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Isolation and Structure Elucidation of Terpene Compound From the Stem Bark of *Couroupita Guianensis* Aubl.(Amyauk-San-Bin)

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Abstract

In this paper, *Couroupita guianensis* Aubl. (Amyauk-San-Bin), was collected from Magway township, Magway Region. Chemical constituent of *Couroupita guianensis* Aubl. (Amyauk-San-Bin) was examined by phytochemical test. The pure organic compounds were isolated by column separation method. The partial structures of pure organic compound (PPH-1) have been described as follows using sophisticated spectroscopic methods such as Proton Nuclear Magnetic Resonance (^{1}HNMR), Carbon Nuclear Magnetic Resonance (^{13}C NMR), Double Quantum Filtered ^{1}H–H Correlation Spectroscopy (DQF-COSY), Heteronuclear Multiple Quantum Coherence (HMQC), Distortionless Enhancement by Polarization Transfer (DEPT) and Heteronuclear Multiple Bond Coherence (HMBC) spectral data respectively.

Key words: *Couroupita Guianensis* Aubl., Thin Layer Chromatography, Column Chromatography,FT-IR

1 INTRODUCTION

The terpenoids called isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from terpenes.About. 60% of known natural products are terpenoids.Plant terpenoids, are used for their aromatic qualities and play a role in traditional herbal remedies.(*Firn R, 2010)*

Medicinal plants have been in use for treatment of various diseases all over the world. Medicinal plants are used as traditional form of providing relief to several diseases. Presently, millions of adults are depending on medicinal plants for their primary health care needs. Medicinal plants have been a major source in the maintenance of health, as well as in the prevention, improvement or treatment of physical and mental illness. (Chandolu Kumar, et al, 2011)

Herbal medicines certainly have over a millenary history, especially through the Orient. Although, the use of these medicines in the western world is decidedly amplifying, most of them are not yet acceptable scientific evidences to support this conviction. Various medicinal plants have been identified and modern scientific approaches have been used to study their authenticity, safety and efficacy of their therapeutic uses. (Al-Dhabi,N.A., et al. 2012)

Couroupita guianensis Aubl. (Myanmar name Amyauk-San-Bin) is also called as “Cannonball tree” or “Sal tree”. The effects of the Cannon ball tree in medical use are strong. As when using any natural medicine, the correct dosage is vital. In medicinal use, the flowers, leaves, bark and fruit flesh are used. The bark of Cannon ball tree malaria, antibiotic, antifungal, antiseptic and analgesic qualities. The trees are used to cure colds and stomach aches. The present study was carried out to scientifically evaluate the efficacy of *Couroupita guianensis* Aubl.(Myanmar name Amyauk-San-Bin) (Lim,2012)

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1.1 Botanical Description

Scientific name	- *Couroupita Guianensis* Aubl.
Family name	- Lecythidaceae
Local name	- Amyauk-San-Bin
Distribution	- Amazonian Colombia, Northen Venezuela, Guyana, Surinan, French Guiana, Amazonian Ecuador, Amazonian Peru, eastern and southwestern Amazonian Brazil and Myanmar
Part of Use	- Bark
Medicinal Use	- malaria, antibiotic, antifungal, antiseptic and analgesic qualities

Figure(1) Plant, flower and bark of *Couroupita Guianensis* Aubl.

2 Experimental

2.1 Instrumentation and Materials

Instrumentation

The occurrence of UV absorption on TLC plate was checked by UV detector and iodine vapor. The apparatus for extraction and chromatography were used with common laboratory equipments.

Materials

Before the research work was taken all the commercially available reagent and solvent were distilled. Analytical and preparative thin-layer chromatography was performed by using precoated silica gel plates. Silica gel (Merck-Co., Inc., Kieselgel 60, 70-230 Mesh ACTM) was used for column chromatography.

2.2 Sample Collection and Preparation

Myanmar medicinal plant Amyauk-San-Bin was collected from Magway Township, Myanmar.

2.3 Phytochemical screening

The air-dried powdered sample was subjected to preliminary phytochemical test in order to find out the types of phytochemical constituents such as alkaloid, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, steroid, polyphenol and terpene present in sample according to appropriate reported methods.

2.4 Column Chromatography Separation

The bark of *Couroupita Guianensis* Aubl.(Lecythidaceae)Myanmar named Amyauk-San-Bin collected from Magway Township, Myanmar.

The air dried bark of *Couroupita Guianensis* Aubl.(675 g) were extracted with 95% EtOH (2.5 L) at room temperature about two months. Ethanol extract was re-extracted with EtOAc (300) ml and then filtered and

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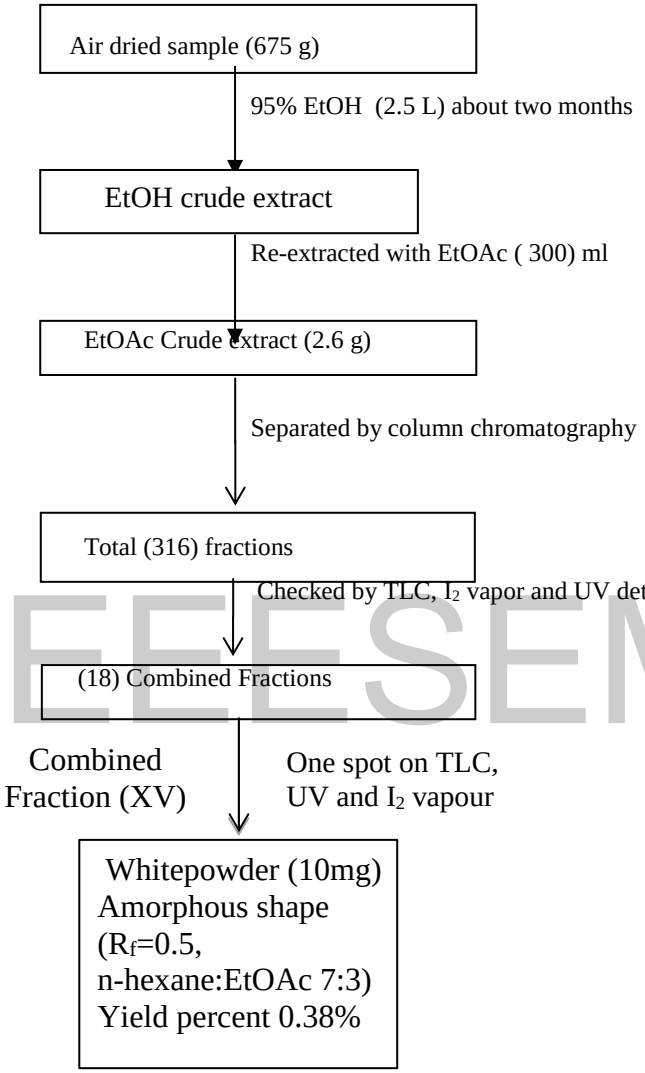
evaporated. Ethyl acetate crude extract (2.6 g) was separated by column chromatography with adsorbent (silica gel 70-230 mesh) and eluent (n-hexane: EtOAc various ratio). Total (316) fractions were obtained. Each fractions were checked by TLC, I_2 vapor and UV detector. The same R_f value fractions were combined. And then 18 combined fractions were obtained. Among them combined fraction XV, this shape is amorphous with R_f value 0.5. n-hexane and EtOAc ratio is 7:3, yield percent 0.38% .

