Isolation and Characterization of Oleanolic Acid Benzoate from the Ethylacetate Leaves Extracts of Vernonia ambigua (Kotschy Ex. Peyr)

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Received 13 July 2020; accepted 27 July 2020, published online 28 August 2020

Abstract

Vernonia ambigua, an annual herb which belongs to the family Asteraceae/Compositae has been used in ethnomedicine to treat different ailments such as fungal infection, diarrhea and intestinal worm among others. The aim of the study was to isolate and characterize compound(s) from the leaves of V. ambigua. Powdered leaf of the plant were gradiently extracted with n-hexane, ethylacetate and methanol using Soxhlet extractor. The ethyl acetate extract was gradiently eluted in a silica gel column and further purified using preparative thin-layer chromatography which led to the isolation of colourless solid substance identified as Oleanolic acid benzoate via chemical tests, 1D-NMR analysis and by comparison with reference spectral data. After thorough exploration of the leaf extract of V. ambigua, using available techniques, it can be concluded that the leaf part of the plant contains a chemical compound suggested to be oleanolic acid benzoate.

Keywords: Vernonia ambigua, leaves, oleanolic acid benzoate, NMR analysis

INTRODUCTION

Vernonia ambigua (Figure 1) commonly known as iron weed is an annual herb which belongs to the family Asteraceae/Compositae. It is called Taba-taba or Tattaba in Hausa and Orungo in Yoruba. The plant is an erect, coarse and bushy in nature which grows up to 600 mm high [1-2]. V. ambigua is widely distributed along the drier parts of Angola, Sudan, Tanzania, Uganda and also dispersed in similar parts of tropical West Africa [2] across Northern part of Nigeria; the plant grows mostly in terrestrial habitat [3-5]. V. ambigua is used traditionally for the treatment of skin diseases, fungal infection, diarrhea and intestinal worm [6]; the plant has also been used in the management of reproductive health issues such as Endometriosis and Sexually Transmitted Diseases (STDs) and the leaves are taken orally for the treatment of male and female infertility [7]; the decoction of the plant, which is bitter, is taken as a remedy for cough and fever and it also has good antimicrobial activity.

Different studies revealed the medicinal and industrial importance of V. ambigua to be attributed to the presence of secondary metabolites in the plant and thus, may be used in the development of drugs for treatment of diseases [8]. The plant have been reported to exhibit antiviral, antibacterial and anti-tumor activities. Studies have also reported that, the plant has the ability to inhibit HIV replication selectively and can be used as diuretic [9]. The leaves (mostly flavonoids constituents) of the plant might have anti-inflammatory, anti-cancer, anti-microbial and anti-allergic activities [10]. Many of these alleged effects of flavonoids are reported to be linked to their strong antioxidants, free radical scavenging and metal chelating properties [11]. V. ambigua has been used by various Traditional Medical Practitioners (TMPs) for the treatment and cure of malaria [12]. Extensive literature search revealed the paucity of research on isolation and characterization of bioactive compounds from V. ambigua, however the class of compounds isolated from other species from the same family include triterpenoids [13], saponins, steroids, polysaccharides, flavonoids, alkaloids, tannins and glycosides [2]. In this paper, we report the isolation and structure elucidation of oleanolic acid benzoate from the leaves of V. ambigua for the first time.
MATERIALS AND METHODS

General experimental procedures
TLC was carried out using silica gel 60 GF\textsubscript{254} pre-coated aluminium sheets by Sigma Aldrich, Germany. Column chromatography was conducted using LOBA Cheme silica gel (60–120 mesh) while preparative TLC was performed using silica gel pre-coated TLC plate 20 cm × 20 cm × 0.25 mm thick. Spots on TLC plates were visualized by spraying with 10 \% H\textsubscript{2}SO\textsubscript{4} followed by heating at 105 °C for 10 min. NMR data were recorded on a Bruker AVANCE III spectrometer (400 MHz) with residual solvent as internal standard. All chemicals and solvents used were of analytical grade.

Collection, identification and preparation of plant sample
The plant sample of *V. ambigua* were collected in June, 2016 at Bagari garden in Gwandu Local Government Area, Kebbi State, Nigeria in the month of June, 2016. The sample was identified and authenticated at Herbarium unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto where voucher specimen number was given as UDUH/ANS/0130. The specimen was preserved in the herbarium unit for future reference. The leaves were shade dried and pulverized using a pestle and mortar and then the powdered sample was stored in separate clean, air-tight polythene bag until required for use.

