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*Research Article*

# Epifriedelanol and Dammara-20,24-dien-3-yl Acetate from Ethiopian *Inula confertiflora* Root Extract

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Phytochemical investigation of the ethanol extract of the root of *Inula confertiflora* afforded two rare triterpenoids, namely, epifriedelanol and dammara-20,24-dien-3-yl acetate, and their chemical structure was elucidated using appropriate techniques.

## 1. Introduction

The herb *Inula confertiflora* (A. Rich, Asteraceae, called *Weynagift* in *Amh.*) is an endemic medicinal plant found in Ethiopia. It is locally applicable to treat skin diseases caused by viruses, wounds, and eczematous lesions [1]. Traditionally dried roots of *I. confertiflora* are smoked as a fumigant during child birth and used in treating leprosy. Maceration of pounded leaves in water is also applied to diseased eyes of cattle and treating asthma and cough [2].Similarly, the roots of *I. racemosa, I. helenium,* and *I. viscosa* are reported for their medicinal use by the indigenous people of their origin and are highly studied for their phytochemical profile. The root of *I. racemosa* is used for handling asthma-like conditions, reducing cholesterol, supporting healthy circulation, and ensuring healthy heart functions. It is also applicable to the management of diabetes. In Chile, *I. helenium* treats wounds and dandruff, bronchitis, and asthma. In Hungarian traditional medicine, the roots of *I. helenium* are used against asthma, cough, bronchitis, lung disorders, tuberculosis, and infectious and helminthic diseases. The root powders of *I. viscosa* are used against cough, hypertension, and diabetes mellitus. Decoction of *I. viscosa* roots was also used in the treatment of skin irritations of allergic origin in Italian traditional medicine [3, 4].

Chemical constituents of several Inula species known elsewhere were well explored, more than 400 compounds were reported, and they are categorized as terpenoids, flavonoids, and glycosides, etc. Of all the isolated compounds, sesquiterpene lactones were the major constituents [5–7]. They are grouped under eudesmanolides, guaianolides, pseudoguianolides, germacranolides, and xanthanolides [8]. These sesquiterpene lactones showed a wide range of biological properties including anticancer, anti-inflammatory, antifungal, and antibacterial activities [5–9]. In this study, the claims of the traditional healers, production of variety of compounds by other *Inula* species, plant endemicity, and the absence of reported work on phytochemical studies of *I. confertiflora* root were of interest.

## 2. Experimental

*2.1. General.* All chemicals and solvents used were of analytical grade. Melting point was determined using Thomas Hoover capillary melting point apparatus. TLC was performed on 0.25 mm thick layer of precoated silica gel GF254 (Merck) hexane/ethyl acetate as eluent with vanillin-sulfuric acid as detecting reagent. Column chromatography was performed on silica gel (Merck). NMR spectral measurements were done on Bruker ACQ 400 AVANCE spectrometer operating at 400 MHz. The IR spectra were recorded using a Perkin-Elmer BX Spectrometer (400–4000 $cm^{-1}$) in KBr. LC-MS/EIMS was done at Korea Research Institute of Chemical Technology.

## 3. Plant Material and Extraction Method

Root of *I. confertiflora* (Figure 1) was collected from Ankober Palace Lodge, identification was made by a professional


Figure 1: *Inula confertiflora* leaf (left) and root (right) parts (photo by MG).

botanist, and voucher specimen (S1152) of the plant was deposited at the National Herbarium of Ethiopia (AAU). Shade dried roots were ground and placed in brown bottles until used. The plant material (100 g) was macerated using ethanol (95%) with occasional shaking for 72 h, and the solvent was removed under vacuum using rotary evaporator. The crude extract was subjected to phytochemical study.

## 4. Separation, Purification, and Structure Elucidation of Compounds

Separation of the gummy crude extract of plant material into several fractions and purification of the fractions into different compounds were done by using combination of chromatographic techniques (TLC, CC). The structures of purified compounds were elucidated using the data generated by appropriate spectroscopic methods. The ethanol crude extract of the root (4 g) subjected to CC separation using hexane and ethyl acetate of increasing polarity afforded nine pooled fractions. Epifriedelanol (96–39H, 5 mg) was recrystallized from Fr2 in EtOAc. Repeated chromatographic analysis of the mixture of Fr1 and Fr 3-4 in hex:EtOAc as mobile phase afforded fourteen fractions of which dammara-20, 24-dien-3-yl acetate (96–56A, 25 mg) was recrystallized from Fr1 in EtOAc. The structures of the isolated compounds were elucidated using appropraite spectroscopic techniques such as 1H and 13C-NMR and IR and MS and compared with data in the reported literature.

## 5. Results and Discussion

Phytochemical studies on the ethanol crude extract of the root revealed the presence of epifriedelanol (**1**) and dammara-20,24-dien-3-yl acetate (**2**). The structures of these triterpenoid compounds were elucidated by using spectroscopic data and through comparison with facts reported in the literature as follows.

