Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Undulaterpene A: A New Triterpene Fatty Acid Ester from *Pulicaria undulata*

Hossam Mohamed Abdallah[1,2], Gamal A. Mohamed[1,3], Sabrin R. M. Ibrahim[4,5], Hani Z. Asfour[6], Maan T. Khayat[7]

[1]Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, [2]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, [3]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut, Egypt, [4]Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Al Madinah Al Munawwarah, Saudi Arabia, [5]Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt, [6]Department of Medical Microbiology and Parasitology, Faculty of Medicine, Princess Al-Jawhara Center of Excellence in Research of Hereditary Disorders, King Abdulaziz University, [7]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia

Submitted: 28-12-2018 Revised: 12-02-2019 Published: 19-09-2019

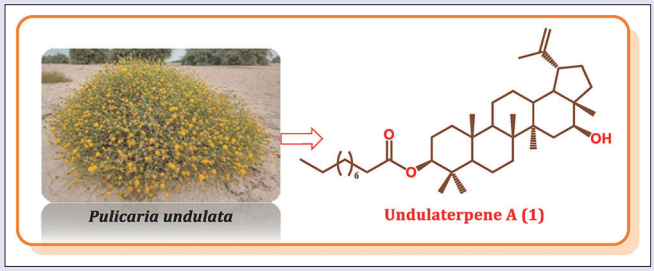
ABSTRACT

Background: Natural products display a remarkable role not only in the synthesis, design, and discovery of new drugs but also as the most prominent source of innovative drugs and bioactive substances. Genus *Pulicaria* (*Asteraceae*) includes about 100 species that are widely distributed in Europe, Asia, and Africa. **Objective:** In this work, the chemical investigation of *Pulicaria undulata* aerial parts was performed. In addition, the cytotoxic activity of the isolated metabolites was estimated toward various cell lines. **Materials and Methods:** Plant extract was subjected to fractionation and different column chromatography to isolate the biometabolites. Their structures were verified using nuclear magnetic resonance, infrared, ultraviolet, and high-resolution mass spectrometry, as well as compared with the literature. The cytotoxic effect was evaluated *in vitro* toward various cell lines: HCT-116 (colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), and A549 (lung carcinoma). **Results:** A new triterpene fatty acid ester, undulaterpene A (1) (3β,16β-dihydroxylup-20 (29)-ene 3-decanoate) and four known metabolites: 3-O-acetyl-pseudotaraxasterol (2), pseudotaraxasterol (3), stigmasterol (4), and tomentosin (5) were separated. Compound 1 displayed cytotoxic potential toward hormone-dependent breast carcinoma cell line (MCF7), colon carcinoma cell line (HCT116), and lung carcinoma cell line (A549) cell lines with half maximal inhibitory concentrations (IC_{50}s) 8.2, 6.9, and 12.4 μM, respectively in comparison to doxorubicin (IC_{50}s 0.14, 0.39, and 1.15 μM, respectively). However, 2, 3, and 4 displayed activity toward HCT-116 with IC_{50}s 13.2, 23.1, and 16.4 μM, respectively. **Conclusion:** This work led to the identification of a new triterpene fatty acid ester (1) and four known metabolites (2–5) from *P. undulata* growing in Saudi Arabia. The new compound showed moderate cytotoxic potential against hormone-dependent breast carcinoma cell line (MCF7), colon carcinoma cell line (HCT116), and lung carcinoma cell line (A549) cancer cell lines.

Key words: *Asteraceae*, cytotoxic activity, *Pulicaria undulata*, triterpenes, undulaterpene A

SUMMARY

- A new triterpene fatty acid ester, undulaterpene A (1), and four known metabolites (2–5) were separated from the aerial parts of *Pulicaria undulata*. Their structural was determined by various spectral analyses. The cytotoxic potential of the isolated metabolites was assessed toward MCF-7, HCT-116, and A549 cell lines using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.



