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Anti-inflammatory Activities of the Leaves Extracts and Compounds Isolated from *Boscia coriacea* Graells, *Uvaria leptocladon* Oliv and *In vitro* Antibacterial Activity Test of the Isolated Compounds

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ABSTRACT: Anti-inflammatory drugs that have been used for the treatment of diseases are associated with numerous adverse effects. Antibacterial drugs resistance has become one of the major challenges of controlling infectious diseases. Therefore, it is necessary to search for safe and effective anti-inflammatory and antibacterial drugs. The objective of this study was to evaluate anti-inflammatory activities of the leaves extracts and compounds isolated from *Boscia coriacea* Graells, *Uvaria leptocladon* Oliv and antibacterial effects of the isolated compounds. Fresh leaves of *B. coriacea* and *U. leptocladon* were collected in April 2021 from Alie and Konso, Southern Ethiopia. The powdered leaves of *B. coriacea* and *U. leptocladon* were extracted using 80% MeOH by maceration extraction method. Compound isolation was performed using column chromatography and structural elucidation of the isolated compounds was done using nuclear magnetic resonance spectrometry (^{1}H NMR and ^{13}C NMR). Carrageenan induced paw-edema model, cotton pellet induced granuloma method and protein denaturation assay were used to test the anti-inflammatory activities of the leaves extracts of the plants. Agar well diffusion assay was used to evaluate antimicrobial activity of the compounds isolated from the leaves of *B. coriacea* and *U. leptocladon*. Analysis of the difference between antibacterial and anti-inflammatory activities of the different groups was performed using one-way analysis of variance with post hoc comparison (Tukey's test). β-sitosterol and lucidine-type compounds were isolated from *B. coriacea.*1-tiacontanol, Beta-sitosterol, Beta-sitosterol glucoside, whereas alpha-humulene were isolated from *U. leptocladon*. All compounds except 1-tricontanol showed anti-inflammatory and antibacterial activities. The leaves extracts of *B. coriacea* and *U. leptocladon* contain compounds, which have potent anti-inflammatory and antibacterial activities. Further study is needed to elucidate the mechanisms underlying the anti-inflammatory and antibacterial actions of βeta-sitosterol, βeta-sitosterol glucoside, alpha-humulene and lucidine type compound.

Key words/phrases: antibacterial activity, anti-inflammatory activity, *Boscia coriacea* Graells, Phytochemicals, *Uvaria leptocladon* Olive

INTRODUCTION

Anti-inflammatory drugs that have been used for the treatment of diseases are associated with numerous adverse effects including gastrointestinal bleeding; suppression of immune response; cardiovascular and renal risks (Hougee, 2008; Harirforoosh *et al*., 2013). Therefore, it is necessary to search for safe and effective anti-inflammatory drugs.

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Antibacterial drugs resistance has become one of the major challenges of controlling infectious diseases. Antimicrobial drugs resistance is estimated to account for 700,000 deaths every year in the world (Shankar, 2016). Thus, it is necessary to search for safe and effective antibacterial drugs.

Medicinal plants are the major sources of chemicals with anti-inflammatory and antibacterial activities. The genus *Boscia* belongs to the family Capparidaceae (Vougat *et al*., 2015), and widely distributed in Ethiopia. It is found in Bale, Sidamo, Kefa, Konso, Gamo Gofa and Hararge regions of Ethiopia (Friis and Persson, 2000). For instance, *B. coriacea Graells* is found in Konso, SNNPR, Ethiopia. The genus *Uvaria* belongs to the family Annonaceae that is also widely distributed in Ethiopia. For example, *U. leptocladon* is found in Sidamo, Kefa, Konso (Alie), and Gamo Gofa regions of Ethiopia (Friis and Persson, 2000).

Some medicinal plants that belong to the genus *Uvaria* and *Boscia* have anti-inflammatory and antibacterial properties (Okwu and Iroabuchi, 2009; Oluremi *et al*., 2010; Bila, 2016; Bamba et al., 2019; Jalil *et al*., 2020; Sintayehu Tsegaye *et al*., 2022A; Sintayehu Tsegaye *et al*., 2022B). Therefore, the objective of this study was to evaluate anti-inflammatory activities of the leaves extracts and compounds isolated from *Boscia coriacea* Graells, *Uvaria leptocladon* Oliv and antibacterial effects of the isolated compounds.

MATERIALS AND METHODS

Plant collection

Fresh leaves of *B. coriacea* and *U. leptocladon* were collected in April 2021 from Alie and Konso, which are located in Southern Ethiopia. Fresh leaves of *B. coriacea* and *U. leptocladon* were collected from Konso and Alie, Southern Nation, Nationalities, and People Region, Ethiopia, in April 2021. The plant materials were authenticated by one of the authors of this manuscript (Mr. Melaku Wondafrash a Botanist at the Department of Plant Biology and Biodiversity Management), and a voucher specimen of each plant (ST001 and ST002, representing *B. coriacea* and *U. leptocladon*, respectively) was deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University (AAU).

Extraction

The leaves of the medicinal plants were dried in shaded condition. The dried leaves of the plants were ground into powder. Following this, the powdered leaves of the plants (one kilogram for each plant) were extracted using 80% methanol (MeOH) maceration extraction method. After filtration using Whatmann no.1 filter paper, Rotary evaporator was used to evaporate the MeOH from the filtrate. A lyophilizer (Christ, ALPHA 2-4-LD Plus) was used to remove the water component of the mixture.

The crude leaf extracts of the plants were fractionated at the Department of Chemistry, CNCS, AAU. For the antibacterial study, the crude extracts were fractionated using methanol (MeOH), chloroform ($CHCl_3$) and $CHCl_3$: MeOH (1:1 v/v). For the anti-inflammatory study of *B. coriacea,* the crude extract was fractionated using $CHCl_3$, MeOH, $CHCl_3$: MeOH (1:1 v/v) and petroleum ether. For the anti-inflammatory studies of *U. leptocladon,* the crude extract was fractionated using $CHCl_3$, ethanol (EtOH), and ethyl acetate (EtOAc). All the extracts were stored in refrigerator at -20°C until experiments were conducted.

Isolation and characterization of compounds from U. leptocladon and B. coriacea

The isolation and characterization of compounds were done at the Department of Chemistry, CNCS, AAU. The characterization of compounds was done using nuclear magnetic resonance spectrometry (^{1}H NMR and ^{13}C NMR). First, the leaves of the plants were dried in shaded condition. Then, the dried grounded leaves of *B. coriacea* and *U. leptocladon* were placed in separate Erlenmeyer flasks then extracted first with chloroform to get the chloroform extract. Then to the marks left after filtering the $CHCl_3$ extracts, $CHCl_3$: MeOH (1:1) were added to obtain the $CHCl_3$: MeOH (1:1) extracts from the two plant materials. Finally the mark left after $CHCl_3$: MeOH (1:1) extracts were extracted with absolute EtOH to get the EtOH extracts. All these extracts were obtained by soaking for 72 h, filtrating, and concentrating the extracts by using rotary evaporator.

The $CHCl_3$ extract of *U. leptocladon* was fractioned on column chromatography packed with silica gel (70-230 mesh). Elution was done by using n-hexane and different combination of n-hexane and EtOAc. From this column, 50 fractions

in which each fraction contain about 40 ml were collected. The fractions were applied on thin layer chromatography (TLC) with different solvent systems and vanillin in H_2SO_4 as a visualizer. The polar EtOH fraction was also purified by applying on column chromatography using solvents $CHCl_3$ and MeOH and their combination. The TLC was analyzed by observing the purity of spots in each track and by comparing the retention factor (RF) value of the spots.

Similarly the $CHCl_3$: MeOH (1:1) extract of *B. coriacea* was purified by using column chromatography packed with silica-gel. Elution was made by using $CHCl_3$ and EtOAc with increasing polarity obtained by mixing different proportion of these two solvents. After analyzing the TLC of the collected fractions, NMR (400 MHz) spectrometry was used for structural elucidation.

Experimental animals

Healthy Swiss albino mice that weighed in the range of 25–45g and aged from 9 to 12 weeks were used as experimental animals for the anti-inflammatory studies (Carrageenan-induced paw edema model and Cotton pellet induced granuloma method). All mice were housed in standard cages and the mice were fed with standard pellet laboratory diet and ordinary clean tap water *ad libitum*.

Evaluation of anti-inflammatory activities of B. coriacea and U. leptocladon

Carrageenan-induced paw edema model

Evaluation of the anti-inflammatory activities of compounds isolated from the leaves extracts of *B. coriacea* and *U. leptocladon* was conducted using a carrageenan-induced paw edema model (Winter *et al*., 1962). The anti-inflammatory test was performed in male Swiss albino mice weighed in the range of 25–45g and aged from six to eight weeks. The Swiss Albino mice were randomly grouped into three groups. Each experimental group consisted of six Swiss albino mice. Thirty minutes before carrageenan injection, the first group was orally given leaves extracts, and the second and third groups were orally treated with normal saline (the negative control) and indomethacin (25 mg/kg, the positive control). After 30 minutes, 50 µL of 1% freshly prepared 1% solution of carrageenan (w/v in normal saline) was injected into the sub-plantar tissue of the left hind paw of each mouse. The diameter of the left hind paw of each mouse was measured in millimeter before and after administration of the leaves extracts, every hour for six consecutive hours using a digital micrometer (Mitu Toyo Corporation).

