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Preliminary phytochemical screening, isolation and structural elucidation of chloroform leaf extracts of *Maesa lanceolata*

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The aim of this study was to screen the phytochemical constituents, isolate and elucidate the structure of chloroform extracts from the leaves of *Maesa lanceolata*. The qualitative phytochemical analysis of the leaf extract of *Maesa lanceolata* was done following standard procedures and the tests revealed the presence of cardiac glycosides and saponins in the crude extracts. The chloroform extract of the leaves of M. lanceolata led to the isolation of one compound, C1. Structure determination was accomplished by means of spectroscopic methods (IR, ^{13}C and ^{1}H NMR). According to the spectral data (IR, ^{1}HNMR, ^{13}CNMR and DEPT), probably Myrsenene was identified which is reported for the first time from *M. lanceolata*.

Capsule Summary: Phytochemical constituent's analysis, isolation and structure elucidation of chloroform extracts from the leaves of *Maesa lanceolata* were investigated.

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INTRODUCTION

Maesa lanceolata (*Myrsinaceae*) is a well-known plant and widely distributed in many parts of Africa especially in Ethiopia (Leulet al., 2010; Dereket al., 2009).The leaves of the plant traditionally used for the treatment of helminthes and bacterial infections in most rural areas of the country since it is rich in biologically active constituents (Takele et al., 2014; Focho et al., 2009).In the previous studies, researchers reported the isolation of hydroxylated-1, 4-benzoquinone derivatives from various parts of plant. Qualitative phytochemical screenings of the different solvent extracts of the plant undertaken by different investigators have revealed the isolation and identification of various acylated triterpene saponins based on oleanane skeleton. Lately, flavonol glycosides and alkaloids have been reported from the plant leaves (Lawrence et al., 2011). In the present study, preliminary phytochemical analysis, isolation and structural elucidation of chloroform extract of the plant leaves has been done.

MATERIAL AND METHODS

Sample collection and preparation

The fresh leaves of *Maesa lanceolata* plant were collected from Awi Zone, Guangua Wereda at Elala forest which is about 220 km away from Bahir Dar City during December 2014 (Fig 1).The collected plant materials were properly

Fig 1: The photograph of *Maesa lanceolata* Leaf

washed with tap water, air dried for two weeks under shad. The dry leaves were ground to obtain a fine powder.

Extraction and isolation of *Maesa lanceolata* leaves

150 g fresh and uncrushed leaves of *Maesa lanceolata* were successively extracted with 250 mL of chloroform at room temperature. The extracts were freed from the solvent under reduced pressure to give crude extract. The combined extract was subjected to column chromatography. As indicated in Table 1, the column was eluted first with hexane then with hexane: EtOAc (5:1, 2:1, 1:1, 1:2 and 1:5) and EtOAc to pure and get ride off any EtOAc retained in the packed column. This gave different fractions and TLC of each fraction was examined using different solvent systems to give four major fractions (I-V). The first two fractions contained oily substances and chlorophyll and were discarded. Fractions III to V were further purified using column chromatography and refractionated using different solvent systems. Purification by preparative TLC was done for each fraction to give pure compound, C_1. The correct structural of pure component (C_1) was elucidated by NMR (^{1}H-NMR, ^{13}C-NMR, DEPT) and IR techniques (Ayoola et al., 2008; Zohra et al., 2012; Tiwari et al., 2011, Carmen-María et al., 2014; Abdulet al., 2015; Harami et al., 2016).

