Original Research Article

Naturally Occurring Triterpenic Acid from the *Olea* species

Nur Nabila Fatini Ab Muib, Hannis Fadzillah Mohsin, Syed Adnan Ali Shah, Ibtisam Abdul Wahab*

Department of Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.

Abstract

Olea europaea is originated in the Mediterranean region. It is also commonly known as European olive and is cultivated at various regions since ancient times. From the literatures, the plant has a lot of benefits for common and biological uses. This research covers the general information of *O. europaea* and the analytical investigations of its chemical compounds. The methodology involved the extraction of the secondary metabolites of the dried leaves, followed by the liquid chromatography of the crude extract and spectroscopic techniques. The characterization of the biomolecule from the chloroform extract revealed the presence of oleanolic acid, a natural triterpenic acid.

Keywords: chromatography, *Olea europaea*, spectroscopy, triterpene

Corresponding author

Ibtisam Abdul Wahab
Level 11, FF1 Building, Faculty of Pharmacy,
UiTM Puncak Alam, Bandar Puncak Alam, 42300,
Selangor, Malaysia
ibtisam@uitm.edu.my

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1.0 Introduction

Olea europaea is the famous member of the *Olea* L. (Oleaceae), as it is the only species used for food (1). There are 256 species for this genus, however, only 42 species names are accepted, while the other names are synonyms (2). The botanical information of *Olea* was revised more than a decade ago (3). Later, the biotechnological advances of this commodity was published (4). Various studies including chemometric analysis (5), *in vitro* culture, micro propagation, DNA fingerprinting and studies on genetic diversity of the plant are underway, worldwide. Nevertheless, there is still limited knowledge on the physiology of the olive tree.

Recent review and toxicity studies suggest that the olive leaves are generally safe, even at high doses (6). They contain a large variety of phenolic derivatives, which consist of simple phenols and flavonoids, including the flavones, flavanones, flavonols and 3-flavanols (7). The leaves also contain secoiridoids which consist of oleuropein, ligstroside, dimethyloleuropein and oleoside. The ultrasound-assisted extraction of this plant part, could provide the phenolics (8). A qualitative and quantitative compositional analysis which was carried out by using high performance liquid chromatography (HPLC), coupled with photo diode array detection, has revealed six major polyphenolics in the olive leaf extract. These compounds included oleuropein (24.5%), verbascoside (1.1%), luteolin-7-Oglucoside (1.4%), apigenin-7-O-glucoside, hydroxytyrosol (1.5%) and tyrosol (0.7%) (9). Meanwhile, gas chromatography was reported as a tool to evaluate leaves' triterpenic components (10). The current study focused on the chemical investigations of the biomolecule of *O. europaea.*

2.0 Materials and methods

2.1 Plant material

The dried leaves were purchased from retails in September 2018. Prior to the extraction method, dirt and foreign materials on the leaves, were brushed off beforehand.

2.2 Preparation of plant extract

The maceration was performed at room temperature, by using chloroform, hexane and methanol (Merck) for three (3) days in separate beakers. All the extracts were filtered using Whatman No.1 filter paper into individual round bottom flasks. Next, the filtrates were concentrated and dried under vacuum using a rotary evaporator with 70 rpm at 40^{o}C to obtain the crude extract.

2.3 Thin Layer Chromatography

The compound separation was performed *via* Thin Layer Chromatographic (TLC) techniques. The silica gel 60 F254 on aluminium sheets and on the glass plates (20 x 20 cm) were purchased from Merck (Germany) (11). The visualization was assisted by an ultraviolet (UV) light (short- and long-wavelength, λ = 254 nm and 366 nm, respectively) from a UVP handheld lamp (4 watts), in a Cole-Parmer UV viewing cabinet. The plate staining was monitored by sulphuric anisaldehyde. The bands from the preparative TLC plates were scrapped to obtain the pure compound. Later, it was dissolved in deuterated chloroform ($CDCl_3$). The solvent was filtered to remove the impurities that may present before it was placed into an NMR tube. The ^{1}H-NMR spectra of the compound was recorded on a Bruker DRX-500 NMR spectrometer. This analysis was held at the NMR spectroscopy facility at Faculty of Pharmacy, Universiti Teknologi MARA (UiTM).

