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ORIGINAL ARTICLE

Commigileadin A: A New Triterpenoid from *Commiphora gileadensis* Aerial Parts

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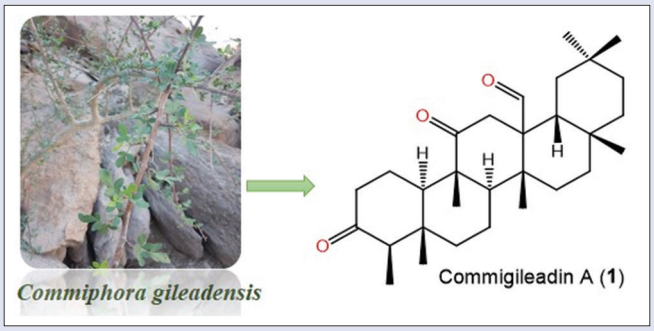
ABSTRACT

Background: Genus *Commiphora* (Burseraceae) includes about 150 species of aromatic shrubs located in Africa, India, and the Arabian region. *C. gileadensis* L. has been used for treating several ailments such as constipation, urinary retention, headache, jaundice, joint pain, liver and stomach diseases, and inflammatory disorders. **Objectives:** In this work, a phytochemical investigation of *C. gileadensis* aerial parts was carried out. Moreover, the antimicrobial potential of the new metabolite was assessed. **Materials and Methods:** The aerial parts were extracted at room temperature with methanol (MeOH). The MeOH extract was subjected to various chromatographic techniques to separate the bio-constituents. Their structures were exhaustively specified by utilizing diversified spectroscopic data and comparing them with the literature. The antimicrobial potential of the new compound **1** was assessed toward *Bacillus cereus, Escherichia coli, Staphylococcus aureus,* and *Clostridium albicans*. **Results:** A new friedelan triterpenoid, commigileadin A [3,11-dioxo-(D: A)-friedo-olean-27-al] (**1**) and four known metabolites, namely, Stigmasterol (**2**), naringenin (**3**), naringenin-4-methyl ether (**4**), and kaempferol (**5**) were obtained. Compound **1** possessed moderate activity toward *B. cereus* (inhibition zone diameter (IZD): 12.6 mm and minimum inhibitory concentration [MIC]: 8.9 µg/mL) in comparison to ciprofloxacin (IZD: 21.1 mm and MIC: 2.5 µg/mL, respectively). **Conclusion:** This work reported the characterization of a new triterpenoid (**1**) and four known constituents (**2-5**). Compound **1** possessed a moderate activity toward *B. cereus*.

Key words: Antimicrobial, Burseraceae, commigileadin, *Commiphora gileadensis*, triterpene

SUMMARY

- A new friedelan triterpenoid (1) and four known constituents (2-5) were separated from the aerial parts of *C. gileadensis*. Their structures were characterized by spectral analyses and comparing with the literature. The antimicrobial potential of the new compound 1 was assessed.



Abbreviations used: 1D: One dimensional; 2D: Two dimensional; CC: Column chromatography; $CDCl_3$: Deuterated chloroform; $CHCl_3$: Chloroform; COSY: Correlation spectroscopy; DMSO: Dimethyl sulfoxide; EIMS: Electronimpact mass spectrometry; EtOAc: Ethyl acetate; HMBC: Heteronuclear multiple bond correlation; HRESIMS: Highresolution electrospray ionization mass spectrometry; HSQC: Heteronuclear singlequantum correlation; IR: Infrared; IZD: Inhibition zone diameter; LTQ: Linear trap quadruple; m.p.: Melting point; MeOH: Methanol; MIC: Minimum inhibitory concentration; NMR: Nuclear magnetic resonance; RP: Reversed phase; SiO_2: Silica gel; TLC: Thinlayer chromatography; UV: Ultraviolet.

