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Phytochemical Investigation on The Root of *Cyphostemma Adenocaule (Steud. Ex A. Rich)*

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Abstract: *Cyphostemma adenocaule (Steud. ex A. Rich)* (family: Vitaceae) is herbaceous climber plant which grows in Ethiopia and has been used as a traditional medicinal plant for the treatment of snake bite, rabies, blackleg and etc. The present study was carried out on the phytochemical investigation and antimicrobial activities on the root extract of C. adenocaule. The powdered root of plant was extracted with methanol. Then, the methanol crude extract was sequentially partitioned with organic solvents in the increasing order of polarity: petroleum ether, chloroform, ethyl acetate and methanol respectively. Phytochemical screening of C. adenocaule root was performed on methanol crude extract and shown the presence of alkaloids, terpenoids, steroids, anthraquinones and glycoside compounds. The crude extracts of petroleum ether, chloroform, ethyl acetate and methanol were tested in-vitro antimicrobial activity by disc diffusion method. The maximum antibacterial activity was exhibitted in chloroform crude extract against S. aureus and E. coli (inhibition diameter 15 mm and 22.50 mm respectively). The solvent extraction (chloroform extract) followed by PTLC of root extract of C. adenocaule yielded one compound which was characterized as **β-sitosterol** using FT-IR, and NMR spectroscopic techniques and by comparing with literature reports.

Keywords: *Cyphostemma adenocaule (Steud. ex A. Rich)* (family: Vitaceae) is herbaceous climber plant which grows

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Approximately 25% of drugs in modern pharmacopoeia were derived from plants and many others were synthetic analogues built on prototype compounds isolated from plants [1]. Medicinal plants are used worldwide, especially in undeveloped nations. More than 80% of populations in these countries use herbal products to treat many diseases. Medicinal plants have become the focus of intense study to correlate their traditional uses with actual pharmacological effects. With the increasing acceptance of traditional medicine as an alternative form of health care, the screening of

medicinal plants for active compounds is very important [2]. Phytochemicals are bio-active chemicals of plant origin. They are regarded as secondary metabolites because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [3].

The genus *Cyphostemma* belongs to the family of vitaceae which consist of a wide range of creeping plants with broadly ovate leaves. It is herbaceous climber or scrambler with stems growing to 6-10 meters long. It is found on wooden grassland, riverine forest and clearings of forest [4]. Even though various species now occur in different parts of the world, they originated from Africa and Madagascar. *Cyphostemma adenocaule* is widely disributed in tropical Africa and grows in Ghana, Ethiopia, Eritrea, Tanzania, Kenya, Gabon, Angola and Uganda [5].

Some literatures reported that it has been used in traditional pharmacopoeia for the treatment of several diseases such as malaria, urinary tract infections, syphilis, inflammatory pain and bloody diarrhea [6]. In Tanzania, the leaves of *C. adenocaule* are used to prepare medicines used in the treatment of sore throat, cough and pneumonia and Aerial parts are macerated in water and applied on skin to lighten the skin. In Ghana, Gabon and East Africa, a paste made from its root is applied to treat abscess and inflammation [5]. The root of the herb is used as a medicine traditionally and was employed as a test material to assess antimicrobial effects and inhibited the growth of *Streptococcus agalactiae* and *Staphylococcus aureus* isolates at 40%-5% concentration levels [7]. It was also reported that *cyphostemma adenocaule* used in the treatment of Peptic ulcers. It Contains carotenoids (carotenes), xanthophylls, Vitamin C, Tocopherols, and Tocotrienols [8]. In Kenya it has been reported that *cyphostemma adenocaule* is used for the treatment of Colorectal, breast and skin cancer in which the leaves are powdered and root bark boiled and taken orally as an infusion half a glass once a day until recovery and has a medicinal use for tuberculosis, arthritis [9-10].

Cyphostemma adenocaule (local name- ‘ Luxa or Hida Bofa ’ in Afan oromo) was reported to be the most widely used plant for treating blackleg in Ada’a District, East Shewa Zone of Oromia Regional State, Ethiopia [11]. Traditional healers around Gondar town, Ethiopia use boiled root of *Cyphostemma adenocaule* with milk, filtered and the filtrate is taken in empty stomach by oral administration to treat rabies [12]. In Hawzen district of Tigray regional state, Northern Ethiopia, the fresh root of *Cyphostemma adenocaule* is also used as anti-snake bite [13].