Abbreviations used: CC: Column chromatography; $CHCl_3$: Chloroform; $CDCl_3$: Deuterated chloroform; COSY: Correlation spectroscopy; DMSO: Dimethyl sulfoxide; EIMS: Electron impact mass spectrometry; EtOAc: Ethyl acetate; GCMS: Gas chromatography coupled with mass spectrometry; HCl: Hydrochloric acid; H_2O: Water; HMBC: Heteronuclear multiple bond correlation experiment; HRESI: High-resolution electrospray ionization; HRMS: High-resolution mass spectrometry; HRESIMS: High-resolution electrospray ionization–mass spectrometry; HSQC: Heteronuclear single quantum correlation; IC_{50}: Half maximal inhibitory concentration; IR: Infrared; KBr: Potassium bromide; KOH: Potassium hydroxide; MeOH: Methanol; NMR: Nuclear magnetic resonance; NOESY: Nuclear Overhauser effect spectroscopy; RP: Reversed phase; SiO_2: Silica gel; TLC: Thin-layer chromatography; VIS: Visible; VLC: Vacuum liquid chromatography; UV: Ultraviolet.

Correspondence:

Prof. Hossam Abdallah Mohamed,
Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia.
Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt.
E-mail: hmafifi@kau.edu.sa, hmafifi2013@gmail.com
DOI: 10.4103/pm.pm_668_18

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Natural products display a remarkable role not only in the synthesis, design, and discovery of new drugs but also as the most prominent source of innovative drugs and bioactive substances. Genus *Pulicaria* (tribe *Inuleae* and family *Asteraceae*) includes about 100 species that are widely distributed in Europe, Asia, and Africa.[1] The plants of this genus have been used in traditional medicines for treating various aliments as back pain, inflammation, menstrual cramps, intestinal disorders, dysentery,

Cite this article as: Abdallah HM, Mohamed GA, Ibrahim SR, Asfour HZ, Khayat MT. Undulaterpene A: A new triterpene fatty acid ester from *Pulicaria undulata*. Phcog Mag 2019;15:671-4.

and diarrhea.[2,3] They possessed various bioactivities such as cytotoxic, antipyretic, antioxidant, antispasmodic, antimicrobial, antihistaminic, analgesic, hepatoprotective, anti-inflammatory, cardioprotective, and nephroprotective.[2,4-6] This genus is known to be rich in sesqui-, di-, and tri-terpenoids, phenolics, and sterols.[2,7] Moreover, many sesquiterpenes isolated from this genus have been shown to exhibit a wide range of biological activities.[2,8,9] In course of our ongoing search for bioactive metabolites from Saudi plants, the chemical investigation of *Pulicaria undulata* aerial parts afforded a new lupeol fatty acid ester: undulaterpene A (1) and four known metabolites (2–5) [Figure 1]. The structural elucidation of these compounds was carried out by extensive spectral data analysis. In addition, the cytotoxic activity of the isolated metabolites was evaluated toward various cell lines.

MATERIALS AND METHODS

General experimental procedures

Shimadzu 400 infrared (IR) and Shimadzu 1601 ultraviolet (UV)/visible spectrophotometers were utilized for measuring IR and UV spectra, respectively. Measuring the optical rotation was performed using JASCO DIP-370 polarimeter. Gas chromatography coupled with mass spectrometry (GCMS) analysis was done using Clarus 500 GC-MS under the same conditions as previously stated.[10] Mass spectrometers, LTQ Orbitrap, and JEOL JMS-SX/SX 102A were used for high-resolution electrospray ionization (HRESI) and electron impact mass spectrometry (EIMS) measurements, respectively. Nuclear magnetic resonance (NMR) spectra were performed using INOVA 850 BRUKER. Chromatographic operations were done using reversed phase (RP)-18 and silica gel (SiO_2) 60 (0.04–0.063 mm). Thin-layer chromatography (TLC) plates (SiO_2 60 F_{254}) were utilized for TLC. The compounds were purified using RP-18 LiChrolut extraction tube (6 mL). The ethyl acetate (EtOAc):*N*-hexane systems: S_1 (5:95), S_2 (10:90), and S_3 (15:85) were used for TLC.