Extraction and Isolation of Pure Compound (PPH-1) from Amyauk-San-Bin



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3 RESULTS AND DISCUSSIONS

3.1 Nuclear Magnetic Resonance Spectrum of Pure Organic Compound (PPH-1)

The ^{1}H NMR (600 MHz) spectrum, Figure (3.1), represents the total numbers of protons present in compound (PPH-1). In this spectrum, total (49) protons could be observed. Their chemical shifts are tabulated in Table (1). (Silversten R.M., et al.,1998)

^{13}C NMR Spectrum of Pure Compound (PPH-1)

^{13}C NMR spectrum, Figure (3), indicates the total number of carbons in this compound to be (30). The chemical shift values of particular carbons and their assignments are tabulated in Table (3.2).(Silversten R.M., et al.,1998)

Table (1) ^{1}H NMR Spectral Data of Pure Compound (PPH-1)

No	Chemical shift (ppm)	No. of protons	Proton assignment
1.	0.75	1 H	CH
2.	0.79,0.96	3H	CH_3
3.	0.83	3H	CH_3
4.	0.87	2H	CH_2
5.	0.87	3H	CH_3
6.	0.88,1.51	3H	CH_3
7.	0.94	3H	CH_3
8.	0.98	3H	CH_3
9.	0.99	3H	CH_3
10.	1.14	3H	CH_3
11.	1.33	2H	CH_2
12.	1.34,1.52	2H	CH_2
13.	1.41,1.55	2H	CH_2
14.	1.42,1.22	2H	CH_2
15.	1.56	1H	CH
16.	1.61,0.82	2H	CH_2
17.	1.63	2H	CH_2
18.	1.66	2H	CH_2
19.	1.77	2H	CH_2
20.	1.94	1H	CH
21.	1.99	2H	CH_2
22.	3.22	1H	CH
23.	5.18	1H	CH
Total			49 protons

Table (2) ^{13}C NMR Spectral Data of Pure Compound (PPH-1)

No.	Chemical Shift Carbonδ(ppm)	Assignment (hybridization)
1.	15.49	sp^3 carbon
2.	15.58	sp^3 carbon
3.	16.81	sp^3 carbon
4.	18.38	sp^3 carbon
5.	23.53	sp^3 carbon
6.	23.69	sp^3 carbon
7.	25.99	sp^3 carbon
8.	26.16	sp^3carbon
9.	26.95	sp^3carbon
10.	27.24	sp^3carbon
11.	28.10	sp^3 carbon
12.	28.39	sp^3 carbon
13.	31.07	sp^3carbon
14.	32.49	sp^3carbon
15.	32.67	sp^3carbon
16.	33.33	sp^3 carbon
17.	34.74	sp^3 carbon
18.	36.96	sp^3carbon
19.	37.15	sp^3carbon
20.	38.60	sp^3 carbon
21.	38.78	sp^3carbon
22.	39.80	sp^3carbon
23.	41.73	sp^3carbon
24.	46.84	sp^3 carbon
25.	47.25	sp^3carbon
26.	47.65	sp^3carbon
27.	55.19	sp^3carbon
28.	79.03	sp^3 carbon
29.	121.73	sp^2carbon
30.	145.19	sp^2carbon
	Total	30 carbons

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DEPT Spectrum of Pure Compound (PPH-1)

DEPT spectrum, Figure (4), gives rise to the number and kinds of carbons as well as protons. The assignment of DEPT spectrum is tabulated as Table (3). (Silversten R.M., et al.,1998)

HSQC Spectrum of Pure Compound (PPH-1)

HSQC spectrum, Figure (5), indicates the direct proton-carbon correlation. The chemical shift values of carbons and their related protons are shown in Table (4). (Silversten R.M., et al.,1998)

Table (3)DEPT Spectral Data of Pure Compound (PPH-1)

No.	Chemical Shift (ppm)	Kinds of carbons	Number of Carbon	Number of Proton
1.	15.49	sp^3methyl carbon	1	3
2.	15.58	sp^3methyl carbon	1	3
3.	16.81	sp^3methyl carbon	1	3
4.	18.38	sp^3methylene carbon	1	2
5.	23.53	sp^3 methylene carbon	1	2
6.	23.69	sp^3methylcarbon	1	3
7.	25.99	sp^3 methyl carbon	1	3
8.	26.16	sp^3methylenecarbon	1	2
9.	26.95	sp^3methylene carbon	1	2
10.	27.24	sp^3methylene carbon	1	2
11.	28.10	sp^3methylcarbon	1	3
12.	28.39	sp^3 methyl carbon	1	3
13.	31.07	sp^3quaternary carbon	1	-
14.	32.49	sp^3quaternary carbon	1	-
15.	32.67	sp^3 methylene carbon	1	2
16.	33.33	sp^3methyl carbon	1	3
17.	34.74	sp^3methylene carbon	1	2
18.	36.96	sp^3quaternary carbon	1	-
19.	37.15	sp^3methylene carbon	1	2
20.	38.60	sp^3 methylene carbon	1	2
21.	38.78	sp^3quaternary carbon	1	-
22.	39.80	sp^3quaternary carbon	1	-
23.	41.73	sp^3quaternary carbon	1	-
24.	46.84	sp^3 methylene carbon	1	2
25.	47.25	sp^3methine carbon	1	1
26.	47.65	sp^3methinecarbon	1	1
27.	55.19	sp^3methinecarbon	1	1
28.	79.03	sp^3methine carbon	1	1
29.	121.73	sp^2methine carbon	1	1
30.	145.19	sp^2quaternary carbon	1	-

Table (3) ^1H-^{13}C Correlation in HSQC Spectrum of Compound (PPH-1)