Phytochemical investigations on the genus are scarce. However, the preliminary screening of the *Cyphostemme. digitatum* extracts showed that it exhibited inhibitory activity against *Bacillus subtilis*, good antioxidant activity and moderate antifungal potential and the presence of alkaloids, flavonoids, saponins, coumarins, steroids, triterpenes and tannins have been reported [14]. Phytochemical investigation on *Cyphostemma adenocaule* and *Cyphostemma maranguense* leaves revealed that both species showed same phytochemical profile with the presence of alkaloids, flavonoids, steroids and tannins in the similar proportions based on the colour intensity observation. However, *C*. *maranguense* contained less amounts of sterols and tannins than *C. adenocaule.* Both plants have been used in Tanzania as traditional beauty for skin brightening. Their application for skin lightening may be associated with the presence of steroids since steroids have catabolic and anti-anabolic effect on the skin which results to the thinning of the skin, making the skin appear lighter. In Tanzania the phytochemical study exhibited that aerial part of *cyphostomma adenocaule* contain flavanoids, phenol and sterol in high amounts as well as alkaloids, steroids and tannins in higher amounts [15]. Investigation of the crude extract of *Cyphostemma greveana* barks yielded five (**1–5)** bioactive compounds (**Figure 1**) bellow [16].

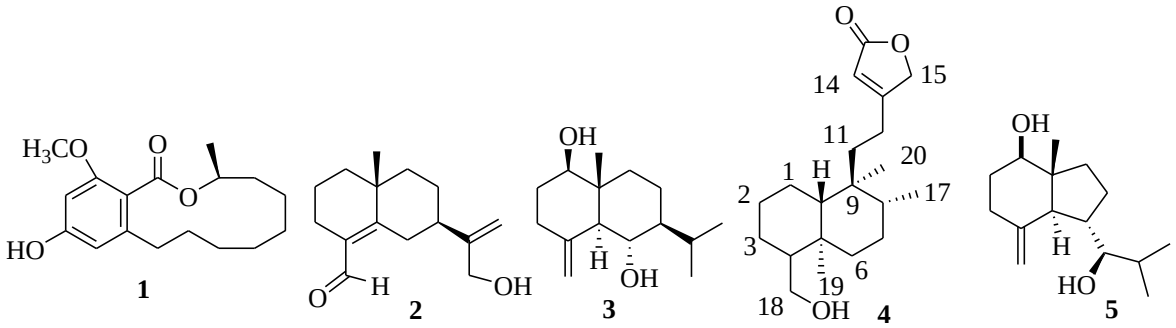
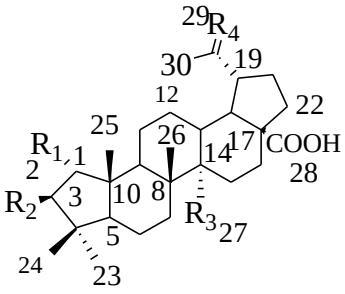
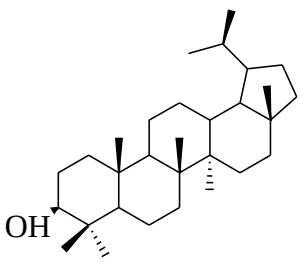


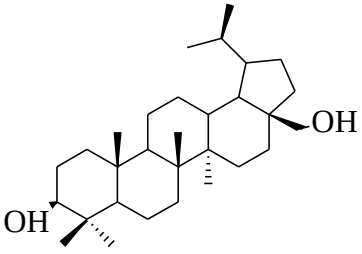
Figure 1 : Structure of 5 Compounds isolated from Cyphostemma greveana

Chouna, J.R. et al conducted phytochemical investigation on the bark and wood methanol extract of *C. adenocaule* and resulted in the isolation, structure elucidation of two new ceanothane-type triterpenoids, cyphostemmic acid **A 6** and cyphostemmic acid **B 7**, epigouanic acid A **8,** zizyberanal acid **9**, lupeol **10**, betulin **11**, betulinic acid **12,** □-sitosterol **13** and its glycoside (□-sitosterol-3-O-b-d-glucopyranoside **14**) and two derivatives (cyphostemmic acid **C 15**, cyphostemmic acid **D 16)** obtained by ozonolysis of cyphostemmic acid **A 6** and epigouanic acid **A 8** respectively. Ozonolysis of of betulin **11** afforded 3b-28-Dihydroxy-30-norlupan-20-one **17**. Several ceanothane type triterpenoids were found to possess antiplasmodial and antimycobacterial activities (**Figure 2**) [6].



