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Inuviscolide and 4α, 5α-Epoxyinuviscolide from *Inula confertiflora* flower extract

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Abstract

The study was focused on isolations of metabolites from the flowers of *Inula confertiflora* A. Rich (Asteraceae), and during the study two sesquiterpene lactones namely inuviscolide and 4α, 5α-epoxyinuviscolide were obtained along with the ubiquitous metabolites of the plant vis-a-vis graveolide, carabrone, carpesiolin and stigmastero.

Keywords: ***I. confertiflora* flower, inuviscolide, 4α,5α-epoxyinuviscolide, sesquiterpene lactones**

1. Introduction

The Inula genus belongs to the Asteraceae family and distributed all over the world including Africa. Globally, these herbs are known traditionally for the treatment of ailments and thus studied for their phytochemicals of which bioactive sesquiterpene lactones are isolated and characterized [1-8]. The Ethiopian endemic plant, *Inula confertiflora,* is traditionally used to treat leprosy (flower), asthma (leaf) and fumigating the room during childbirth (root) [9-11]. Previous chemical investigations of the root and/or leaf of this plant afforded sterols, triterpenes and sesquiterpene lactones [12,13] and sesquiterpene acids and lactones [8]. Therefore, sesquiterpene lactone isolation and characterization from the flowers of the plant was the focus of this study.

Materials and Methods

Sample Plant Collection

I. confertiflora flower from Ankober Palace Lodge, North Shoa Zone, Ethiopia, was authenticated by professional botanist (Dr. Abiyou Tilahun, Debre Berhan University), and placed at the voucher specimen No. S1152, National Herbarium of Ethiopia, AAU.

Fig. 1 Aerial part of *I. confertiflora* flower (Photo by Minbale Gashu)

1) Chemicals and Instruments

Petroleum ether or n-hexane, chloroform, methanol and ethyl acetate were used as solvent. A TLC (Precoated silica-gel (GF254, 0.25 mm)) was developed using hex and EtOAc mobile phase, and detected by vanillin-sulfuric acid. Mesh size of silica gel (60-120) (Merck) was also used for separation using column. UV light, Perkin-Elmer BX Spectrometer recorded in the range 400-4000 cm^{-1} and Bruker ACQ 400 AVANCE NMR spectrometer (400 MHz) were also used.

Extraction methods

Maceration (3 days) of ground flowers (100 g) with ethanol (95%) at room temperature followed by rotary-evaporator under vacuum gave 10 g extract for further use.

Isolation and characterization of compounds

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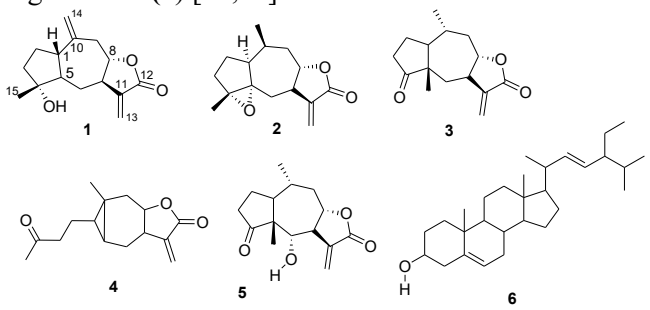
The extract was column chromatographed using hexane: Ethyl acetate as gradient eluent, and 25 pooled fractions (30 ml) were collected by monitoring using TLC. NMR analysis of crystals recrystallized in diethyl ether from Fr13 (12 mg), and white solid obtained from Fr19 (18 mg) showed the presence of three known sesquiterpene lactones. Further purification of Fr11 by column chromatography using hexane: chloroform: Ethyl acetate yielded 20 mg of sterol, 15 mg of **1** and 10 mg of **2**. Characterizations of these compounds made using available techniques and literature reports.

Results and Discussion

Phytochemical analysis of ethanol extract of *Inula confertiflora* flower afforded six compounds; of which the first five are sesqiterpene lactones, namely, inuviscolide (**1**), 4α,5α-epoxyinuviscolide (**2**), graveolide (**3**), carabrone (**4**), carpesiolin (**5**), and stigmasterol **(6)** [12,13].



The first two were isolated for the first time from the flower and the last four were commonly obtained from all parts of the plant**.** The structures of **1** and **2** were elucidated using UV, IR and NMR spectral data analysis as follows.

Characterization of inuviscolide

The gum like component (15 mg, Rf 0.25 in Ethyl acetae: Hexane (1:1)) obtained from the flowers extract had no significant absorption in the UV range in ethanol. The IR bands at (cm^{-1}) 3448 (-O-H), 2949 (-C-H), 1753 (-C=O) and 1660 (C=C) were clearly observed. Two groups of exocyclic methylene protons, α-methylene-γ-lactone moiety and terminal double bond protons at C-10/ C-14, were observed at δ4.33 (H-8, *ddd*, *J*=6.0, 9.2, 12.0), δ6.23 (1H, *J*=3.6 Hz), 5.55 (1H, *J*=3.2Hz), 5.10 (1H,s)/ 4.97(1H, s), respectively. Methyl proton signal (δ1.22) coupled to quaternary carbon were also apparent.