The percentage reduction of paw edema was calculated by the following formula:

$$\frac{[(Vt\text{-}V0 \text{ in negative control group})\text{-}(Vt\text{-}V0 \text{ in treatment group })] \times 100}{Vt\text{-}V0 \text{ in negative control group}}$$

Where,

Vt= is the mean diameter of paw in treated and negative control groups after carrageenan injection at time t and

V0 = is the mean diameter of paw before injection of carrageenan in treated and negative control groups

Cotton pellet induced granuloma method

Cotton pellet-induced granuloma method was used to test the effect of the extracts on the transudative and the proliferative (granulomatous) features of chronic inflammation (Afsar *et al*., 2013). The cotton pellet-induced granuloma study was performed using Swiss Albino mice. The Swiss Albino mice were randomly grouped into four groups. Each experimental group consisted of five Swiss albino mice. Male Swiss albino mice that weighed in the range of 30–45g were fasted for 3 hours with free access to water prior to the beginning of the experiment. Crude extract, indomethacin (10 mg/kg) and negative control (normal saline) were given to the test, standard, and control groups of mice, respectively. Each experimental group consisted of five Swiss albino Mice. Pieces of rolled cotton pellets weighing 10 mg were sterilized in an autoclave for 30 min at 121°C. Twenty minutes after administration of the extracts, indomethacin (10 mg/kg) and the negative control (normal saline), the mice were sedated with ketamine (200 mg/kg) intraperitoneally (0.4 mL for each mouse). Then, a subcutaneous tunnel was created using a surgical blade on both parts of the recently shaved parts of each mouse. Then, a chromic catgut (40 mm ½ curved cutting, CMTANGE, China) was used to stitch two sterilized cotton pellets into a subcutaneous tunnel on either side. The extracts (200 mg/kg), indomethacin (10 mg/kg) and the negative control were given for seven consecutive days (once a day). On the 8th day, the mice were

sacrificed and the pellets enclosed by granuloma tissue were carefully dissected. Just after removing the cotton pellets, the wet weights of the cotton pellets were measured. Finally, the wet pellets were dried at 60°C for 24 h, and the dry weights of the cotton pellets were measured. The granuloma tissue formation (in mg), exudate amount (in mg), and percent inhibitions of granuloma tissue and exudate formation were calculated by using the following formula:

Exudates inhibition (%) = 1- [Exudates in treated group]X 100
Exudate in control group

Granuloma inhibition (%) = 1- [Granuloma in treated group] X 100
Granuloma in control group

Where: Measure of exudates formation=immediate wet weight of pellet-constant dry weight of pellet.
Measure of granuloma tissue formation=Constant dry weight-initial weight of cotton pellet.

Protein (egg albumin) denaturation inhibition assay

In vitro anti-inflammatory activity of the leaves extracts of *B. coriacea* and *U. leptocladon* was evaluated using an inhibition of protein (egg albumin) denaturation assay (Afsar *et al*., 2013; Banerjee *et al*., 2014). A reaction mixture was prepared by mixing 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (pH 6.4), and 2 mL of extract (different concentrations: 100, 250, and 500 µg/mL). The mixture was incubated for 15 minutes at 37°C and then heated for 5 minutes at 70°C. Finally, the reaction mixture was cooled, and absorbance was measured at 660 nm using a spectrophotometer (UV-UV/Vis/NIR spectrophotometer, Perkin Elemer, Lambda 950). Diclofenac (with different concentrations: 100, 250, and 500 µg/mL) was used as a reference. Distilled water was used as a negative control. The experiment was done in triplicate, and the average was recorded. The inhibition of egg albumin denaturation by the leaves extracts of the plants was expressed as a percentage inhibition of egg albumin denaturation and an IC_{50} of egg albumin denaturation. The IC_{50} of the extracts was calculated based on the standard curve (regression equation: Y=mx + b) derived from the percentage inhibition of egg albumin denaturation by the different concentrations of extracts (100, 250, and 500 µg/mL).

The percentage inhibition of egg albumin denaturation was calculated as follows:-
% protein denaturation inhibition=100 x ([Vt/Vc]-1)
Where, Vt is the absorbance of the test sample, and Vc is the absorbance of the negative control.

Antibacterial study

Test bacteria species

Escherichia coli (ATCC25922), *Klebsiella pneumonia* (ATCC700603), *Listeria monocytogenes* (ATCC19115), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhimurium* (ATCC14028), *Staphylococcus aureus* (ATCC25923) were obtained from the Ethiopian Public health institute (EPHI), Addis Ababa, Ethiopia.

Isolation of test bacteria species

Each bacterium species was allowed to grow in nutrient broth at 37°C for 24 h. In order to get pure colony of each bacterium species, some colonies were streaked from nutrient broth to their selective media. Ceterimide agar, Hektone enteric gar, MacConkey agar, Palcam agar and Mannitol salt agar were used for isolation of *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Listeria momocytogenes* and *Staphylococcus aureus*, respectively.

Reference drugs (positive control)

Amoxicillin (25 µg/ml) was used as standard drug against *S. aureus* and *L. monocytogenes*. Whereas, ciprofloxacin (5 µg/ml) was used as reference drug against *E. coli*, *S. typhimurium, K. pneumoniae* and *P. aeruginosa*. Both the amoxicillin and the ciprofloxacin were obtained from the Ethiopian Pharmaceuticals Manufacturing Company SC (EPHARM), Addis Ababa, Ethiopia.

Agar well diffusion assay

The antimicrobial activities of the leaves extracts of *B. coriacea* and *U. leptocladon* against was determined against above stated bacteria using agar-well diffusion assay (Dey *et al*., 2011). Inoculums were standardized by comparing with 0.5 McFarland turbidity standards.

A Mueller-Hinton agar (MHA) medium was streak with 100 µL of the test bacteria using a sterile cotton swab. One hundred µL of the test bacteria were inoculated on Petri dishes that contain solidified Mueller-Hinton agar (MHA)

media using a sterile cotton swab. Then, a sterile cork borer was used to prepare wells (6 mm in diameter wells) in the MHA media. Fifty µL of the compounds at a concentration of 1mg/mL (dissolved in 10% Dimethyl sulfoxide (DMSO)) were dispensed into the wells and allowed to stand for about 30 minutes to allow diffusion of metabolites. Similarly, 50 Fifty µL of the negative (10% DMSO) and positive controls positive controls (Amoxicillin (25 µg/ml) and ciprofloxacin (5 µg/ml) were also loaded in the agar wells. Then, the plates were incubated for 24 h at 37ºC. The negative control was the vehicle (10% DMSO). A Vernier caliper was used to measure the diameter of the zone of inhibition (in millimeters) to determine the sensitivity of the test bacteria to the extracts and the standard drugs. The experiments were performed in triplicate.

Data analysis

Data on percent of anti-inflammatory assay and diameter of antibacterial activity of plant extracts assay were performed using one-way analysis of variance (ANOVA) and expressed as mean ± SEM. A post hoc comparison of means of the biological activities of the two plants species were done using Tukey's test whenever necessary. Association between in vitro and in vivo anti-inflammatory activities of the leaves extracts of *B. coriacea* and *U. leptocladon* was analyzed by using linear regression model. $P<0.05$ was considered statistically significant.

RESULTS

Compounds isolated from the leaf extract of B. coriacea and U. leptocladon

Five compounds were isolated from the biologically active fractions of *B. coriacea* and *U. leptocladon*. Two of these compounds (namely β-sitosterol and lucidine type compound) (Benzopyranyl sequiterpene type compound) were isolated from the leaf extract of *B. coriacea.* Whereas, four compounds were isolated from the leaves extract of *U. leptocladon*, which are namely:- 1-Triacontanol, β-sitosterol, β-sitosterol glucoside and α-humulene (Figure 1). The exact structural elucidations of the lucidine type compound require further investigations. The spectrums of the five compounds are found in Appendix 1.

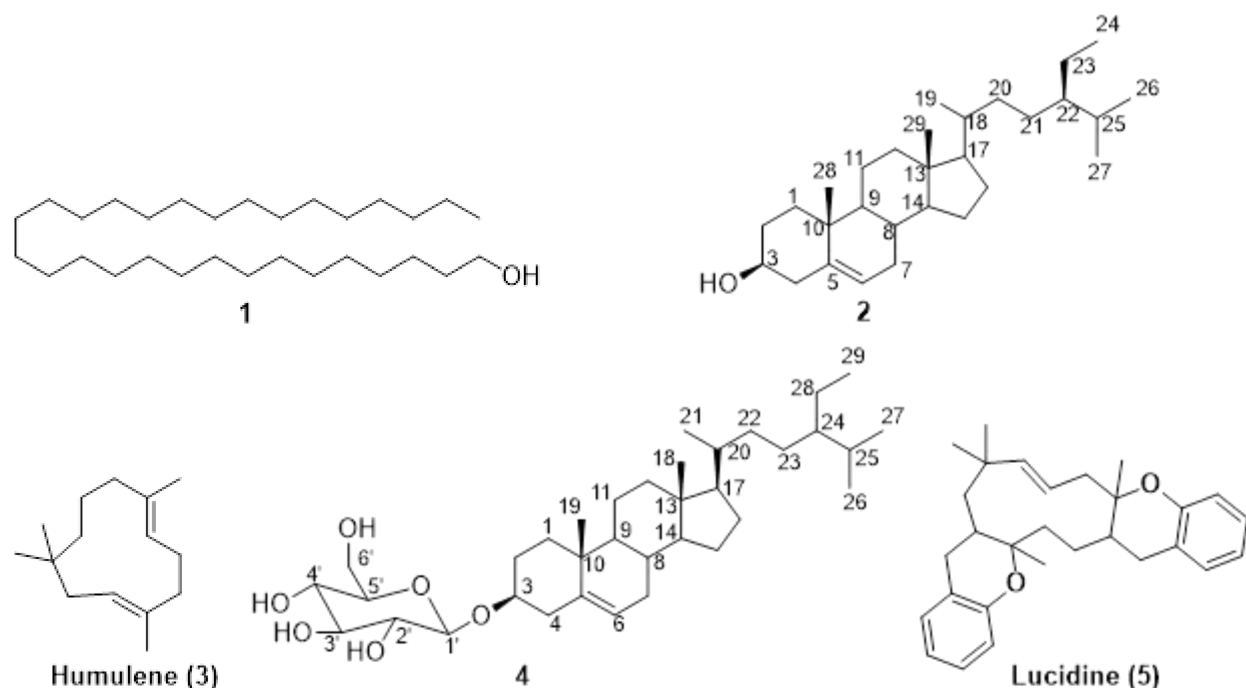

Figure 1: Structure of the isolated compounds: Triacontanol (1), β-sitosterol (2), Humulene (3), β-sitosterol glucoside (4), and Lucidine (5).