Table 1: Solvent system (*n*-hexane/EtOAc) used to separate compound C_1

Solvent system (ratio)	Total amount taken (mL)
100 %	30
5:1	30
2:1	30
1:1	30
1:2	30
1:5	30
100%	30

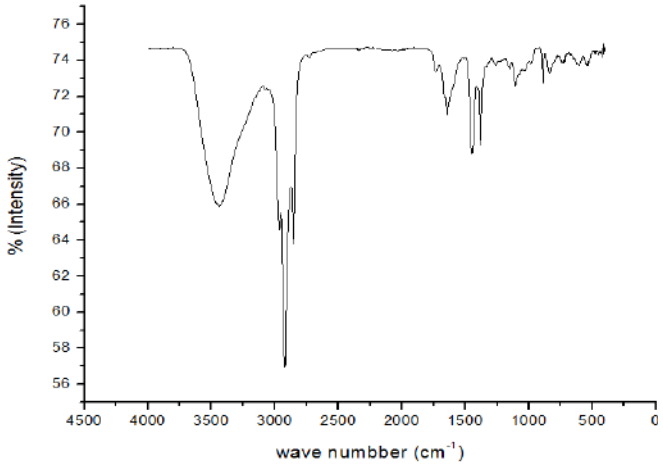

Fig 2: FTIR spectra of compound C_1

Phytochemical screening of the extract

The preliminary phytochemical screening of the extracts of *Maesa lanceolata* leaves have been done following standard procedures. The leaf extract was subjected to qualitative chemical investigation using specific reagents for each group of constituents (Margeretha et al., 2012; Beatrice et al., 2002). Compound C_1. R_f value of 0.75cm ($CHCl_3$); light yellow, IR (KBr) ν_{max} 3300, 3000, 2900, 1600, 1460, 1380, 1250 and 720 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) δ 0.80, 1.05, 1.30, 1.45, 1.65, 1.70, 1.95, 2.1, 5.15 and 4.55 – 4.85. ^{13}CNMR and DEPT spectrum of compound C_1 is summarized in Table 2.

RESULTS AND DISCUSSION

Phytochemical analysis of the extract

The results of phytochemical screening test performed on crude leaf extracts of *Maesa lanceolata* plant are summarized in Table 3 below. Phytochemical analysis of chloroform crude extracts of *Maesa lanceolata* leaf extract revealed the presence of cardiac glycosides and saponins. The presence of these substances in the investigated plant accounts for its usefulness as medicinal plant. This information obtained is used to facilitate quantitative estimation and qualitative separation of constituents from the leaves. In addition to the phytochemical screening of the plant extract, we have checked the anthelmintic activities and the extract showed the prominent activity towards aquatic leech; Lymnatis nilotica (Wondu and Alemayehu, 2016).