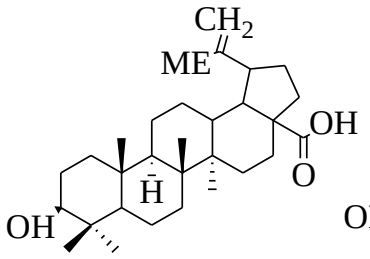


lupeol (**10**)

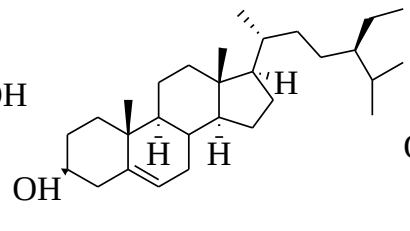


betulin (**11**)

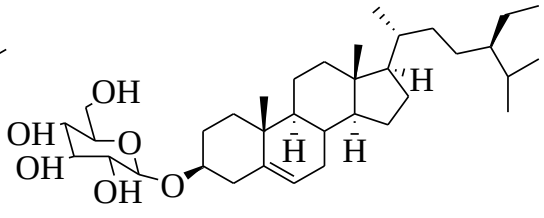
	R1	R2	R3	R4
6	CH_2OH	OH	COOH	CH_2
7	CH_2OH	OH	CH_2OH	CH_2
8	CH_2OH	H	COOH	CH_2
9	COH	H	COOH	CH_2
15	CH_2OH	OH	COOH	O
16	CH_2OH	H	COOH	O



betulinic acid (**12**)



β-sitosterol (**13**)



β-sitosterol glycoside (**14**)

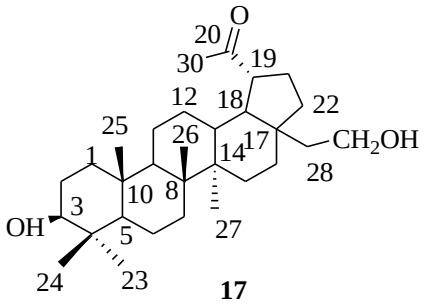


Figure 2 : Structure of Compounds 6-17 isolated from the bark and wood of c. adenocaule

Yet, a close look at the literature review indicated that no study has been done on phytochemical investigation of the root of *Cyphostemma adenocaule*. Therefore, the main aim of this work was to evaluate the anti-microbial activity of crude extract, isolate and elucidate the structure of possible bioactive chemical(s) from the root of *cyphostemma adenocaule* by using various chromatographic techniques such as thin layer chromatography and preparative thin layer chromatography. The structure of the pure isolated compound was elucidated by means of spectroscopic techniques.

II. Experimental Section

II.1. General Experimental Procedures

The solvents used in extraction and isolation including: *n*-hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol are Analytical grade reagent and are products of Sigma Aldrich. Milling machine and whatmann number 1 filter paper (110x100 circles) were used for powdering sample and filtration respectively. Rotary Evaporator (Heidolph Laborata 4000) was used for solvent evaporation from extracts. TLC and PTLC analyses were carried out on TLC plates 0.2 mm thick layer of Merck silica gel 60 (mesh) coated on aluminum foil and precoated Silica gel plates (Merck, Kiesel gel 60 F254, 0.25mm). Compounds on TLC were detected using UV light (254 and 365 nm). Separatory funnel was used in the partition of the crude methanol extract using solvents of increasing polarity. The FT-IR samples were prepared using spectral grade KBr and made into pellets and measured on Perkin-Elmer IR spectrophotometer in the range between 4000 cm^{-1} and 400 cm^{-1}. 1D ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra were recorded on a Bruker advance spectrometer at 400 MHz (^{1}H) and 100 MHz(^{13}C) at room temperature using $CDCl_3$ as solvent and TMS as the internal standard. The chemical shifts were reported in δ (ppm) units relative to TMS signal. Standard antibiotic drug (ciprofloxacin 500 mg), Mueller Hinton agar was used as a culture medium during antibacterial test.

