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Original Research Article

Separation and structural description of aloin and microdontin from leaf of *Aloe yavellana* reynolds

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Abstract

Employment of succulent leaf of *Aloe yavellana* (Rey.), a ubiquitous plant species restricted to Sidamo floristic region, of Ethiopia, through preparative thin layer chromatography and using spectroscopic techniques led to the separation of two core tricyclic aromatic hydrocarbon (anthrones) compounds known as aloin (**1**), a light yellow powder; R_f = 0.351 (chloroform/methanol: 4:1); UV λ_{max} (methanol): 357, 299, 208 nm; IR ν_{cm}^{-1}: 1085, 1291, 1618, 1631, 3400; ESIMS (-ve mode) with mass-to-charge ratio: 417 $[M\text{-}H]^-$, representing molecular formula $C_{21}H_{22}O_9$ and molecular mass: 418 mu; and microdontin (**2**), a light yellow powder; R_f = 0.582 (chloroform/methanol 4:1); UV λ_{max} (methanol): 311, 302, nm; IR ν_{cm}^{-1}: 1453, 1603, 1168, 1297, 1709, 3415; ESIMS (-ve mode)) with mass-to-charge ratio: 563 $[M - H]^-$, representing molecular formula $C_{30}H_{28}O_{11}$ and molecular mass: 564 mu.

Keywords: Ethiopia, spectroscopic techniques, tricyclic aromatic hydrocarbon, *Aloe yavellana* Reynolds, R_f

1. Introduction

Aloes are perennial plants that exhibit a spacious variety of habitats. They occur in range of sizes from a dwarf rosette about 30 cm high to the tall trees about 12-15 m in height and associate of the genus diverge from small herbs; shrubs and climbers to trees [1].

The genus aloe is principally originated in Africa and it is also indigenous to the Arabian- Peninsula and Jordan, Madagascar and African coast. More than 500 identified species including trees, shrubs and perennials of aloe are known in the world [2-3].

According to the analysis based on the flora account about 40 species of *Aloe* have been described in Ethiopia and Eritrea so far [4] and later research [5-6], 35 (i.e., 87%) of the 40 species of *Aloe* found in the flora area are endemic or near endemic (i.e., have limited allotment in one or few neighboring countries.

The assorted nature of *Aloe* yield may contribute to the varied organic, biological and healing activities that have been experimentally seen as anti-inflammatory and immune-modulator properties and served as bactericidal, virucidal, and fungicidal. It provides a stimulant effect for wound healing, serves as a fuel for proliferating cells and dressing for open wounds [7].

Aloe yavellana (Fig. 1) is a narrow endemic restricted to two localities in Sidamo floristic region, near Yavello town and in the north-eastern slopes of Mega Mountain, Ethiopia, where it occurs in great numbers in forest, in clearings, and on rocks. The specific essence name 'yavellana', orients to the place of growth, Yabello, in Sidamo floristic region from where the species was collected. *Aloe yavellana* belongs to a group of caulescent aloes characterized by erect, ascending or sprawling stems [8].

Fig. 1. Individuals of *Aloe yavellana*; photograph taken 2 Km outside the town of Yabello

A variety of *Aloe* species are used throughout Ethiopia in traditional medicine and for other purposes, given that to its high degree of biodiversity in southern Ethiopia region. *A. yavellana* is by tradition used in the treatments of various disorders [9]. A larval packet test method used for the acaricidal effect of *A. yavellana* in opposition to cattle ticks

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shows its bioacaricidal potential in the treatments of external parasites in animals [10].

Thus, the intention of the present study is to isolate and structurally describe the two core components of tricyclic aromatic hydrocarbons (anthrons) from the succulent leaf of *A. yavellana* a ubiquitous species claimed to possess various biological activities due to the synergistic and individual effects of these two core compounds and other non-separated constituents.

2. Materials and methods

2.1 Plant material

A. yavellana plant was collected on 25/2/2015; within the range of ten to fifteen kilometer around the urban city of Yabello on the main road from Addis Ababa to Moyale, Ethiopia. The plant material was collected for authentication and voucher specimen was kept at Addis Ababa University, Department of Biology, National Herbarium (collection number: TH/03/05) by Professor Demissew Sebsebe.

2.2 Instruments and apparatus

Thin layer chromatography plates used for separation purpose were, a plate spreader silica-gel based PTLC with 0.25 and 0.5 mm thickness. The instruments used for recording ^{1}H NMR, ^{13}C NMR and DEPT-135 running by tow-channel DMX400 FT-NMR spectrometer and ESI-MS were measured by ULTIMATE ^{TM} 3000 standard binary system LC-MS.