Compound **1** was obtained as colorless solid (Rf0.46 in hex: EtOAc (1 : 1)) from the root of *I. confertiflora*. The IR spectrum showed characteristic absorption bands at 3490 $cm^{-1}$(O-H), 2925/2870 $cm^{-1}$ (C-H). Its $^1$H NMR spectrum contained only one downfield doublet at $\delta$3.75 (1H, $J = 2.8$ Hz) indicating the presence of a proton on an oxygenated carbon (H-3). Although the proton spectrum is complex, there is a proton observed at $\delta$1.92 (1H *dt*, $J = 9.2$, 2.8 Hz, H-2a) showing its correlation with H-3. The spectrum also showed doublet at $\delta$ 0.90 (3H, *d*, $J = 6.8$ Hz, H-23) due to methyl protons attached to a tertiary carbon (C-4) which is related with multiplet signal at $\delta$1.76 (H-4). The seven singlets at $\delta$ 0.87, 0.91, 0.94, 0.95, 0.97, 1.01, and 1.18 are attributed to methyl attached to quaternary carbons. The $^{13}$C-NMR spectrum showed 30 carbon resonances due to six quaternary, five methine, eleven methylene, and eight methyl groups. The presence of a hydroxyl group inferred from IR spectrum was evident in the$^{13}$C NMR spectrum from the appearance of an oxymethine (C-3) carbon signal at $\delta$72.8. The $^1$H and $^{13}$C-NMR spectral data indicate that the compound belongs to the friedelane group of triterpenes. It was identified as epifriedelanol by comparing the spectroscopic data with those reported in the literature [10–12] (Table 1).

The EIMS also showed a molecular ion peak at m/*z* 428 corresponding to the molecular formula $C_{30}H_{52}O$ in agreement with compound **1**.

Compound **2** was isolated as white crystals (Rf 0.44 in EtOAc : Hex (1 : 1) from extract of *I. confertiflora* root. Its molecular formula was determined as $C_{32}H_{52}O_2$ using EIMS analysis (m/*z* 468 ($M^{+\cdot}$)). The IR spectrum exhibited absorption bands at 1728 $cm^{-1}$(C=O) and 2963/2850 $cm^{-1}$ (C-H). The $^1$H NMR spectrum ($CDCL_3$) showed the presence of olefinic methylene proton signals (H-21) at $\delta$ 4.72 (1H, s) and 4.76 (1H, s), vinyl proton (H-24) at $\delta$5.16 (1H, m), and oxymethine proton (H-3) at $\delta$4.50 (1H,m). Two singlets at $\delta$1.63 (H-26) and 1.71 (H-27) due to allylic methyl groups were also observed. Six additional singlets at $\delta$1.31, 1.27,0.89, 0.87, 0.99, and 2.05 corresponding to H-18, H-19, H-28, H-29, H-30, and acetyl methyl protons were also evident, respectively. The $^{13}$C-NMR spectrum ($CDCl_3$) showed thirty-two resolved carbon signals and DEPT-135 spectrum identified them as eight methyl, seven quaternary, eleven methylene, and six methine carbon signals. In the $^{13}$C-NMR spectrum, seven singlets at $\delta$16.3 (C-18), 15.7 (C-19), 25.7 (C-26), 17.7 (C-27), 28.0 (C-28), 15.9(C-29), and 16.5 (C-30) were due to methyl groups.

The $^{13}$C-NMR spectrum also showed the presence of one terminal double bond resonating at $\delta$152.7 (C-20) and 107.5 (C-21) and a substituted olefin at $\delta$124.5 (C-24) and 131.4(C-25). Downfield oxymethine carbon signal ($\delta$80.9) along with

Table 1: $^{13}$C-NMR data of compound **1** and the data reported in the literature for epifriedelanol ($CDCl_3$,$\delta$ in ppm).

| C no | Exp results $\delta$C | Lit results $\delta$C |
|---|---|---|
| **1** | 15.8 | 15.80 |
| **2** | 35.2 | 35.18 |
| **3** | 72.8 | 72.78 |
| **4** | 49.1 | 49.16 |
| **5** | 37.1 | 37.11 |
| **6** | 41.7 | 41.72 |
| **7** | 17.5 | 17.56 |
| **8** | 53.2 | 53.20 |
| **9** | 38.4 | 38.38 |
| **10** | 61.3 | 61.34 |
| **11** | 35.3 | 35.35 |
| **12** | 30.6 | 30.65 |
| **13** | 37.8 | 37.84 |
| **14** | 39.7 | 39.68 |
| **15** | 32.3 | 32.33 |
| **16** | 36.1 | 36.08 |
| **17** | 30.0 | 30.04 |
| **18** | 42.8 | 42.81 |
| **19** | 35.5 | 35.56 |
| **20** | 28.2 | 28.20 |
| **21** | 32.8 | 32.81 |
| **22** | 39.3 | 39.30 |
| **23** | 11.6 | 11.66 |
| **24** | 16.4 | 16.42 |
| **25** | 18.2 | 18.27 |
| **26** | 18.7 | 18.69 |
| **27** | 20.1 | 20.15 |
| **28** | 31.8 | 31.81 |
| **29** | 35.0 | 35.06 |
| **30** | 32.1 | 32.11 |

$\alpha$-methyl ($\delta$21.3) and carbonyl ($\delta$171.0) signals showed the existence of acetyl group. The overall spectroscopic data were in close agreement with the reported values of dammara-20,24-dien-3-yl acetate (**2**) [12, 13] (Table 2).

