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Cyclooxygenase inhibitory compounds from *Gymnosporia heterophylla* aerial parts

Charles O. Ochieng[a,*], Sylvia A. Opiyo[a], Edward W. Mureka[a], Ismail O. Ishola[b]

[a] Department of Chemistry, Maseno University, Private Bag, 40105, Maseno, Kenya
[b] Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria

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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.

* Corresponding author.
E-mail addresses: otieno.charles9@gmail.com, cochieng@maseno.ac.ke (C.O. Ochieng).

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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