Compound 1 (1-tricontanol) was isolated as a white powder from $CHCl_3$ fraction of *U. leptocladon*. The TLC (Rf 0.61) developed using n-hexane and EtOAc (1:1) as a mobile phase gave

light pink spot after spraying with vanillin in H_2SO_4. The most down field triplet signal at δ 3.66 and the triplet at δ 1.59 in the ^{1}H-NMR spectrum assigned for oxymethylene and aliphatic methylene protons situated between two methylene groups respectively. The oxymethylene protons were supported by the presence an oxygenated carbon signal at δ 63.1 in ^{13}C-NMR and DEPT spectra. The broad singlet at δ 1.27 was due to many overlapping methylene protons (27 CH_2), which was also supported by the presence an intense carbon signal at δ 29.7 and other signals between 22.7–32.8 ppm. The most up field triplet signal at δ 0.90 showed the presence of only one terminal methyl group and which was also confirmed by the presence of carbon signal at δ 14.2. The ^{1}H-NMR and ^{13}C-NMR spectra of compound 1 were in agreement with literature value for 1-triacontanol (Valgas *et al*., 2007) (Table 1).

Table 1. 1H- and ^{13}C- NMR values of compound 1 and literature report for 1-triacontanol.

Experimental result		Literature report (Valgas *et al*., 2007)	
δ_H, mult	δ_C (Position)	δ_H, mult	δ_C (Position)
3.66 (t, 2H, H-1)	63.1 (C-1)	3.63 (t, 2H, H-1)	63.1 (C-1)
1.59 (m, 2H, H-2)	32.8 (C-2)		32.9 (C-2)
	25.8 (C-3)		25.8 (C-3)
	29.4 (C-4)		29.4 (C-4)
1.27 (br s, 52H,	29.5 (C-5)	1.26 (br s, 56H,	
H-3 to H-28)	29.6 (C-6)	H-2 to H-29)	29.7 (C-5 to C-27)
	29.7 (C-7 to C-27)		
	32.0 (C-28)		32.0 (C-28)
1.47 (br s, 2H, H-29)	22.8 (C-29)		22.7 (C-29)
0.90 (t, 3H, H-30)	14.2 (C-30)	0.88 (t, 3H, H-30)	14.1 (C-30)

Compound 2 (β-sitosterol) was isolated as a white solid from $CHCl_3$ fraction of *U. leptocladon* and MeOH: $CHCl_3$ fraction of *B. coriacea*. The TLC (Rf 0.54) developed using n-hexane and EtOAc (1:1) as a mobile phase. The ^{1}H-NMR spectrum of compound 2 suggested the presence of six methyl (6-CH_3) resonating at δ 0.70 (3H, s), 0.85 (9H, m), 0.94 (3H, m) and 1.03 (3H, s). The oxygenated proton was represented by signal at δ 3.55 (1H, tt). The resonance at δ 5.37 (1H, dd) was due to the presence of an olefinic proton. The ^{13}C-NMR and DEPT-135 spectra of compound 2 revealed the presence of 28 well resolved signals assignable for 29 carbon atoms. The spectral data suggested that compound 2 was a β-sitosterol. The spectroscopic data (^{13}C-NMR) was also in a close agreement with the literature report for β-sitosterol (Ruangrungsi *et al*., 1987) (Table 2).

Table 2. ^{13}C NMR chemical shift values and literature report for β-sitosterol

Position	Experimental result	Literature report (Ruangrungsi *et al*., 1987)
1	37.2	37.5
2	31.9	31.9
3	71.8	72.0
4	42.3	42.5
5	140.8	140.9
6	121.8	121.9
7	31.9	32.1
8	31.9	32.1
9	50.1	50.3
10	36.5	36.7
11	21.1	21.3
12	39.8	39.9
13	42.3	42.6
14	56.8	56.9
15	26.0	26.3
16	28.3	28.5
17	56.0	56.3
18	36.2	36.3
19	19.0	19.2
20	33.9	34.2
21	26.0	26.3
22	45.8	46.1
23	23.0	23.3
24	12.0	12.2
25	29.1	29.4
26	19.8	20.1
27	19.4	19.6
28	18.8	19.0
29	11.9	12.0

Compound 3 was isolated from $CHCl_3$ fraction of *U. leptocladon* . 13C-NMR analyzed with the DEPT-135 spectrum showed the presence of six olefinic carbon atoms, two of which are quaternary, four methylene groups and four methyl groups. When comparing the NMR data generated for compound 3 with the literature report for α-humulene, they were found to agree well (Chaturvedula and Prakash, 2012; Neuenschwander *et al*., 2012) (Table 3).

Table 3. ^{13}C NMR chemical shift values compound 3 and literature report for alpha-humulene.

Position	Experimental Data ($CDCl_3$)	Literature Report (Chaturvedula and Prakash, 2012)
	δ_C	δ_C
1	124.98	124.8
2	139.14, 17.95	139.9, 17.8
3	40.42	40.5
4	127.72	127.6
5	140.98	141.0
6	37.36	37.6, 30.3
7	42.00	41.3
8	125.87	124.8
9	133.08, 15.09	133.2, 15.1
10	39.77	39.6
11	23.37	23.1

Compound 4: was isolated from EtOH fraction of *U. leptocladon* and found as a white solid with melting point 265-267°C. The ^{1}H-NMR spectrum showed the presence of six methyl groups at δ 0.65 (3H), 0.80 (9H) and 0.95 (3H). One olefinic proton situated at δ 5.32 (1H, br s) and one proton on oxygenated carbon at δ 3.65. The spectrum showed one anomeric proton (H-1′) of glucose at δ 4.22 (1H, d, J = 7.6). The other sugar protons are situated between 3.45-3.54 (2H, m), 3.12 (1H, m), 3.05 (1H, m) and 2.90 (1H, m). The ^{13}C-NMR showed 35 well resolved signals for 35 carbons. Two of the signals were due to olefinic carbons, C-5 (δ 140.9) and C-6 (δ 121.6). The signal at δ 77.5 was assigned to C-3. The signal at δ 101.3 was due to anomeric carbon of the glucose unit. The other five oxygenated signal of the glucose unit were appeared at δ 77.2, 77.2, 73.9, 70.5 and 61.6. The rest 26 signals were assigned to 26 carbon atoms of the sitosterol unit. The spectroscopic data (^{13}C-NMR) is in a close agreement with the literature report for β-sitosterol-D-glucoside (Neuenschwander *et al*., 2012; Khanna and Kannabiran, 2008) (Table 4).

Table 4: ^{13}C NMR chemical shift values and literature report for β-sitosterol glucoside.

Position	Experimental Result	Literature Report (Neuenschwander *et al*., 2012)
1	36.7	36.8
2	29.7	29.1
3	77.5	78.6
4	42.3	42.1
5	140.9	140.0
6	121.6	121.5
7	31.9	31.4
8	31.9	31.5
9	50.1	49.8
10	36.0	36.3
11	21.1	20.2
12	37.3	38.2
13	38.8	41.9
14	56.7	56.4
15	24.4	23.8
16	28.3	27.8
17	55.9	55.7
18	12.1	11.3
19	20.2	19.1
20	34.4	35.7
21	19.6	18.7
22	33.8	33.5
23	25.9	25.6
24	45.6	45.5
25	29.2	28.7
26	19.4	18.7
27	19.1	18.4
28	23.1	22.6

Compound 5 was isolated from MeOH: $CHCl_3$ fraction of *B. coriacea,* which has a structure similar to that of lucidine (Mgani, 2012). The ^{13}C-NMR spectrum was analyzed together with the DEPT-135 spectrum and showed 29 well-resolved signals representing 29 carbon atoms. Of these, 11 are methylene, six are methine, five are methyl, and seven are quaternary. Of the seven quaternary

carbon atoms, six are olefinic quaternary atoms and one is an oxygen-containing carbon atom. Based on the 1D-NMR data of compound 5 and a close observation of the structure of lucidine, it was tentatively suggested that the structure of the isolated compound is as shown in Figure 1.

Anti-inflammatory activity

I. Inhibition of cotton pellet induced granuloma by leaves extracts of the plants

The 80% MeOH extracts of *B. coriacea* and *U. leptocladon* inhibited the formation of granuloma mass and inflammatory exudates (Table 5). *B. coriacea* caused 17.5 % and 53.8 % inhibition of inflammatory exudate and granuloma mass formation, respectively. The inhibition of inflammatory exudate and granuloma were 20.1 % and 53.8 %, respectively, by *U. leptocladon* leaf extract. Comparison among groups indicated that the inhibition of formation of granuloma mass by the 80% MeOH leaf extracts of *B. coriacea* and *U. leptocladon* were comparable with the standard drug (indomethacin). Whereas, the inhibition of formation of inflammatory exudates by the 80% MeOH leaf extract of *U. leptocladon* is significantly higher than that of *B. coriacea* and the standard indomethacin.

Table 5. Anti-inflammatory activity of the 80% MeOH leaves extracts of *B. coriacea* and *U. leptocladon* on cotton pellet induced granuloma in Swiss albino mice.

Test group	Percent (%) exudate inhibition	Percent (%) granuloma inhibition
B. coriacea	17.5 b*	53.8
U. leptocladon	20.1 ac*	53.8
Indomethacin	17.5 b*	53.8

Note: Indomethacin (10 mg/kg) was used as positive control. a: compared with B. coriacea, b: compared with U. leptocladon, c: compared with Indomethacin. * indicates the mean difference is significant at $p < 0.05$.

II. Inhibition of egg albumin denaturation by leaves extracts of the plants

The leaf extracts of *B. coriacea* and *U. leptocladon* have egg albumin denaturation inhibition activity, which is comparable with the standard drug (diclofenac) (Table 6). The results showed that different concentrations of the leaf extracts of *B. coriacea* and *U. leptocladon* had a dose-dependent egg albumin denaturation inhibition activity. Figures 2-4, show the concentration dependent inhibition of protein denaturation by *B. coriacea*, *U. leptocladon* and diclofenac, respectively.

Table 6. Inhibition of egg albumin denaturation by leaves extracts of *B. coriacea* and *U. leptocladon*.

Concentration (µg/mL)	Test groups *B. coriacea* (%) (mean ±SEM)	*U. leptocladon* (%) (mean ±SEM)	Diclofenac (positive control) (%) (mean ±SEM)
100	15.2 ±0.08	15 ±0.087	15±0.17
250	24.3 ±0.08	24.1 ±0.17	24±0.08
500	45 ±0.17	44.9 ±0.17	44.8±0.09
IC_{50}	541 µg/mL	542.7 µg/Ml	542.8 µg/mL

Note: SEM stands for standard error of the mean.