II.2. Collection of Plant Material and Preparation of extracts

The root of *cyphostemma adenocaule* was collected in March, 2017. The basic operations including steps, such as pre-washing to remove dirt, drying of plant materials, grinding of the dried root to obtain a homogenous sample and increasing the contact of sample surface with the solvent system to facilitate the extraction process. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds n-hexane, petroleum ether and chloroform can be used.

II.3. Crude Extraction

The air dried and ground root of *C.adenocaule* (603gm) was soaked in 2L of methanol for 72 hr. After 3 days the mixture was filtered using whatmann No.1 filter paper by using gravity filtration and the filtrate was concentrated by using rotary evaporator to obtain the crude extract. 43gm of crude methanol extract was obtained after solvent recovery.

II.4. Phytochemicals Screening on root of *cyphostemma adenocaule*

The phytochemical report on the roots of the plant under investigation is scarce. Therefore, the preliminary phytochemical screening for the roots of *c.adenocaule* was carried out according to standard methods to analyze the presence of compounds namely: Saponins, Anthraquinones, Flavonoids, Phenols, Alkaloids, Tannins, Terpenoids, Steroids, Phlobatannins and Glycosides [17].

II.5. Liquid-liquid partition of methanol crude extract

The appropriate eluent (mobile phase) for chromatographic separation was selected by checking the TLC of crude methanol extract for the mixture of n-hexane and ethyl acetate in the increasing order of the polarity (decreasing the amount of n-hexane while increasing the amount of ethyl acetate). The complex spots were observed at 7:3, 6:4 and 1:1 ratio of n-hexane and ethyl acetate. But the clear spot with best separation was obtained using n-hexane/ ethyl acetate (2:3) ratio. Due to the fact that plant extracts usually occur as a combination of various types of bioactive Compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds.
Furthermore, methanol is a polar solvent and hence, it has the ability to extract polar and non-polar compounds. Therefore, about 32 gm of methanol crude extract was partitioned into different solvent extracts of increasing polarities (petroleum ether, chloroform, ethyl acetate and methanol) using separator funnel. The crude methanol extract was first dissolved in 150 mL of petroleum ether and the petroleum ether extract was collected by a pipette. The non- dissolved part (bottom layer) was then dissolved in 150 mL of chloroform and the chloroform extract was collected by opening the stop cock. Likewise, the non-dissolved part (top layer) was dissolved in 150 mL of ethyl acetate and the ethyl acetate extract was collected by a pipette. Finally, the non-dissolved part (bottom layer) was dissolved in methanol and the white precipitate labelled as **GF-2** was settled at the bottom of separatory funnel. The methanol extract was collected by using a pipette. The white crystal (**GF-2**) was again washed by methanol, then with diethyl ether. The outline of liquid-liquid partition was shown in (**Figure 3**).