No.	Chemical Shift of carbon δ (ppm)	Chemical shift of Proton δ (ppm)	Kinds of carbons
1.	15.49	0.94	sp^3methyl carbon
2.	15.58	0.79,0.96	sp^3methyl carbon
3.	16.81	0.98	sp^3methyl carbon
4.	18.38	1.41,1.55	sp^3methylene carbon
5.	23.53	0.87	sp^3 methylene carbon
6.	23.69	0.87	sp^3methylcarbon
7.	25.99	1.14	sp^3 methyl carbon
8.	26.16	1.77	sp^3methylenecarbon
9.	26.95	1.99	sp^3methylene carbon
10.	27.24	1.61,0.82	sp^3methylene carbon
11.	28.10	0.99	sp^3methylcarbon
12.	28.39	0.83	sp^3 methyl carbon
13.	31.07	-	sp^3quaternary carbon
14.	32.49	-	sp^3quaternary carbon
15.	32.67	1.34,1.52	sp^3methylene carbon
16.	33.33	0.88,1.51	sp^3methyl carbon
17.	34.74	1.33	sp^3methylene carbon
18.	36.96	-	sp^3quaternary carbon
19.	37.15	1.42,1.22	sp^3methylene carbon
20.	38.60	1.63	sp^3 methylene carbon
21.	38.78	-	sp^3quaternary carbon
22.	39.80	-	sp^3quaternary carbon
23.	41.73	-	sp^3quaternary carbon
24.	46.84	1.66	sp^3 methylene carbon
25.	47.25	1.94	sp^3methine carbon
26.	47.65	1.56	sp^3methinecarbon
27.	55.19	0.74	sp^3methinecarbon
28.	79.03	3.22	sp^3methine carbon
29.	121.73	5.18	sp^2methine carbon
30.	145.19	-	sp^2 quaternary carbon

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3.2 Preliminary Phytochemical Test for the bark of *Couroupita guianensis*Aubl.

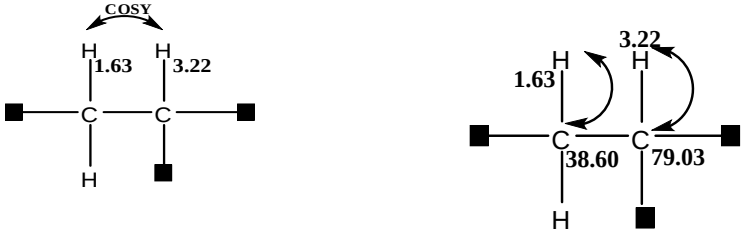
Table 5. Phytochemical Test for the bark of *Couroupita guianensis* Aubl.

No.	Constituents	Reagent used	Observation	Results
1.	Alkaloid	Dragendroff's reagent	Yellow ppt	+
		Wagner reagent	Orange ppt	+
2.	Flavonoid	EtOH, Mg ribbon, Conc: HCl	Pink colour	+
3.	Steroid	EtOH, acetic anhydride,$CHCl_3$ Conc: H_2SO_4	Greenish blue colour	+
4.	Terpene	Petether, acetic anhydride, $CHCl_3$, Conc; H_2SO_4	Reddish brown colour	+
5.	Glycoside	10% lead acetate solution	White ppt	+
6.	Reducing Sugar	Benedict's solution	Brick red ppt	+
7.	Polyphenol	1% $FeCl_3$, 1% $[K_3Fe(CN)_6]$ solution	Greenish blue colour	+
8.	Saponin	H_2O, shaking	Frothing	+
9.	Phenolic	H_2O, Δ , 10 min, 10% $FeCl_3$ solution	Brown colour	+

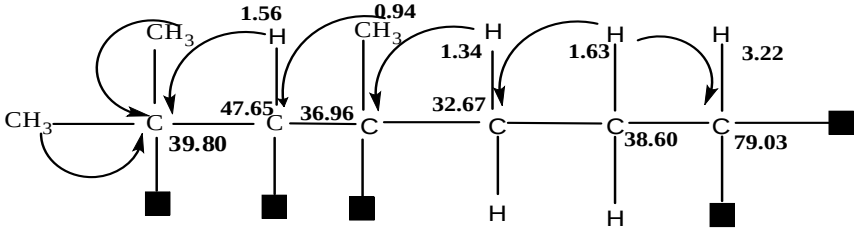
3.3 Terpene Structure Elucidation of Pure Compound (PPH-1)

The partial structure of compound (PPH-1) was elucidated by using HSQC, HMBC, DEPT, DQF-COSY Spectral data.In DQF-COSY spectrum Figure (6), the medium graphic area of correlation between methylene and methine protons at δ 3.22 ppm and δ 1.63 ppm .

The direct connection of these methylene and methine protons at δ 3.22 ppm and δ 1.63 ppm with their corresponding carbons at δ 38.60 and 79.03 ppm in HSQC spectrum, Figure (5), gives rise to the fragment .



In HMBC spectrum, Figure (7), the methylene proton at δ 1.63 ppm have α ^1H-^{13}C long range coupling with methine carbon at δ 79.03 ppm and with methylene carbon at δ 32.67 ppm which implies the following fragment. The observation of α ^1H-^{13}C long range coupling between sp^3 methylene proton at δ 1.34 ppm and quaternary carbon at δ 36.96 ppm gave rise to the following fragment . According to the Figure (7), the methyl proton at δ 0.94 ppm has α ^1H-^{13}C long range coupling with methine carbon at δ 47.65 ppm and then the methine proton at δ 1.56 ppm has α ^1H-^{13}C long range coupling with quaternary carbon at δ 39.80 ppm which implies the following fragment which implies the following fragment . Moreover the occurrence of α ^1H-^{13}C long range signals of methyl proton at δ 0.98 ppm and also methyl proton δ 0.79 ppm with quaternary carbon at δ 39.80 ppm leads to the following fragment in HMBC spectrum Figure (7).



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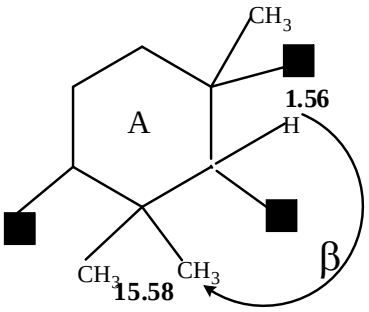
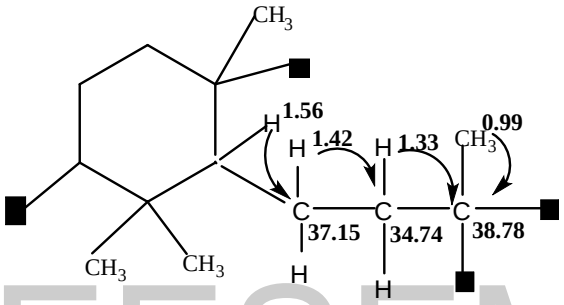
On the other hand, the attachment of gemdimethyl to sp^3 quaternary carbon at δ 39.80 ppm could be proved by FT-IR spectral data at 1382.04 and 1361.79 cm^3in IR spectrum Figure (3.7).The formation of ring A could be confirmed by β ^1H-^{13}C long range signal of sp^3 methine carbon at δ 1.56 with sp^3 methyl carbon at δ 15.58 ppm.

