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Cyclooxygenase inhibitory compounds from *Gymnosporia heterophylla* aerial parts

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ABSTRACT

Gymnosporia heterophylla (Celastraceae) is an African medicinal plants used to treat painful and inflammatory diseases with partial scientific validation. Solvent extractions followed by repeated chromatographic purification of the *G. heterophylla* aerial parts led to the isolation of one new β-dihydroagarofuran sesquiterpene alkaloid (**1**), and two triterpenes (**2**–**3**). In addition, eight known compounds including one β-dihydroagarofuran sesquiterpene alkaloid (**4**), and six triterpenes (**5**–**10**) were isolated. All structures were determined through extensive analysis of the NMR an MS data as well as by comparison with literature data. These compounds were evaluated for the anti-inflammatory activities against COX-1 and -2 inhibitory potentials. Most of the compound isolated showed non selective COX inhibitions except for 3-Acetoxy-1β-hydroxyLupe-20(29)-ene (**5**), Lup-20(29)-ene-1β,3β-diol (**6**) which showed COX-2 selective inhibition at 0.54 (1.85), and 0.45 (2.22) IC_{50}, in mM (Selective Index), respectively. The results confirmed the presence of anti-inflammatory compounds in *G. heterophylla* which are important indicators for development of complementary medicine for inflammatory reactions; however, few could be useful as selective COX-2 inhibitor.

1. Introduction

Inflammatory complications are as serious causes of morbidity and limitation of physical activity, especially in the elderly [1]. Although there is no cure, medications including steroids, non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are commonly used for the management of inflammatory disorders. Since most of these drugs are associated with undesirable side effects such as gastrointestinal disturbances [2], complementary and alternative anti-inflammatory drugs are needed [3]. NSAIDs are well known as COX inhibitors, which are markedly up regulated at the inflammatory sites [3], opening the possibility for discovering selective inhibitors with reduced gastrointestinal side effects.

The plants of the genus *Gymnosporia* (syn. to *Maytenus*) are widely used in folk medicine as an antiseptic, antiasthmatic, fertility regulating agent, antitumor, as well as for stomach problems [4]. The genus *Gymnosporia* are notably used in Brazilian traditional medicine for the treatment of gastric ulcers [5], inflammation and diarrhea [6] as antimicrobial [7], and antitumor [8]. In south-central Zimbabwe, *G. buxifolia* is particularly used as a remedy for abdominal pains and the aerial parts reported for antiplasmodial and anti-inflammatory activities [9]. Similarly, *G. heterophylla* and *G. senegalensis* are two African medicinal plants used to manage painful and inflammatory diseases [10], a claim confirmed through an in *vivo* experiment using leaf extracts of the two plants on mice that showed a significant anti-inflammatory activity [10]. *G. heterophylla* is further claimed by different African communities to be used against malaria [11]; sexually transmitted diseases (antimicrobial effects); breathing difficulties and chest pains [12]; in the management of livestock diarrhea [13]; and eradication of ticks from animals [14]. Phytochemical studies on the plant *G. heterophylla* reported the isolations of a dihydroagarofuran alkaloid, heterophyllin together with β-amyrin, 3α-hydroxy-2-oxofriedelane-20α-carboxylic acid, lupeol, lup-20(29)-ene-1β,3β-diol, (−) - 4′-methylepigallocatechin and (−) epicatechin from EtOH extracts of the aerial parts [15]. Pristimerin isolated from *G. heterophylla* stem bark showed potent anticytomegalovirus properties against human cytomegalovirus (HCMV) [16] whereas maytenfolic acid showed moderate antimicrobial activity [15].

In *vivo* anti-inflammatory activities of ethanol leaf extracts *G. heterophylla* and *G. senegalensis* were evaluated against carrageenan-induced paw oedema on Wistar albino rats, which indicated significant anti-inflammatory activity of up to 35% and 51% oedema reductions, respectively [10]. However, while *G. heterophylla* extracts at 1200 mg/kg showed no toxicity, *G. senegalensis* extracts indicated some toxicity.

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Such results indicated significant safe anti-inflammatory effects of *G. heterophylla* against acute inflammation and suggested the absence of acute and sub-acute toxicity signs of the *G. heterophylla* leaf extract [10]. Since anti-inflammatory activities of the compounds from *G. heterophylla* responsible for such activities remained uncertain, establishment of medicinal efficacies of the plant were thus partial. The present study was undertaken to investigate COX inhibitory potential of the compounds from aerial parts the plant to contribute to an understanding of their possible mechanism in treatment of inflammatory disorders.