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INTRODUCTION

Genus *Commiphora* (family Burseraceae) includes about 150 species of aromatic shrubs located in Africa, India, and the Arabian region.[1] Flora of Saudi Arabia comprises six species of *Commiphora* located mainly in the southwest regions.[2] *Commiphora* resin has been used as a fragrance, food additive, and traditional medicine.[3] Moreover, its essential oils can be utilized in functional foods and cosmetic and pharmaceutical preparations due to the antioxidant potential in the oil substrates.[4] *Commiphora gileadensis* L.[5] is a small non-thorny shrub known as an expensive perfume due to its volatile oil constituents. Its shrub gives oleo-gum resin with a nice fragrant odor after removal of the bark or pressing of its stems.[6] It is known in Arabic as Ood-e-balsam, Bechan, Balessan, Bisham, Balm of Mecca, or Balsam.[3] In Bible, it was given the name "Balm of Gilead" and in Hebrew as "Apharsemon." It produces a white-to-buff oleo-gum resin characterized by its strong aromatic odor. In traditional Arabian medicine, it is used to treat constipation, urinary retention, headache, jaundice, joint pain, liver and stomach diseases, and inflammatory disorders, whereas its twigs are used for teeth brushing.[1] Externally, the exudate from its bark is utilized for treating skin disorders such as burns, wounds, and infections in Oman and Yemen.[7,8] In the Middle East, the aerial parts' decoction is given as a laxative,

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pain reliever, and diuretic.[5] It possesses diversified bio-activities such as anti-cancer, anti-hypertensive, anti-inflammatory, hepato-protective, anti-ulcer, antioxidant, anti-diabetic, antiviral, and antibacterial.[8-12] Its fresh stem oil contains mainly α-calacorene, terpinen-4-ol, δ-cadinene, viridiflorol, and cadalene.[1] The methanol (MeOH) extract of its aerial parts revealed the existence of syringic acid, triterpenoids (oleanonic acid, canophyllal, and friedelin), and flavonols (quercetin and mearnsetin).[13] Moreover, chemical investigation of its oleo-gum resin yielded cycloartane triterpenes and eudesmane and guaiane sesquiterpenes that showed cytotoxic activity on prostate cancer cell lines.[14,15] Herein, the separation and characterization of one new triterpene, Commigileadin A (**1**) and four known compounds (**2**-**5**) from *C. gileadensis* aerial parts have been discussed. Moreover, the antimicrobial potential of the new compound was estimated utilizing a disc diffusion assay.

MATERIALS AND METHODS

General experimental procedures

Ultraviolet (UV) and infrared (IR) spectroscopy were carried out utilizing the UV-Vis Shimadzu 1601 and Infrared Shimadzu 400 spectrophotometers, respectively. A 9100 Digital Electrothermal Melting Point instrument was utilized to get the melting points. Orbitrap LTQ and JMS-SX/SX 102A JEOL spectrometers were used for electronimpact mass spectrometry (EIMS) and High resolution electrospray ionization mass spectrometry (HRESIMS) measurements, respectively. The optical rotation was carried out utilizing the DIP-370 JASCO polarimeter. BRUKER 850 INOVA was utilized for measuring the NMR spectra. Chromatographic separation was accomplished using SiO_2 60 and RP-18. Thin-layer chromatography (TLC) analysis was performed using TLC plates (SiO_2 60 F_{254}).

Plant material

From Al-Baha governorate, *C. gileadensis* aerial parts were collected (May 2017). Dr. Emad Al-Sharif established the plant authentication (Plant Ecology, Biology Department, Faculty of Science and Arts, Khulais, KSA). A Reg. no. CG-0442 specimen was preserved at the Department of Natural Products and Alternative Medicine`s herbarium (KAU, Faculty of Pharmacy).