Plant material

P. undulata aerial parts were obtained from Gabal Al-Ateeq, Al Madinah Al Munawwarah, Saudi Arabia, in April 2016. The plant identification was carried out based on the library database and morphological features.[11] This was proved by Emad Al-Sharif (Associate Professor of Plant Ecology, King Abdulaziz University). A specimen (PU 2016-1) was stored at the herbarium (Department of Natural Products and Alternative Medicine).

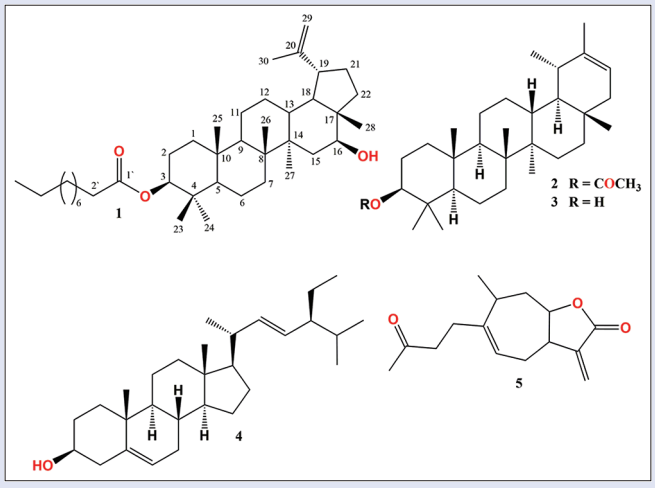


Figure 1: Structures of isolated compounds 1–5

Extraction and isolation

Dried powdered aerial parts (270 g) were extracted with MeOH (4 × 2.5 L). The total concentrated extract (16.4 g) was mixed with distilled water (H_2O) (200 mL). Successively, it was fractionated using chloroform ($CHCl_3$) (4 × 500 mL) and EtOAc (4 × 500 mL). Each fraction was evaporated to obtain $CHCl_3$ (7.3 g), EtOAc (2.3 g), and aqueous (5.5 g) fractions. SiO_2 column of the $CHCl_3$ fraction (7.3 g) using *n*-hexane: EtOAc gradient gave eight subfractions: *Pulicaria undulata* chloroform PUC-1 to PUC-8. Subfraction PUC-3 (590 mg) was submitted to SiO_2 column chromatography (CC) using *n*-hexane:EtOAc gradient to give 1, which was purified on RP-18 LiChrolut extraction tube using gradient H_2O:acetonitrile to get 1 (11.4 mg). SiO_2 CC of PUC-4 (1.2 g) using gradient *n*-hexane:EtOAc afforded impure 2 and 3. They were separately purified on RP-18 LiChrolut extraction tube with gradient H_2O:acetonitrile to yield 2 (21.9 mg) and 3 (13.2 mg). PUC-5 (910 mg) was subjected to SiO_2 CC using gradient *n*-hexane:EtOAc to afford 4, which was purified by repeated SiO_2 CC to obtain 4 (41 mg). PUC-6 (1.1 g) was chromatographed using EtOAc:*N*-hexane: (2:98–30:70) over SiO_2 CC to give 5, which was purified on RP-18 CC using gradient H_2O: MeOH to get 5 (24.7 mg).

Spectral data

Undulaterpene A (1)

White amorphous powder R*f* = 0.54 (S_1); $(\alpha)_D$ + 35.7 (*c* 0.2, MeOH); UV λ_{max} (log ε): 221 (4.21), 239 (3.98) nm; IR (potassium bromide) γ_{max}: 3436, 2954, 1697, 1665, 887, 728 cm^{-1}; NMR data are presented in Table 1; HRESI–mass spectrometry (HRESIMS): M/z 597.5251 $[M+H]^+$ (calcd for 597.5247, $C_{40}H_{69}O_3$).