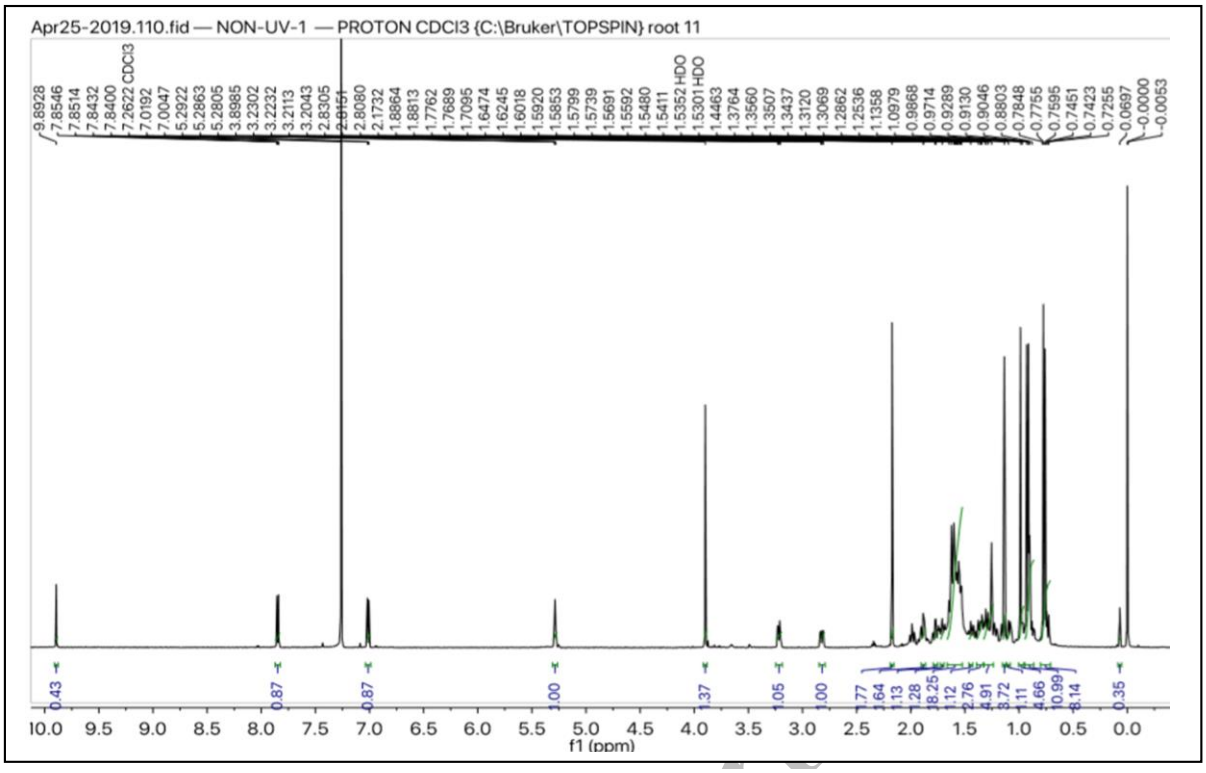

Figure 1: ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) for compound 2 from the chloroform extract of *Olea* leaves.

3.0 Results

The analysis of the olive leaves includes chromatographic approach (12), where the TLC profile was obtained. It is observed that significant amount of compounds can be detected, after the plate was sprayed with sulphuric anisaldehyde. A mixture of two organic solvents, consisting of hexane: ethyl acetate, could be utilized for the spot development, in contrast to the tertiary mobile system of methanol: ethyl acetate: benzene (4:4:2) (11). Dominant spots were detected at retardation factor (Rf) values of 0.5 and above, in a mobile system consisting of hexane:ethyl acetate = 6:4. Next, an optimized method could be discovered, after developing the TLC plate in hexane:ethyl acetate = 7:3.

The chloroform extract was chosen for the preparative TLC technique. The mobile phase was hexane and ethyl acetate, in a ratio of 6:4. Six compounds (**1**-**6**) were separated. However, only compounds **2**, **3**, **4** and **6** were subjected to ^{1}H-NMR spectroscopic analysis. Further data observation on each NMR spectra led to the examination of compound **2** for a detailed discussion. The compound in *O. europaea* chloroform extract was possibly oleanolic acid (13-15).

4.0 Discussion

Referring to the ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) for compound **2**, few signals could be seen, indicating the presence of *p*-anisaldehyde, the staining reagent used during the detection of the terpenes. The peak that appeared in the region of δ_H 9.89 ppm (singlet, 1 H), (Figure 1, Table 1) could indicate the presence of an aldehyde. Furthermore, two peaks were observed in the region of δ_H 7.00 and 7.85 (doublets, J = 6/7 Hz, 2 H), which correspond to the *ortho*-coupled aromatic protons, as in *p*-anisaldehyde. The methoxy group was detected when a singlet was recorded at a chemical shift of δ_H 3.89, equivalent to 3 H. These observations were most probably due to the chemical infusion on the preparative TLC plate.