FTIR analysis

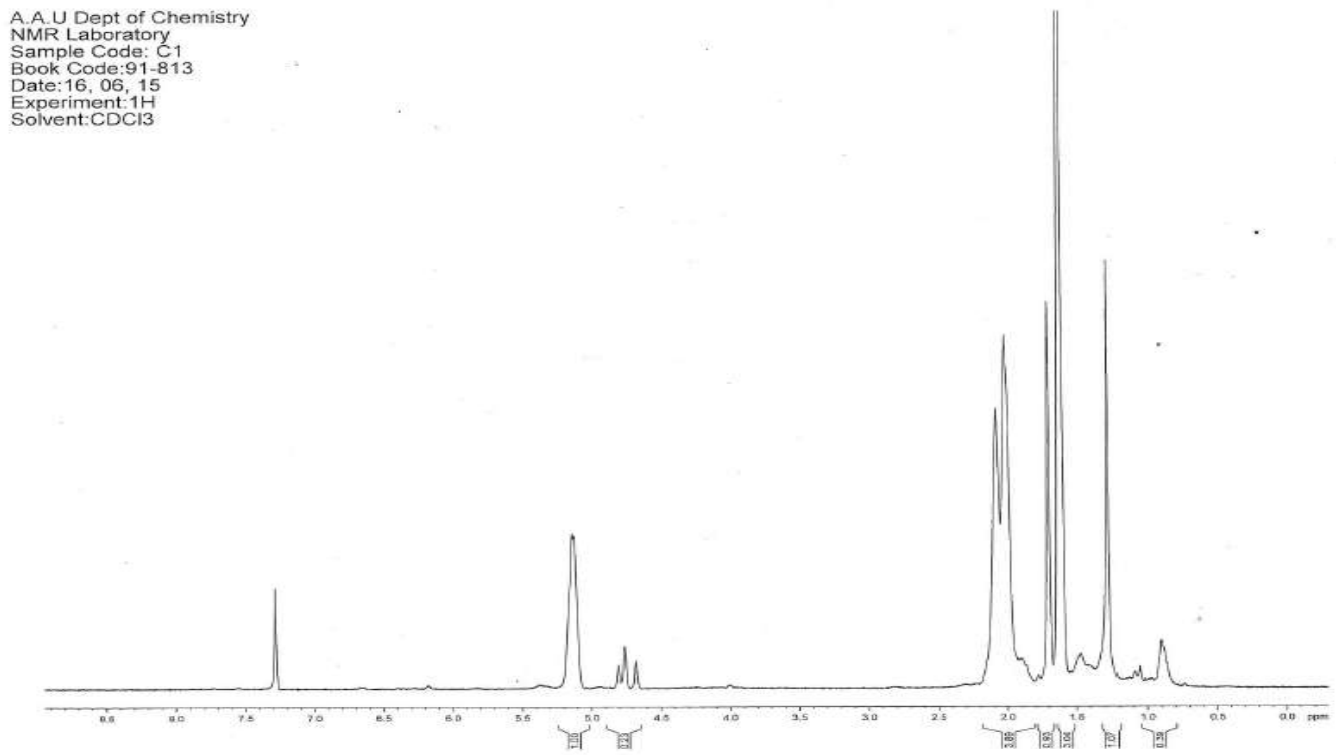

Fig 3: ^{1}H-NMR spectra of C_1

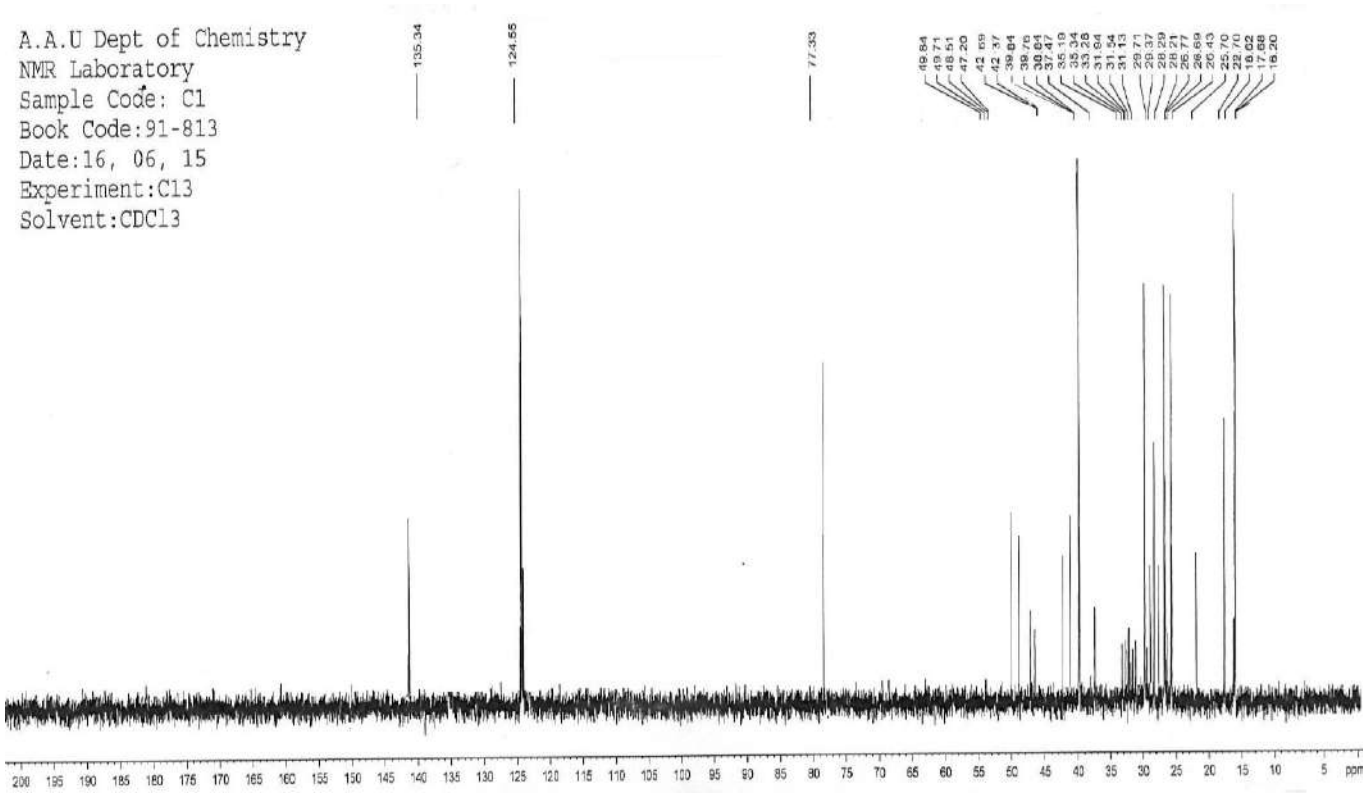