2. Experimental

2.1. General instrumentation

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were measured using $CHCl_3$ on a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. IR spectra were recorded as glassy film of KBr on Perkin Elmer 200 FT-IR spectrometer. The HRTOF-MS spectra were recorded on Finnigan Mat SSQ 7000 direct probe mass spectrometer. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) spectra were recorded on Bruker's Avance-400 FT 400 MHz using deuterated solvents and referenced to residual TMS signal. Silica gel ((Merck Keiselgel 60, 0.063–0.200 mm) used for gravity column chromatography together with analytical thin layer chromatography on Merck 60 F_{254} (0.25 mm) plates, which were visualized by UV inspection and/or spraying with 5% H_2SO_4 in EtOH and/or 4% anisaldehyde-sulphuric acid reagent, followed by heating at 110 °C for 10 min.

2.2. Plant materials

The leaves and stem bark of *G. heterophylla* were collected in April 2015 from Kitale KCC forest (1°01′27.14″N 35°00′55.04″E) Trans Nzoia County in western Kenya and authenticated in the National Museum of Kenya where the voucher specimen (MEW/03/2014) was deposited.

2.3. Solvent extraction

The air-dried and crushed aerial parts of *G. heterophylla* (2.6 kg) were extracted three times with MeOH (64 l) at room temperature (20–26 °C) for 24 h. A dark brown residue was obtained after removing the solvent under reduced pressure, which was suspended in H_2O, and then partitioned with *n*-hexane, EtOAc and *n*-butanol to yield 2.2 g, 23.6 g 11.6 g and 21.6 g, respectively. The EtOAc-soluble extract (150.0 g) was chromatographed over a silica gel column and eluted with *n*-hexane-CH_2Cl_2 (100:0–0:100, v/v) followed by CH_2Cl_2-MeOH (100:0–5:95, v/v) to give thirteen fractions (Fr. 1–Fr. 6). Fractions 1–4 yielded 3-hydroxycamphane (**7**) (71 mg) [3,4-seco-olean-3,11β-olide (**8**)] (35 mg), and lupeol (**9**), respectively as white amorphous compounds after recrystallization in CH_2Cl_2-MeOH. Further column chromatographic separation on fraction 5 (3.74 g) followed by preparative TLC yielded 3-Acetoxy-1β-hydroxyLupe-20(29)-ene (**5**) (64 mg), 2-Methoxy-4-deoxyzeylasterone (**3**) (96 mg) and pristimerin (**10**) (34 mg). Further separation of fraction 6 (3.4 g) on silica gel column chromatography followed by fractional recrystallization of the two major eluates yielded lup-20(29)-ene-1β,3β-diol (**7**) (21 mg, R_f 0.35) and 3,4-*seco*- 1-hydroxy-21-oxo-olean-3, 11-olide (**2**) (83 mg).

The *n*-butanol extract showed positive results for presence of alkaloids with Dregendoff reagents which prompted extraction of alkaloids. The methanol extract (20 g) was dissolved in H_2O and acidified with H_2SO_4 to pH 3–4, then extracted with petroleum ether to remove acidic and neutral compounds which on drying yielded 8.5 g (alkaloid-free extracts). The aqueous solution was further basified to pH 9–10 with NH_4OH (25% ml/ml) followed by dichloromethane extraction. The extract was washed with distilled water, dried with anhydrous Na_2SO_4 followed with concentration to dryness under reduced pressure using a rotatory evaporator to afforded 9.3 g crude alkaloid extracts. The alkaloid extracts (8 g) which showed two spots on TLC was separated by column chromatography on silica gel (50 g, activated grade) in *n*-hexane-dicloroemthane-dicloromethane-5% methanol-dichloromethane. 1β,2β-diacetoxy-9β-benzoyloxy-6α-nicotinoyloxy-β-dihydroagarofuran (**1**) (100 mg) was obtained from the dichloromethane (100%) eluent after crystallization from dichloromethane-*n*-hexane mixture (1:1). Elution with 2% methanol in dichloromethane furnished 1β-acetoxy-9β-benzoyloxy-4α-hydroxy-6α-nicotinoyloxy-β-agarafuran (**4**), (81 mg) after recyrstallization.

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compound **1** ($CDCl_3$).