Extraction and isolation

The powdered *C. gileadensis* aerial parts of (210 g) were extracted at room temperature with methanol (4 × 2 L) using Ultra Turrax. Under reduced pressure, the total MeOH extract was concentrated to produce brown residue (13 g). The residue was slurred with silica gel and partitioned using SiO_2 CC (column chromatography) by $CHCl_3$, EtOAc, and MeOH to produce 4.9 g of $CHCl_3$, 2.1 g of EtOAc, and 5.4 g of MeOH fractions. The $CHCl_3$ fraction (4.5 g) was submitted to SiO_2 CC, eluting with gradient *n*-hexane: EtOAC to obtain four subfractions, namely, CGC-1 (75:25), CGC-2 (50:50), CGC-3 (25:75), and CGC-4 (100% EtOAc). Subfraction CGC-2 (960 mg) was separated on SiO_2 CC (*n*-hexane: EtOAc gradient) to get three fractions, namely, CGC-2A to CGC-2C. SiO_2 CC of CGC-2A (204 mg) (*n*-hexane: EtOAC gradient) afforded **1** (10.2 mg). Fraction CGC-2C (191 mg) was treated similarly to CGC-2A to obtain **2** (9 mg). Subfraction CGC-4 (1.19 g) was subjected to SiO_2 CC (*n*-hexane: EtOAc gradient) to produce two fractions CGC-4A and CGC-4B. Fraction CGC-4A (420 mg) was chromatographed on SiO_2 CC (*n*-hexane: EtOAc gradient) to get **3** (22 mg). Similarly, fraction CGC-4B (501 mg) was treated as CGC-4A to obtain **4** (27 mg). SiO_2 CC of the EtOAC fraction (2.0 g) ($CHCl_3$:MeOH gradient) afforded four subfractions, namely, CGE-1 (90:10), CGE-2 (75:25), CGE-3 (50:50), and CGE-4 (20:80). Subfraction CGE-1 (240 mg) was chromatographed on SiO_2 CC (gradient $CHCl_3$:MeOH) to obtain **5** (14.6 mg of a yellow powder).

Spectral data

Commigileadin A (**1**): White amorphous powder; m.p. 213–215°C (decompose); $[\alpha]^{20}_D$ + 16.8 (*c* 0.2, MeOH); IR 2946, 1720, 1707 cm^{-1}; NMR spectral data, see Table 1; HRESIMS *m*/*z* 455.3451 $[M + H]^+$ (calcd for 455.3447, $C_{30}H_{47}O_3$).

Antimicrobial activity

The antimicrobial capacities of **1** were assessed toward *Candida albicans, Staphylococcus aureus, Escherichia coli*, and *Bacillus cereus* as previously stated procedures.[16-19] Sterile 6-mm filter paper discs were impregnated with tested compounds (20 µL in 5% v/v DMSO) at a concentration of 20 µg/disc. Moreover, the minimum inhibitory concentrations (MICs) were determined using a concentration range of 128–1 µg/mL. Dimethyl sulfoxide (DMSO) 5% v/v was used as a negative control. Ciprofloxacin and clotrimazole were utilized as antibacterial and antifungal standards, respectively.

RESULTS AND DISCUSSION

Isolated metabolite purification

Chemical investigation of the MeOH extract of *C. gileadensis* yielded one new triterpenoidal compound (**1**), along with four known metabolites (**2**-**5**) [Figure 1]. Their structures were elucidated utilizing

Table 1: NMR spectral data of compound 1 ($CDCl_3$, 850 and 212.5 MHz)