Alkaline hydrolysis of compound 1

Six milliliters of 3% potassium hydroxide in MeOH was added to 5 mg of 1 and left to stand for 25 min at room temperature. Then, the mixture was neutralized with 1 N hydrochloric acid in MeOH, extracted with $CHCl_3$, and finally concentrated. SiO_2 CC of the residue using gradient *n*-hexane: EtOAc gave a methyl ester of decanoic acid, which was identified by GC-MS and EIMS m/z: 186 $[M]^+$ and GC-MS: t_R 36.6 min.[10]

Cytotoxic assay

The cytotoxic potential of 1 toward MCF-7, A549, and HCT-116 cell lines was assessed as previously. Dimethyl sulfoxide and doxorubicin were the negative and positive controls, respectively.[12-15]

RESULTS AND DISCUSSION

Purification of metabolites

The known metabolites such as 3-O-acetyl-pseudotaraxasterol (2),[16] pseudotaraxasterol (3),[17] stigmasterol (4)[18] and tomentosin (5)[19] were assigned by comparing of their spectral data with literature [Figure 1].

Structural characterization of 1

Compound 1 was separated as white amorphous powder and had a positive Liebermann–Burchard reaction, indicating its triterpenoidal nature.[20] It had a pseudo-molecular ion peak at m/z 597.5251 $[M+H]^+$ (calcd for 597.5247, $C_{40}H_{69}O_3$) in the HRESIMS compatible with $C_{40}H_{68}O_3$ molecular formula, indicating seven double bond equivalent. It displayed UV maxima at 221 and 239 nm. Its IR revealed bands at 1697 (C=O), 3436 (OH), 1665 and 887 (exocyclic double bond) and 728/cm (long aliphatic chain).[21,22] The NMR and MS spectra indicated that 1 was a dihydroxy lupene derivative with a fatty acid moiety [Figures S1-S6].[23-25] The ^{13}C and heteronuclear single-quantum correlation (HSQC) spectra suggested the existence

Table 1: Nuclear magnetic resonance spectral data of compound 1 ($CDCl_3$, 850 and 214 MHz)

Number	δ_H (multiple, J (Hz)	δ_C (multiple)	HMBC
1	1.64, 1.26 m	37.9 CH_2	3
2	1.63 m	23.7 CH_2	4, 10
3	4.46 dd (11.1, 5.1)	80.5 CH	2, 4, 5, 23, 24, 1`
4	-	37.7 C	-
5	0.77 m	55.4 CH	3, 4, 7
6	1.51 m	18.2 CH_2	8, 10
	1.38 m		
7	1.54 m	36.9 CH_2	5, 9
	1.27 m		
8	-	41.0 C	-
9	1.27 m	49.9 CH	5, 8, 10, 12
10	-	37.3 C	-
11	1.42 m	20.9 CH_2	9, 13
	1.20 m		
12	1.68 m	24.7 CH_2	9, 14
	1.03 m		
13	1.28 m	37.2 CH	-
14	-	44.1 C	8, 18
15	1.65 m	38.4 CH_2	8, 17
	0.98 m		
16	3.61 dd (11.1, 4.3)	76.9 CH	18, 22, 28
17	-	48.6 C	-
18	1.38 m	47.6 CH	13, 14, 16, 17
19	2.49 td (11.1, 6.0)	47.7 CH	13, 21, 30
20	-	150.0 C	-
21	1.96 m	29.9 CH_2	17, 20, 22
	1.38 m		
22	1.64 m	37.1 CH_2	17, 19
23	0.84 s	28.0 CH_3	3, 4, 5, 24
24	0.83 s	16.2 CH_3	3, 4, 5, 23
25	0.85 s	16.0 CH_3	1, 5, 9
26	1.03 s	16.6 CH_3	7, 8, 9, 14
27	0.98 s	16.1 CH_3	8, 14, 15
28	0.79 s	11.7 CH_3	16, 17, 22
29	4.70 d (1.7)	109.9 CH_2	19, 21, 30
	4.60 d (1.7)		
30	1.68 s	19.3 CH_3	19, 20, 29
1`	-	173.8 C	-
2`	2.28 t (7.8)	34.1 CH_2	1`, 3`
3`	1.61 m	25.2 CH_2	
4`-7`	1.23-1.26 m	29.1-29.7 CH_2	
8`	1.23 m	31.9 CH_2	15`, 16`
9`	1.27 m	22.7 CH_2	14`, 16`
10`	0.88 t (6.8)	14.1 CH3	14`, 15`

