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Isolation of ursolic acid from the leaves of *Ocimum lamiifolium* collected from Addis Ababa Area, Ethiopia

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Ocimum lamiifolium **is a well known medicinal plant in Ethiopia. Fresh leaves of the plant are squeezed and sniffed to treat colds and coughs and as an eye rinse for eye infections, while crushed leaves are used to arrest nose bleeding. In this study phytochemical investigation on the methanol extracts of this plant collected from Addis Ketema subcity, Addis Ababa. A triterpene, namely ursolic acid, was isolated using column chromatography over silica gel. The structure of this compound was identified using one dimensional Nuclear Magnetic Resonance (NMR) spectroscopic techniques such as proton NMR (^{1}H NMR), carbon-13 NMR (^{13}C NMR) and distortionless enhancement by polarization transfer (DEPT), two dimensional NMR spectroscopic method such as HMBC, HMQC and COSY and literature survaye.**

Key words: *Ocimum lamiifolium*, Ethiopia, phytochemical investigation, ursolic acid, Nuclear Magnetic Resonance (NMR) spectroscopy.

INTRODUCTION

Natural products are believed to play vital roles in the physiology and ecology of the plants that produce them, particularly as defense elements against pests and pathogens or as attractants for beneficial organisms such as insect pollinators. Because of their biological activities, some plant natural products have been exploited by human beings as pharmaceuticals, stimulants, and poisons (Hadush et al., 2016). Natural products are organic compounds that are formed by living systems. The elucidation of their structures and their chemistry, synthesis and biosynthesis are major areas of organic chemistry. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites. (Hadush et al., 2016).

People living in the villages of Africa, Asia and other parts of the developing world are forced to resort traditional practitioners and to use traditional medicine for the continued maintenance of their health and to alleviate their diverse sufferings. The World Health Organization (WHO) estimated about 80% of the people in the developing countries relies on traditional medicine for primary health care needs, of which a major proportion corresponds to plant extracts (Sarah et al., 2010). Herbal remedies have been used for centuries but more recently, the compounds that are active have been identified, extracted and purified. Synthetic organic chemists have then been able to produce the molecules *in vitro* and so produce them on large scales (Daniel et al., 2012).

Ocimum lamiifolium is a plant which belongs to the

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genus *Ocimum* in the family lamiaceae and it is an erect, hairy perennial, robust branching shrub or herb growing to 3 m tall. It grows beside roads and streams, in bush land and at forest edged and on grassland between 1200-2900 m (Raimo and Yvonne, 1999). *O. lamiifolium* has been used in folk medicine worldwide since time immemorial. It is used to treat diseases like hepatitis, gonorrhea, gastric ulcer, asthma and dropsy. It is used as food additive in Tanzania (Runyoro et al., 2010). In Ethiopia, the plant is known by its vernacular name as 'dama-kassie'. Fresh leaves of *O. lamiifolium* are squeezed and sniffed to treat colds and coughs; and as an eye rinse for eye infections, while crushed leaves are used to arrest nose bleeding. It is also used for the treatment of inflammatory conditions and infections and to treat ailments associated with pyrexia, eye disease, cough, cold, cutaneous leishmaniasis, headache, herpes (kusil). Mich is also treated traditionally by squeezing and sniffing fresh leaves of *O. lamiifolium* (Runyoro et al., 2010).

In Ethiopia there are 12 *Ocimum* species namely *Ocimum stirbeyi, Ocimum forskolei, Ocimum basilicum, Ocimum americanum, Ocimum ciricinatum, Ocimum jamessi, Ocimum spicatum, Ocimum cufodontii, Ocimum gratissimum, Ocimum urticifolium, Ocimum tricodon* and *Ocimum lamiifolium* (Edwards et al., 2000). Among these species *O. lamiifolium* and *O. basilicum* are widely used in traditional medicine and as culinary herb, respectively.

Literature survey showed that the isolation of only a flavonoid which is Quercetin3-*O* xylosyl (1'''→2'') Galactoside from the *O. lamiifolium* plant found in Ethiopia (Grayer, 2002). To the best of my knowledge no other phytochemical investigation was reported. In this thesis research attempt will be made to isolate phytochemicals from the leaves of *O. lamiifolium*. The main objective of this work is to undertake phytochemical study on the methanol extract of the leaves of *O. lamiifolium* and to elucidate structures of the components of the methanol extract of the plant. The plant was selected for this study because of limited study on the phytochemical constituents of the plant and importance of the plant in traditional medicine in Ethiopia.