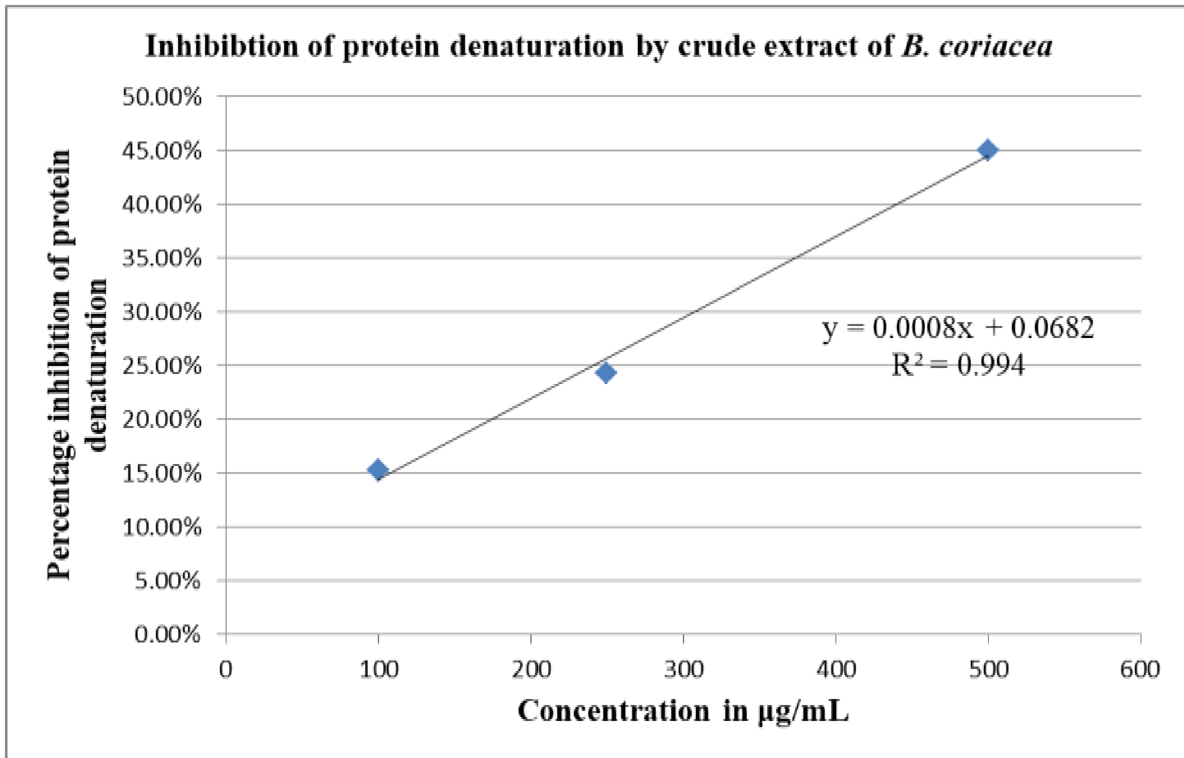

Figure 2: Inhibition of protein denaturation by the crude extract of *B. coriacea*

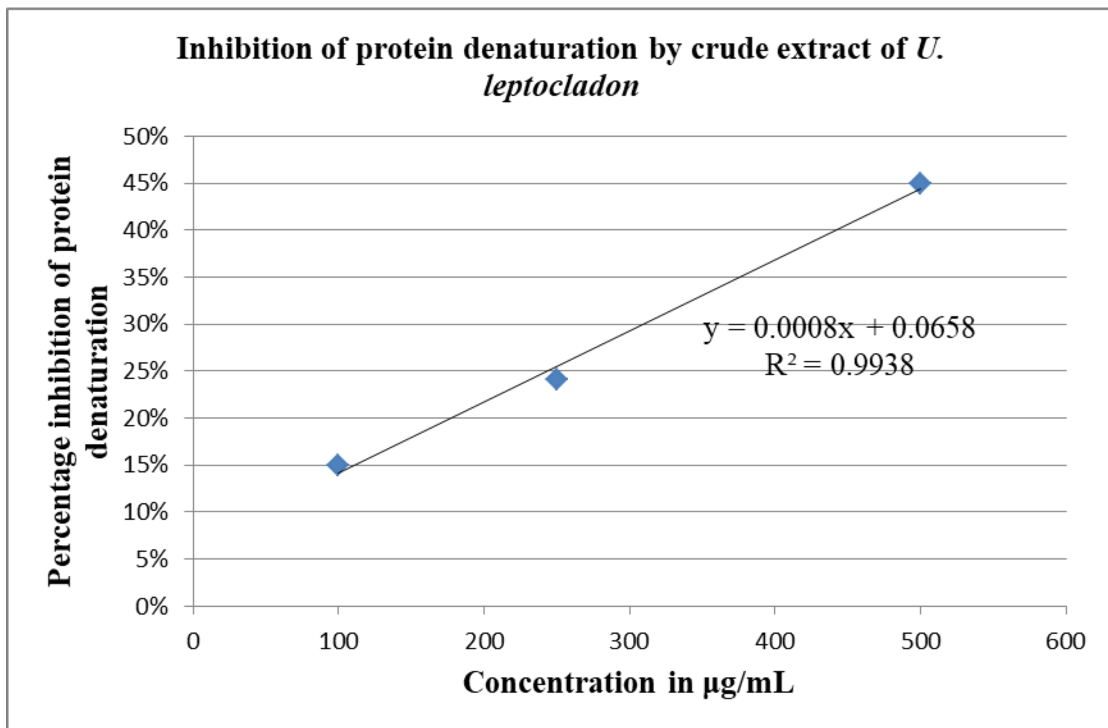

Figure 3: Inhibition of protein denaturation by the crude extract of *U. leptocladon*

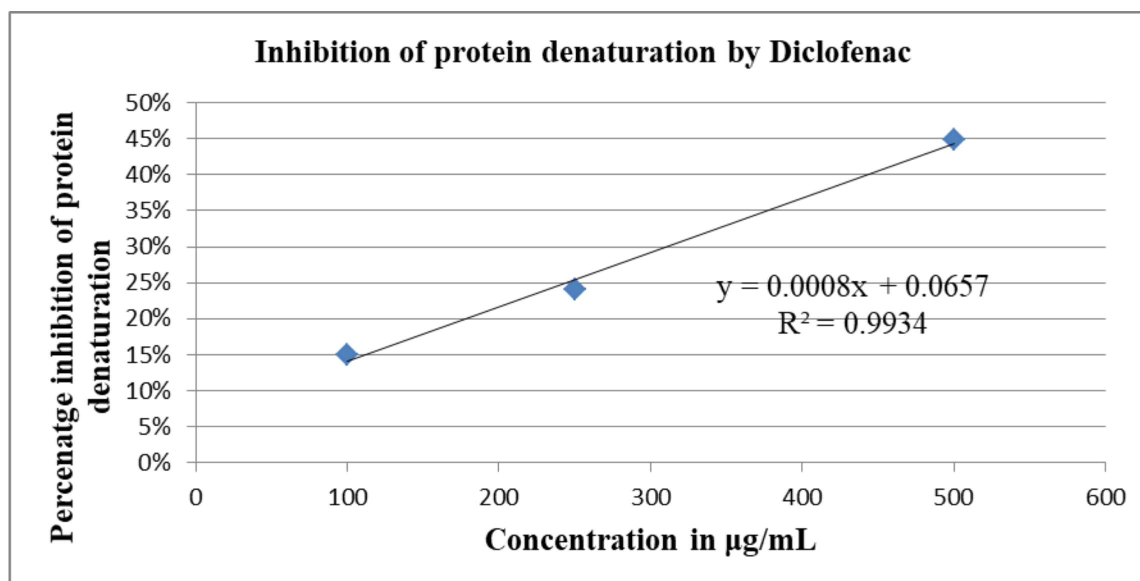

Figure 4: Inhibition of protein denaturation by Diclofenac

III. Anti-inflammatory activity of compounds isolated from the leaves extracts of the plants

All of the compounds except 1-tricontanol that were isolated from the leaf extracts of *B. coriacea* and *U. leptocladon* showed anti-inflammatory activity using carrageenan-induced paw edema model (Table 7).

Table 7. Anti-inflammatory activities of compounds isolated from the leaf extract of *B. coriacea* and *U. leptocladon* (25 mg/kg) using carrageenan induced paw edema model.

Test group	% Inhibition of paw edema (mean diameter of paw edema (mm) ±SEM)					
	1h	2 h	3 h	4 h	5 h	6 h
Indomethacin	8% c* (3.35±0.02)	28% ef* (3.05±0.02)	45% def* (2.8±0)	57% def* (2.9±0)	72% bdef* (2.48±0.01)	87.8% bdef* (2.23±0.021)
β-sitostrol glucoside	5% c* (3.48±0.04)	17% cf* (3.31±0 .04)	34% f* (3.06±0. 06)	52% def* (2.86±0.05)	58% adef* (2.86±0.05)	73% af* (2.6±0.04)
β-sitostrol	28% abef* (3.05±0.02)	38% bdef* (2.9±0.036)	42% ef* (2.85±0. 02)	54% def* (2.71±0.01)	68% adef* (2.56±0.02)	80.9% ef* (2.36±0.021)
α-Humulene	17.8% f* (3.3±0.04)	18.9% cf* (3.28±0.04)	24% acf* (3.21±0.05)	38% abcf* (3.1±0.04s)	43.3 abcf* %(3.08±0.04)	71.8% af* (2.65±0.06)
Lucidine type compound	10.9% c* (3.3±0.025)	14.3% acf* (3.23±0.02)	21.2% acf* (3.16±0.03)	40% abcf* (2.98±0.01)	42% abcf* (2.98±0.01)	63.8% acf* (2.68±0.01)
1-Tricontanol	3.4% cd* (3.51±0.05)	3.4% abcde* (3.51±0.05)	3.4% abcde* (3.51±0.05)	3.6% abcde* (3.66±0.03)	9.2% abcde* (3.66±0.04)	16.4% abcde* (3.66±0.04)
Normal saline	3.7±0	3.7±0	3.7±0	3.86±0.04	3.96±0.04	4.11±0.04

*Note: Mean diameter of paw edema was expressed in millimeter (mm). SEM stands for standard error of the mean. Indomethacin (25 mg/kg) was used as positive control. Normal saline was used as negative control. a: compared with indomethacin, b: compared with β-sitostrol glucoside, c: compared with β-sitostrol, d: compared with a-humulene type compound, e: compared with lucidine type compound, f: compared with 1-Tricontanol. * indicates the mean difference is significant at p<0.05.*

Antibacterial activities of compounds isolated from the leaves extracts of B. coriacea and U. leptocladon

β-sitosterol and β-sitosterol glucoside caused zone of growth inhibition against all the tested bacteria species using agar wall diffusion assay. Whereas, the α-humulene and the lucidine type compound showed antibacterial effects against all the tested bacteria species except *S. auerus* (Table 8). Whereas, the α-humulene and the lucidine type compounds effectively inhibited the growth of *S. typhimurium*, *P. aeruginosa*, *E. coli* and *L. monocytogenes* (Table 8). In contrast, 1-tricontanol did not show antibacterial activity against all the tested bacteria species (Table 8).