Atom	δ_H (multiplicity, *J*, Hz)	δ_C (ppm)
1	5.60 d (4)	76.3
2	4.83 m	69.4
3	1.60 m/1.92 m	22.2
4	2.25 m	36.8
5	–	88.1
6	5.94 s	76.7
7	2.23 dd (4.3, 3)	48.2
8	2.28 m/2.56 m	31.0
9	5.68 d (6.8)	76.4
10	–	51.5
11	–	81.5
12	1.57 s	25.0
13	1.56 s	26.7
14	1.16 d (8)	20.7
15	1.24 s	17.2
1-OC=O	–	170.5
1-O=CCH_3	2.02 s	20.8
2-OC=O	–	169.9
2-O=CCH_3	1.95 s	22.3
6-OC=O	–	165.9
2′	9.43 s	153.6
3′	–	123.8
4′	8.84 d (7)	137.4
5′	7.58 d (7.2)	128.7
6′	8.57 d(7.2)	155.6
9-OC=O	–	165.3
1	–	129.1
2′6′	8.17 d (8.8)	130.4
3′5′	7.45 d (8)	128.7
4′	7.55 dd (8, 8)	133.3

Assignments were aided by 2D NMR COSY, HMQC and HMBC experiments.

2.4. Physical and spectroscopic data of isolated of compounds from G. heterophylla stem back

Compound **1** (1β,2β-diacetoxy-9β-benzoyloxy-6α-nicotinoyloxy-β-dihydroagarofuran) white crystalline solids. Mp. 130–132 °C; $[\alpha]_D$ + 73.0° (c, 1.00, $CHCl_3$); UV λ_{max} (logε) ($CHCl_3$): 270 (3.94), 260 (3.62), 229 (3.90) nm; IR (KBr) ν cm^{-1}: 1759, 1721, 1611, 1459, 1039, 715; ^{1}H NMR and^{13}C NMR ($CDCl_3$, 400 MHz and 100 MHz; Table 1); HRTOFMS: (*m/z*) 602.7435. ($C_{32}H_{37}NO_9Na$) [M + Na]$^+$.

Compound **2** (3,4-*seco*- 1-hydroxy-21-oxo-olean-3, 11-olide): white amorphous solid, m.p 268–270 °C; IR (KBr) ν cm^{-1}: 2990, 1740, 1371, 1277, 1248 cm^{-1}.^{1}H NMR and ^{13}C NMR ($CDCl_3$, 400 MHz and 100 MHz; Tables 2 and 3); ESIMS *m/z* 470.68 (M^+).

Compound **3** (2-Methoxy-4-deoxyzeylasterone): pale yellow amorphous solid, m.p 252–254 °C. TLC (purple spot R_f1.6) Uncorrected; IR (KBr) ν cm^{-1}: 3400, 2900, 1742, 1721, 1642, 1298 and 717 cm^{-1}. UV (MeOH) λ_{max}: 241 nm and 304 nm; ^{1}H NMR and^{13}C NMR ($CDCl_3$, 400 MHz and 100 MHz; Tables 2 and 3); HRESIMS *m/z* (482.4196 [M]$^+$, 505.4316 [M + Na]$^+$).

2.5. Cyclooxygenase inhibitory assay

Inhibitory activities of the compounds towards COX-1 and COX-2

Table 2
^{1}H NMR (400 MHz) and **^{13}C** NMR (100 MHz) data for compounds **2**, and **3** ($CDCl_3$).

Atom	**2** δ_H ppm, multiplicity (*J*)	δ_C (ppm)	**3** δ_H ppm, multiplicity (*J*)	δ_C (ppm)
1	4.34 dd (4.8, 7.9)	79.6	6.82 s	109.5
2α	3.19 dd (7.9, 14.5)	44.5		150.8
2β	2.79 dd (4.8, 14.5)			
3	–	172.4		152.8
4	2.75 m	56.0	7.61 s	113.2
5	2.05 m	85.0		141.9
6α	1.69 m	33.2		201.2
6β	2.13 dt (3.5, 13.8)			
7α	1.59	27.6	2.53 dd (17, 3)	43.8
7β	1.16 t (6.6)		2.14 dd (12, 17)	
8	–	43.2	2.38 dd (3, 12)	35.4
9	2.03 d (9.2)	55.2		38.6
10		44.5		123.5
11	4.54 d (9.2)	67.3	1.36 dt (5, 13)	32.4
			2.18 dt (4, 14)	
12	5.76 d (2.9)	133.5	1.43 dt (5, 12)	29.9
			1.99 dt (3, 11)	
13		139.8		36.8
14		42.6		35.9
15α	1.57 m	19.8	1.58 m	30.3
15β	1.56 m		1.62 m	
16α	1.55 m	34.1	1.43 m	
16β	1.43 m		1.56 m	
17	–	51.4	–	35.7
18	1.60 t (3.5)	37.8	1.86 t (6)	40.4
19α	1.65 dd (3.5, 14.6)	40.5	1.46 d (6)	29.5
19β	1.48 dd (7.2, 14.6)		1.58 d (6)	
20		47.7		39.1
21α	–	215.9	1.47 dt (4, 11)	29.6
21β	–		1.24 dt (5, 13)	
22α	2.36 d (14.5)	40.4	1.19 dt (4, 12)	28.2
22β	3.01 d (14.5)		1.39 dt (5, 13)	
23	1.01 d (6.8)	21.0	1.08 s	31.9
24	1.13 d (6.7)	20.3	1.02 s	25.3
25	1.30 s	13.8	0.78 s	16.7
26	1.07 s	19.1	0.99 s	15.2
27	1.22 s	22.6	1.16 s	31.5
28	1.45 s	26.8	–	179.4
29	1.19 s	28.4		
30	1.23 s	21.3		
OCH_3			3.87 s	55.7
$COOCH_3$			3.62 s	51.5

