EXTRACTION, ISOLATION, ANTIMICROBIAL STUDY AND STRUCTURAL ELUCIDATION OF A PURE COMPOUND ISOLATED FROM THE TUBER OF *STEPHANIA GLABRA* (ROXB.) MIERS

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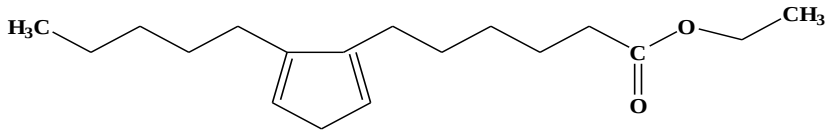
Abstract

The tuber of *Stephania glabra* (Roxb.) Miers, one of Myanmar indigenous medicinal plants was selected for this research work. The sample was chopped in to small pieces and dried in air. The air dried tuber sample was extracted with 95% of ethanol for about one month. Moreover, it was further extracted with ethyl acetate and ran by different solvents polarities using thin layer and column chromatography. A biologically active pure compound (KLT-1) could be isolated from n-hexane and ethyl acetate solvent ratio (n-hex 19:1EtOAc, 40 mg, and R_f value 0.9). The antimicrobial activity of this pure compound was examined by agar well diffusion method on six selected organisms. It was highly responded on three microorganisms such as *Bacillus subtilis, Bacillus pumilus and E. coli*. The molecular formula of pure compound (KLT-1) was assigned as $C_{18}H_{30}O_2$ (ester compound) by FT IR (Fourier Transform Infrared), ^{1}H NMR (Proton Nuclear Magnetic Resonance), ^{13}C NMR (Carbon Nuclear Magnetic Resonance), DEPT (Distortion Enhancement by Polarization Transfer), HSQC (Heteronuclear Single Quantum Coherence) and DART (Direct Analysis of Real Time Mass Spectroscopy) mass spectral data. Moreover, the complete structure of pure compound could be elucidated by using advanced spectroscopic methods such as DQF-COSY (Double Quantum Filtered Correlation Spectroscopy) and HMBC (Heteronuclear Multiple Bond Coherence) spectral evidences. The prominent functional groups containing in this compound was assigned and its complete structure was described as follows.



Ethyl-6-(5-pentyl) cyclopentadienyl hexanoate

Keywords: extraction, isolation, thin layer and column chromatography, antimicrobial activities, isolated compound, spectral evidences

Introduction

Medicinal plants serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines (Motaleb *et al.*, 2011). Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization (Sasidharan *et al*., 2011). Successful determination of biologically active compounds from plants material is largely dependent on the type of solvent used in the extracting procedure (Tiwari, 2011). Column chromatography is generally used as a purification technique to isolate desired compounds from a mixture (Kenkel, 2003). Thin layer chromatography (TLC) is a simple, quick and a support material which is used to identify of a compound in a mixture. Additional tests involve the detection of I_2 vapour and UV lamp which cause color spots on TLC sheet (Sasidharan *et al*., 2011).

The tuber of *Stephania glabra* (Roxb.) Miers, Myanmar name is *Taung-kya* having high medicinal value was selected for this research paper. It has long been used in traditional practices

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such as for treatment of asthma, tuberculosis, dysentery, hyperglycemia, cancer, fever, intestinal complaints, sleep disturbances and inflammation (Semwal and Semwal, 2015). The antimicrobial activity on six microbial strains using agar well diffusion method was prepared by its procedure. The structural elucidation of this pure compound (KLT-1) was assigned by using FT IR, NMR spectroscopic and DART MS techniques.

Morphology and Distribution of *Stephania glabra* (Roxb.) Miers

Stephania glabra (Roxb.) Miers (Figure 1) a species under the genus of climbers belonging to family menispermaceae (Semwal and Semwal, 2015). It has greenish yellowish flowers and large tubers weighing as much as 30 kg (Vashist *et al.*, 2012). It is mainly observed the flowering within April to May (Rai, 2018). Roots are tubers with fibrous roots below, round or oval also with irregular shapes (Thakur, 2016). This plant mainly grows in the tropical regions of India, Myanmar and China (Titova *et al*., 2012). It is herbaceous vines. Stems striate, glabrous, hollow. (Xianrui *et al*., 1996).