MATERIALS AND METHODS

Phytochemical investigation

General

^{1}H, ^{13}C, DEPT and 2D NMR experiments were recorded using a Bruker 400 MHz Advance NMR spectrometer in $CDCl_3$ and deuutrated methanol. Thin layer chromatography (TLC) was performed on a 0.25 mm thick silica gel GF_{254} (Merck). Melting points were recorded using Thomas HOOVER capillary melting point apparatus. Components of the *O. lamiifolium* on TLC were detected by their UV fluorescence and by spraying with 1% vanillin in H_2SO_4.

Plant material

Leaves of *O. lamiifolium* were collected from home grown plant around Addis Ketema subcity, Addis Ababa. A voucher specimen (AAOL001) was deposited at the National Herbarium (ETH), Department of Biology, Addis Ababa University, Addis Ababa.

Isolation and analysis

990 g of powdered leaf of *O. lamiifolium* was first extracted with petroleum ether. The marc from the extract was then soaked with methanol.

Methanol extract

40 g of the crude extract from methanol was applied to a silica gel (251 g) column chromatography and eluted with solvent system changing continuously from pet. Ether: EtOAc (9:1) to pet. Ether: EtOAc (4:6) and finally with net ethyl acetate and net methanol. 34 fractions were collected and TLC analysis was made. According to their TLC profile, the 34 fractions were reduced to 5 fractions. This is made by comparison of their R_f value, fractions having similar Rf value are mixed.

RESULTS AND DISCUSSION

Phytochemical investigation

Ground aerial parts (990 g) of *O. lamiifolium* were subjected to exhaustive extraction successively with petrol ether, chloroform and methanol. The solvent from each extract was recovered under reduced pressure using rotavapor to obtain a petrol extract (PE,54 g), a chloroform extract (CE, 52 g) and a methanol extract (ME, 40 g) respectively. Chromatographic purification of the methanol extract gave a compound coded; OLM-1. The structure of this compound has been elucidated on the basis of spectroscopic evidences and in comparision with literature data for similar compounds. Characterizations of compound OLM-1

Compound OLM-1 was a pale yellow crystalline solid with melting point of 250-251°C obtained from methanol extract and its characterization was determined using spectroscopic techniques. The COSY spectrum did not give much information as most of the hydrogens are overlapping in the chemical shift region of δ 1.26-1.71 ppm. So it was impossible to unambiguously assign all the signals for each hydrogen by this method. Therefore, most of the NMR assignments were based on HMBC correlations. Only unambiguously assigned signals were taken into consideration when determining this structure. These correlations are shown in Table 1.

The ^{1}H spectrum (Table 1) indicated the presence of quaternary methyl groups [(δ 0.79, 0.86, 0.99, 1.13, 3H each, s) and (0.87 and 0.95, 3H each, d)]. A downfield doublet integrated for one proton at δ 5.24 coupled with the methylene multiplet at δ 1.93 was assigned to the methane proton on the olefinic carbon. A downfield

Table 1. ^{1}H, ^{13}C and COSY ($^{1}H \leftrightarrow {}^{1}H$) NMR data of compound 1 (in MeOH, δ in ppm).

C no.	δ_C	δ_H	COSY
C_1	38.45	1.68	$H^1 \leftrightarrow H^2$
C_2	26.51	1.64	$H^2 \leftrightarrow H^1$, $H^2 \leftrightarrow H^3$, $H^2 \leftrightarrow H^{20}$
C_3	78.31	3.16	$H^3 \leftrightarrow H^2$
C_4	39.17		-
C_5	55.35	0.75	$H^5 \leftrightarrow H^6$
C_6	18.09	1.37	$H^6 \leftrightarrow H^5$
C_7	32.95	1.54	-
C_8	39.39		-
C_9	46.24	1.56	$H^9 \leftrightarrow H^{11}$, $H^9 \leftrightarrow H^{15}$
C_{10}	36.78		
C_{11}	22.67	1.93	$H^{11} \leftrightarrow H^9$, $H^{11} \leftrightarrow H^{12}$, $H^11 \leftrightarrow H^{15}$
C_{12}	125.50	5.24	$H^{12} \leftrightarrow H^{11}$
C_{13}	138.23		-
C_{14}	41.84		-
C_{15}	27.82	1.09	$H^{15} \leftrightarrow H^9$, $H^{15} \leftrightarrow H^{12}$, $H^{15} \leftrightarrow H^{16}$
C_{16}	23.93	2.03	$H^{16} \leftrightarrow H^{15}$, $H^{16} \leftrightarrow H^{22}$
C_{17}	46.97		-
C_{18}	52.97	2.20	$H^{18} \leftrightarrow H^{19}$
C_{19}	39.03	1.36	$H^{19} \leftrightarrow H^{18}$
C_{20}	38.61	1.67	$H^{20} \leftrightarrow H^2$
C_{21}	30.38	1.49	$H^{21} \leftrightarrow H^{22}$
C_{22}	36.72	1.00	$H^{22} \leftrightarrow H^{21}$
C_{23}	27.36	0.99	$H^{23} \leftrightarrow H^{24}$
C_{24}	14.94	0.79	$H^{24} \leftrightarrow H^{23}$
C_{25}	15.002	0.95	-
C_{26}	16.27	0.86	-
C_{27}	23.14	1.13	-
C_{28}	180.21		-
C_{29}	16.33	0.87	-
C_{30}	20.19	0.95	-