Fig 4: ^{13}C-NMR spectra of compound C_1

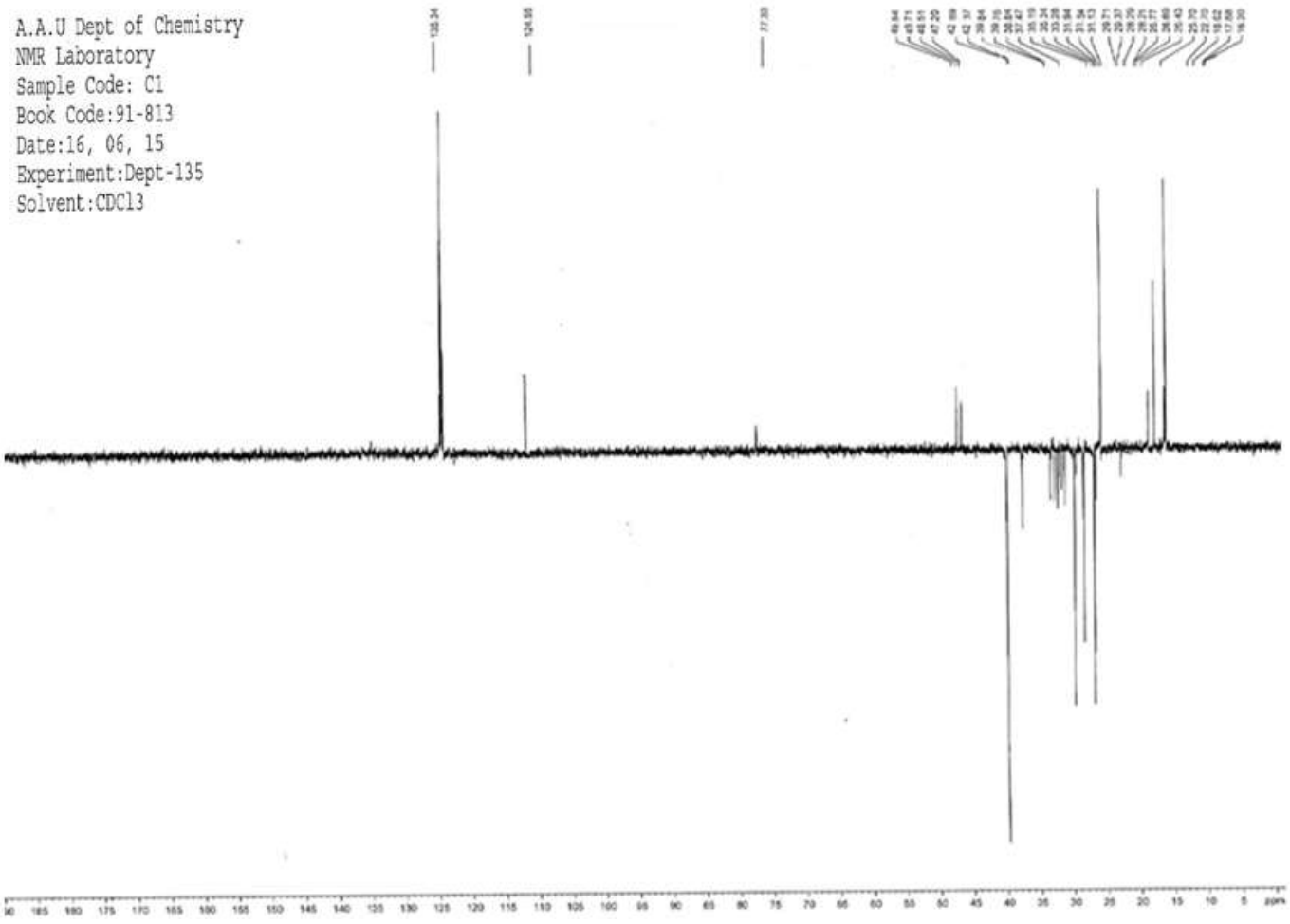

Fig. 5: The DEPT Spectra of C_1