Figure 1 *Stephania glabra* (Roxb.) Miers (Source by Researcher)

Materials and Methods

Plant Material and Preparation

The tuber of *S. glabra* to be analyzed was collected from Kalay Township, Myanmar on September, 2015. This plant specimen was identified with the help of an expert, Pro-rector. Dr Soe Myint Aye, Myitkyina University. After washing and cleaning, the tuber was chopped in to small pieces and dried in air for 25 days. The dried sample was weighed and stored in a well stopper bottle and made to be ready for experiment.

Preparation of Extract

The plant sample (650 g) of *S. glabra* was occasionally percolated with 95% ethanol (6000 mL) for one month. After that, the ethanol extract was filtered by using Whatman No.1 filter paper and the filtrate was evaporated to obtain the viscous mass. The prepared extract 10.75 g (1.65 %) was obtained and stored at 4°C for further analysis. The extract yield percent (Adhikari *et al.*, 2015) was expressed as follows.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the dry extract (g)}}{\text{Weight of the sample used for the extraction (g)}} \times 100$$

Isolation

A few spots of ethyl acetate crude extract were checked on TLC plate by using n-hexane : EtOAc - 9:1, 1:1, 3:7 ratios for further separation. Common laboratory tools and commercially grade solvents were used for isolation of pure compound (KLT-1). The ethyl acetate extract sample (8.5 g) was fractioned by column chromatography over SiO_2 (70-230 mesh) eluting with n-hexane : ethyl acetate in various ratios from non-polar to polar (Figure 2). Totally (458) fractions were obtained and the resulted fractions were frequently checked by TLC with each relative solvent ratio system. Moreover, UV lamp and I_2 vapour were used as color visualizing materials for identification of the constituent compounds. The fractions of the same R_f values were combined and obtained (11) combine fractions. Among them, pure compound (KLT-1)

(40 mg and R_f = 0.9) was isolated from fraction 1 and it showed UV active as well as I_2 vapour on TLC plate (only one black spot) from the fraction of n-hexane : ethyl acetate (19:1) solvent ratio.