$CDCl_3$: Deuterated chloroform; NMR: Nuclear magnetic resonance; HMBC: Heteronuclear multiple bond correlation experiment

of 40 carbon resonances, including eight methyls, 18 methylenes, and seven methines: two of them for oxymethines at δ_C 80.5 (C-3) and 76.9 (C-16) and seven quaternary carbons including carbonyl (δ_C 173.8, C-1`). The ^1H and ^{13}C NMR showed characteristic signals for six tertiary methyls at δ_H 0.84/δ_C 28.0 (H-23/C-23), 0.83/16.2 (H-24/C-24), 0.85/16.0 (H-25/C-25), 1.03/16.6 (H-26/C-26), 0.98/16.1 (H-27/C-27), and 0.79/11.7 (H-28)/C-28) and an allylic methyl at δ_H 1.68/δ_C 19.3 (H-30/C-30) reminiscent of a lupeol-type triterpene.[10] Their assignment was deduced from the heteronuclear multiple bond correlation (HMBC) cross-peaks of H-26 to C-9, C-7, C-8, and C-14, H-24 and H-23 to C-5 and C-4, H-25 to C-9, C-5, and C-1, H-27 to C-15, C-14, and C-8, H-28 to C-22, C-17 and C-16, and H-30 to C-20, C-19, and C-29. Moreover, two doublet signals at δ_H 4.70 and 4.60 (each d, J = 1.7 Hz, H-29) having HSQC cross-peaks to carbon at δ_C 109.9, together with carbon at δ_C 150.0 (C-20), characterized the existence of an exocyclic methylene. It had cross peaks to C-30, C-19, and C-21 in the HMBC. Furthermore, two oxymethines groups at δ_H 4.46/δ_C 80.5 (H-3/C-3) and 3.61/δ_C 76.9 (H-16/C-16) were observed. Their position was secured by the HMBC cross-peaks of H-1, H-5, H-23 and H-24/C-3 and H-18 and H-28/C-16 [Figure 2], suggesting 1 was a 3β,16β-dihydroxy lup-20 (29)-ene derivative.[24,26,27] In addition, the triplet methylene at (δ_H 2.28, H-2`), terminal methyl ($\delta_H$ 0.88, H-10`) and methylene signals (δ_H 1.23–1.26) characterized a fatty acyl moiety in 1. Further, this was also aided by the ^{13}C signals at δ_C 14.1, 173.8, and 29.1–29.7 for terminal methyl, ester carbonyl group, and long chain of methylene groups, respectively. The fatty acid moiety comprised a C10 chain by alkaline hydrolysis of 1 to afford a methyl ester of decanoic acid that was specified by a molecular ion peak at m/z 186 $[M]^+$ in GC-MS and EIMS and assured by a characteristic peak at m/z 442.3896 $[M + H\text{-}C_{10}H_{19}O]^+$ in HRESIMS. The cross peak of H-3/C-1` in the HMBC and the H-3/C-3 downfield shift confirmed the linkage of the long chain fatty acid moiety at C-3. The relative configuration at C-16 and C-3 was assigned by comparison of the ^{13}C and ^1H NMR shifts with those of related metabolites[24,26,27] and further confirmed by the nuclear Overhauser effect spectroscopy peaks of H-3/H-24, H-5 and H-27 and H-16/H-18 and H-27. Thus, 1 was 3β,16β-dihydroxy lup-20 (29)-ene derivative, having a decanoic acid unit at C-3. From the above evidence and by comparison with literature, 1 was identified as 3β,16β-dihydroxylup-20(29)-ene 3-decanoate and named undulaterpene A.