No.	δ_H (m, *J* in Hz)	δ_C	HMBC
1	1.96 m	22.3 CH_2	3, 5, 10
2	2.39 dd (13.9, 4.3)	41.5 CH_2	1, 3, 4, 10
	2.28 m		
3	–	213.1 C	–
4	2.22 q (6.8)	58.2 CH	3, 5, 10, 24
5	–	42.0 C	–
6	1.74 dd (12.8, 5.1)	41.1 CH_2	7, 8, 10, 24, 25
	1.42 m		
7	1.36 m	18.1 CH_2	9
	1.32 m		
8	1.38 m	52.8 CH	9, 11
9	–	52.0 C	–
10	1.51 m	59.3 CH	1, 2, 4, 5, 11, 24, 25
11	–	213.2 C	–
12	2.25 d (14.5)	38.8 CH_2	13, 14, 18, 27
	2.07 d (14.5)		
13	–	47.8 C	–
14	–	37.1 C	–
15	1.21 m	32.3 CH_2	13
	0.96 m		
16	1.40 m	33.4 CH_2	14, 28
	1.34 m		
17	–	37.7 C	–
18	2.18 dd (12.8, 4.3)	36.5 CH	12, 13, 17, 27, 28
19	1.42 m	35.0 CH_2	27
	1.23 m		
20	–	28.4 C	–
21	1.36 m	32.4 CH_2	17, 29, 30
	1.22 m		
22	1.45 m	30.6 CH_2	
23	0.87 d (6.8)	6.8 CH_3	3, 4, 5
24	0.71 s	14.6 CH_3	4, 5, 6, 10
25	0.84 s	17.2 CH_3	8, 9, 10, 11
26	0.67 s	20.0 CH_3	8, 13, 14, 15
27	9.47 s	209.2 CH	12, 13, 14, 18, 26
28	1.07 s	18.8 CH_3	17, 18, 22
29	0.98 s	29.4 CH_3	19, 20, 21, 30
30	0.95 s	34.5 CH_3	19, 20, 21, 29

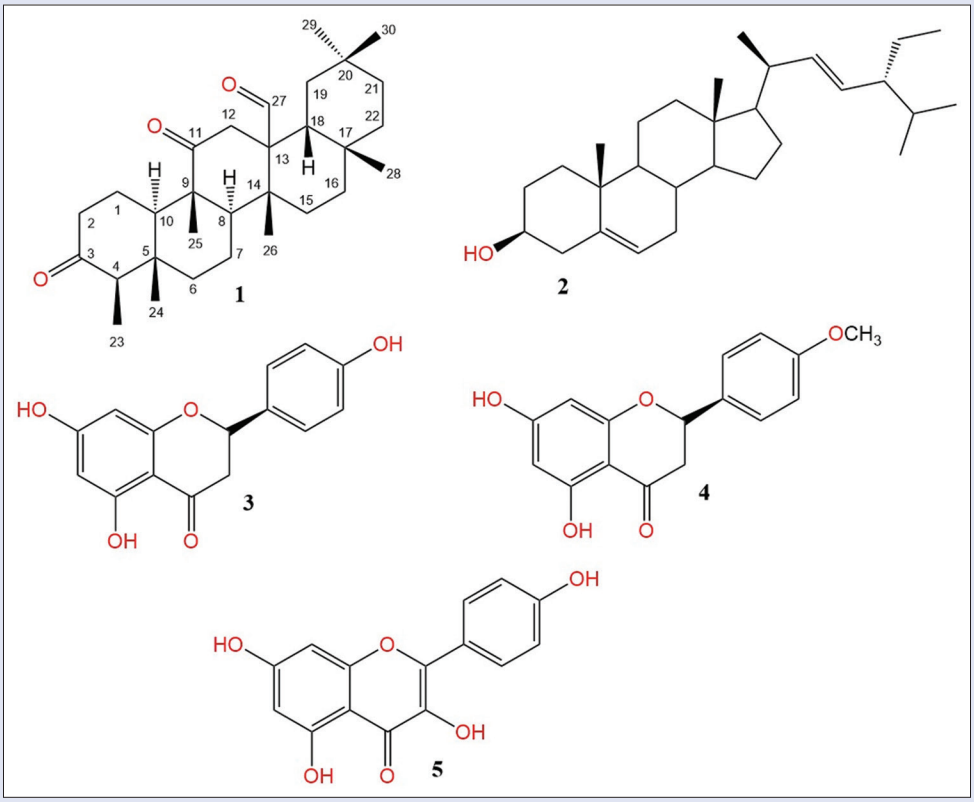

Figure 1: Chemical structures of compounds 1-5

one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR), as well as compared with literature data. The known compounds were identified as stigmasterol (**2**),[13,20] naringenin (**3**),[21] naringenin-4-methyl ether (**4**), (21), and kaempferol (**5**).[22]