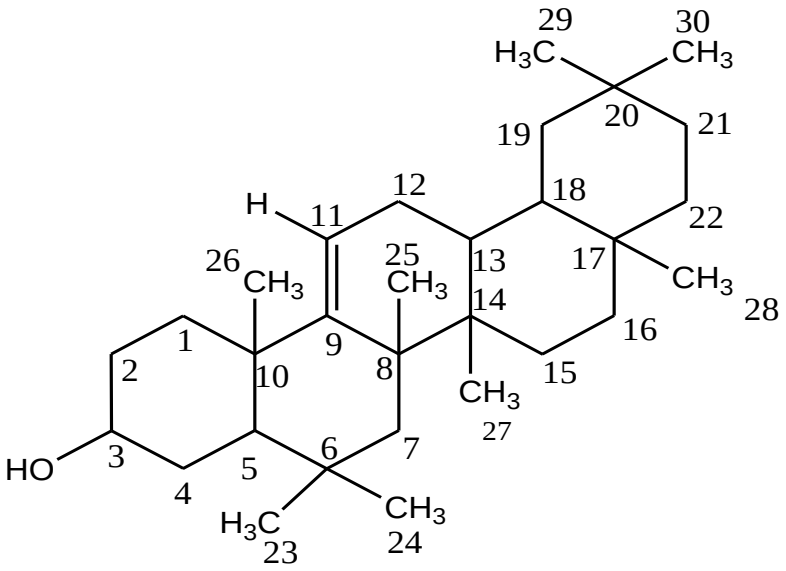

Fig 6: Possible structure of C_1 (Myrsenene) isolated from the plant extract.