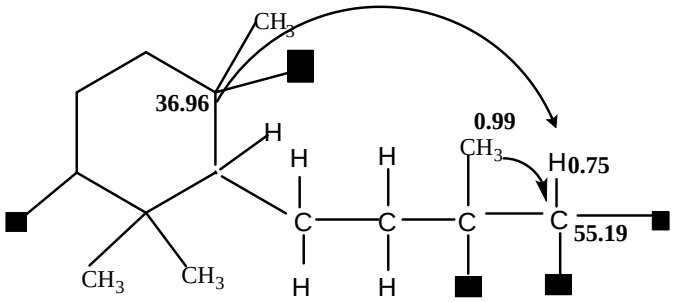


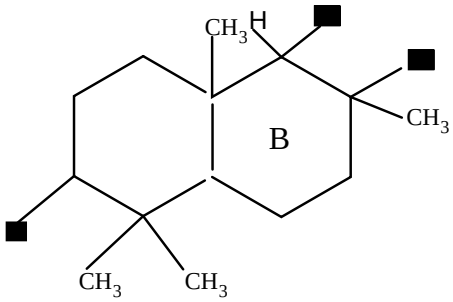
Fragment (A)

In HMBC spectrum, Figure (7), the methine proton at δ 1.56 ppm have α ^1H-^{13}C long range coupling with methylene carbon at δ 37.15 ppm and also methylene proton at δ 1.42 ppm is connected to the methylene carbon at δ 34.74 ppm. Moreover the quaternary carbon at δ 38.78 ppm have α ^1H-^{13}C long range coupling with methylene proton at δ 1.33 ppm and methyl proton at δ 1.33 ppm which produce the following fragment.



Futhermore the occurrence of α ^1H-^{13}C long range signals of methine proton at δ 0.75 ppm with quaternary carbon at δ 39.69 ppm .In addition the methyl proton at δ 0.99 ppm has β ^1H-^{13}C long range signal with methine carbon at δ 55.19 ppm leads to the following fragment(B) in HMBC spectrum Figure (7).





Fragment (B)

In HMBC spectrum, Figure (7), the methylene carbon at δ 46.84 ppm have α ^1H-^{13}C long range coupling with two methine protons at δ 0.75 ppm and at δ 5.18 ppm which implies the following fragment. The observation of β ^1H-^{13}C long range coupling of quaternary carbon at δ 145.19 ppm are connected to the methylene proton at δ 1.66 ppm and methyl proton at δ 1.14 ppm gave rise to the following fragment . According to the Figure (7), the methyl proton at δ 1.14 ppm has α ^1H-^{13}C long range coupling with quaternary carbon at δ 41.73 ppm which implies the following fragment which implies the following fragment . On the other hand the occurrence of β ^1H-^{13}C long range signals of methyl proton at δ 1.14 ppm with quaternary carbon at δ 38.78 ppm .In addition the methyl proton at δ 0.99 ppm has β ^1H-^{13}C long range signal with quaternary carbon at δ 41.73 ppm leads to the following fragment(C) in HMBC spectrum Figure (7).

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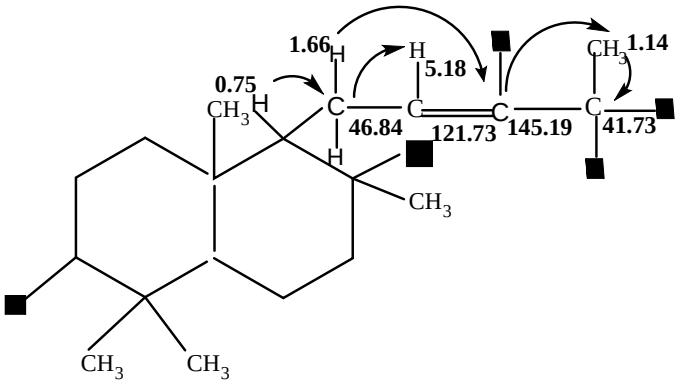
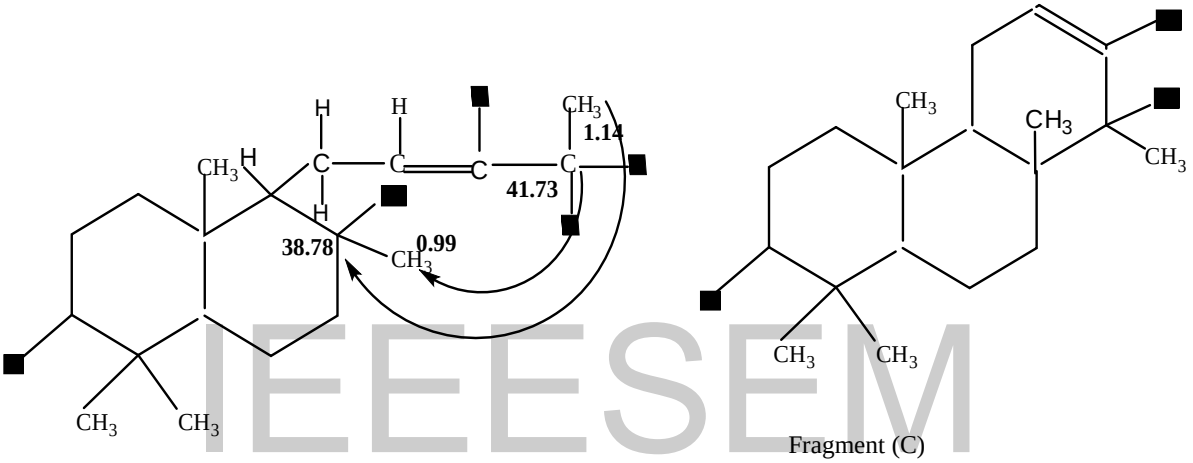
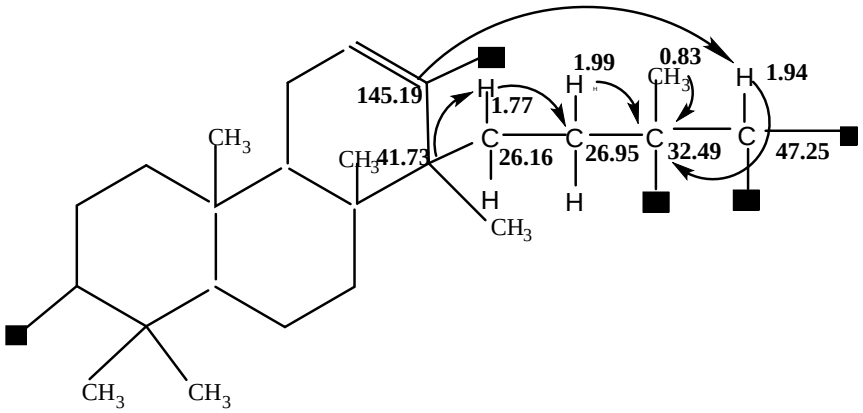


