_{30}H_{50}O_2$ was determined on the basis of EI-MS. (calcd. 442 for $C_{30}H_{50}O_2$). The ^{1}H NMR data showed the signals for six tertiary methyl groups at δ 0.78, 0.81, 0.86, 0.97 and 0.98 indicating a lupane skeleton.[14] ^{13}C NMR (Table 3) spectra showed the presence of two terminal double bonds δ 155.8 and 106.8 which are characteristic peaks for lupane type of skeleton assigned to C-28 and C-29 respectively . The oxygen deshielding chemical shift at 78.2 was assigned to C-3. ^{1}H and ^{13}C NMR patterns for compound (III) were similar to those of lupueol as reported in the literature[15,16] except for the additional hydroxyl resonance in the ^{1}H and ^{13}C NMR spectra (δ_H 4.10 and δC 64.7). Compound (III) was thus identified as lup-20(29)-en-3-ol and reported for the first time in the genus of *Grewia*.

Table 1: Comparison of ^{1}H and ^{13}C NMR data for compound (I) and literature.[12]

Carbon atom	^{13}C Experimental	^{13}C Literature	^{1}H Experimental	^{1}H Experimental
C-1	37.26	36.72		
C-2	29.13	29.71		
C-3	71.66	71.97	3.34 (m,1H)	3.53 (m, 1H)
C-4	42.32	42.35		
C-5	140.95	140.94		
C-6	121.46	121.32	5.24 (s, IH)	5.38 (s, 1H)
C-7	31.7	31.71		
C-8	29.13	29.24		
C-9	50.88	50.9		
C-10	36.13	36.16		
C-11	24.27	24.12		
C-12	39.81	39.82		
C-13	40.5	40.45		
C-14	56.06	56.03		
C-15	25.38	24.32		
C-16	28.2	28.9		
C-17	56.06	56.03	1.02 (d,3H)	1.29 (d, 3H)
C-18	12.03	12.06	0.72 (d,3H)	0.74 (d, 3H)
C-19	19.18	19.06		
C-20	39.7	39.82		
C-21	23,01	23.12	1.15 (d,3H)	1.20 (d, 3H)
C-22	138.4	138.4		
C-23	129.21	129.34	5.26 (m,1H)	5.20 (m, 1H)
C-24	51.26	51.26		
C-25	33.89	34.01		
C-26	21.06	21.01		0.84 (d, 3H)
C-27	19.5	22.82	0.93 (d,3H)	0.97 (d, 3H)
C-28	25.38	25.32		
C-29	11.9	12.06		1.04 (t, 3H)



I II III

Figure 1: Structures of stigmasterol (I), butelin (II) lup-20(29)-en-3-ol (III) isolated from the stem bark of *G. plagiophylla*.

Cytotoxicity

Stigmasterol exhibited low cytotoxicity at the concentrations tested (Table 4). Butelin and Lup-20(29)-en-3-ol were highly cytotoxic. It has been reported that structurally related triterpenes and steroids showed moderate to strong cytotoxic effect.[17]

Table 2: Comparison of ^{1}H and ^{13}C NMR data for compound (II) and literature.[13]Adenium obesum. Methods: The stem-bark, after air-drying and powdering, was subjected to sequential hot-continuous extraction using petroleum spirit (60 - 80 oC

Carbon atom	^{13}C Experimental	^{13}C Literature	^{1}H Experimental	^{1}H Literature
1	38.6	38.9		
2	27.4	27.5		
3	78.7	79.2	3.08(dd)	3.79 (dd)
4	38.6	38.8		
5	55.2	55.4		
6	18.3	18.4		
7	34.2	34.3		
8	40.9	41		
9	50.3	50.5		
10	37.3	37.4		
11	20.8	20.9		
12	25.2	25.3		
13	37.1	37.2		
14	42.7	42.8		
15	27.0	27.1		
16	29.7	29.2		
17	47.8	47.9		
18	48.7	47.9.		
19	47.8	48.8		
20	150.6	150.6		
21	29.7	29.8		
22	34.2	34.1		
23	27.7	28..1		
24	15.1	15.4	0.74s	0.75 s
25	15.7	16.2	0.90s	0.80 s
26	15.9	16.1	0.85s	
27	14.5	14.7	0.96 s	0.99 s
28	60.2	60.6	3.70,3.20d	3.33 d
29	109.3	109.4	4.60 d	4.58 d
30	18.8	19.4		

Antiplasmodial activity

Antiplasmodial activity was classified as follows: high at IC_{50} ≤10 µg/ml, moderate at 10–50 µg/ml, low at 50–100 µg/ml and inactive at >100 µg/ml. The IC_{50} values for compound II was moderate and to both strains while compound I and III exhibited no activity to both W2 and D6 *P. falciparum* strains. (Table 5)