doublet of doublet at δ 3.16 was assigned for the methine proton on the carbon which bears the hydroxyl group. Five other methine protons appeared at δ 0.75 (1H, d, $J_{5, 6}$ = 11.4Hz), 1.36 (1H, m), 1.56 (1H, m), 1.67 (1H, m), 2.20 (1H, $J_{18, 19}$ = 11.4 Hz). Nine CH_2 methylene protons appeared at δ 1.00 (1H, dd, $J_{21, 22}$ = 3.6 Hz, 13.2 Hz), 1.09 (1H, m), 1.37 (1H, m), 1.49 (1H, m), 1.54 (1H, m), 1.57 (1H, m), 1.68 (1H, m), 1.93 (1H, m) and 2.03 (1H, m). The ^{13}C NMR and DEPT-135 (Table 2) indicated that OLM-1 has a total of 30 carbon atoms : seven methyls (δ_C 15.00, 16.27, 16.33, 14.97, 20.19, 23.14, 27.36), nine sp^3 methines (δ_C 38.45, 39.03, 47.61, 52.97, 55.35, 78.31), one sp^2 methine (125.50), five quaternary sp^3 carbons (δ_C 39.17, 37.78, 39.39, 41.84, 48.25) and two quaternary sp^2 carbon (138.23, 180.21). The three sp^2 carbons (δ_C 125.5, 138.23, and 180.21) indicate a double bond functionality and carbonyl. (Figure 1) was propsed for compound OLM-1 from the spectroscopic data obtained for the compound and in comparision with published data for similar compound (Runyoro et al., 2010). The 2D NMR spectra of OLM-1 further supported the propsed struscture (Figure 1).

The positions of hydrogens on carbons were deduced from the HMQC correlation spectrum. The 2D $^{1}H \leftrightarrow {}^{1}H$ COSY spectrum showed correlation between the methane proton on C-3 with the methylene proton C-2. In the ^{1}H NMR spectrum, the hydrogens at δ 3.16 showed multiplicity of doublet of doublet with coupling constant of 4.2 and 11.4 Hz. The methylene hydrogen at δ 1.64 also showed COSY correlation with the methylene hydrogen at δ 1.67(δ 38.45).

In the COSY spectrum, the methylene hydrogens on C-11 showed correlations with the methine hydrogen on C-12. In the ^{1}H NMR spectrum, the methine hydrogen at δ 5.24(C-12) showed multiplicity of triplet with a coupling constant of δ 3.6 Hz. The chemical shift of the methine

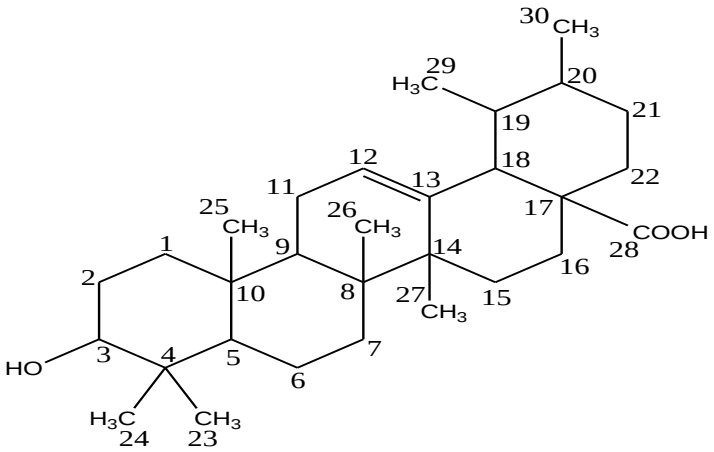

Figure 1. Proposed structure of OLM-1.