The presence of 15 carbon signals including $6CH_2$, 4CH and $1CH_3$ (δ24.1, C-4) were observed from ^{13}C NMR spectrum. The structural unit named α-methylene-γ-lactone was identified from CH signals (δ45.4 (C-7), δ82.4 (C-8)), signals of exocyclic double bond (C-11, δ139.6 and C-13, 120.5) and lactone carbonyl (C-12, δ170.2). The signals of C-14 (δ111.7) and C-10 (146.6) showed another exocyclic double bond. The C-O signal (δ80.4) of the alcohol function is also described in IR. Guaianolide-type sesquiterpene lactone was predicted from the whole data as proved by ^{1}H-^{1}H COSY spectrum. The correlations of H-13 (δ5.55, 6.23) with H-7 (δ2.67), H-9 (δ2.56, 3.20) with H-14 (δ4.97, 5.10), H-8 (δ4.33) with H-7 and H-9, H-5 (δ1.68) with H-6 (δ1.21, 2.31) and H-15 (δ1.22), and H-7 with H-8 and H-13. The overall data match with inuviscolide (**1**) [8,14] (Table 1) reported from *I. viscosa* and *I. graveolens*.

Table 1. ^{13}C-NMR record of **1** and **2** with reported value of Inuviscolide and epoxyinuviscolide ($CDCl_3$, *δ* in ppm, Mult, *J* in Hz)

Carbon no	1	Inuviscolide	2	Epoxyinuviscolide
1	46.9	46.9	47.7	47.7
2	26.3	26.3	30.6	30.6
3	41.1	41.1	32.7	32.7
4	80.4	80.4	69.9	69.9
5	59.1	59.1	69.7	69.7
6	29.9	29.9	28.9	29.0
7	45.4	45.3	44.4	44.4
8	82.4	82.3	82.6	82.6
9	40.7	40.7	40.4	40.4
10	146.6	146.6	34.6	34.6
11	139.6	139.6	139.1	139.1
12	170.2	170.1	170.0	170.0
13	120.5	120.4	119.8	118.9
14	111.7	111.7	14.6	14.7
15	24.1	24.1	15.5	15.6

Characterization of 4α, 5α-epoxyinuviscolide

Jelly material (10 mg, Rf 0.42 in Ethy acetate:Hexane (1:1)) **(2)** from flowers of *I. confertiflora* showed UV λmax(EtOH) at 275 nm. The strong IR bands at (cm^{-1}) 2937 (-C-H), 1765 (-C=O) and 1656 (C=C) together with ^{1}H and ^{13}C NMR spectra signals indicated similar reported data with 4α, 5α-epoxyinuviscolide. The CH signals (δ 4.05 (1H, *ddd, J*=12.0, 9.0, 6.0, 4.0 Hz)) (oxymethine), δ 2.99 (1H, m), 2.58 (1H,*d*, *J*=8.8 Hz) and 2.11(1H, m), methyl peaks (δ 0.95 (3H, *d*, *J*=9.6 Hz) and 1.38 (3H, s) and doublet proton (δ 5.51 and 6.22) showed the molecular fragments of the molecule. A set of exocyclic methylene group observed as terminal double bond carbon NMR signals (δ 139.1 & 119.8) and the presence of α-methylene-γ-lactone moiety signals were indicated at δ 170.0 and 82.6.

Among 15 signals of the ^{13}C NMR spectrum 4 quaternary carbons, one lactone carbonyl and substituted terminal double bond were detected using DEPT experiment. The δ 69.7 and δ69.9 NMR signals were also quaternary and proposed the existence of an epoxide ring. The ^{1}H-^{1}H COSY correlations between H-13 (δ 5.51, 6.22) and H-7 (δ 2.99), H-7 with H-6 (δ 1.27, 1.86), H-8 (δ 4.05) and H-13 indicated exocyclic α-methylene-γ-lactone moiety. The data represented 4α, 5α-epoxyinuviscolide (**2**) [8,13,14] (Table 1).

In conclusion this study demonstrates the presence of valuable terpenoids in the ethanol extract of the flowers of *I. confertiflora* in common with similar extracts of its leaves and roots. Hence, this medicinal plant as a whole is rich source of important sesquiterpene lactones of medicinal importance.

Conflict of interest

There is no conflict of interest.