Epifriedelanol is a triterpenoid natural product previously isolated from the root bark of *Vitis trifolia* with antitumor activity [14] and also obtained from *Anoectochilus chapaensis* showing protein tyrosine phosphatase 1B inhibiting property [15]. *Ulmus davidiana* is also a source of epifriedelanol potent to reduce cellular senescence in human primary cells that control tissue aging or aging-associated diseases [11]. *Quercus variabilis* [16] also contains this bioactive metabolite. Anticancer activity of the compound is also reported [17]. According to the report [12], *I. confertiflora* is rich in sesquiterpene lactones such as graveolide, carabrone, and carpesiolin. In the course, the occurrence of stigmasterol and thymol was also verified. In relation to these metabolites, different studies [3, 4] reported the isolations and characterizations of graveolide from *I. graveolens*, *I. hupehensis*, *I. sericophylla*, *I. hookeri*, and *I. falconeri*; carabrone from *I. viscosa*, *I. falconeri*, *I. cappa*, *I. hookeri*, *I. royleana*, *I. hupehensis*, and *I. helenium*; and carpesiolin from *I. hupehensis*, *I. falconeri*, *I. sericophylla,* and *I. hookeri*. Similar phytochemical investigations also show the presence of stigmasterol in *I. helenium*, *I. cappa*, and *I. salsoloides* [3, 4, 18] and thymol and its derivatives in *I. helenium, I. cuspidate, I. cappa*, and *I. ensifolia* [3, 4]. To the best of the author's knowledge, epifriedelanol and dammara-20,24-dien-3-yl acetate are not ubiquitous in all species of the genus and they are previously reported only from *I. cappa* [3, 4]. In contrast, Ethiopian *I. confertiflora* contained these rare bioactive natural products.

Table 2: $^{13}$C-NMR data of compound **2** and the data reported for dammara-20,24-dien-3-yl acetate ($CDCl_3$,$\delta$ in ppm).

| C no | Exp results $\delta$C | Lit results $\delta$C |
|---|---|---|
| **1** | 38.8 | 37.9 |
| **2** | 23.7 | 23.7 |
| **3** | 80.9 | 80.9 |
| **4** | 37.9 | 38.8 |
| **5** | 56.0 | 56.0 |
| **6** | 18.2 | 18.2 |
| **7** | 35.4 | 35.4 |
| **8** | 40.5 | 40.5 |
| **9** | 50.9 | 50.9 |
| **10** | 37.2 | 37.2 |
| **11** | 21.4 | 21.4 |
| **12** | 24.9 | 25.0 |
| **13** | 45.3 | 45.3 |
| **14** | 49.4 | 49.5 |
| **15** | 31.4 | 31.4 |
| **16** | 27.1 | 27.1 |
| **17** | 47.8 | 47.9 |
| **18** | 16.3 | 16.3 |
| **19** | 15.7 | 15.7 |
| **20** | 152.7 | 152.7 |
| **21** | 107.5 | 107.5 |
| **22** | 34.2 | 34.2 |
| **23** | 28.9 | 28.9 |
| **24** | 124.5 | 124.5 |
| **25** | 131.4 | 131.4 |
| **26** | 25.7 | 25.7 |
| **27** | 17.7 | 17.7 |
| **28** | 28.0 | 28.0 |
| **29** | 15.9 | 15.9 |
| **30** | 16.5 | 16.5 |
| C=O | 171.0 | 170.9 |
| $CH_3$ | 21.3 | 21.3 |

## 6. Conclusion

Chemical investigations on ethanol crude extract of *I. confertiflora* root afforded two rare triterpenes namely, epifriedelanol and dammara-20,24-dien-3-yl acetate. In the same study, the presence of stigmasterol and thymol was also noticed in the plant's root. This study confirms the potential of *I. confertiflora* as the source of bioactive compounds of human importance related to other members of the genus.

## Data Availability

The data generated or analyzed during this study are included within the supplementary information file and can be accessed from https://etd.aau.edu.et/handle/123456789/19320.

## Conflicts of Interest

The author declares that there are no conflicts of interest.

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## Supplementary Materials

NMR data of compounds 1 and 2. (*Supplementary Materials*)

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