2.3 Succulent mass preparation

Succulent mass of *A. yavellana* plant was prepared by slant cutting of the leaves in order to drop out the fluid and leaving it in a light protected shade at room temperature for two to four days to yield dried light yellow powder material.

2.4 Compounds separation

Separation of compounds were performed by dissolving previously prepared dried light-yellow material in pure analytical grade methanol and by means of direct application to PTLC plates (20 cm × 20 cm; 0.5 mm thickness; silica gel). Separated compounds were cleansed by repeated PTLC. Purity of the isolated compound was monitored by pre-coated analytical TLC aluminum sheets silica gel 60 F_{254} (10 cm × 10 cm; 0.25 mm thickness). The solvent system used for both analytical TLC and PTLC was chloroform and methanol (4:1). The chromatograms were observed by UV-VIS spectrometry under 254 and 366 nm wavelengths and the visualized zones were coded as compound **2** and compound **1** based on the descending order of R_f values 0.582 and 0.351 respectively (Fig. 2). Each compound was taken away separately from the plate and dissolved in chloroform:methanol (1:1), then filtered, dried and kept in a tight container for the next experimental use.

2.5 Spectroscopic techniques

Ultraviolet (UV) spectra were recorded under room temperature by dissolving the separated compounds in analytical grade methanol and spectra measured at the range of 200-400nm. IR spectra were measured using KBr pellet method and spectra obtained in the range of 400-4000 cm^{-1}. Electrospray ionization with negative mode method of ESU-Mass spectra measurement also carried out to indicate the relative molecular weight of separated compounds. NMR spectra were recorded on spectrometer operating using duterated methanol. **Aloin** (**1**): light yellow powder; TLC: R_f = 0.351 in chloroform:methanol (4:1); UV λ_{max} (MeOH): 357, 299, 208 nm; IR ν_{cm}^{-1}: 1085, 1291, 1618, 1631, 3400; ESIMS (-ve mode) mass-to-charge ratio: 417 $[M-H]^{-}$, corresponding to a molecular formula $C_{21}H_{22}O_9$ and molecular mass 418 mu; ^{1}H NMR ppm: 2.85-3.55 (*m*, H-1'-H-6'), 4.56 (*s*, H-10), 4.66 (*s*, CH_2, H-11), 6.86 (*brs*, H-7), 6.87 (*brs*, H-2), 7.04 (*brs*, H-4), 7.05 (*brs*, H-5), 7.47 (*t*, *J*=7.9 Hz, H-6), 11.88/11.80 (*s*, OH-8), 11.90/11.82 (*s*, OH-1); ^{13}C NMR ppm: 43.92/44.22 (C-10), 61.44 (C-11), 62.44/62.48 (C-6'), 70.16/70.27 (C-2'), 70.30/70.32 (C-4'), 78.18/78.25 (C-3'), 80.76/80.88 (C-5'), 85.08/85.20 (C-1'), 112.39/112.70 (C-2), 115.41/115.76 (C-7), 115.86 (C-1a), 116.28/117.86 (C-4), 117.09/117.41 (C-8a), 118.92/120.28 (C-5), 135.22/136.08 (C-6), 141.85/142.06 (C-4a), 145.65/145.85 (C-5a), 151.37/152.19 (C-3), 160.82/160.94 (C-8), 160.96/161.17 (C-1), 193.42/193.44 (C-9).

Microdontin (2): Light yellow powder; TLC: R_f = 0.582 in chloroform:methanol (4:1): UV λ_{max} (MeOH): 311, 302, nm; IR ν_{cm}^{-1}: 1453, 1603, 1168, 1297, 1709, 3415; ESIMS (-ve mode) mass-to-charge ratio: 563 $[M - H]^{-}$, corresponding to a molecular formula $C_{30}H_{28}O_{11}$ and molecular mass 564 mu; ^{1}H NMR ppm: 3.11-4.36 (*m*, H-1'-H-6'), 4.55 (*s*, H-10), 4.69 (*s*, CH_2, H-11), 5.92 (*d*, *J*=15.9 Hz, H-8"), 6.82 (*d*, H-3"/H-5"), 6.90 (*brs*, H-7), 6.91 (*brs*, H-2), 7.09 (*brs*, H-4), 7.24 (*d*, *J*=15.9 Hz, H-7"), 7.28 (*brs*, H-5), 7.36 (*d*, H-2"/H-6"), 7.50 (*t*, *J*=7.4 Hz, H-6), 12.01 (*s*, OH-1), 12.17 (*s*, OH-8); ^{13}C NMR ppm: 46.52/46.55 (C-10), 63.11/63.15 (C-6'), 64.55/64.75 (C-11), 71.50 (C-4'), 72.90 (C-2'), 78.30 (C-3'), 81.8 (C-5'), 85.18 (C-1'), 114.21 (C-2), 114.67 (C-8"), 116.68 (C-1a), 116.81 (C-3"/ C-5"), 117.3 (C-7), 117.40 (C-8a), 118.80 (C-4), 121.10 (C-5), 127.24 (C-1"), 131.31 (C-2"/ C-6"), 137.10 (C-6), 143.53 (C-4a), 145.81 (C-5a), 146.81 (C-7"), 152.43 (C-3), 161.25 (C-4"), 163.67 (C-8), 163.78 (C-1), 167.73 (C-9"), 194.80 (C-9).