Table 2. HMBC ($^{1}H\leftrightarrow^{13}C$) NMR correlation data of compound **1.**

C	δ_C	HMBC
C_1	38.45	$C^1\leftrightarrow H^2$, H^4, H^5
C_2	26.51	$H^2\leftrightarrow C^1$, C^4, C^{10}
C_3	78.31	$H^3\leftrightarrow C^{23}$,$C^{24}$
C_4	39.17	$C^4\leftrightarrow H^2$, H^{23}, H^{24}
C_5	55.35	$H^5\leftrightarrow C^1$, C^6, C^9
C_6	18.09	$C6\leftrightarrow H^5$
C_7	32.95	$C^7\leftrightarrow H^{26}$
C_8	39.39	$C^8\leftrightarrow H^{15}$, H^{26}, H^{27}
C_9	46.24	$H^9\leftrightarrow C^1$,C^5,C^8,C^{11},C^{14},C^{25},C^{26}
C_{10}	36.78	$C^{10}\leftrightarrow H^{25}$
C_{11}	22.67	$H^{11}\leftrightarrow C^8C^9$,$C^{12}$,$C^{13}$,$C^{14}$, C^{18}
C_{12}	125.50	$H^{12}\leftrightarrow C^9$, C^{11} ,C^{14},C^{18}
C_{13}	138.23	$C^{13}\leftrightarrow H^{15}$, H^{18}
C_{14}	41.84	$C^{14}\leftrightarrow H^{12}$, H^{18}
C_{15}	27.82	$H^{15}\leftrightarrow C^{17}$
C_{16}	23.93	$H^{16}\leftrightarrow C^{18}$,$C^{28}$
C_{17}	46.97	$C^{17}\leftrightarrow H^{21}$, H^{22}
C_{18}	52.97	$H^{18}\leftrightarrow C^{10}$, C^{29}
C_{19}	39.03	$H^{19}\leftrightarrow C^{20}$
C_{20}	38.61	$C^{20}\leftrightarrow H^{19}$
C_{21}	30.38	$C^{21}\leftrightarrow H^{30}$
C_{22}	36.72	$H^{22}\leftrightarrow C^1$
C_{23}	27.36	$H^{23}\leftrightarrow C^3$,$C^4$,$C^5$,$C^{24}$
C_{24}	14.94	$H^{24}\leftrightarrow C^3$,$C^4$,$C^5$,$C^{23}$
C_{25}	15.002	$H^{25}\leftrightarrow C^5$,$C^9$
C_{26}	16.27	$H^{26}\leftrightarrow C^7$, C^8,C^9,C^{10},C^{14}
C_{27}	23.14	$H^{27}\leftrightarrow C^8C^{13}$,$C^{14}$,$C^{15}$
C_{28}	180.21	$C^{28}\leftrightarrow H^{18}$, H^{26}
C_{29}	16.33	$H^{29}\leftrightarrow C^{18}$,$C^{19}$
C_{30}	20.19	$H^{30}\leftrightarrow C^{19}$,$C^{21}$

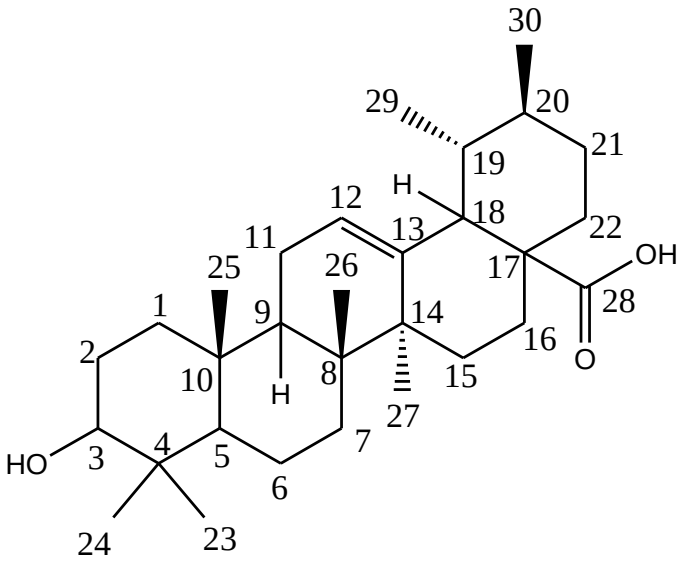

Figure 2. Structure of ursolic acid.

carbon at δ 125.50 indicated that it must be attached to the sp^2 quaternary carbon at δ 138.23. The methine proton on C-19 showed COSY correlation with the methine proton C-18. In the ^{1}H NMR spectrum, the methine hydrogen on C-18 showed multiplicity of doublet with coupling constant 11.4Hz. This indicated that both the methine hydrogens on C-18 and C-19 are in an axial-axial relationship (Table 1).