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References

1. Sun, C-P.; Jia, Z-L.; Huo, X-K.; Tian, X-G.; Feng, L.; Wang, C.; Zhang, B-J.; Zhao, W-Y. & Ma, X-C. (2021). Medicinal *Inula* Species: Phytochemistry, Biosynthesis, and Bioactivities. *American Journal of Chinese Medicine* 49,315-358; DOI: org/10.1142/S0192415X21500166.
2. Yang, L., Wang, X., Hou, A., Zhang, J., Wang, S., Man, W. & Kuang, H. (2021). A review of the botany, traditional uses, phytochemistry, and pharmacology of the Flos Inulae. *Journal of Ethnopharmacology*, 276, 114125; DOI: 10.1016/j.jep.2021.114125.
3. Bessada, S.M.F; Barreira, J.C.M. & Oliveira, M.B.P. (2015). Asteraceae species with most prominent bioactivity and their potential applications: A review. *Industrial Crops and Products* 76, 604–615; DOI: 10.1016/j.indcrop.2015.07.073.
4. Seca, A. M. L; Grigore, A; Pinto, D. C. G. A. & Silva, A. M. (2014). Review: The genus *Inula* and their metabolites: From ethnopharmacological to medicinal uses. *Journal of Ethnopharmacology* 154, 286–310; DOI: 10.1016/j.jep.2014.04.010.
5. Zhao, Y-M; Zhanga, M-L; Shi, Q-W. & Kiyota, H. (2006). Chemical Constituents of Plants from the Genus *Inula*. *Chemistry and. Biodiversity,* 3, 371-384; DOI: 10.1002/cbdv.200690041.
6. Seca, A. M; Pinto, D. C. & Silva, A. M. (2015). Metabolomic Profile of the Genus *Inula. Chemistry and Biodiversity 12, 859-906;* DOI: org/10.1002/cbdv.201400080
7. Chadwick, M; Trewin, H; Gawthrop, F. & Wagstaff, C. (2013) Sesquiterpenoids Lactones: Benefits to Plants and People. *International Journal of Molecuar Sciences* 14, 12780-12805; DOI: 10.3390/ijms140612780.
8. Ahmed, B.H; Desta, M.A.; Wami, A.A.; Beyene, G.T. & Bedane, K.G. (2024). Sesquiterpene Acids and Lactones from Inula Confertiflora with their Antibacterial Activities. *Chemistry and Biodiversity*, *21*, e202400265; https://doi.org/10.1002/cbdv.202400265
9. Mesfin, T. (2004). Asteraceae (Compositae): Flora of Ethiopia and Eritrea 4 (2), 121-122.
10. Gebre-Mariam, T; Neubert, R.; Schmidt, P.C; Wutzler, P. & Schmidtke, M. (2006). Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders. *Journal of Ethnopharmacology* 104, 182–187; DOI: 10.1016/j.jep.2005.08.071.
11. Beressa, T.B; Deyno, S; Mtewa, A.G; Aidah, N; Tuyiringire, N; Lukubye, B; Weisheit, A; Tolo, C.U. & Ogwang, P.E. (2021). Potential Benefits of Antiviral African Medicinal Plants in the Management of Viral Infections: Systematic Review. *Frontiers of Pharmacol*ogy 12, 682794; DOI: 10.3389/fphar.2021.682794.
12. Gashu, M. (2022). Epifriedelanol and Dammara-20, 24-dien-3-yl Acetate from Ethiopian Inula confertiflora Root Extract. *Journal of Chemistry*. DOI: org/10.1155/2022/2114595
13. Gashu, M., Wolde, E. & Awoke, S. (2023). Sesquiterpene lactones from Inula confertiflora A. Rich (Asteraceae) Leaf. *Letters of Applied NanoBiosci*ence 12(4), 95. DOI: org/10.33263/LIANBS124.095

1. Mossa, J. S; El-Feraly, F. S; Muhammad, I; Zaw, K; Mbwambo, Z .H; Pezzuto, J .M. & Fong, H. H S. (1997). Sesquiterpene Lactones and Thymol Esters from *Vicoa pentanema. Journal of Natural Products* 60, 550-555; DOI: org/10.1021/np960506oSaeed-Ul-Hassan S, Muhammad TA, Imran T, Arshad AS, Sohail A: Evaluation of physicochemical parameters of selected brands of pharmaceutical oils sold in punjab, pakistan. *Latin American Journal of Pharmacy* 2014, 33(1): 115-22.
2. Ixtaina VY, Martínez ML, Spotorno V, Mateo CM, Maestri DM, Diehl BW, Nolasco SM, Tomás MC: Characterization of chia seed oils obtained by pressing and solvent extraction. *Journal of Food Composition and Analysis* 2011, 24(2):166-174.
3. Mehanni AE: Chemical and technological studies on some vegetable oils (Doctoral dissertation, M.

Sc. Thesis, Food Sci. Dept. Fact of Agric. Minia University, Minia, Egypt.

4. Zahir E, Saeed R, Hameed MA, Yousuf A: Study of physicochemical properties of edible oil and evaluation of frying oil quality by Fourier Transform-Infrared (FT-IR) Spectroscopy. *Arabian Journal of Chemistry* 2017, 10: S3870-S3876.