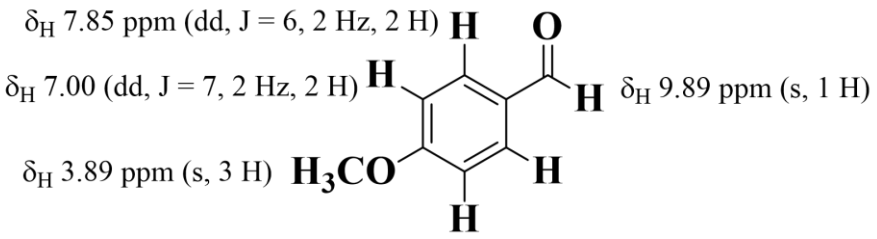

Figure 2: The [1]H-NMR chemical shifts of the protons in *p*-anisaldehyde.

Table 1. The [1]H-NMR data for compound 2 of the chloroform leaf extract.

Multiplicity (J, Hz)	δ_H (ppm)	Integration	Interpretations
s	9.89	1 H	aldehyde proton in *p*-anisaldehyde
dd, J = 6, 2 Hz	7.85	2 H	aromatic protons, *ortho*-coupling in *p*-anisaldehyde
	7.26		deuterated chloroform
dd, J = 7, 2 Hz	7.00	2 H	aromatic protons, *ortho*-coupling in *p*-anisaldehyde
t, J = 3 Hz	5.29	1 H	-C**H**-, H-12, the olefinic proton of oleanolic acid
s	3.89	3 H	methoxy group in *p*-anisaldehyde
dd, J = 9, 3 Hz	3.22	1 H	-C**H**OH-, H-3 of oleanolic acid
dd, J = 8, 3 Hz	2.82	1 H	-C**H**-, H-18 of oleanolic acid
ddd	1.98	1 H	-C**H**$_2$-, H-16 of oleanolic acid
m	1.77		-C**H**$_2$-, H-2 of oleanolic acid
m	1.88	1 H	-C**H**$_2$-, H-11 of oleanolic acid
m	1.71		-C**H**-, H-9 of oleanolic acid
m	1.76		-C**H**$_2$-, H-19 of oleanolic acid
m	1.77		-C**H**$_2$-, H-22 of oleanolic acid
m	1.54		-C**H**$_2$-, H-7 of oleanolic acid
m	1.44		-C**H**$_2$-, H-6 of oleanolic acid
m	1.43		-C**H**$_2$-, H-21 of oleanolic acid
s	1.35	C-27	-CH_3, methyl group
m	1.10		-C**H**$_2$-, H-15 of oleanolic acid
s	0.99	C-23	-CH_3, methyl group
s	0.93	C-30	-CH_3, methyl group
s	0.91	C-25	-CH_3, methyl group
s	0.90	C-29	-CH_3, methyl group
m	0.88	1 H	-C**H**-, H-5 of oleanolic acid
s	0.76	C-26	-CH_3, methyl group
s	0.78	C-24	-CH_3, methyl group

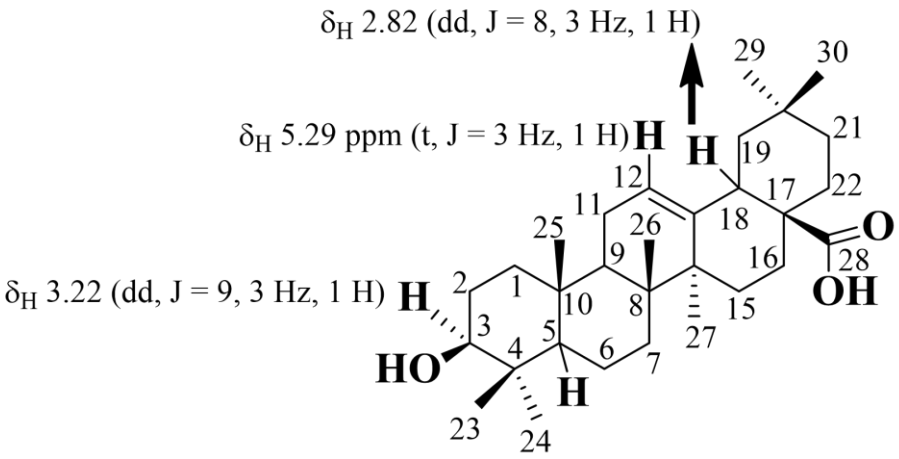

Figure 3: The ^{1}H-NMR chemical shifts of the protons in oleanolic

There is a possibility of an instant absorption of *p*-anisaldehyde from both sides of the plate into the middle part where the band was scrapped out during the purification step.

5.0 Conclusion

A number of articles mentioned the benefit of the oil from *O. europaea*. It is hoped that the phytochemical investigations on *Olea* should be further extended, in order to explore the therapeutic potential of this plant, particularly *in vivo* studies. The spectroscopic analysis of the isolated biomolecule has revealed the presence of oleanolic acid. It is anticipated that this sacred medicinal plant could be utilized for clinical therapy.

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Conflicts of interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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