On the other hand the occurrence of β ^{1}H-^{13}C long range signals of methyl proton at δ 1.14 ppm with quaternary carbon at δ 38.78 ppm .In addition the methyl proton at δ 0.99 ppm has β ^{1}H-^{13}C long range signal with quaternary carbon at δ 41.73 ppm leads to the following fragment(C) in HMBC spectrum Figure (7).



Fragment (C)

According to the HMBC spectrum, Figure (7), the methylene proton at δ 1.77 ppm have α ^{1}H-^{13}C long range coupling with methylene carbon at δ 26.95 ppm and quaternary carbon at δ 41.73 ppm which implies the following fragment. And then the quaternary carbon at δ 32.49 ppm have α ^{1}H-^{13}C long range coupling with methylene proton at δ 1.99 ppm , methyl proton at δ 0.83 ppm and methine proton at δ 1.94 ppm gave rise to the following fragment .Moreover methine proton at δ 1.94 ppm is connected to the quaternary carbon at δ 145.19 ppm which produces the following fragment (D) .



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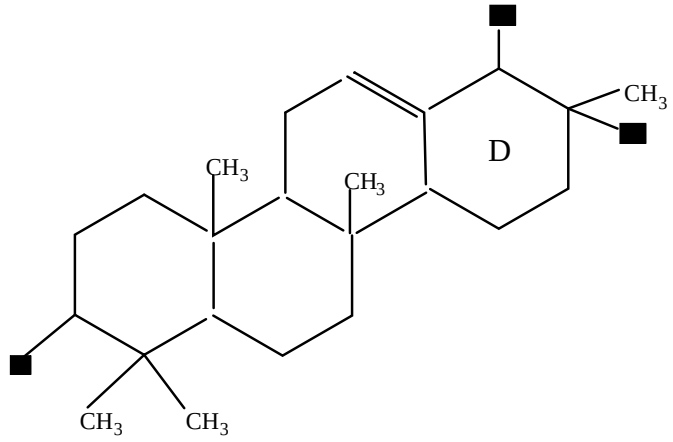
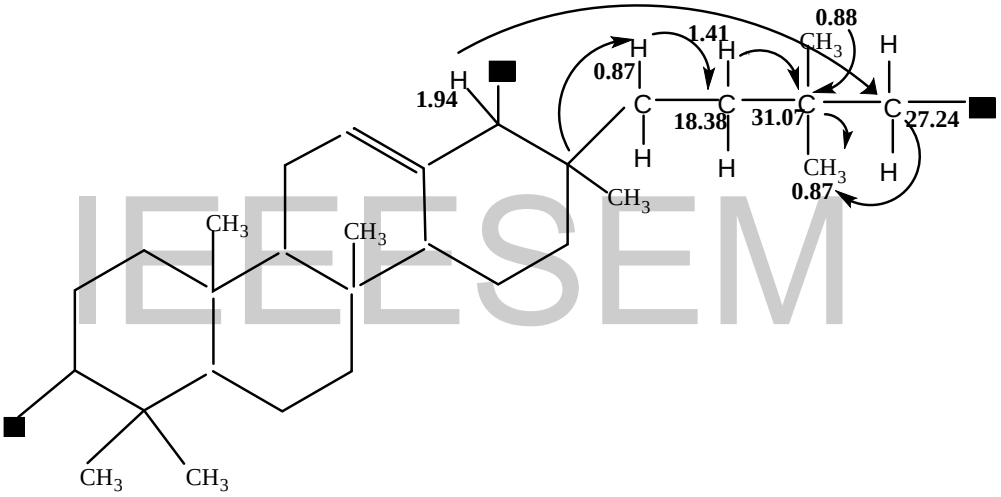
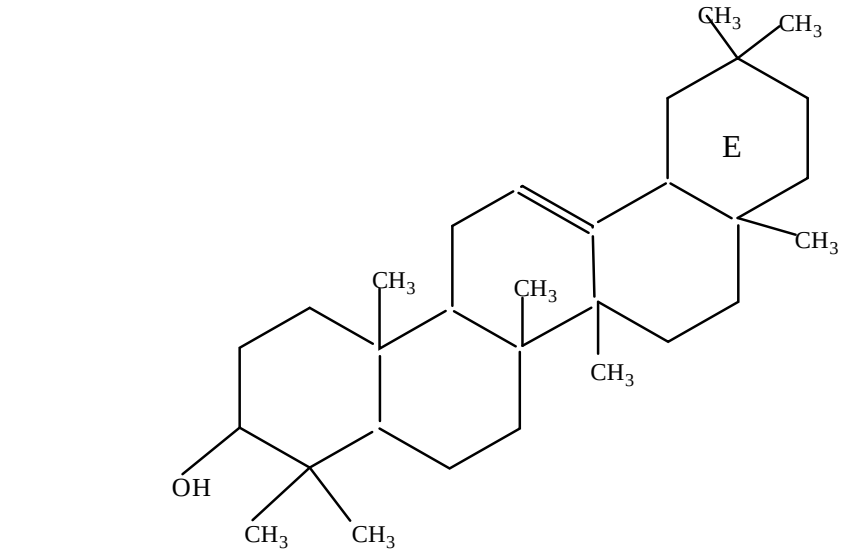


Fragment (D)

In HMBC spectrum, Figure (7), the methylene proton at δ 0.87 ppm have α ^{1}H-^{13}C long range coupling with quaternary carbon at δ 32.49 ppm and methylene carbonat δ 18.38 ppm which implies the following fragment. The observation of α ^{1}H-^{13}C long range coupling of quaternary carbon at δ 31.07 ppm are connected to the methylene proton at δ 1.41 ppm , two methyl protons at δ 0.87 ppm and δ 0.88 ppm gave rise to the following fragment . In addition, the methylene carbon at δ 27.24 ppm have α ^{1}H-^{13}C long range coupling with methyl proton at δ 0.87 ppm and methine proton at δ 1.94 which confirm the following fragment (E) .