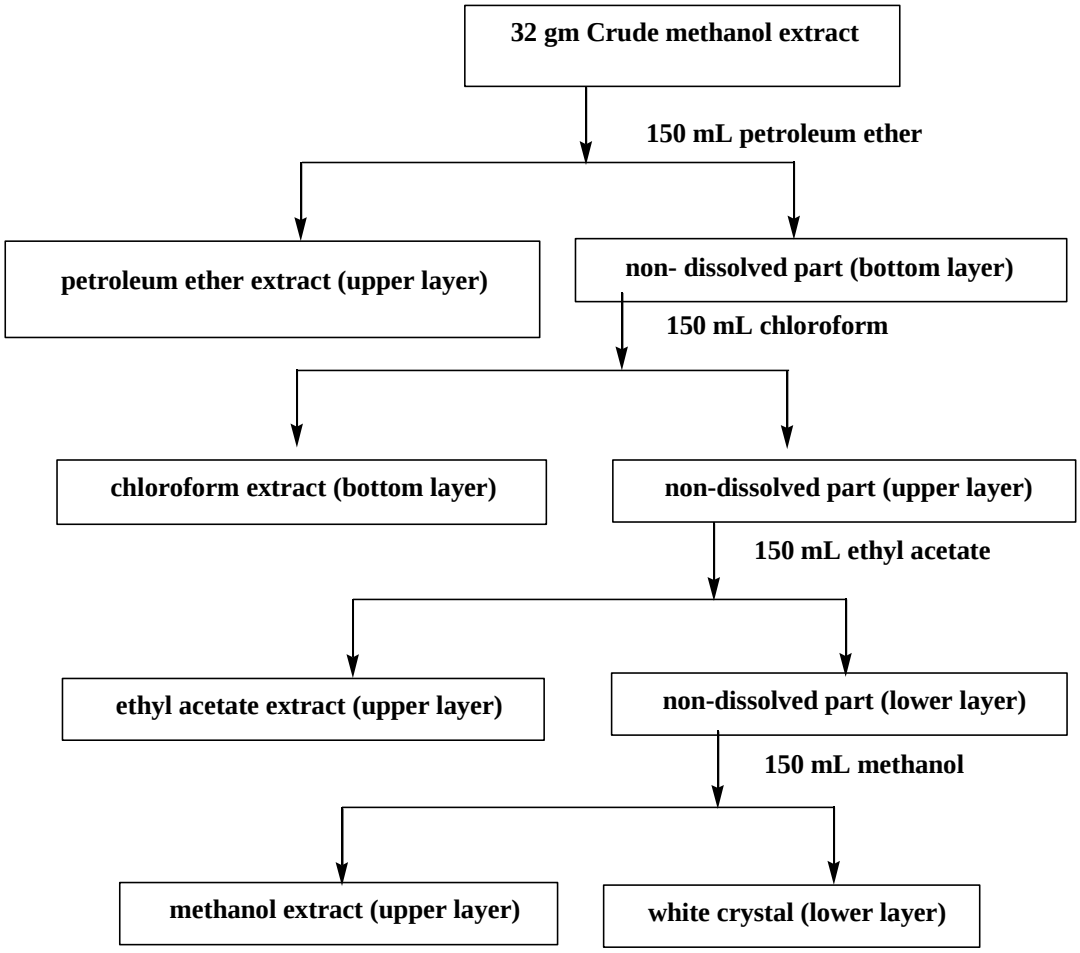


Figure 3 : *Flow chart of Liquid-Liquid partition of crude methanol extract of roots of C.adenocaule*

II.6. Antibacterial assay

II.6.1. Preparation of test solutions and bacterial strains for preliminary activity

The test solutions were prepared by dissolving 50 mg of crude extract in 1 mL of dimethyl sulfoxide (DMSO) to achieve final stock concentration of 50 mg/mL solution of the test sample. Microorganisms used for evaluation of antibacterial activities of the crude extracts were Gram-positive (*Staphylococcus aureus)* and Gram-negative *(Escherichia coli).* These standard bacterial strains were obtained from the Department of Bioechnology, University of Gondar.

II.6.2. Preparation of fresh inoculums for bioactivity test of crude extracts

The antibacterial activity test was done using disc diffusion method standard procedures [18]. Muller Hinton Agar culture media was used for growing of organisms. The culture media was boiled in distilled water to dissolve the media and autoclaved at 121°C for 50 min, and poured into sterile Petri dishes. After the culture media solidified, organisms were uniformly seeded with it. Two well isolated colonies of the same morphological type were selected from an agar plate culture and the top of each colony was touched with a loop, and the growth was transferred into a tube containing 4.5 mL of a suitable nutrient broth medium. Inoculums of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. 50 mg/mL concentration of 20 µL of the working suspension of the same concentration of the sample plant gradient extracts were impregnated using cork method (diameter 6 mm) with the help of

micropipette. Positive control using Ciprofloxacin was assayed simultaneously. Plates were left for 10 minutes till the extract diffuse in the medium with the lid closed and incubated at 37°C for 24 hours. After overnight incubation, the plates were observed for the zone of inhibition (ZI) and the diameter of the inhibition zone was measured using ruler and mean was recorded.

II.7. Isolation and Purification of compounds from chloroform extract

The eluent for use as mobile phases for PTLC were experimentally determined by TLC experiments and by successive trials with increasing order of polarity. The TLC of each extract was checked for eluents of n-hexane: ethyl acetate (3:7, 2:3, and 1:1) and about three spots were observed for chloroform and ethyl acetate extracts. However, the components with clear bands were seen in chloroform extract. Therefore, the chloroform extract was first concentrated to dryness and the oily crude was applied on PTLC to isolate and purify the compounds of interest using the eluent of n-hexane: ethyl acetate in the ratio of (2:3). About six bands were observed and these bands were different in color under UV lamp. Each band was collected separately and dissolved in chloroform and acetone (band 1 in chloroform while bands: 2,3,4,5 and 6 in acetone). Out of six bands, only band 1, which was labelled as **GF-1** (10mg) was characterized by spectroscopic techniques. It is slightly white crystal.