Assignments were aided by 2D NMR COSY, HMQC and HMBC for ^{1}H NMR and experiments DEPT, HMQC and HMBC for ^{13}C NMR.

activity was determined using colorimetric COX (ovine) inhibitor screening assay kit (Cayman, No. 760111) following manufacturer's instructions. The assay was conducted by monitoring the appearance of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-phenediamine (TMPD) at 590 mm. Aspirin and Celecoxib served as positive controls. The test compounds were dissolved in 1% DMSO (v/v) in distilled water make up a 10 mM concentration for each compound. The same stock solution was serial diluted into four different concentrations (1.0, 0.5, 0.25 and 0.125 mM) that were used to estimate the IC_{50} for each compound. The colorimetric substrate solution TMPD (20 µl) was added to all of the wells containing graded concentration of the compounds followed by addition of 20 µl of arachidonic acid. The plate was shaken for a few seconds and incubated for 5 min at 25 °C. The absorbance was measured at 590 nm using a microplate reader. Average absorbance was calculated for all the samples (n = 3) in order to determine the percentage of inhibition.

2.6. Statistical analysis

Results of COX inhibition activity were expressed as the mean standard error of mean (SEM). Data analyzed by using one way ANOVA followed by Turkey's multiple comparison as post-hoc test. The limit of statistical significance was set at $P < 0.05$.

3. Results and discussion

Repeated column chromatography on the extract of *G. heterophylla* stem bark yield a dihydroagarofuran sesquiterpene alkaloids [1β,2β-diacetoxy-9β-benzoyloxy-6α-nicotinoyloxy-β-dihydroagarofuran (**1**) and], and two triterpenes [3,4-*seco*- 1-hydroxy-21-oxo-olean-3, 11-olide (**2**) and 2-Methoxy-4-deoxyzeylasterone (**3**)] together with seven known compounds (Fig. 1). The molecular identity of the known compounds were established based on comparison of physical (mp and $[\alpha]_D$) and their ^{1}H and ^{13}C NMR data with those previously reported information as 1α-acetoxy-9β-benzoyloxy-4α-hydroxy-6α-nicotinoyloxy-β-agarafuran (**4**) [17], 3-Acetoxy-1β-hydroxyLupe-20(29)-ene (**5**) [18], Lup-20(29)-ene-1β,3β-diol (**6**) [19], 3-hydroxycampane (**7**) [20], 3,4-*seco*-olean-3,11β-olide (**8**) [21], lupeol (**9**) [22–24], and Pristimerin (**10**) [16].

Compound **1** was isolated as a crystalline solid analyzed for [$C_{32}H_{37}NO_9Na$] based on *m/z* 602.7435 by HRTOFMS. ^{1}H NMR and ^{13}C NMR (Tables 1) spectra suggested the presence of a nicotinoyl, a benzoyl and an acetyl ester groups alongside six methyls including two acetyl methyls, three methylenes, four oxygenated methines, three quaternary, eleven aromatic and four ester carbonyl carbons. The ^{1}H

Table 3
Inhibitory activities of isolated compounds from *Gymnosporia heterophylla* on COX assay.