Table 2: ^{13}C NMR and DEPT spectrum of compound C_1

Carbon Number	Multiplicity (DEPT)	^{13}C-NMR	Remark
1	CH_2	35.9	Methylene
2	CH_2	29.71	Methylene
3	CH	77.33	Methine
4	C	37.47	Quaternary Carbon
5	CH	47.20	Methine
6	CH_2	28.29	Methylene
7	CH_2	49.5	Methylene
8	C	39.76	Quaternary Carbon
9	C	135.34	Quaternary Carbon
10	C	39.84	Quaternary Carbon
11	CH	124.55	Methine
12	CH_2	28.21	Methylene
13	CH	49.71	Methine
14	C	42.35	Quaternary Carbon
15	CH_2	31.94	Methylene
16	CH_2	33.25	Methylene
17	C	38.84	Quaternary Carbon
18	CH	48.51	Methine
19	CH_2	42.56	Methylene
20	C	29.37	Quaternary Carbon
21	CH_2	31.54	Methylene
22	CH_2	35.54	Methylene
23	CH_3	26.43	Methyl
24	CH_3	25.70	Methyl
25	CH_3	18.62	Methyl
26	CH_3	22.70	Methyl
27	CH_3	17.68	Methyl
28	CH_3	26.77	Methyl
29	CH_3	31.13	Methyl
30	CH_3	26.69	Methyl

The FTIR analysis of the sample (C_1) revealed the presence of polycyclic compound with alcohol functional group (Fig 2).Accordingly from the FTIR spectrum, the prominent absorption band at 3300cm^{-1} indicates the O-H stretch. The absorption band at 3000 cm^{-1}indicates sp2 C-H stretch with weak intensity and the absorption band at 2900 cm^{-1}revealsthe presence of sp3 C-H stretch with weak intensity. The FTIR spectrum also showed the presence of olefinic carbon (C=C) at 1600 $cm^{-1}$with medium intensity and the presence of -CH_2out of plane bending with weak intensity at 1460 cm^{-1}.Furthermore the absorption band at 1380cm^{-1}indicates-CH_3bending with medium intensity and there is weak absorption band of C-O stretch at 1250 cm^{-1}. The weak absorption band of -CH_2 rocking was also observed at 720 cm^{-1}.

^1H-NMR spectrum of the compound showed eight singlet signals due to the presence methyl groups at δ 0.80, 1.05, 1.30, 1.45, 1.65, 1.70, 1.95 and 2.1, which are is due to the hydrogen atoms attached to C-29, C-30, C-28, C-27, C-25, C-26, C-24 and C-23, respectively. One proton signal at δ 5.15 is due to methine proton at C-11. A triplet proton signal at δ4.55 – 4.85 is due to the presence of protons at C-12. The ^1H-NMR data, therefore, clearly indicates compound C_1 is triterpene.

^{13}C NMR and DEPT spectrum

The proton decoupled ^{13}C-NMR spectrum (both 1D ^{13}C-NMR and DEPT) showed the signals of 30 carbon atoms(**Table 3**) of eight methyl (C-23 to C-30), ten methylene (C-1, C-2, C-4, C-7, C-12, C-15, C-16, C-19, C-19, C-21 and C-22), five methine (C-3, C-5, C-11, C-13 and C-18) and seven quaternary carbons

(C-6, C-8, C-9, C-10, C-14, C-17 and C-20). In DEPT spectrum, the data are collected in such way that the resulting signal is either positive (CH & CH_3) or negative (CH_2) depending on the number of protons attached. Accordingly, ten signals are pointing down (negative) indicating that there are ten methylene groups in the C_1 and eight signals pointing upward (positive) indicating eight carbons attached with either one or three hydrogens. Based on the information obtained, the most likely structure of the isolated compound (C_1) is found to *Myrsenene* (Fig. 6).

Table 3: Preliminary phytochemical analysis of chloroform extracts of *Maesa lanceolata*

S.No	Phytochemicals	Result
1.	Tannins	-
2.	Cardiac Glycosides	+
3.	Alkaloids (Wondu and Alemayehu, 2016)	+
4.	Flavonoids(Wondu and Alemayehu, 2016)	+
5.	Steroids	-
6.	Anthraquinones	-
7.	Saponins	+
8.	Proteins	-

CONCLUSIONS

Qualitative preliminary phytochemical investigation of chloroform extracts of the leaf of *Maesa lanceolata* revealed that the presence of saponins and cardiac glycosides. Upon isolation and structural elucidation of the extract, one compound was found with the name of *Myrsenene*.

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