II.8. Identification and characterization of the active compounds

The isolated compounds (**GF-1 and GF-2**) were characterized using FT/IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectroscopic techniques.

Iii. Results and Discussion

III.1. Determination of the Yield of Extraction

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. The percentage yield of crude extract in respective solvent was recorded in (**Table 3. 1)**. It was calculated according to the method as follows [19].

$$\% \text{ Yield} = \frac{Weight\ of\ crude\ extract}{Weight\ of\ plant\ sample}\ x\ 100$$

Table 1*: Comparison on yield of each solvent crude extract at room temperature*

Crude solvent extract	**Amount (gm)**	**% Yield**
Methanol before extraction	43	7.13
Post partition		
Petroleum ether	4.5	14
Chloroform	6.4	20
Ethyl acetate	10	30.23
Methanol	11	34

In general as shown in (**Table 1)** the yield of methanol extract is the highest compared to any other solvent extracts. However, the yield of petroleum ether extract is the least one. To get the high amount of crude extract, ethyl acetate and methanol are better solvents for extraction. This indicates that the plant under study has more amounts of polar compounds.

III.2 Phytochemical Analysis

The medicinal value of traditionally important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, terpenes, flavonoids, saponins, etc [20]. To promote the proper use of phytomedicine and to determine their potential as sources for new drugs, it is essential to study phytochemical constituents present in the plant species in order to prop up species from traditional location to world medicinal plants category. In the present study the qualitative analysis of *C.adenocaule* root extract was carried out for dried root sample. The preliminary phytochemical screenings on root (methanol) extract was tested in this study. Terpenoids, steroids, anthraquinones, alkaloids and glycosides compounds were revealed to be present in root extract of *C.adenocaule* (**Table 2**). This could be responsible for the versatile medicinal properties of plant.

Table 2: *Phytochemical Screening of crude methanol root extract of c.adenocaula*

S/No	Constituents	Methanol crude extract
1	Alkaloids	+
2	Flavonoids	-
3	Glycosides	+
4	Anthraquinine	+
5	phenols	-
6	Tannins	-
7	Saponin	-
8	Terpenoids	+
9	Steroids	+

+ = presence of phytochemical constituents

- = absence of phytochemical constituents

III.3. Antibacterial screening of crude extracts of root of *cyphostemma adenocaule*

The air dried and powdered root of *Cyphostemma adenocaule* were extracted with methanol. Then, the crude methanol extract was partitioned with solvents of increasing polarity. The solvents used were petroleum ether, chloroform, ethyl acetate and methanol. The crude extracts were subjected to preliminary antibacterial screening. The bacterial strains used for the test were *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with the ruler and the result of antibacterial activity was recorded. Chloroform and ethyl acetate crude extracts have shown comparable antibacterial activities, but less active than the observed activity of the reference drug (Ciprofloxacin) as demonstrated by the observed inhibition zone values (**Table**

3). On the other hand, the petroleum ether and methanol extract were less active against the test strains. When antibacterial activities of crude extracts observed, chloroform extract was found to have better activity against *Escherichia coli* than *Staphylococcus aureus*. Therefore, the crude chloroform extract is potent against gram negative bacteria (*Escherichia coli*). However, the crude extracts are almost less active against Gram- positive bacteria (*S. aureus*).

Table 3: *Antibacterial inhibition zones (mm) of crude extracts of C.adenocaule*

Strain	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	ciprofloxacin
E. coli	11	22.5	18	15	25
S. aureus	9	15	12	10.5	24.5

III.4. Structural elucidation of the isolated compounds

Chromatographic separation of the chloroform root extract and the methanol partition gave two compounds labeled as **GF-1** and GF**-2** respectively. Two compounds were fully characterized using with the help of spectroscopic methods (FT-IR and NMR). The details of structural elucidation of the compounds are discussed in the sub-sections below.