CONCLUSION

Fractionation and purification of the CH_2Cl_2: MeOH extract of stem bark of *G. plagiphylla* collected from Kilifi led to the isolation of three compounds: stigmasterol (I), butelin (II) and lup-20(29)-en-3-ol (III). All these compounds were for the first time reported from this species. This paper report for the first time the isolation of compound (III) from *Grewia*. Compound (II) displayed moderate antimalarial activity while compound I and III did not depict antimalarial activity. Stigmasterol (I)

Table 3: Comparison of ^{1}H and ^{13}C NMR data for compound (III) and lupeol.[15]

Carbon atom	^{13}C NMR Experimental	^{13}C NMR Literature	^{1}H NMR Experimental	^{1}H NMR literature
C-1	38.74	38.7		
C-2	27.46	27.4		
C-3	78.71	78.9	3.14s	3.20 dd
C-4	38.74	38.8		
C-5	55.17	55.3	0.71s	0.68m
C-6	18.26	18.3	0.76s	1.50m,1.40m
C-7	34.25	34.2		1.42m,1.32m
C-8	40.81	40.8		
C-9	50.34	50.4	1.28s	1.29m
C-10	37.09	37.1		
C-11	20.96	20.9	1.40s	1.40m,1.20m
C-12	----	25.1		1.68m,1.07m
C-13	38	38		
C-14	42.73	42.8		
C-15	27.46	27.4		
C-16	35.41	35.6		1.48m,1.37m
C-17	42.95	43		
C-18	48.77	48.2	1.46 s	1.37m
C-19	-----	47.9		
C-20	155.18	150.9		
C-21	29.66	29.8	1.26s	
C-22	----	40		
C-23	27.72	28		
C-24	15.15	15.4	0.95s	0.76s
C-25	15.88	16.1	1.03s	0.83s
C-26	15.74	15.9	0.74s	1.03s
C-27	14.74	14.5		0.94s
C-28	17.42	18		0.74s
C-29	106.28	109.3	4.89 d	4.67 s,4.54 s
			4.91d	
C-30	----	19.3		1.68s
	63.54		4.08s	
	31.71			

Table 4: Cytotoxicity of the isolated compounds.

Compounds	CC_{50} (µg/ml), $n = 3$
Stigmasterol	66.54
Butelin	0.76
Lup-20(29)-en-3-ol	0.15

Table 5: *In vitro* antiplasmodial activity of isolated compounds from *G. plagiophylla*, against D6 and W2 strains of *P. falciparum*.

	Compounds	IC_{50} (µg/ml) W2 (CQ sensitive)	SI=CC_{50}/IC_{50}	IC_{50} (µg/ml) D6 (CQ resistant)	SI=CC_{50}/IC_{50}
1	Compound I	> 100	N/A	> 100	N/A
2	Compound II	33.96±4.10	0.022	30.81167±0.31	0.025
3	Compound 111	> 100	N/A	> 100	N/A
4	CQ control	78.99±3.83		6.34±1.12	

N/A - Not applicable

displayed minimal cytotoxicity while triterpenoids II and III were highly cytotoxic.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS

CQ: Chloroquine; **NMR:** Nuclear Magnetic Resonance; **TLC:** Thin Layer Chromatography.

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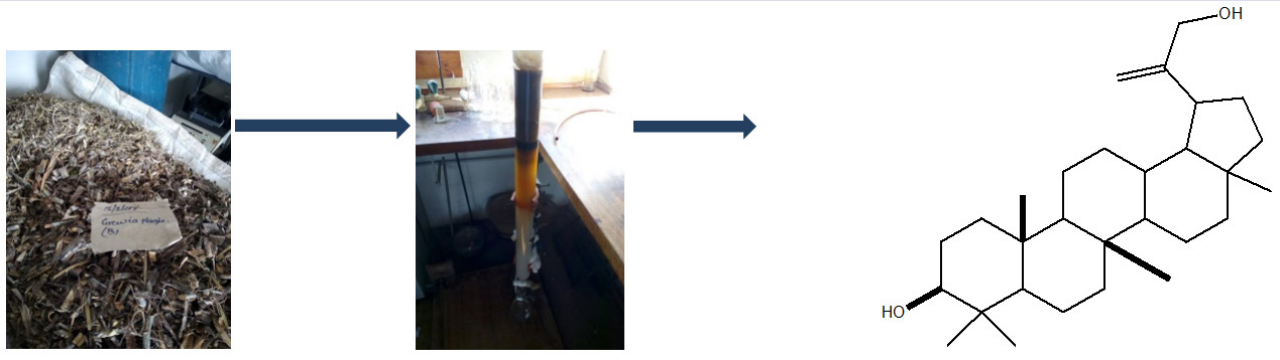
PICTORIAL ABSTRACT



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SUMMARY

- Three triterpenoids were isolated from stem back of *Grewia plagiophylla*
- The compounds were elucidated using 1D and 2D NMR
- The compounds were tested for cytotoxicity and anti-plasmodial activities.