Fragment (E)

Futhermore, according to the FT-IR spectrum figure (8)this compound PPH-1 containing OH group (appear 3303.21 cm^{-1}). Therefore, fragment (E) compound is triterpene($C_{30}H_{50}O$) or β-sisterol.

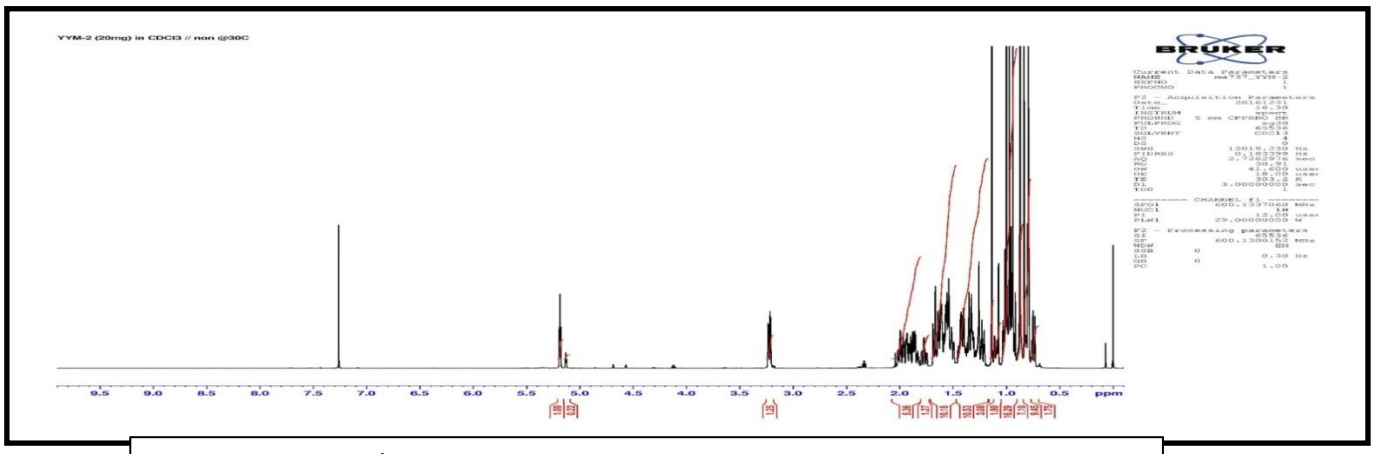


Figure (2) ^{1}H NMR Spectrum of Pure Compound (PPH-1)

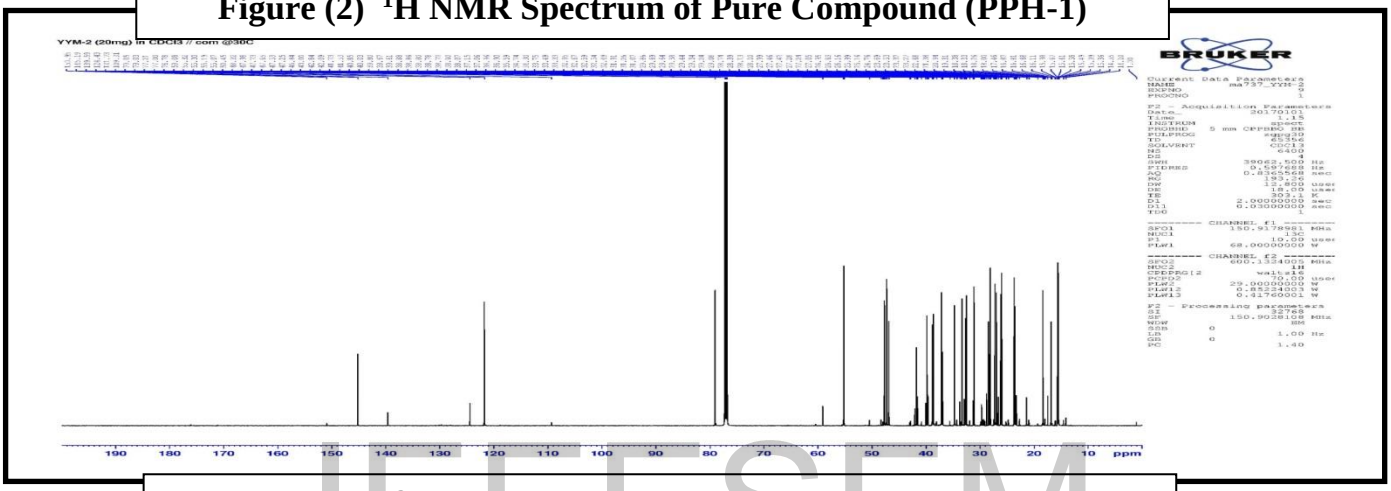


Figure (3) ^{13}C NMR Spectrum of Pure Compound (PPH-1)

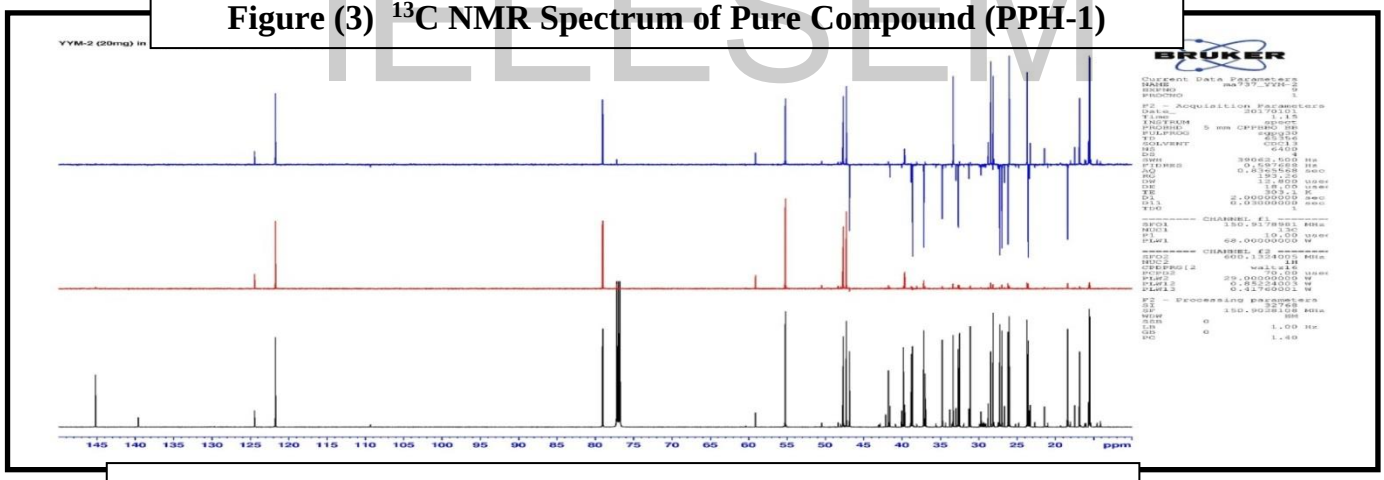


Figure (4) DEPT Spectrum of Pure Compound (PPH-1)