In the HMBC spectrum (Table 2) the quaternary carbon signal appearing at δ 39.39 showed correlation with the methylene hydrogen on C-11, methyl haydrogens on C-26 and C-27. The quaternary carbon signal appearing at 41.84 had HMBC correlations with the methine hydrogens on C-12 and C-18 indicating the position of C-26 methyl group is likely to be on C-8. The quaternary carbon signal appearing at δ 180.21 showed HMBC correlations with methane hydrogen on C-18 and the methylene hydrogen on C-16 indicating that C-28 is attached to C-17. HMBC correlations were also observed between the methine hydrogen on C-18 with C-10 and C-29. This indicated that C-29 is likely to be attached to C-10. This is supported by the multiplicity of doublet in the ^{1}H NMR spectrum. The methine carbon signal appering at δ_C 36.78 showed HMBC correlation with the methyl hydrogen on C-30. HMBC correlations were also observed between the methyl hydrogens on C-30 with the methylene carbons C-21 and C-22. In the ^{1}H NMR spectrum, the C-30 methyl hydrogen showed multiplicity of doublet, which indicates that it is attached to C-20. The ^{1}H and ^{13}C NMR data obatained for OLM-1 is comparable with the data for Ursolic acid reported in literature. Based on spectroscopic data and literature survaye the compound OLM-1 is characterized to be ursolic acid (Figure 2 and Table 3).

Spectral data

Compound OLM-1: pale yellow crystalline solid, ^{1}H NMR; δ 0.75 (1H, d, H-5), 0.79 (3H, s, Me-24), 0.86 (3H, s, Me-26), 0.87 (3H, d, Me-29), 0.95 (3H, s, Me-27), 0.95 (3H, s, Me-25), 0.99 (3H, s, Me-23), 1.00 (1H, dd, H-22β), 1.09 (1H, m, H-15β), 1.13 (3H, s, Me-27), 1.32 (1H, m, H-21α), 1.34 (1H, m, H-7β), 1.36 (1H, m, H-19), 1.37 (1H, m, H-6β), 1.49 (1H, m, H-21α), 1.54 (1H, m, H-7α), 1.54 (1H, m, H-6α), 1.56 (1H, m, H-9), 1.57 (1H, m, H-2β), 1.64 (1H, m, H-2α), 1.64 (1H, m, H-1β), 1.64 (1H, m, H-16β), 1.67 (1H, m, H-22α), 1.67 (1H, m, H-20), 1.68 (1H, m, H-1α), 1.93 (1H, m, H-11α), 1.93 (1H, m, H-11β), 1.93 (1H, m, H-15α), 2.03 (1H, td, H-16α), 2.20 (1H, d, H-18), 3.16 (1H, dd, H-3), 5.24 (1H, t, H-12); ^{13}C NMR, 14.94 (C-24), 15.002 (C-25), 16.27 (C-26), 16.33 (C-29), 18.09 (C-6), 20.19 (C-30), 22.67 (C-11), 23.14 (C-27) 23.93 (C-16), 26.51 (C-2), 27.36 (C-23), 27.82 (C-15), 30.38 (C-21), 32.95 (C-7), 36.72 (C-22), 36.78 (C-10), 38.45 (C-1), 38.61 (C-20),$_0$ 39.03 (C-19), 39.17 (C-4), 39.39 (C-8), 41.84 (C-14), 46.24 (C-9), 46.97 (C-17), 52.97 (C-18), 55.35 (C-5), 78.31 (C-3), 125.50 (C-12), 138.23 (C-13), 180.21 (C-28).

Conclusions

In this thesis research the methanol extract results one triterpen; ursolic acid (OLM-1). This compound was identified by comparing thesis ^{1}H and ^{13}C NMR spectroscopic data for similar compounds from literature.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

Table 3. Comparison of ^{13}C NMR of compound OLM-1 with that of ursolic acid.

Carbon	^{13}C NMR compound OLM-1	^{13}C NMR Ursolic acid [80]
1	38.45	39.8
2	26.51	27.8
3	78.31	79.6
4	39.17	39.9
5	55.35	56.7
6	18.09	19.4
7	32.95	34.3
8	39.39	40.7
9	46.24	47.6
10	36.78	38.1
11	22.67	24.3
12	125.50	126.8
13	138.23	139.6
14	41.84	42.8
15	27.82	27.36
16	23.93	25.3
17	46.97	47.6
18	52.97	54.3
19	39.03	40.4
20	38.61	40.4
21	30.38	31.7
22	36.72	38.1
23	27.36	28.7
24	14.94	16.0
25	15.002	16.3
26	16.27	17.6
27	23.14	24.0
28	180.21	181.6
29	16.33	17.8
30	20.19	21.5

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