Table 8. Zone of growth inhibition by compounds isolated from the leaves extracts of *B. coriacea* and *U. leptocladon* (1mg/mL) against the bacterial species using agar well diffusion assay.

Bacteria species	Zone of growth inhibition (expressed in millimeter (mm±SEM)					
	β-sitosterol	β-sitosterol glucoside	α-Humelene	Lucidine type compound	Standard drug	
					Amoxicillin	Ciprofloxacin
P. aeruginosa	18±0.57 d*	17±1.15	16 ±0	15±1.15 a*	Not tested	33±0.57
E. coli	15 ±1.15	15±0.57	15±0	15 ± 0.57	Not tested	30 ±0
S. aureus	15±0.57 cd*	15±1.15 cd*	0±0 ab*	0±0 ab*	35±0	Not tested
S. typhimurum	15±1.15	15±0.57	15±0.57	15±0	Not tested	40±0
L. monocytogenes	15±0.57	15±1.15	15±0.57	15±0	35±1.73	Not tested

Note: Zone of growth inhibition is expressed in millimeter (mm ±SEM). Amoxicillin (25 μg/mL) was used as standard drug against L. monocytogenes and S. aureus. Ciprofloxacin (5 μg/mL) was used as reference drug against S. typhimurium, P. aeruginosa, and E. coli. a: compared with β-sitosterol, b: compared with β-sitosterol glucoside; c: compared with α-humelene; d: compared with lucidine type compound. SEM stands for standard error of the mean. * indicates the mean difference is significant at $p<0.05$.

DISCUSSION

This study evaluated the anti-inflammatory and antibacterial activities of the leaves extracts and compounds isolated from *B. coriacea* and *U. leptocladon.* With the exception of 1-tricontanol, the other four compounds that were isolated from the leaves extracts of *B. coriacea* and *U. leptocladon* (including β-sitosterol, β-sitosterol glucoside, α-humulene and the lucidine type compound) showed anti-inflammatory and antibacterial activities.

The phytochemical analysis of the leaves extracts of *B. coriacea* and *U. leptocladon* revealed the presence of tannins, alkaloids, cardiac glycoside, flavonoids, phenols, quinones, and saponins (Sintayehu Tsegaye *et al*., 2022A). The fact that steroids, saponins, alkaloids, tannins, cardiac glycosides, and flavonoids have anti-inflammatory activities (Hosseinzadeh and Younesi, 2002), may explain the anti-inflammatory activities of the leaves extracts of *B. coriacea* and *U. leptocladon* observed in this study.

In agreement with the findings of this study, other researchers also showed that β-sitosterol and β-sitosterol glucoside possess antibacterial activities against gram-positive and gram-negative bacteria (Mgani, 2012; Sen *et al*., 2012; Nyanchoka, 2016; Ododo *et al*., 2016; Luhataab and Usukia, 2021 and Anwar *et al*., 2022).

The antibacterial activity of the compounds noted in this study was in line with our previous study (Sintayehu Tsegaye *et al*., 2022B), which demonstrated antibacterial activities of the crude extracts and fractions of the leaves extracts of *B. coriacea* and *U. leptocladon*.

In this study, β-sitosterol and β-sitosterol glucoside have also shown anti-inflammatory activities. The anti-inflammatory activitiy of β-sitosterol observed in this study are comparable with the findings of other studies that also reported antioxidant and anti-inflammatory activities of β-sitosterol (Gupta *et al*., 2006; Ayaz *et al*., 2017; Baskar *et al*., 2012; Anwar *et al*., 2022). β-sitosterol exerts an anti-inflammatory effect by reducing the expression of inflammatory factors such as interleukin-6 (IL-6), TNF-α, and COX-2 (Anwar *et al*., 2022).

In line with the findings of this study, other researchers have also demonstrated that α-humulene possesses antibacterial and anti-inflammatory activities (Fernandes *et al*., 2007; Rogerio *et al*., 2009; Ali *et al*., 2017; Sun *et al*., 2020).

The lucidine-type compound also showed antibacterial and anti-inflammatory activities.

Carrageenan (a sulfated polysaccharide) causes acute inflammation by the inducing production of various inflammatory factors, including bradykinin, serotonin, histamine, prostaglandins, pro-inflammatory cytokines (such as TNF-α, and interleukin-1β), neutrophil infiltration, neutrophil-derived free radicals, and nitric oxide (Halici *et al*., 2007; Souto *et al*., 2011). Therefore, the inhibition of the carrageenan-induced mice paw edema observed in this study could be due to the inhibition of the release of prostaglandins, histamine, serotonin, bradykinin, pro-inflammatory cytokines, neutrophil infiltration, neutrophil-derived free radicals, and nitric oxide by the phytochemicals that are found in the leaf extracts of *B. coriacea* and *U. leptocladon.*

The anti-inflammatory activity of the compounds noted in this study is in agreement with our previous study (Sintayehu Tsegaye *et al*., 2022A), which demonstrated anti-inflammatory activities of the crude extracts and fractions of the leaves extracts of *B. coriacea* and *U. leptocladon*.

Cotton pellet induced granuloma model was used to evaluate the proliferative and transudative components of chronic inflammation (Carey *et al*., 2010; Patil *et al*., 2012). The inflammatory processes that occur during the cotton pellet induced inflammatory response can be broadly classified into two phases, transudative phase and the proliferative phase. During the transudative phase, an increase in the cotton pellet's wet weight occurs as a result of leakage of fluid from blood vessels produced by an increase in vascular permeability (Sengar *et al*., 2015). Whereas, during the proliferative phase, the formation of granuloma tissue occurs, this brings about an increase in the cotton pellet's dry weight (Carey *et al*., 2010; Wilches *et al*., 2015). The formation of granuloma tissue arises from an increase in fibroblasts, the production of collagen and mucopolysaccharides, and the penetration of proliferating fibroblasts into exudate. The suppression of the formation of granuloma tissue arises from the suppression of the proliferative phase (by suppressing the proliferative agents involved in formation of granuloma tissue) (Anwar *et al*., 2022). Therefore, the decrease in granuloma tissue formation noted in this study, which is indicated by the decrease in the dry weight of cotton pellet granuloma tissue in the mice treated with extracts of *B. coricea* and *U. leptocladon* might be due to the suppression of the proliferative phase of inflammation by β-sitosterol, β-sitosterol glucoside, α-humulene, and lucidine type compounds

Protein denaturation induces inflammation (Pingsusaen *et al*., 2015). Therefore, inhibition of protein denaturation may result in inhibition of inflammation. The *in vitro* anti-inflammatory study showed that the leaf extracts of *B. coriacea* and *U. leptocladon* have inhibitory activity on protein (egg albumin) denaturation. The egg albumin denaturation inhibition activities of the leaf extracts of *B. coriacea* and *U. leptocladon* are comparable with that of the Diclofenac (the standard drug), suggesting that the leaf extracts of *B. coriacea* and *U. leptocladon* are promising sources of chemicals that can be used for the development of effective anti-inflammatory drugs.

Verma *et al* (2011) demonstrated that flavonoids obtained from plants could inhibit the denaturation of protein. The interaction of proteins with phenolic compounds can improve the thermal stability of proteins (Ghasemzadeh and Ghasemzadeh, 2011; Verma *et al*., 2011). Thus, the inhibition of protein denaturation by the leaf extracts of *B. coriacea* and *U. leptocladon* may be due to the high phenolic and flavonoid content found in the leaf extracts of *B. coriacea* and *U. leptocladon.*

CONCLUSION

The leaves extracts of *B. coriacea* and *U. leptocladon* contain compounds, which have potent anti-inflammatory and antibacterial activities. Further study is needed to elucidate the mechanisms underlying the anti-inflammatory and antibacterial actions of β-sitosterol, β-sitosterol glucoside, α-humulene and lucidine type compound. The exact structural elucidation of the lucidine type compound requires further investigations.

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Appendix 1: Spectra of the isolated compounds.