Isolated metabolite characterization

Compound **1** was obtained as a white amorphous powder and possessed a positive Liebermann–Burchard reaction, suggesting its triterpenoidal nature.[23,24] It showed a HRESIMS pseudo-molecular peak at *m/z* 455.3451 $[M + H]^+$ (calcd for 455.3447, $C_{30}H_{47}O_3$), compatible with $C_{30}H_{46}O_3$ molecular formula. This formula indicates eight unsaturation degrees that are attributed to three carbonyl groups and a five-ring system. The IR displayed absorptions at 2946, 1720, and 1707 cm^{-1}, compatible with the existence of aliphatic C-H as well as aldehyde and ketone carbonyls, respectively. The ^{13}C and ^{1}H NMR revealed signals for six singlet methyl groups at δ_H 1.07 (H-28)/δ_C 18.8 (C-28), 0.67 (H-26)/20.0 (C-26), 0.95 (H-30)/34.5 (C-30), 0.98 (H-29)/29.4 (C-29), 0.84 (H-25)/17.2 (C-25), and 0.71 (H-24)/14.6 (C-24) and a doublet methyl at δ_H 0.87 (H-23)/δ_C 6.8 (C-23) [Table 1]. The HMBC cross-peaks of H-28/C-17, C-18, and C-22; H-26/C-8, C-13, C-14, and C-15; H-30/C-19, C-20, C-21, and C-29; H-29/C-19, C-20, C-21, and C-30; H-25/C-8, C-9, C-10, and C-11; H-24/C-4, C-5, C-6, and C-10; and H-23/C-5, C-4, and C-3 established the assignment and location of these methyl groups [Figure 2]. Moreover, the aldehydic proton at δ_H 9.47 (H-27) and four methine protons at δ_H 2.22 (H-4), 1.38 (H-8), 1.51 (H-10), and 2.18 (H-18) were noticed. They related to the carbons at δ_C 209.2, 58.2, 52.8, 59.3, and 36.5, respectively, in heteronuclear singlequantum correlation (HSQC). Their locations were secured based on the noticed heteronuclear multiple bond correlation (HMBC) and ^{1}H-^{1}H correlation spectroscopy (COSY) relations [Figure 2]. The ^{1}H NMR displayed signals for two methylene groups at δ_H 2.39 (H-2A) and 2.28 (H-2B) and 2.25 (H-12A) and 2.07 (H-12B). The ^{13}C NMR and HSQC spectra showed 30 carbons; among them, 3 carbonyl carbons at δ_C 209.2, 213.1, and 213.2 were observed. The NMR data indicated that **1** had friedelan-3,11-dione derivative, carrying an aldehyde group.[25,26] The HMBC correlations of the aldehyde proton at δ_H 9.47 to C-12 (δ_C 38.8), C-13 (47.8), C-14 (37.1), C-18 (36.5), and C-26 (20.0) confirmed the positioning of the aldehyde group at C-27. Moreover, the NMR of **1** was consistent with those in the literature for kokoonal[25,27] with the existence of an extra ketonic group at C-11. The placement of the ketone carbonyls at C-3/C-11 was assured based on the correlations of H-4, H-2, H-1, and H-23 to C-3 (δ_C 213.1) and H-8, H-10 and H-25 to C-11 carbonyl (δ_C 213.2) in the HMBC. Based on comparing the *J* values and NMR shifts with those in the literature, the relative configuration of **1** was assigned to be the same as that of kokoonal.[26,27] From the above evidence, **1** was identified as 3,11-dioxo-(D: A)-friedo-olean-27-al and named commigileadin A. This name was given to the compound based on the plant's genus and species names.