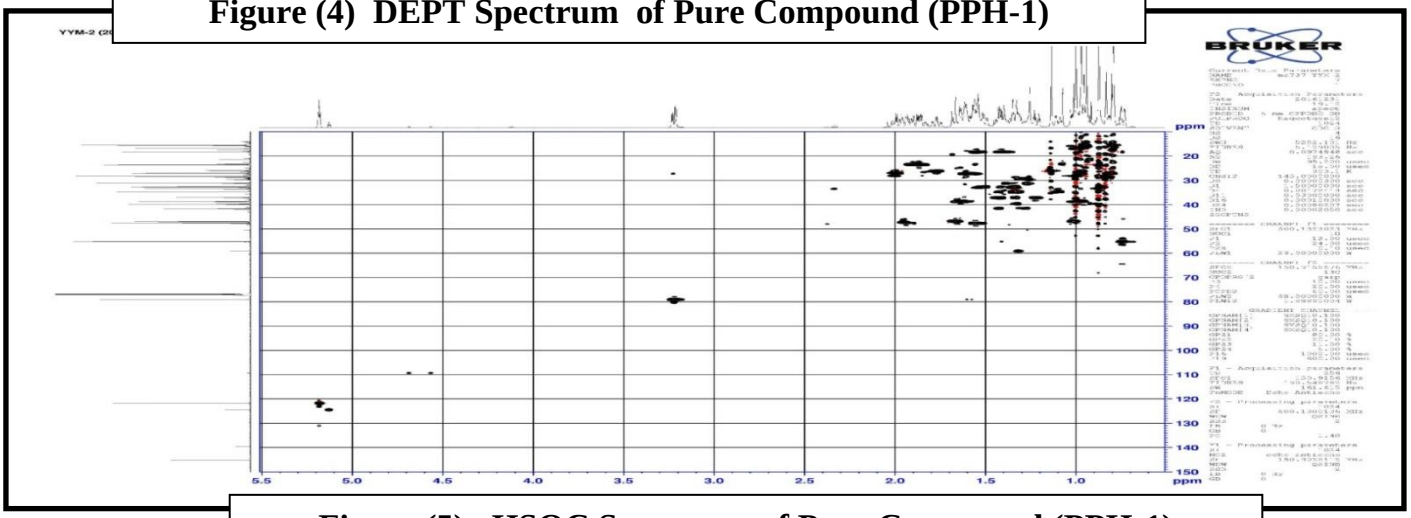


Figure (5) HSQC Spectrum of Pure Compound (PPH-1)

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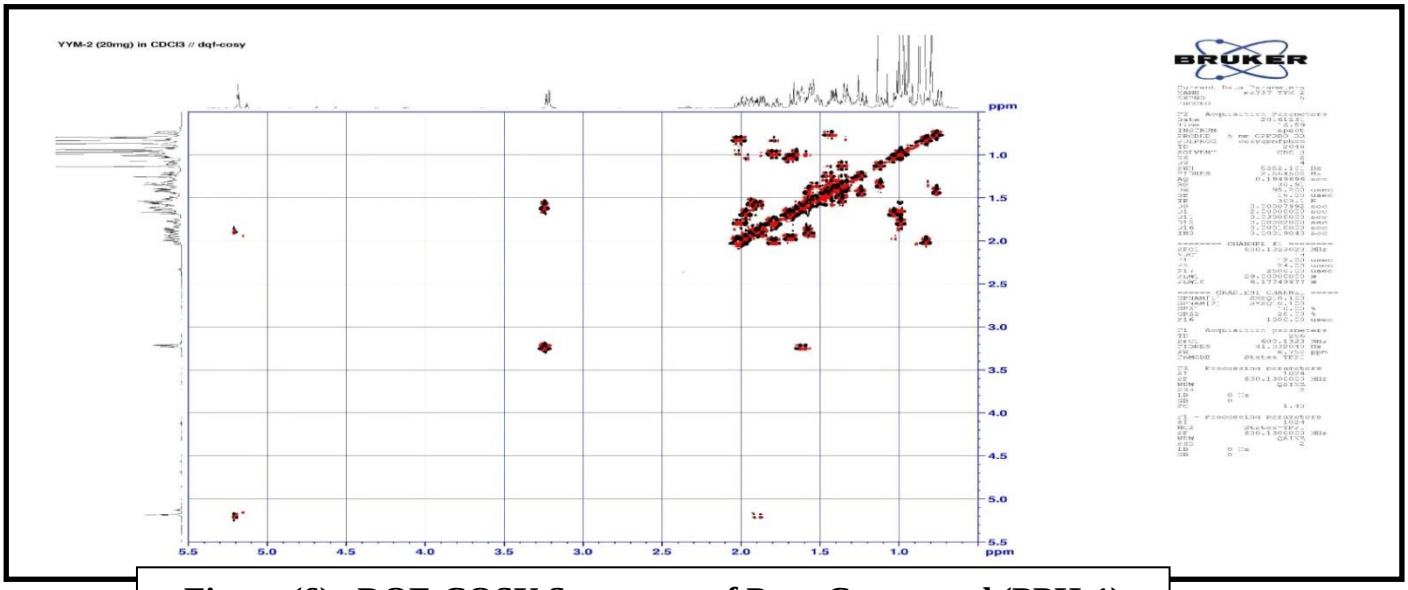

Figure (6) DQF-COSY Spectrum of Pure Compound (PPH-1)