Compound	(%) Inhibitions[a]		IC_{50} (mM)[b]		SI
	COX-1	COX-2	COX-1	COX-2	
1β,2β-Diacetoxy-9α-benzoyloxy-6α-nicotinoyloxy-β-dihydroagarofuran (**1**)	60.58 ± 4.90*	52.23 ± 7.56*	0.65 ± 0.02	0.56 ± 0.09*	1.16
1α-Acetoxy-9α-benzoyloxy-4α-hydroxy-6α-nicotinoyloxy-β-agarafuran (**4**)	55.95 ± 4.21	60.16 ± 6.25*	0.58 ± 0.02	0.51 ± 0.02*	1.14
3, 4-*seco*- 1-Hydroxy-21-oxo-olean-3, 11-olide (**2**)	63.35 ± 6.50*	49.72 ± 9.06	0.46 ± 0.07*	0.46 ± 0.02*	1
2-Methoxy-4-deoxyzeylasterone (**3**)	29.92 ± 7.14	43.06 ± 8.52	0.61 ± 0.04	0.66 ± 0.02	0.92
3-Acetoxy-1β-hydroxyLupe-20(29)-ene (**5**)	27.63 ± 7.01	50.62 ± 5.57	> 1	0.54 ± 0.02	1.85
Lup-20(29)-ene-1β,3β-diol (**6**)	25.13 ± 5.55	54.64 ± 5.63*	> 1	0.45 ± 0.04*	2.22
3-Hydroxycampane (**7**)	30.08 ± 4.89	33.98 ± 4.07	> 1	> 1	–
3,4-*seco*-Olean-3,11β-olide (**8**)	64.96 ± 5.91*	43.85 ± 5.11	0.53 ± 0.07*	0.65 ± 0.04	0.82
Lupeol (**9**)	25.82 ± 6.44	35.03 ± 5.40	> 1	> 1	–
Pristimerin (**10**)	64.33 ± 5.45*	54.44 ± 6.54*	0.48 ± 0.04*	0.43 ± 0.03*	1.12
Aspirin	65.27 ± 4.00		0.41 ± 0.04	> 1	0.41
Celecoxib		67 ± 3.31	> 1	0.43 ± 0.08	2.32

[a] Inhibition (%) values were tested at 10 mM and expressed as the mean S.D. (n = 3).
[b] IC_{50} values are the mean S.D. (n = 3). Meaning the 50% inhibition concentration calculated from regression using four different concentrations (1.0, 0.5, 0.25, 0.125 mM). Values with (*) are statistically similar to the respective positive controls ($P < 0.05$). SI (selectivity Index) = IC_{50} (COX-1)/IC_{50} (COX-2) SI > 1 are selective to COX-2.