The methanol extract of the roots of *C. adenocaule* was subjected to a series of liquid-liquid partition techniques, leading to the isolation of a compound **GF-2** yield (150 mg). Compound **GF-2** is white crystal and obtained from a successive liquid-liquid extraction of methanol extract using solvents of increasing polarity. For purification purpose, the white crystal was washed by methanol again and again. Then, the mixture was filtered by suction filtration and finally washed by diethyl ether. The purity of compound **GF-2** was checked by TLC (1:1 chloroform/methanol). The compound gave only one spot. Its Rf value was determined as 0.68 and sent to Addis Ababa university for characterization. Unfortunately, the compound was found to be inorganic compound because its ^{1}H-NMR spectrum showed no signal.

III.4.1 Structural elucidation of GF-1

GF-1 crystalized from chloroform extract and gave white crystal and its solubility was checked. This crystal was easily soluble in chloroform and sent to Addis Ababa University for characterization. It was isolated as white crystal with Rf value of 0.60 (2:3 n-haxane: ethyl acetate). The IR spectral analysis of GF-1 (**Fig.3.1**), showed a strong broad band at 3500 cm^{-1} for the hydroxyl (OH) stretching vibration. Other characteristic observation for olefinic C=C double bond and aliphatic C-H stretching vibrations was also seen around 1620 cm-1 and 2945 cm-1 respectively. The bending vibrations at 1400 cm^{-1}, 1300 and 1100 cm^{-1} were observed for -CH_2, -CH_3 and C-O respectively.

The ^{1}H-NMR spectrum of compound GF-1 (**Fig. 3.2**) was found to be consistent with reported data for **β–sitosterol** (**Fig.3.5**). The observed ^{1}H-NMR and ^{13}C-NMR as well as DEPT-135 spectral data of **GF-1** along with reported ^{1}H-NMR data of **β–sitosterol** was given in Table (**4** and **5**) below.

Table 4: *Observed 1H- NMR (400 MHz, $CDCl_3$) spectral data (**GF-1**) along with reported ^{1}H-NMR data of **β–sitosterol**.*

Hydrogen No	**Observed data(GF-1)**	**Reported data β–sitosterol** [21]	**Remark**
H-3	3.55 (m)	3.51 (m)	1H,OH
H-6	5.37 (d, J=4.8 Hz)	5.34 (d, J=5.2 Hz)	1H
H-18	0.70 (s)	0.67 (s)	Me-18
H-19	1.03 (s)	1.00 (s)	Me-19
H-21	0.94 (d, J=6.4 Hz)	0.92 (d, J=6.0 Hz)	Me-21
H-26	0.85 (d, J=6.8 Hz)	0.83 (d, J=7.2 Hz)	Me-26
H-27	0.84 (d, J=7.2 Hz)	0.79 (d, J=7.2 Hz)	Me-27
H-29	0.89 (t, J=6.9 Hz)	0.85 (t, J=8.0 Hz)	Me-29
11 CH_2	1.03-1.42 (complex)	1.02-1.1 (complex)	22H
7 CH	1.7-2.45 (complex)	1.8-2.3 (complex)	7H

Table 5: *The observed ^{13}C-NMR and DEPT-135 (100 MHz, $CDCl_3$) spectral data of **GF-1** along with reported ^{13}C-NMR data of β-sitosterol.*

Carbon No.	**^{13}C-NMR of GF-1 (ppm)**	**DEPT-135 of GF-1** (ppm)	**^{13}C-NMR of *β*-sitosterol** (ppm) [21]	**Remark**
1	37.26	37.25	37.22	CH_2
2	30.90	30.91	31.55	CH_2
3	71.83	71.83	70.61	CH
4	42.33	42.27	42.30	CH_2
5	140.75	-	140.71	C
6	121.72	121.73	121.70	CH
7	31.64	31.64	31.60	CH_2
8	31.92	31.92	31.80	CH
9	50.15	50.14	50.10	CH
10	36.51	-	36.50	C
11	21.09	21.08	21.00	CH_2
12	39.78	39.78	39.70	CH_2
13	42.28	-	42.20	C