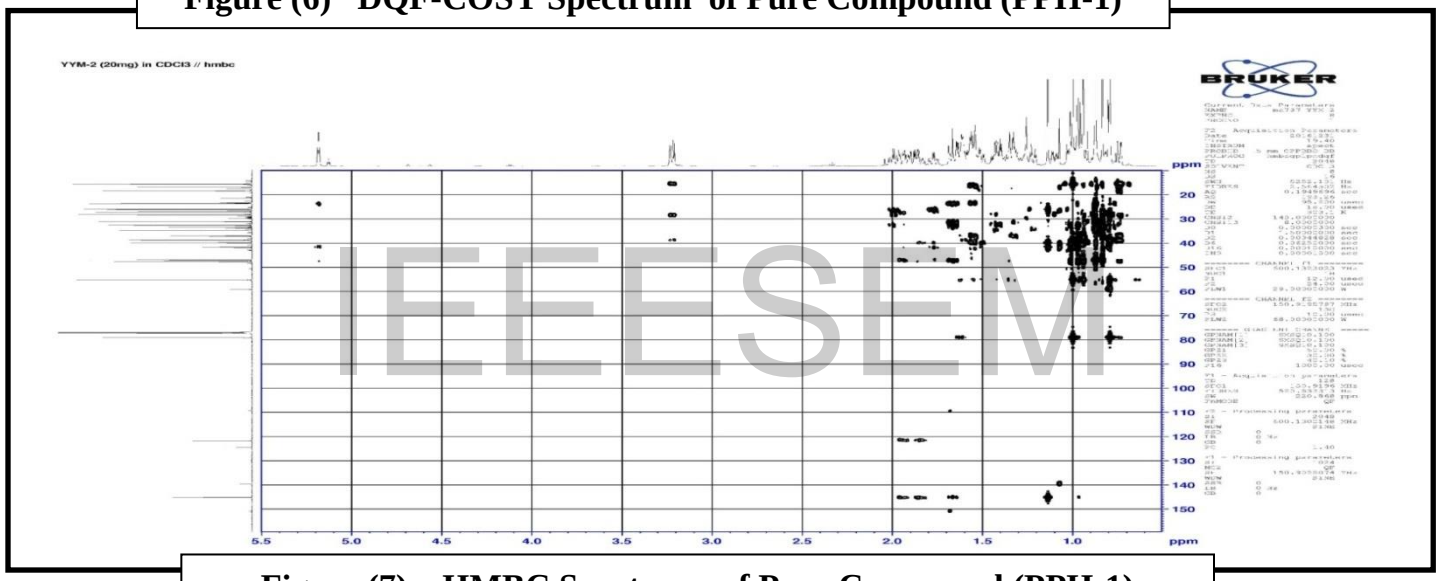

Figure (7) HMBC Spectrum of Pure Compound (PPH-1)

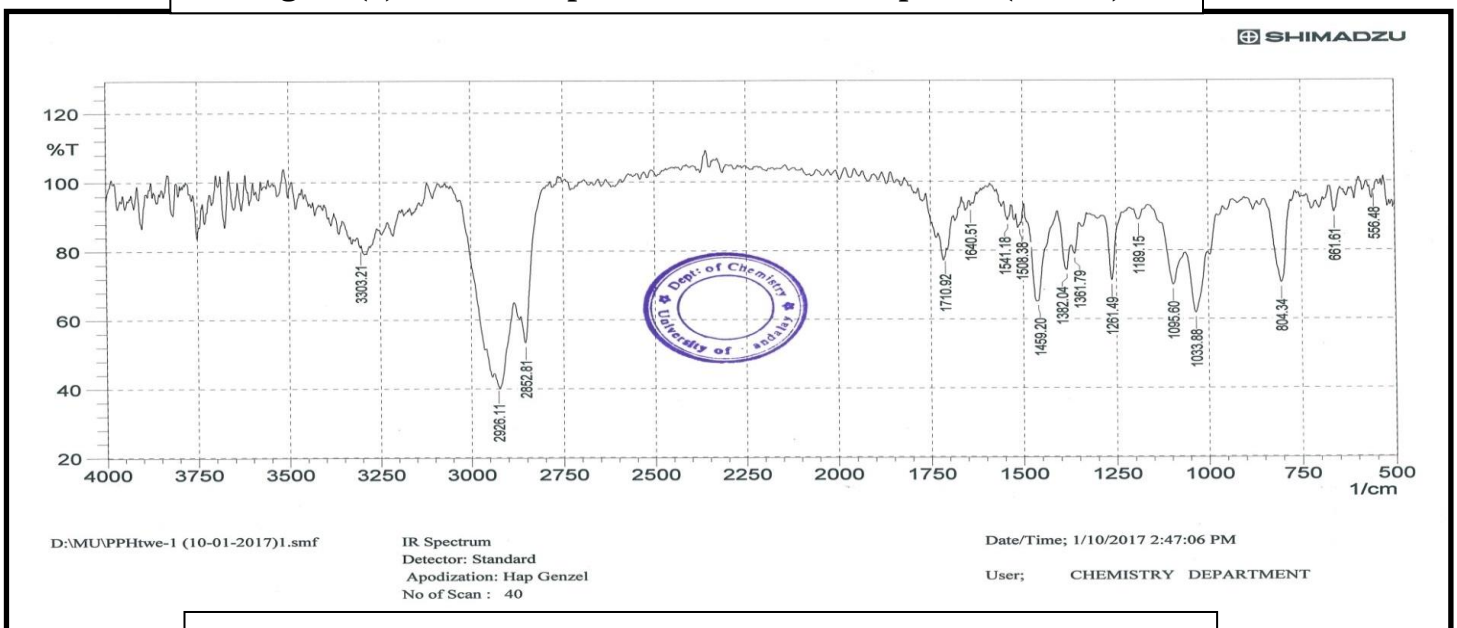

Figure (8) FT-IR Spectrum of Pure Compound (PPH-1)

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4 Conclusion

The bark of *Couroupita Guianensis* Aubl.(Lumbini Ingyin), was used to determine the phytochemical constituents, to isolate the pure organic compound . The bark of this plant contains alkaloid, flavonoid, steroid, terpene, glycoside, reducing sugar, polyphenol, saponin,and phenolic compound respectively. Phytochemical test for pure compound (PPH-1) was done and it showed the positive test for terpene. The pure compound (PPH-1) was isolated from the bark of *Couroupita Guianensis* Aubl.(Lumbini Ingyin) by using thin layer and column chromatographic separation techniques. The partial structures of compound (PPH-1)was elucidated by ^{1}HNMR , ^{13}CNMR , DEPT, DQF-COSY , HSQC , HMBC spectral data respectively. Therefore pure compound (PPH-1) was confirmed the triterpene(β-sisterol) compound.

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Onlines Materials

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