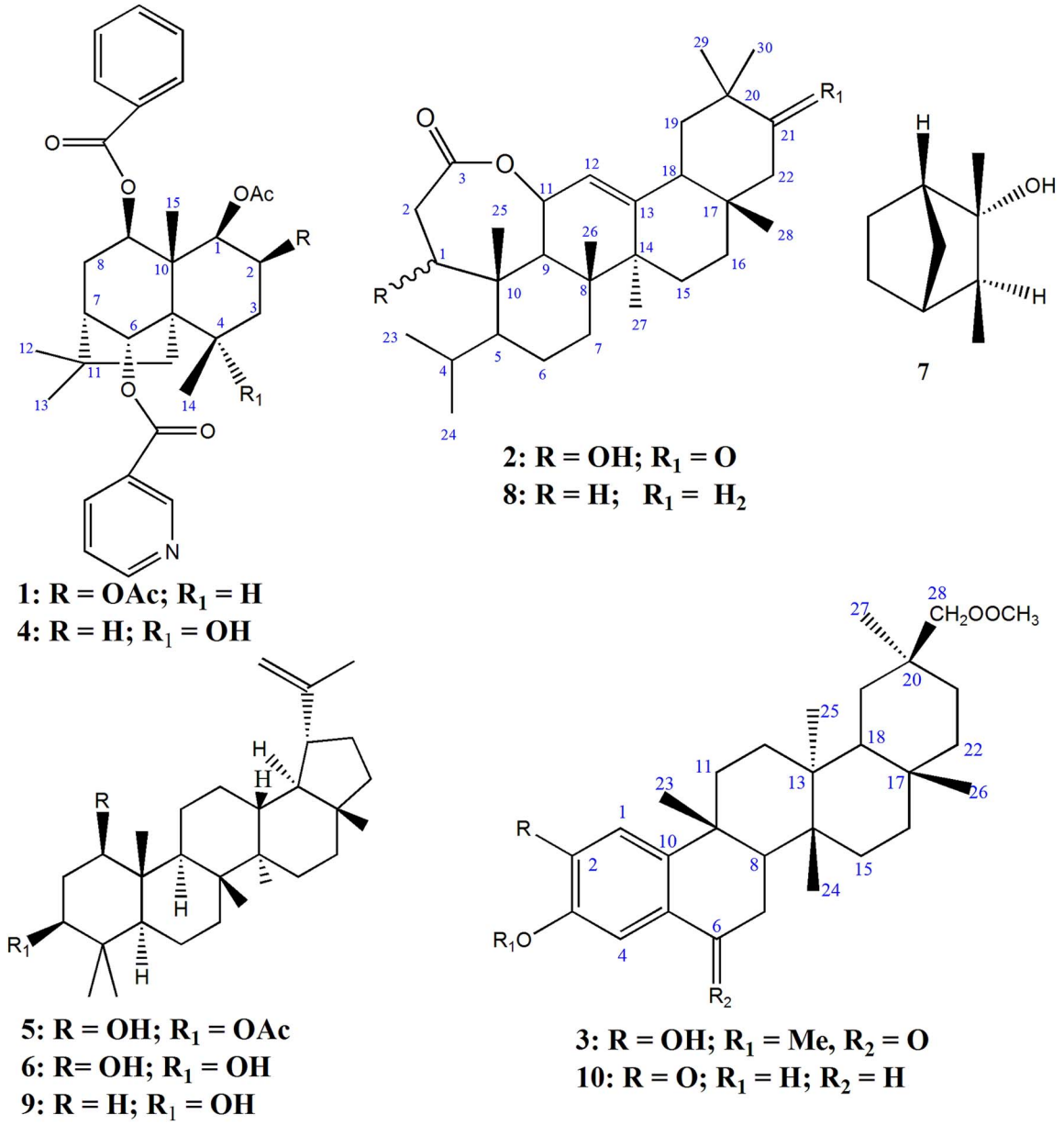

Fig. 1. Compounds isolated from the aerial parts of *Gymnosporia heterophylla*.

NMR signals observed at δ_H 5.94 (1H, br s), 5.68 (1H, d, J = 6.8 Hz), 5.60 (1H, d, J = 4 Hz) and 4.83 (1H, m) were assigned to the four protons attached to the carbons bearing secondary esters which were almost identical with those of 1β-acetoxy-9β-benzoyloxy-2β,6α-dinicotinoyloxy-β-dihydroagarofuran except for the absence of a second nicotinoyl esters [15]. Thus compound **1** was deduced as 1,2,6,9-tetraesterified dihydro-β-agarofuran sesquiterpene alkaloid whose proton and carbon signal were confirmed by the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 2), which established correlations between δ_H 5.60 for H-1 and δ_C 170.5 (1-OC═O) which in turn correlated with methyl signal at δ_H 2.02 and another ^2J correlation to δ_C 69.4 (C-2). Proton on C-2 was assigned as δ_H 4.83 based on its COSY correlation with methylene at δ_H 1.60 and 1.92 (H-3), which in turn coupled with a methine proton (δ_H 2.25, H-4) that coupled with a secondary methyl group (δ_H 1.16, Me-14). This proton (H-1) similarly showed ^3J correlation to another carbonyl carbon at δ_C 169.9 (OC = O) which in turn correlated with methyl ester protons (δ_H 1.95) and a ^2J correlation to (δ_C 76.3, C-1). Generally, in this class of compounds ring B is characterized by an axially configured H-6 which always appears as a singlet [25] and in this particular compound such as singlet showed long range correlation with C-5, C-6, C-8, C-10, and C-11. Overall relative configuration was established based on the NOESY (Fig. 2) spectra which displayed strong cross peaks which showed cross peaks between H-9 and H-15 and between H-6 and H-14/H-15, indicating that H-6 and H-9 are axial and equatorial, respectively. Based on the forgoing facts and compassion of the data with that of heterophyline previously isolated from the leaves of the same plant, compound **1** was identified 1β,2β-diacetoxy-9β-benzoyloxy-6α-nicotinoyloxy-β-dihydroagarofuran.

Compound (**2**) was analyzed for molecular formula $C_{30}H_{46}O_4$ based on the HRTOFMS m/z 493.2536 (cald. for 493.2543 $[M + Na]^+$). The $^{13}C^-$ DEPT-135° (Table 3) spectrum revealed the presence of thirty carbon atoms including eight methyls, seven methylenes and seven methines. Existence of eight methyl peaks suggested a pentacyclic triterpene [26] with two hydroxyl and a vinylic moiety revealed by the ^{1}H NMR (Table 2) signals [δ_H 4.34 (1H, dd, J = 4.8, 7.9 Hz) and 4.54 (1H, d, J = 9.2 Hz)] and δ_H 5.76 (1H, d, J = 2.9 Hz), respectively. Contrary to ^{1}H NMR signals for triterpenes compound **3** displayed a unique pair of doublets for methyl groups at δ_H 1.01 (3H, d, J = 6.8 Hz)