Extraction and Isolation
Powdered sample (200 g) was sequentially subjected to Soxhlet extraction using 1000 cm\textsuperscript{3} each of n-hexane, ethylacetate and methanol in a round bottom flask for 12 hours. The extracts were freed from solvents *in-vacuo* using rotary evaporator at 40 °C to yield n-hexane, ethylacetate and methanol leaf extracts. The ethylacetate extract of *V. ambigua* was subjected to column chromatography using silica gel (60-120 mesh) in a glass column of 60 cm by 3.5 cm. The column was packed using 100 \% n-hexane and it was gradiently eluted with solvent mixtures such as n-hexane and ethylacetate (9:1; 8:2; 7:3; 6:4 and 1:1) and a total of 121 collections were made and pooled based on their TLC profile to afford 7 major fractions coded A – G.

Preparative Thin Layer Chromatography (PTLC) of fraction F
Fraction F obtained from the column chromatographic separation of the ethylacetate leaf extract of *V. ambigua* was subjected to further purification using PTLC. The fraction was dissolved in ethylacetate and a baseline was drawn about 2.5 cm from the bottom of the TLC plate and the sample was manually applied uniformly with the aid of capillary tube onto the plate and allowed to dry. The dried plate was placed in an air tight chromatographic tank containing n-hexane: ethylacetate (8:2) as mobile phase, the plate was allowed to developed and the area containing band of interest was then marked and scrapped off with clean blade. The scrapped sorbent was transferred into a beaker and dissolved with mixture of n-hexane and ethylacetate. It was then filtered and rinsed with the two solvents which led to the isolation of compound ZC. The compound gave a single homogenous spot using n-hexane: ethylacetate (8:2) and it was subjected to physicochemical tests to elucidate its structure.
RESULTS AND DISCUSSION

Compound ZC was obtained as white powder which gave a brown single homogenous spot on TLC plate with an Rf value of 0.60 (Plate I). It was found to be soluble in n-hexane, chloroform and ethylacetate and it tested positive to Salkowski’s and Liebberman-Buchard’s reagent suggesting the presence of steroids/triterpenes [14]. The 1H-NMR (400 MHz, CDCl3) of compound ZC exhibited chemical shift values typical of triterpenes [15-16]. It indicated the presence of an olefinic proton at δH 5.26 and a carbinolic proton at δH 3.64 which suggests the axial and alpha orientation of the compound and the presence of a proton signal at δH 2.79 alongside seven singlet methyl protons at δH 1.30, 1.28, 1.25, 0.92, 0.89, 0.88 and 0.84 was also observed, typical of triterpenes and the chemical shift values were in close agreement to what was reported for oleanolic acid by Ayatollahi et al. [15]. The chemical shift values at δH 7.26 – 7.72 showed pattern typical of a benzoate group [17]. The 13C NMR (400MHz, CDCl3) of compound ZC was in consistence with the proton NMR; it exhibited chemical shift values typical of triterpenes having five rings composed of an olefinic and one acidic carbonyl carbon atoms within the molecule. The spectrum indicated the presence of 37 seven carbon atoms which include 8 quaternary, 5 tertiary, 10 secondary and 6 methyl carbon signals typical of oleanolic acid [15]; other 7 additional aromatic carbons were due to the presence of benzoic ester ring [17]. Prominent signals at δC 172.30 (C-28) and 122.77 (C-12) were due to the carbonyl carbon of carboxylic acid and olefinic carbon atoms respectively, typical of an oleanolic acid [15].

The presence of a carbinolic carbon atom was observed at δC 79.95 which was assigned to position 3 and the chemical shift value at δC 166.75 was due to the carbonyl carbon of a conjugated ester. The aromatic signals at δC 129.86, 129.20, 129.20, 128.98, 131.39 and 138.83 corresponds to the benzoic ester ring within the molecule [17]. Comparing the chemical shift value at position 3 with several related data in the literature, revealed a relatively higher chemical shift value which presumably suggested that the benzoate group is more likely attached to position C-3 of the oleanolic acid [15-17]. Based on the 1H-NMR, 13C NMR and comparison with available literature by Rodríguez-Gamboa et al. [17] and Ayatollahi et al. [15] (Table 1), the structure of compound ZC was proposed as an oleanolic acid benzoate (Figure 2).

Oleanolic acid and its related derivatives which are pentacyclic triterpenes mostly found in fruits, leaves and stem bark of different edible and medicinal plants have been reported to exhibit a number of pharmacological actions including anticancer, antidiabetic, antimicrobial, anti-parasitic, antioxidant antihypertensive, Hepatoprotective ability, and anti-inflammatory. Determination of their safety, dosage, adverse effects and pharmacokinetic profile in different phases of clinical trials have also been documented [13, 18]. There is no doubt that, oleanolic acid benzoate isolated from V. ambigua contributes to the pharmacological effects of the plant.