Figure 2: Key heteronuclear multiple bond correlation correlations of compound 1

Cancer is one of the major causes of death worldwide. The major treatments of cancer are radiotherapy and chemotherapy, which unfortunately proved to be toxic to other living cells of the body. Thus, several studies have focused on natural products as an ideal target for the discovery of potential bioactive metabolites or lead structures for new cytotoxic agents. Triterpenes group is among the bioactive metabolites, which exhibit cytotoxic capacities toward different tumor cells with low effect toward normal cells.[28] Therefore, the isolated metabolites were assessed for their cytotoxic potential toward A549, HCT-116, and MCF-7 cell lines using MTT assay [Figure S7]. Interestingly, 1 displayed cytotoxic potential toward MCF-7, HCT-116, and A549 with half maximal inhibitory concentrations (IC_{50}) 8.2, 6.9, and 12.4 μM, respectively, in comparing with doxorubicin (IC_{50} 0.14, 0.39, and 1.15 μM, respectively). However, 2, 3, and 4 displayed cytotoxic activity toward HCT-116 (IC_{50}s 13.2, 23.1, and 16.4 μM, respectively). Compound 5 exhibited only activity against MCF-7 (IC_{50} 13.1 μM). Moreover, 3 and 4 had moderate activity toward A549 and MCF-7 with IC_{50} values of 22.3 and 17.2 μM and 15.3 and 12.6 μM, respectively, compared to doxorubicin (IC_{50} 1.15 and 0.14 μM, respectively).

CONCLUSION

This study led to the identification of a new triterpene fatty acid ester (1) and four known metabolites (2–5) from the aerial parts of *P. undulata* growing in Saudi Arabia. The new compound showed moderate cytotoxic activity against A549, HCT-116, and MCF-7 cancer cell lines.

Acknowledgements

Authors acknowledge with thanks Deanship of Scientific Research (DSR) for technical and financial support.