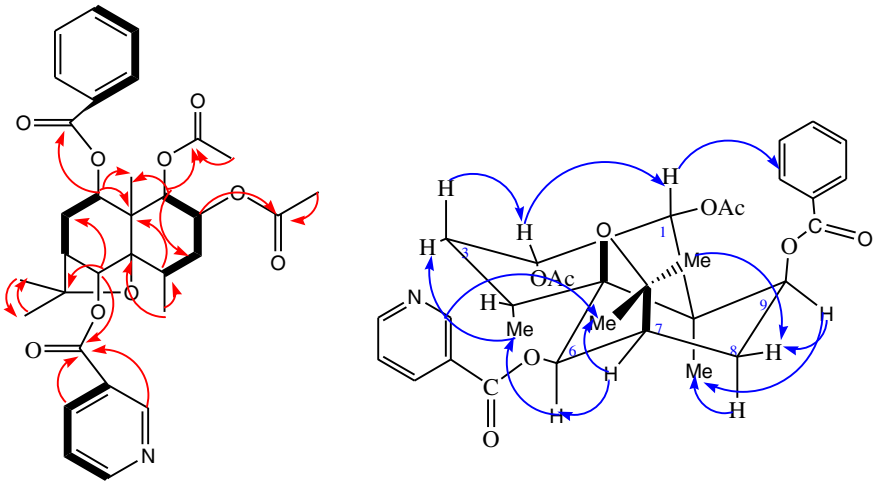

Fig. 2. (A) Main ^{1}H–^{13}C long-range correlation HMBC (→) and ^{1}H–^{1}H correlation COSY (−) and (B) NOESY correlation (→) for compound **1**.

and 1.13 (3H, d, J = 6.7 Hz) both coupled to a multiplet for the methine at δ_H 2.75 (1H, m) and in turn showed mutually HMBC (Fig. 3) correlations indicating the presence of two geminal methyl groups to an aliphatic chain. The above spectral data indicated that compound **2** highly resembled 3,4-*seco*-friedelan-3,11β-olide [21] expect for the presence of an oxymethine proton δ_H 4.34 showing HMBC correlation to δ_C 172.4 (C-3 lactone carbonyl) in **2**. Moreover, HMBC correlation of methylene protons on C-2 [δ_H 3.19 (dd, J = 14.5, 7.9 Hz) and 2.79 (dd, J = 14.5, 4.8 Hz)] to carbonyl C-3 at δ_C 172.4 which in turn showed ^{3}J correlation with proton on C-11 (δ_H 4.54 d) indicated the presence of a C−CH(OH)−CH_2−CO−O−CH a lactone moiety cyclising with an hydroxyl group at C-11 forming an Ɛ-caprolactone. The location of the ketone (δ_H 216.1) was determined based on its HMBC correlation with two methyl groups C-29 and C-30 [δ_H 1.19 s, 1.23 s] and lack of HMBC correlation with H-18 confirmed the ketone functionality at C-21 not C-19. Following the observed spectroscopic evidence, the structure of compound **2** was established as in Fig. 1. The relative stereochemistry was deduced by the NOESY correlations corroborated by the biosynthetic pathway of *seco*-oleanolide. Compound **2** was thus established as 3,4-*seco*- 1-hydroxy-21-oxo-olean-3,11β–olide.

Compound (**3**) assigned a molecular formula $C_{30}H_{42}O_5$ based on ^{1}H and ^{13}C NMR (Tables 2 and 3, respectively) and HRTOFMS observed at m/z at 482.4196. Its IR (KBr) showed bands at 1742 cm^{-1} (Ester carbonyl), 1621 cm^{-1} (α, β – unsaturated carbonyl), 3400 cm^{-1} (OH) and 1298 cm^{-1} (aromatic C−H stretching frequency). The UV spectrum showed maximum absorptions at 241 nm and 304 nm characteristic of an aromatic nucleus and a conjugated ketone, respectively [27]. ^{1}H NMR (Table 2) spectrum showed the presence of a tetra-substituted benzene ring with a *para* oriented singlet protons at δ_H 6.82 (1H, s) and 7.61 (1H, s), two methoxy protons and five aliphatic methyl protons. In support of the data hitherto ^{13}C DEPT-135° spectra showing seven methyls, eight methylenes, four methine and eleven quaternary carbons confirmed a 6-oxophenolic triterpenoid skeleton [28]. A singlet at δ_H 7.61 (H-4) showed HMBC (^{3}J correlation) with keto carbon at δ_C 201.2 (C-6), confirmed the α, β – unsaturation indicated by the IR spectrum. One methoxy proton [δ_H 3.87 (3H, s)] showed a correlation with an aromatic carbon at δ_C 152.8 (C-3) indicating connectivity to the aromatic moiety whereas the other methoxy protons attached on carbon at δ_C 51.5 showed a correlation with a carboxyl carbon at δ_C 179.4, indicating the presence of a carboxymethyl esters (O═$COCH_3$). The carboxyl ester placement was confirmed by the HMBC correlation (Fig. 3) between the methyl proton (H-27) and the carbonyl carbon, which in turn showed ^{3}J correlation with two sets of methylene protons δ_H 1.46/1.58 (both s) and 1.47/1.58 (both s) (H-19 and H-21, respectively). The complete connectivity of the functional groups supported by the literature data, confirmed 2-Methoxy-4-decarboxydihydrozeylasterone (**3**) as a new compound a derivative of zeylasterone isolated from *Gymnosporia blaphorodes* and *Kokoona zeylanica* [27].