3. Results and discussion

The application of silica gel based PTLC and make use of analytical TLC for purification on dried light yellow powder material of *A. yavellana* effected in the separation of two core compounds with (R_f) 0.351 and 0.582 in chloroform and methanol (4:1) shown in Fig. 2 as compound 1 and compound 2.

As seen in the thin layer chromatography (Fig. 2), other minor compound(s) were also present with the same anthrone (tricyclic aromatic hydrocarbon) moiety, with compound **1** and compound **2.** It is also expected to be seen the difference in the retention factor (R_f) 0.351 compound **1** and 0.582 compound **2**, leads to the prediction of compound **2** being less polar than compound **1** even if no conclusive evidence can be provided. As well as their appearance and R_f value further differentiation by way of spectroscopic techniques incorporating ESI-MS, ^{1}H, ^{13}C NMR and DEPT-135 spectral data, were given below.

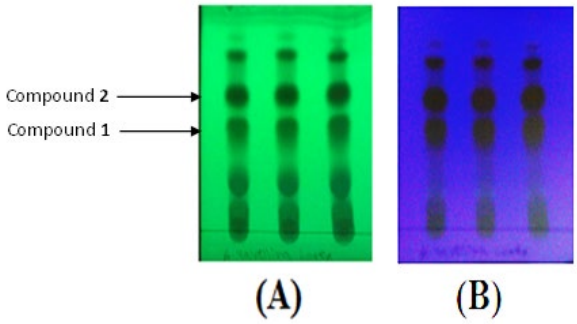

Fig. 2. TLC of the dried light yellow unstructured powder material of *Aloe yavellana* when viewed under UV light of (A): wavelength 254 nm and (B): wavelength 366 nm using chloroform and methanol (4:1) as mobile phase

Compound **1** was separated as a light-yellow powder with R_f value of 0.351 in chloroform and methanol (4:1). A mass–to-charge ratio 417 $[M-H]^-$ was recorded for compound **1** matching to relative molecular weight of 418 mu and molecular formula of $C_{21}H_{22}O_9$ was deduced**.** The appearance of compound **1** either in pairs or overlapping one another propose that the compound is combination of two closely related compounds in both ^{1}H and ^{13}C NMR spectra, for this reason compound **1** was distinctly recognized as **aloin** (Fig. 3) [11].

Compound **2** was also separated as a light-yellow powder with R_f value of 0.582 in chloroform and methanol (4:1). A mass–to-charge ratio 563 $[M-H]^-$ was recorded for compound **2** matching to relative molecular weight of 564 mu and molecular formula of $C_{30}H_{28}O_{11}$ was deduced, which was correlating well with ^{1}H and ^{13}C NMR spectral data as that of compound **1** except additional signal due to *p*-coumaric ester in compound **2** for this reason, compound **2** was distinctly recognized as **microdontin** (Fig. 3) [11].

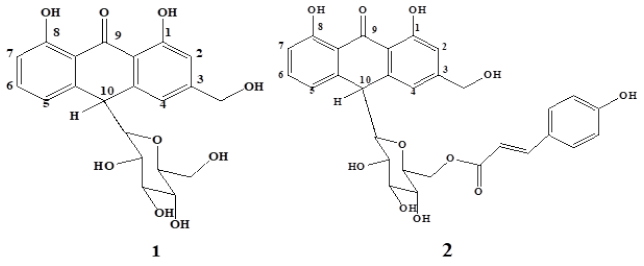

Fig. 3. Chemical structures of the separated compounds from dried light-yellow powder material of *Aloe yavellana* aloin (**1**) and microdontin (**2**)

4. Conclusion

The present study brings to substantiate the separation and structural characterizations of two core compounds aloin **(1)** and microdontin **(2)** from dried light-yellow powder material separated from *A. yavellana* plant species endemic to Southern Ethiopia. Although these two compounds have been separated from different plant species, this is the first report on the separation and structural illustrations of the two core compounds which exemplify that the exposition of biological activity of *A. yavellana.*

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