Plate I: TLC profile of Oleanolic acid benzoate

Figure 2: Oleanolic acid benzoate C37H52O4
Table 1: $^{13}$C NMR Data of compound ZC ($\text{CDCl}_3$) compared with Literature

| Position | $^{13}$C NMR(ppm) (Reference) | Position | Benzoate $^{13}$C NMR(ppm) (Reference) | $^{13}$C NMR(ppm) (ZC) | Type | Position | Benzoate (ZC) |
|----------|--------------------------------|----------|----------------------------------------|-----------------------|------|----------|---------------|
| 1        | 39.10t                         | 1'       | 129.60                                 | 39.30t                | CH$_2$ | 1'       | 129.86       |
| 2        | 27.90t                         | 2'       | 129.60                                 | 27.88t                | CH$_2$ | 2'       | 129.20       |
| 3        | 79.70d                         | 3'       | 128.4                                  | 79.95d                | CH    | 3'       | 129.20       |
| 4        | 40.00s                         | 4'       | 128.5                                  | 40.12s                | C     | 4'       | 128.98       |
| 5        | 55.90d                         | 5'       | 132.80                                 | 54.32d                | CH    | 5'       | 131.39       |
| 6        | 19.10t                         | 6'       | 128.80                                 | 21.67t                | CH$_2$ | 6'       | 138.83       |
| 7        | 33.40t                         | COO'     | 166.00                                 | 34.10t                | CH$_2$ | COO'     | 166.75       |
| 8        | 40.25s                         |          |                                        | 40.30s                | C     |          |               |
| 9        | 48.32d                         |          |                                        | 49.27d                | CH    |          |               |
| 10       | 37.80s                         |          |                                        | 37.66s                | C     |          |               |
| 11       | 24.00t                         |          |                                        | 23.24t                | CH$_2$ |          |               |
| 12       | 123.30t                        |          |                                        | 122.77t               | CH    |          |               |
| 13       | 143.3d                         |          |                                        | 144.20d               | C     |          |               |
| 14       | 42.40s                         |          |                                        | 41.60s                | C     |          |               |
| 15       | 28.40t                         |          |                                        | 28.64t                | CH$_2$ |          |               |
| 16       | 24.10t                         |          |                                        | 21.96t                | CH$_2$ |          |               |
| 17       | 46.60s                         |          |                                        | 47.61s                | C     |          |               |
| 18       | 41.90d                         |          |                                        | 42.17d                | CH    |          |               |
| 19       | 46.60d                         |          |                                        | 47.50d                | CH$_2$ |          |               |
| 20       | 30.40s                         |          |                                        | 30.90s                | C     |          |               |
| 21       | 34.50t                         |          |                                        | 34.50t                | CH$_2$ |          |               |
| 22       | 31.30t                         |          |                                        | 30.88t                | CH$_2$ |          |               |
| 23       | 28.80q                         |          |                                        | 29.30q                | CH$_3$ |          |               |
| 24       | 16.20q                         |          |                                        | 15.68q                | CH$_3$ |          |               |
| 25       | 16.00q                         |          |                                        | 15.40q                | CH$_3$ |          |               |
| 26       | 17.70q                         |          |                                        | 17.70q                | CH$_3$ |          |               |
| 27       | 26.60q                         |          |                                        | 27.10q                | CH$_3$ |          |               |
| 28       | 172.00s                        |          |                                        | 172.30s               | COOH  |          |               |
| 29       | 33.70t                         |          |                                        | 34.20t                | CH$_3$ |          |               |
| 30       | 24.20q                         |          |                                        | 22.69q                | CH$_3$ |          |               |

(Rodríguez-Gamboa et al., 2001; Ayatollahi et al., 2011; Onoja and Ndukwe, 2013)

**Conclusion**

A known compound Oleanolic acid benzoate was isolated from ethylacetate leaf extract of *Vernonia ambigua* and its chemical structure elucidated using spectroscopic method. To the best of our knowledge this is the first report of isolation of this compound from the plant. Further bioactivity-guided isolation of more bioactive compounds from the plant is recommended.

**Acknowledgements**

We acknowledged the efforts of Natasha October, Department of Chemistry, University of Pretoria, South Africa for running the 1D-NMR analysis.

**Conflict of interest**

None declared.

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