3.1. Cyclooxygenase inhibitory activities

Previous anti-inflammatory studies using extracts of *G. heterophylla*

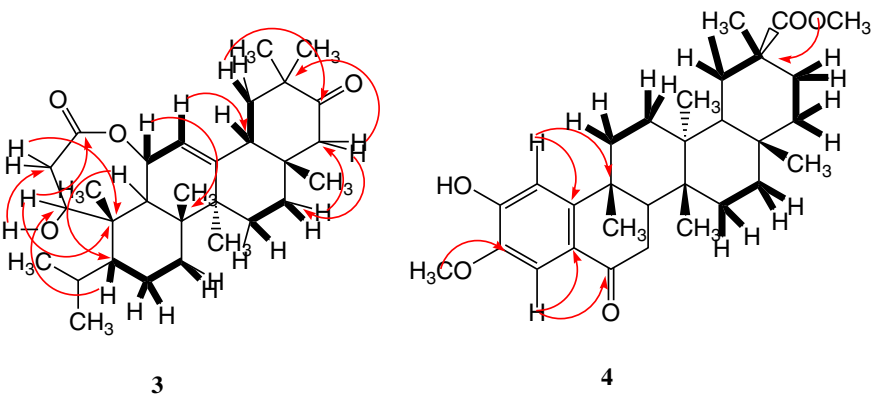

Fig. 3. Main ^{1}H–^{13}C long-range correlation HMBC (→) and ^{1}H–^{1}H correlation COSY (−) for compounds **2** and **3**.

showed up of 51% reduction of paw oedema [10] that was very comparable to indomethacin (positive control) pointing to the potential anti-inflammatory principles which the current study has thus far established (Table 4) using cyclooxyegenase-1 and 2 (COX-1 and -2) inhibitory activity. Except for 3-Hydroxycampane (**7**) and lupeol (**9**), nine of the pure isolates showed varied COX inhibition in the functional assay (Table 3). At concentration of 10 mM dihydro-β-agarofuran sesequiterpene alkaloids **1**, **2** and the triterpenoids **2–10** showed significant COX inhibition compared to the respective controls (Aspirin and Celecoxib). However, only (**1**), (**2**), (**5**), (**6**), (**9**), and (**10**) selectively inhibited COX-2 with selectivity index > 1 (Table 3) based on Griswold and Adams [29] model. The results of this study confirmed the previously reported anti-inflammatory activity for maytenic and pristimerin typical compounds associated with this genus [30,31] and particularly the in *vitro* activity of the 500 μg/ml crude extracts against COX-1 which indicated an overall anti-inflammatory potential [32]. In this study, most of the compounds may have been good inhibitors of COX-2, but still showed COX-1 inhibitions thus may lead to many unwanted side effects. Most compounds with no carboxyl presented better COX-2 selectivity index as would be noticed for lup-20(29)-ene-1β,3β-diol (**6**) (SI 2.22) showing comparable selectively index to NSAIDS (non-selective COX inhibitors) [33]. Most NSAIDs show different effects against COX-1 compared with COX-2, such that low potency against COX-1 compared to COX-2 imply a lower selectivity index and such compounds from *G. heterophylla* would be suggested as good anti-inflammatory activity with reduced side effects on the stomach and kidney [33,34].

4. Conclusion

In conclusion, the study confirmed the presence anti-inflammatory principles from *G. heterophylla* which was in support of the previously established anti-inflammatory activities of the crude extracts of the same plant aerial parts. The results indicated that dihydro-β-agarofuran sesequiterpene alkaloids, triterpenoids are the major anti-inflammatory compounds from the extracts of *G. heterophylla* aerial parts. Such results are important towards development of complementary management alternatives of inflammatory reactions and other disorders besides establishment of the botanical potentials for the traditionally used plant.

Declaration of interest

The authors have declared that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2017.04.015.

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