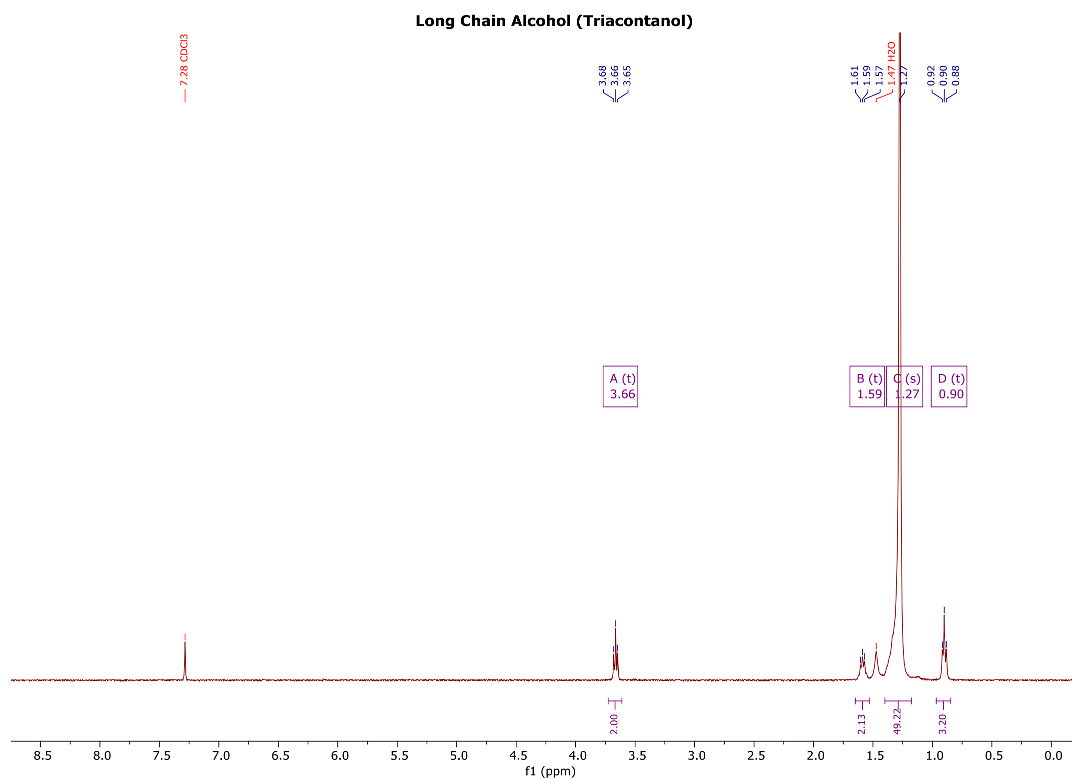

Figure 1. ^{1}H NMR spectrum of 1-Triacontanol.

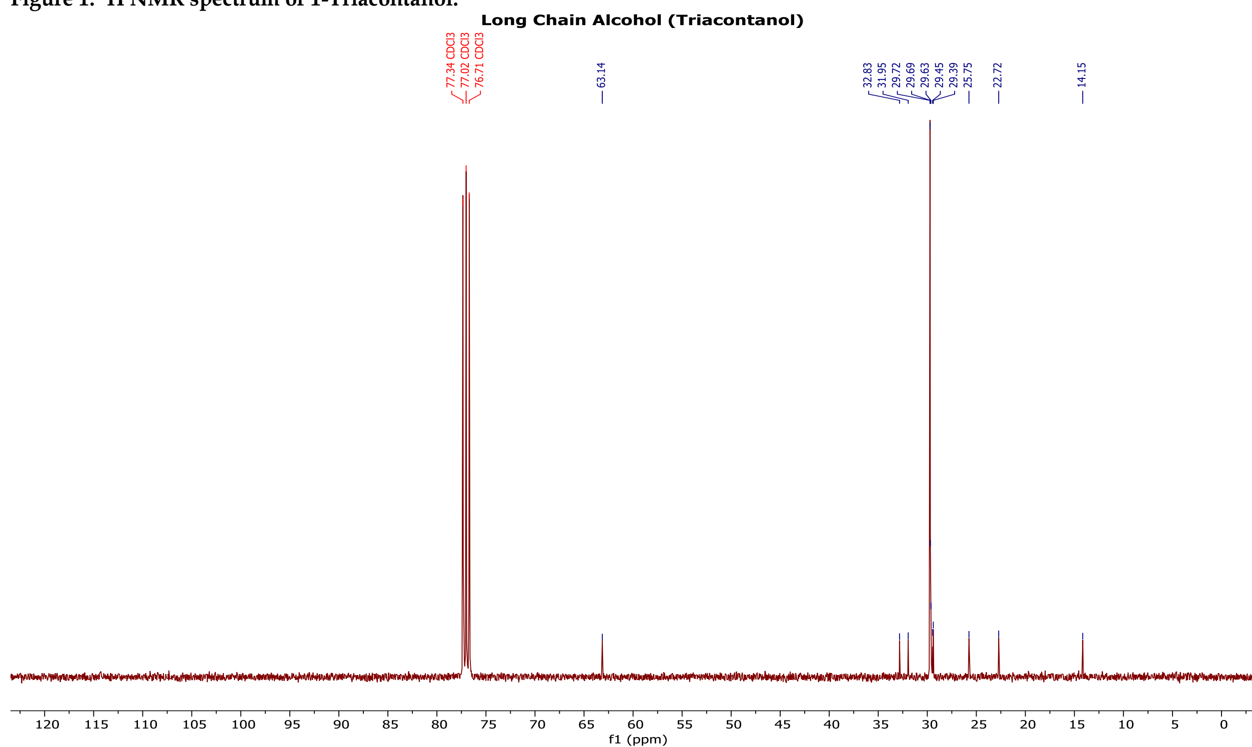

Figure 2. ^{13}C NMR spectrum of 1-Triacontanol.

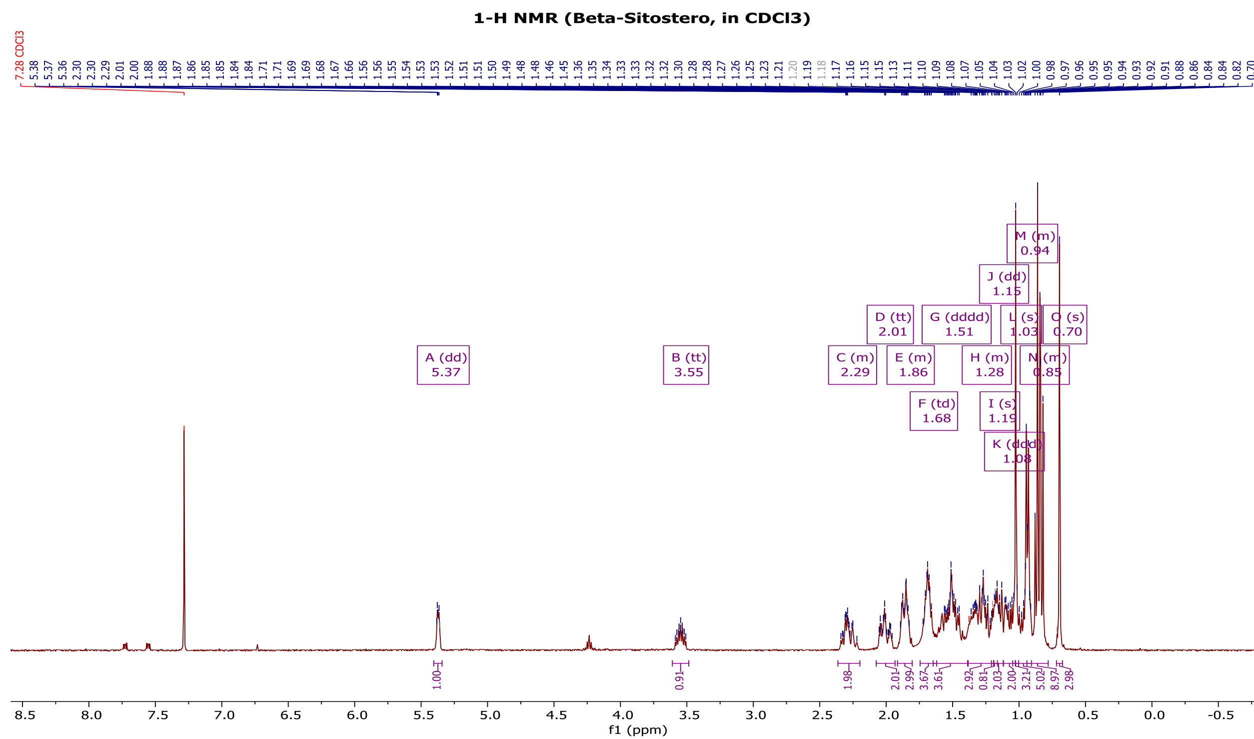

Figure 3. ¹H NMR spectrum of β-Sitosterol.

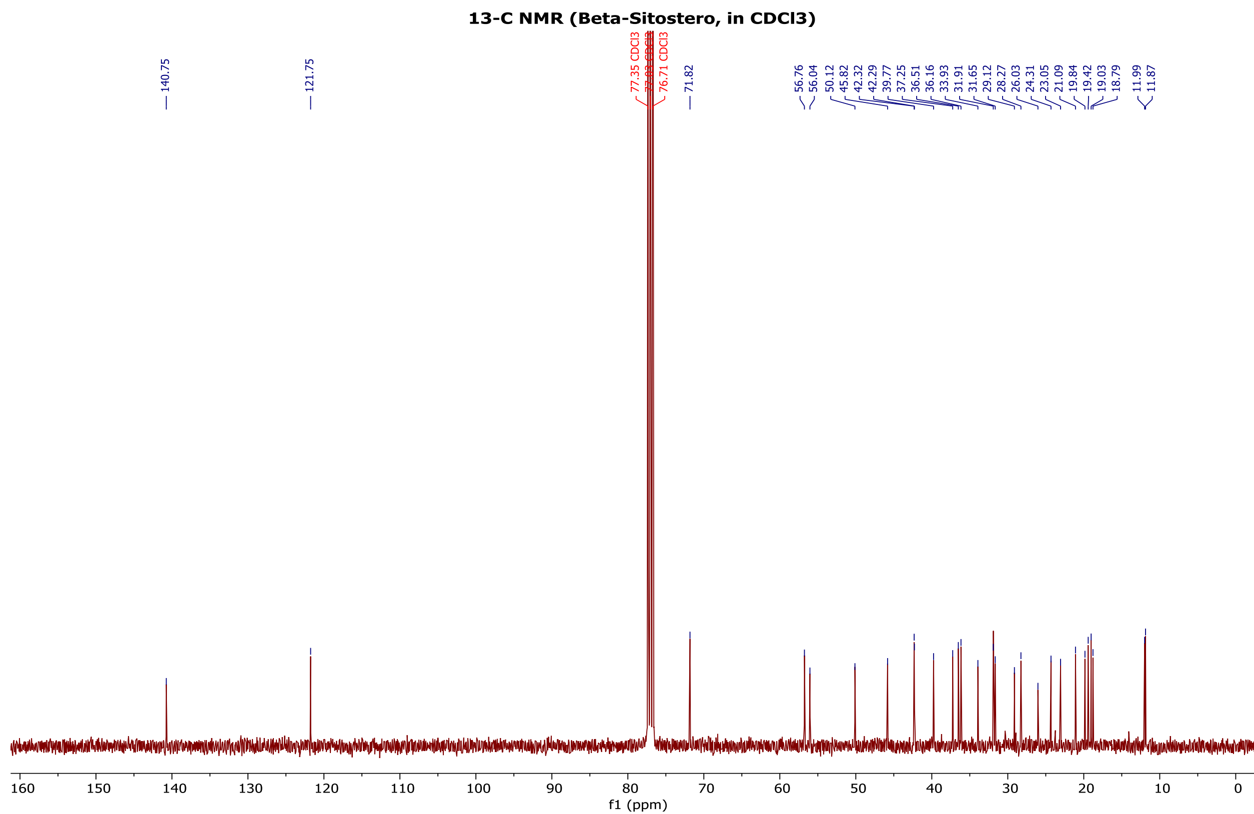

Figure 4. ¹³C NMR spectrum of β-Sitosterol.