Financial support and sponsorship

This project was funded by the DSR at King Abdulaziz University, Jeddah, under grant no. (G-43-166-1439). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Williams CA, Harborne JB, Greenham JR, Grayer RJ, Kite GC, Eagles J. Variations in lipophilic and vacuolar flavonoids among European *Pulicaria* species. Phytochemistry 2003;64:275-83.
2. Liu LL, Yang JL, Shi YP. Phytochemicals and biological activities of *Pulicaria* species. Chem Biodivers 2010;7:327-49.
3. Stavri M, Mathew KT, Gordon A, Shnyder SD, Falconer RA, Gibbons S. Guaianolide sesquiterpenes from *Pulicaria crispa* (Forssk.) oliv. Phytochemistry 2008;69:1915-8.
4. Yusufoglu HS. Analgesic, antipyretic, anti-inflammatory, hepatoprotective and nephritic effects of the aerial parts of *Pulicaria arabica* (Family: *Compositae*) on rats. Asian Pac J Trop Med 2014;7S1:S583-90.
5. Yusufoglu H, Foudah A, Alam A, Soliman G. Cardioprotective and nephroprotective activities of methanolic extracts from *Pulicaria somalensis* herbs against carbon tetrachloride induced toxicity in rats. Planta Med 2016;82:P869.
6. Foudah AI, Alam A, Soliman GA, Salkini MA, Yusufoglu H. Pharmacognostical, antibacterial and antioxidant studies of aerial parts of *Pulicaria somalensis* (Family: *Asteraceae*). Asian J Biol Sci 2016;9:19-26.
7. Ezoubeiri A, Gadhi CA, Fdil N, Benharref A, Jana M, Vanhaelen M. Isolation and antimicrobial activity of two phenolic compounds from *Pulicaria odora* L. J Ethnopharmacol 2005;99:287-92.
8. Rodriguez E, Towers G, Mitchell J. Biological activities of sesquiterpene lactones. Phytochemistry 1976;15:1573-80.
9. Picman AK. Biological activities of sesquiterpene lactones. Biochem Syst Ecol 1986;14:255-81.
10. Al-Musayeib NM, Mohamed GA, Ibrahim SR, Ross SA. Lupeol-3-O-decanoate, a new triterpene ester from *Cadaba farinosa* Forssk. growing in Saudi Arabia. Med Chem Res 2013;22:5297-302.
11. Collenette S. Wild flowers of Saudi Arabia. King of Saudi Arabia: National Commission for Wild Life Conservation and Development (NCWCD). Kingdom of Saudi Arabia: King Fahd National Library; 1999. p. 220.
12. Elkhayat ES, Ibrahim SR, Mohamed GA, Ross SA. Terrenolide S, a new antileishmanial butenolide from the endophytic fungus *Aspergillus terreus*. Nat Prod Res 2016;30:814-20.
13. Borenfreund E, Babich H, Martin-Alguacil N. Rapid chemosensitivity assay with human normal and tumor cells *in vitro*. *In Vitro* Cell Dev Biol 1990;26:1030-4.
14. Ibrahim SR, Abdallah HM, Mohamed GA, Ross SA. Integracides HJ: New tetracyclic triterpenoids from the endophytic fungus *Fusarium* sp. Fitoterapia 2016;112:161-7.
15. Ibrahim SR, Elkhayat ES, Mohamed GA, Fat'hi SM, Ross SA. Fusarithioamide A, a new antimicrobial and cytotoxic benzamide derivative from the endophytic fungus *Fusarium* chlamydosporium. Biochem Biophys Res Commun 2016;479:211-6.
16. Abreu VG, Takahashi JA, Duarte LP, Piló-Veloso D, Junior PA, Alves RO, *et al.* Evaluation of the bactericidal and trypanocidal activities of triterpenes isolated from the leaves, stems, and flowers of *Lychnophora pinaster*. Rev Bras Farmacogn 2011;21:615-21.
17. Mahato SB, Kundu AP. ^{13}C NMR spectra of pentacyclic triterpenoids – A compilation and some salient features. Phytochemistry 1994;37:1517-75.
18. Mohamed GA, Ibrahim SR. Eucalyptone G, a new phloroglucinol derivative and other constituents from *Eucalyptus globulus* Labill. Arkivoc 2007;15:281-91.
19. Kim MR, Lee SK, Kim CS, Kim KS, Moon DC. Phytochemical constituents of *Carpesium macrocephalum* F (R). Et S (AV). Arch Pharm Res 2004;27:1029-33.
20. El-Shanawany MA, Sayed HM, Ibrahim SR, Fayed MA. Stigmasterol tetracosanoate, a new stigmasterol ester from the Egyptian *Blepharis ciliaris*. Drug Res (Stuttg) 2015;65:347-53.
21. Chumkaew P, Kato S, Chantrapromma K. A new triterpenoid ester from the fruits of *Bruguiera parviflora*. Chem Pharm Bull (Tokyo) 2005;53:95-6.
22. Furukawa S, Takagi N, Ikeda T, Ono M, Nafady AM, Nohara T, *et al.* Two novel long-chain alkanoic acid esters of lupeol from alecrim-propolis. Chem Pharm Bull (Tokyo) 2002;50:439-40.
23. Dantanarayana AP, Kumar NS, Sultanbawa MU, Balasubramaniam S. Structures of four new oxygenated lupanes from *Pleurostylia opposita* (Celastraceae). J Chem Soc Perkin 1 1981. p. 2717-23.
24. Hwang BY, Chai HB, Kardono LB, Riswan S, Farnsworth NR, Cordell GA, *et al.* Cytotoxic triterpenes from the twigs of *Celtis philippinensis*. Phytochemistry 2003;62:197-201.
25. Pech GG, Brito WF, Mena GJ, Quijano L. Constituents of *Acacia cedilloi* and *Acacia gaumeri*. Revised structure and complete NMR assignments of resinone. Z Naturforsch C 2002;57:773-6.
26. Zdero C, Bohlmann F, Niemeyer H. Diterpenes from *Nardophyllum lanatum*. Phytochemistry 1990;29:1227-30.
27. Yürüker A, Orjala J, Sticher O, Rali T. Triterpenes from *Rhus taitensis*. Phytochemistry 1998;48:863-6.
28. Zuco V, Supino R, Righetti SC, Cleris L, Marchesi E, Gambacorti-Passerini C, *et al.* Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer Lett 2002;175:17-25.