Antimicrobial activity

Natural products are a substantial fountainhead of new chemical variety. Many presently accessible antibacterial and antifungal agents have undesired toxicity and their prevalent use has resulted in rapid development of drug-resistant strains, which are the major cause of failure in both agricultural and clinical applications. Thus, the discovery of a novel scaffold for the synthesis and design of new antimicrobial agents for helping in the battle toward pathogenic microorganisms is necessary to overcome the rapidly developed resistance to the existing antimicrobial drugs. It is noteworthy that the MeOH extract of *C. gileadensis* possessed a significant antibacterial potential

Table 2: Results of antimicrobial activity of compound 1

Compd	*S. aureus*		*B. cereus*		*E. coli*		*C. albicans*	
	IZD[c]	MICs[d]	IZD	MICs	IZD	MICs	IZD	MICs
1	9.3±0.19	23.3±1.21	12.6±0.22	8.9±0.11	10.3±0.39	21.1±1.02	7.1±0.09	>100
Cipro.[a]	15.7±0.13	3.7±0.25	21.1±0.29	2.5±0.09	25.1±0.78	3.9±0.18	n.a.	n.a.
Clot.[b]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	17.2±0.13	3.8±0.09

[a]Ciprofloxacin as an antibacterial standard; [b]Clotrimazole as an antifungal standard; [c]IZD, inhibition zone diameter (mm); [d]MICs, minimal inhibitory concentrations (μg/mL); n.a., not applied

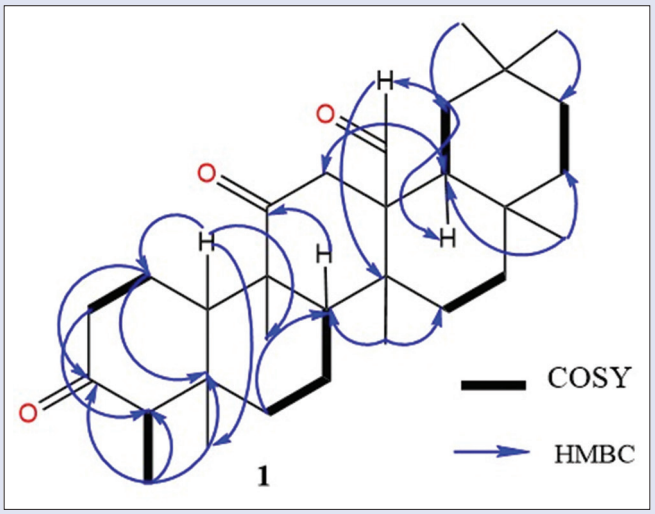

Figure 2: Some key HMBC and COSY correlations of compound 1

toward methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa*.[28] Moreover, it had antimicrobial activity against *Streptococcus salivarius*, *Lactobacillus casei*, *Streptococcus mutans*, *Staphylococcus epidermidis*, and *Fusobacterium nucleatum*.[6] Iluz *et al.*[8] reported that *C. gileadensis* sap possessed an inhibitory potential toward *Bacillus cereus* and blocked the lectins of *P. aeruginosa* that validate the historical uses of the balsam's sap as an antiseptic agent. Therefore, the antimicrobial potential of the new compound **1** was assessed toward *B. cereus*, *E. coli, S. aureus*, and *C. albicans*. The results revealed that **1** had moderate activity toward *B. cereus* (inhibition zone diameter [IZD]: 12.6 mm and MIC: 8.9 μg/mL) compared to ciprofloxacin (IZD: 21.1 mm and MIC: 2.5 μg/mL) and weak activity toward *S. aureus* and *E. coli*, whereas it was inactive toward *C. albicans* [Table 2].

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Conflicts of interest

There are no conflicts of interest.

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