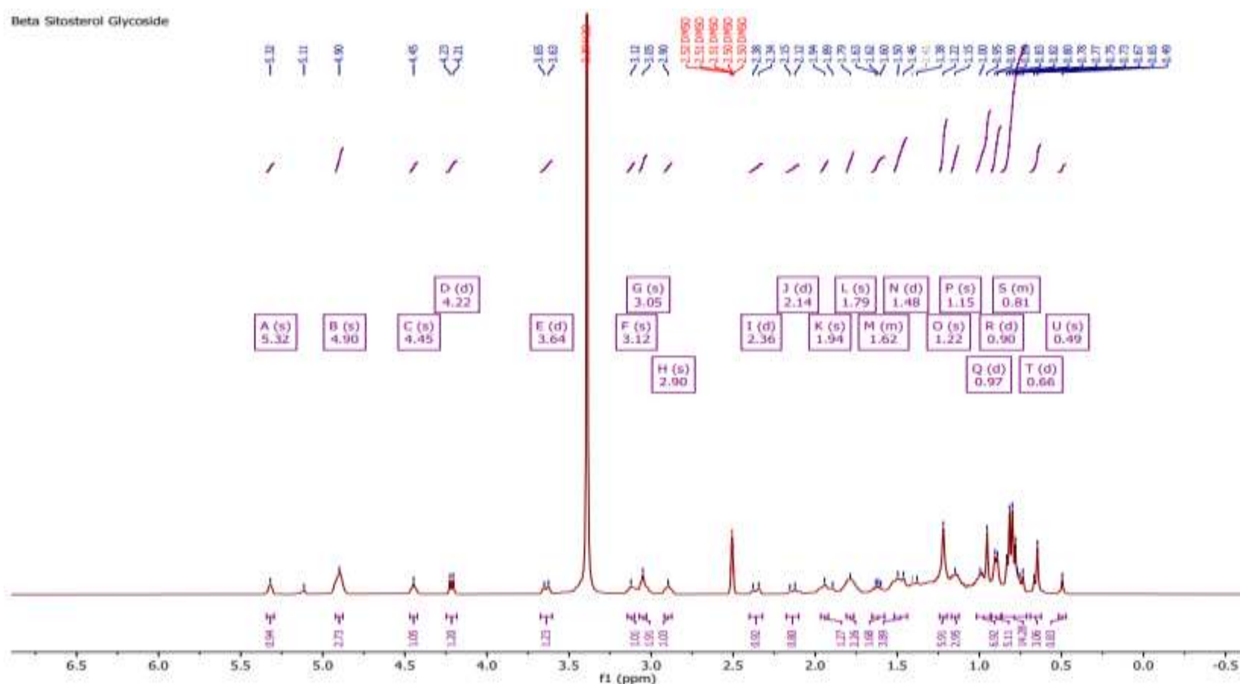

Figure 5. ¹H NMR spectrum of β-Sitosterol glucoside.

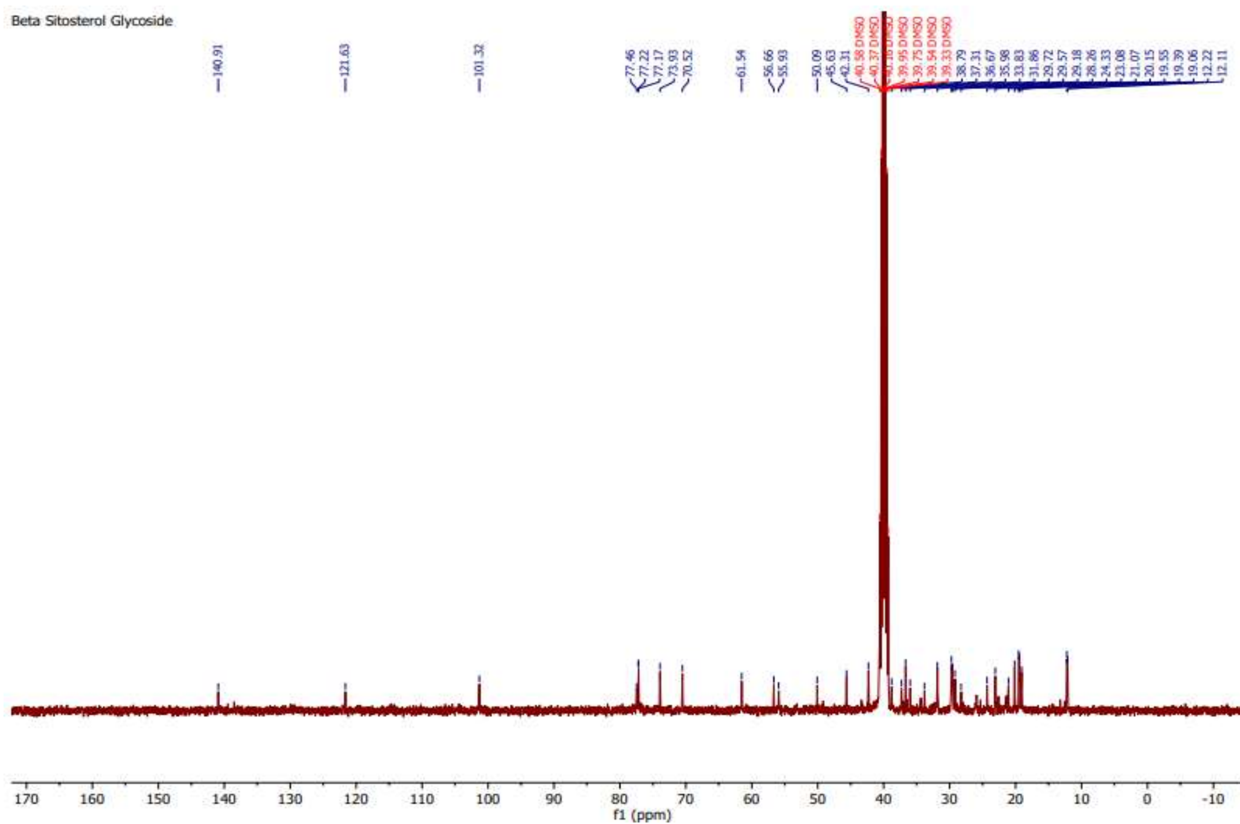

Figure 6. ¹³C NMR spectrum of β-Sitosterol glucoside.

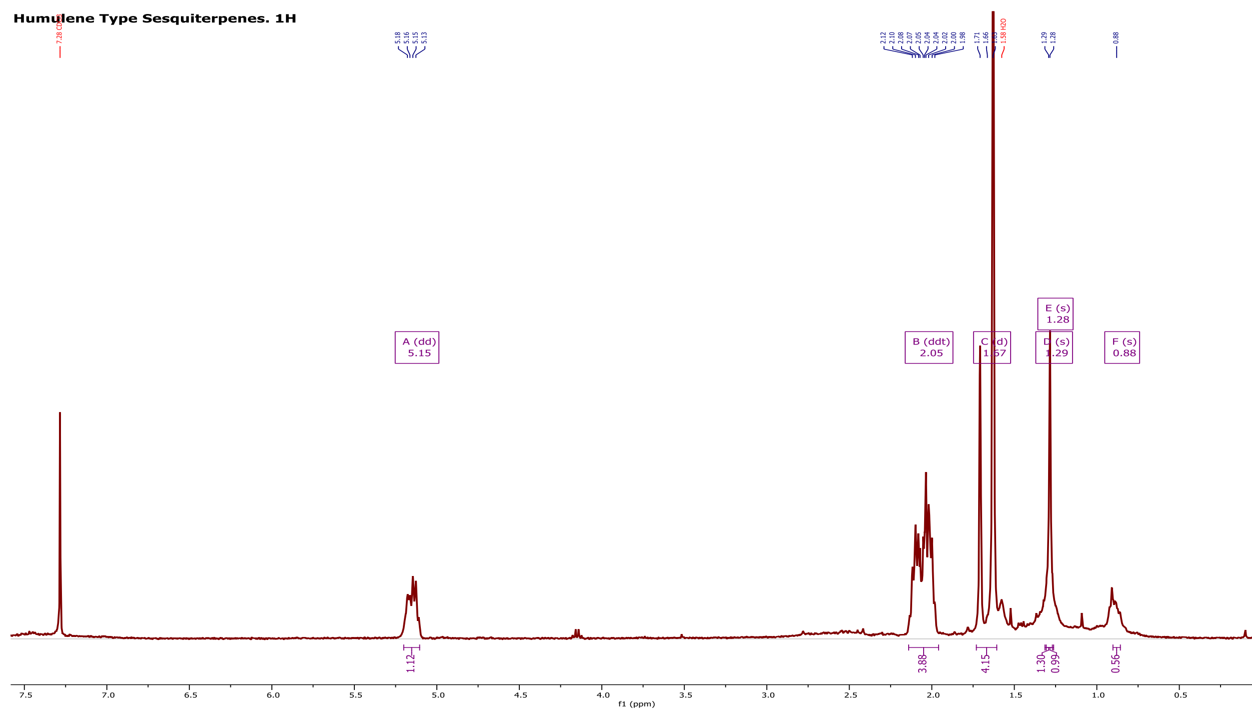

Figure 7. ^{1}H NMR Humulene type compound.