14	56.78	56.77	56.80	CH
15	24.30	24.30	24.30	CH_2
16	28.24	28.24	28.30	CH_2
17	56.07	56.06	55.90	CH
18	11.85	11.85	11.80	CH_3
19	19.39	19.39	19.40	CH_3
20	36.14	36.14	36.10	CH
21	18.78	18.77	18.60	CH_3
22	33.96	33.95	33.89	CH_2
23	26.10	26.09	26.03	CH_2
24	45.85	45.85	45.78	CH
25	29.17	29.16	29.11	CH
26	21.09	21.08	21.20	CH_3
27	19.04	19.03	19.00	CH_3
28	23.08	23.07	23.00	CH_3
29	11.98	11.98	12.00	CH_3

The ^{1}H-NMR spectrum showed one proton multiplet at δ 3.55 and one proton broad doublet at δ 5.37 typical for H-3 and H-6 of a steroidal nucleus. One olefinic proton appeared as characteristic downfield signal at δ 5.37 (1H, d, J = 4.8 Hz) in the 1H -NMR spectrum which was identical with the chemical shift of H-6 of β-sitosterol [21]. The spectrum also showed two three proton singlets at δ 1.03 and δ 0.70 assignable for H-19 and H-18 respectively. In addition, two doublets at δ 0.82 (3H, d, 7.2 Hz) and 0.80 (3H, d, 7.2 Hz) which described the two methyl groups at H-26 and H-27 and another three-proton doublet at δ 0.94 (3H, d, 6.4 Hz) for H-21. On the other hand, one three-proton triplet at δ 0.89 (3H, t, 6.9 Hz) could be assigned to the primary methyl group attached to H-29 [22]. The ^{13}C-NMR spectrum showed 29 carbons including an oxymethine carbon at δ 71·83, which is the characteristics of spirostene and two olefinic carbons appeared at δ 140.75 and 121.72 which were identical with the chemical shift of C-5 and C-6 respectively of β-sitosterol [49]. By comparing DEPT-135 experiment for **GF-1** we confirmed that this compound was having six methyl (CH_3) groups, eleven methylene (CH_2), nine methine (CH) and three quaternary carbons (CQ) groups. The spectral data of the compound was in complete agreement to the reported data in literature value [21-24].

Based on the above spectroscopic data and comparison of this data with the literature, the compound **GF-1** was identified to be the same as ***β***-sitosterol (**Figure 4**). The presence of ***β*-**

sitosterol in *C.adenocaule* was reported in the bark and wood extract [5]. However, there is no report of isolation of ***β*-sitosterol** from the root of *C.adenocaule.* Therefore, this is the first report of isolation and structural elucidation of ***β*-sitosterol** from the root of *C.adenocaule*. Beta-sitosterol (BS) is a phytosterol which is widely distributed throughout the plant kingdom and known to be involved in the stabilization of cell membranes. BS could be obtained from different plants, but the total biosynthetic pathway as well as its exact physiological and structural function in plants, have not been fully understood. Different pharmacological effects have been studied, but most of the mechanisms of action have not been studied in detail. Clinical trials with BS have shown beneficial effects in different diseases, but long-term study results are not available [25].

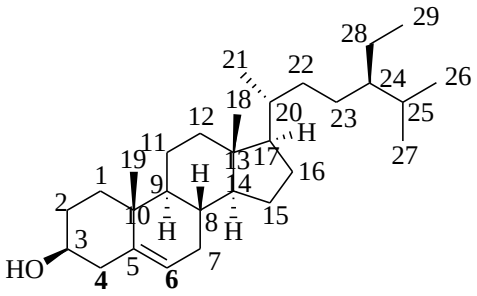


Figure 4 : *Structure of β-sitosterol*

IV. Conclusion

From the above findings, *β*-sitosterol was isolated from chloroform extract of the roots of *C.adenocaule* and its chemical structure was elucidated. It was carried out by means of various physical (solvent extraction, TLC, PTLC) and spectral techniques. After repeated successive solvent extraction and chromatography different compounds were isolated from the plant roots. Among the isolated compounds, one compound labelled as **GF-1** was characterized as *β*-sitosterol. Other bands contain mixed compounds even after attempting to purify. To the best of our knowledge one compound is structurally elucidated here for the first time in the root of plant under study. Screening of the antimicrobial activity of chloroform crude extract exhibited maximum activity against *E.coli.* Further phytochemical investigation is recommended for those interested chemists to isolate and characterize phytochemical constituents from ethyl acetate and methanol extracts.

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VI. References

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