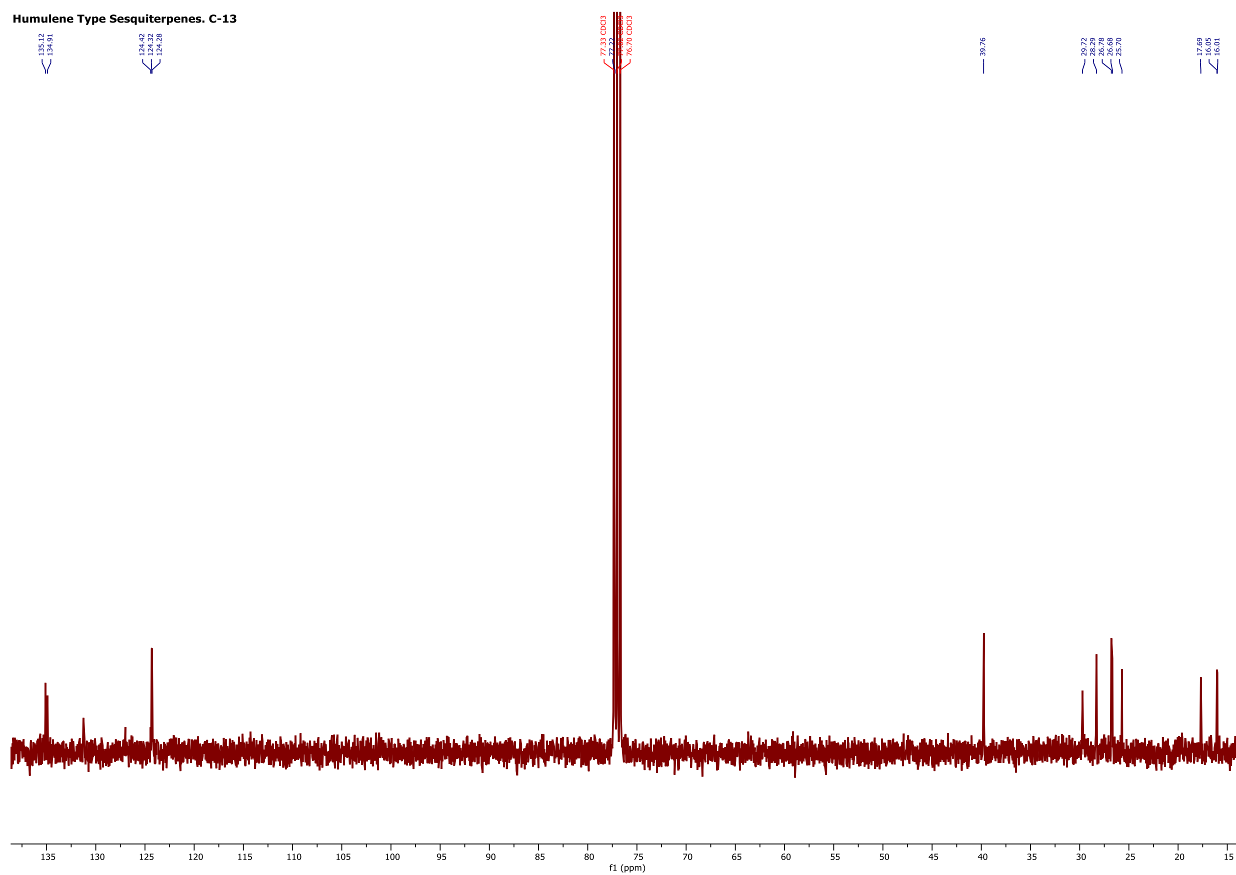

Figure 8. ^{13}C NMR Humulene type compound.

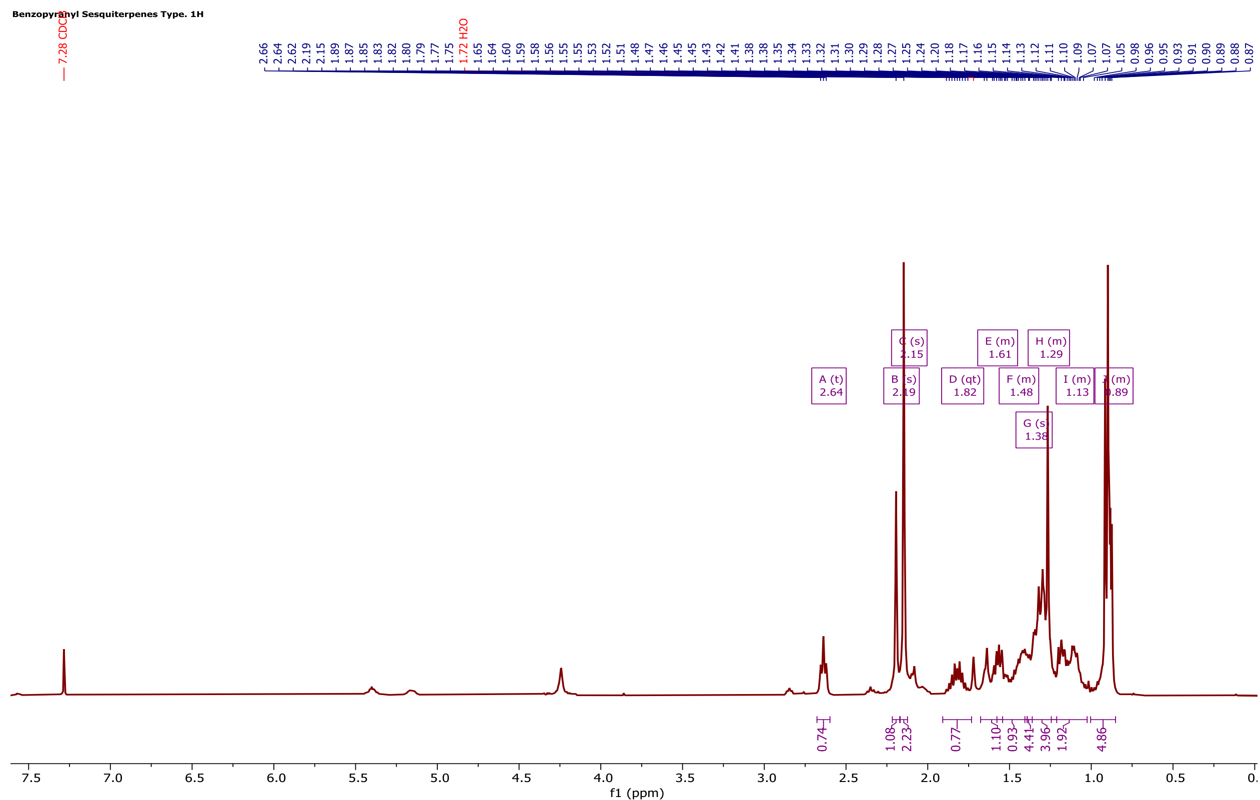

Figure 9. ^{1}**H NMR Lucidine type compound.**

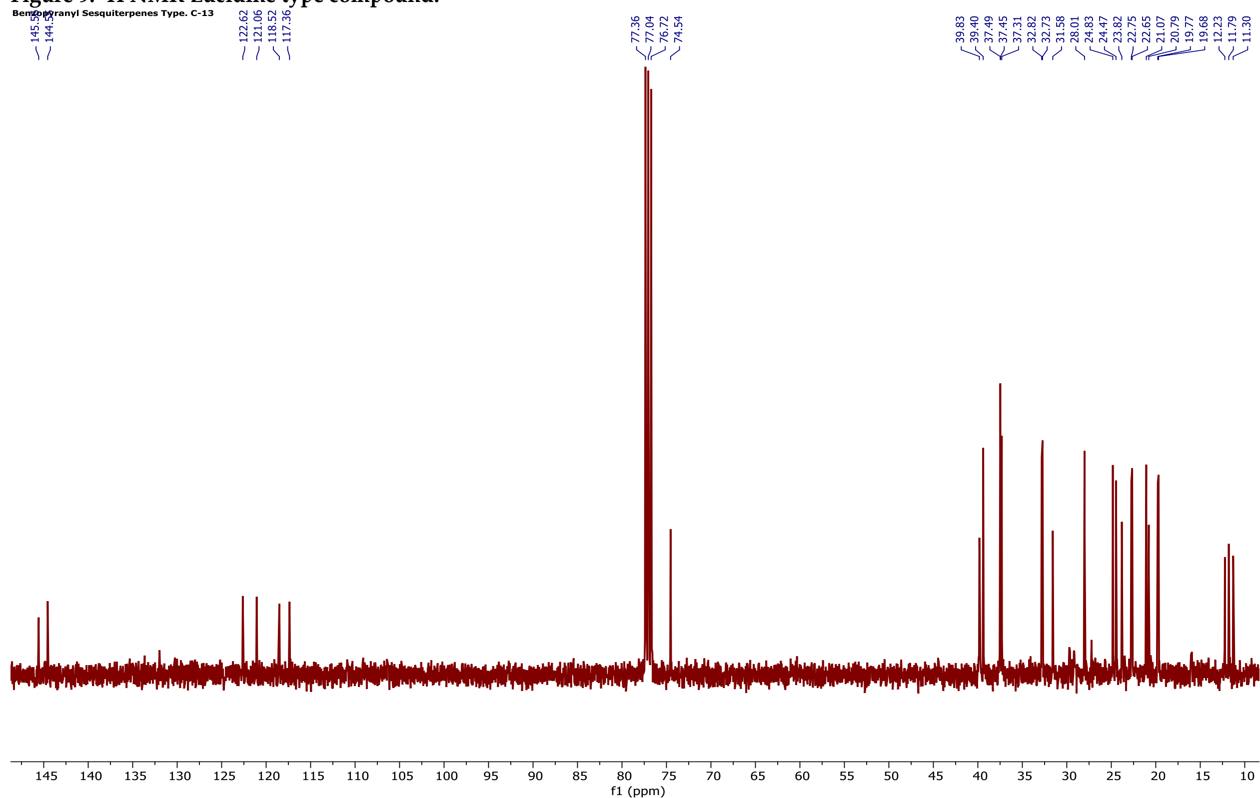

Figure 10. ^{13} **C NMR Lucidine type compound.**