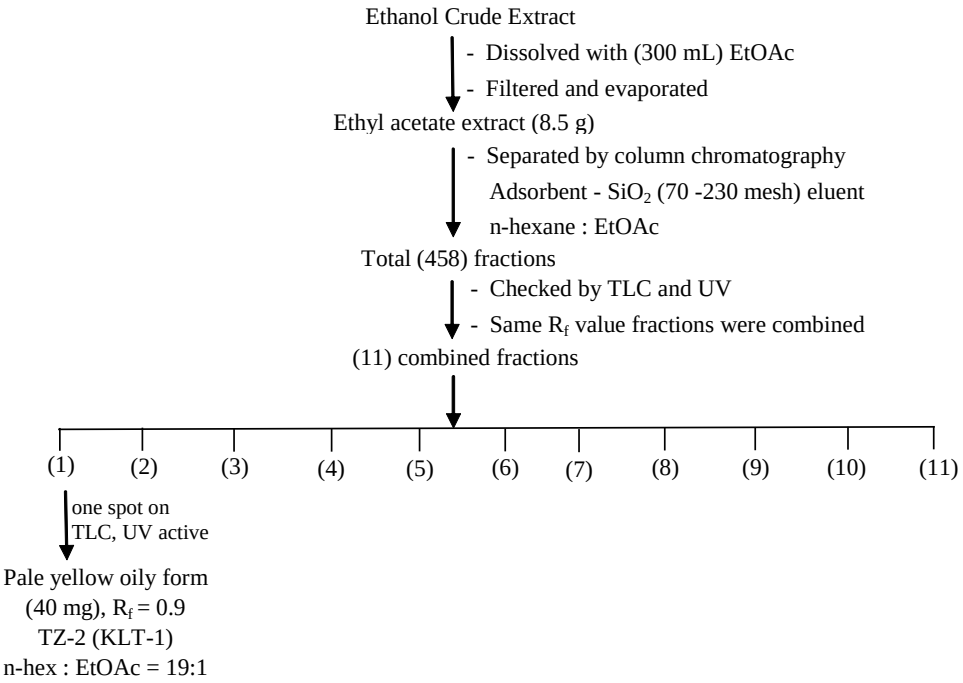


Figure 2 Isolation of pure compound (KLT-1) from the tuber of *S. glabra*

Test of The Pure Compound (KLT-1) for Antimicrobial Activity

The screening of antimicrobial activity of pure compound (KLT-1) was evaluated by Agar well diffusion method at Myanmar Pharmaceutical Industrial Enterprise, Ministry of Industry, Insein Township, Yangon. Totally, six microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* were used to assess their susceptibility to this compound (KLT-1)

Results and Discussion

Antimicrobial Activity of Pure Compound (KLT-1)

The antimicrobial activity of pure compound (KLT-1) was investigated against on six microorganisms; *Bacillus subtilis*(A), *Staphylococcus aureus*(B), *Pseudomonas aeruginosa* (C), *Bacillus pumilus* (D), *Candida albicans* (E), *Escherichia coli* (F). The results are shown in Table 1 and Figure 3.

Table 1 Antimicrobial Activity of Pure Compound (KLT-1) in Ethyl acetate

Compound	Inhibition zone diameter (mm)					
	A	**B**	**C**	**D**	**E**	**F**
KLT-1	20 **(+++)**	19 **(++)**	16 **(++)**	20 **(+++)**	17 **(++)**	20 **(+++)**

(+) Low = 10 ~14 mm (++) Medium = 15mm ~ 19 mm (+++) High = 20 mm above

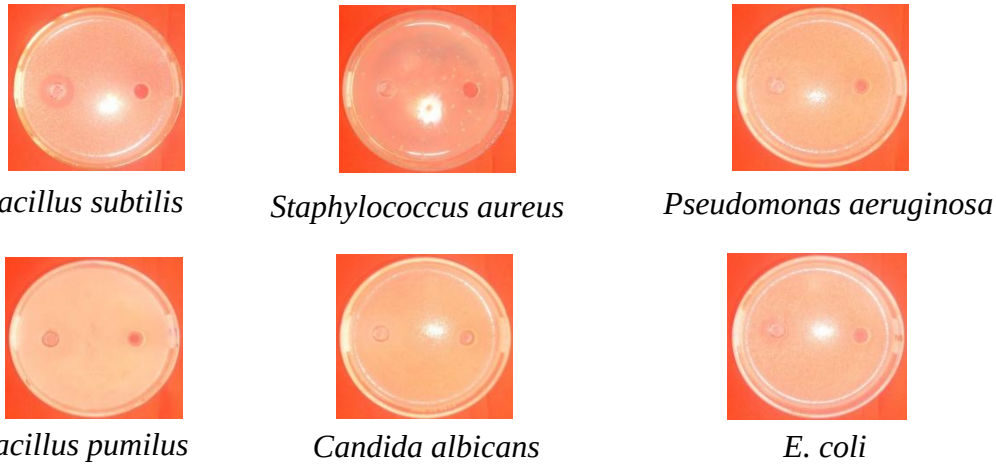


Figure 3 Antimicrobial activity of pure compound (KLT-1)

According to above table, the pure compound (KLT-1) was observed to have good antimicrobial activities on testing all organisms which especially showed high activity on three organisms such as *Bacillus subtilis*, *Bacillus pumilus* and *E.coli*. From these high microbial inhibitions, pure compound (KLT-1) could be considered as a biologically active compound for some medicinal effects on stomach disorders, skin, eye, respiratory tract and urinary tract diseases.

Determination of Molecular Formula of Pure Compound (KLT-1)

The molecular formula of isolated pure compound (KLT-1) was could be assigned by some spectroscopic methods such as FT IR, ^{1}H NMR (600MHz), ^{13}C NMR (150MHz), DEPT, HSQC and DART MS spectrometry.

Functional Groups Determination of Pure Compound (KLT-1)

FT IR spectrum of pure compound (KLT-1) is described in Figure 4 and their assignments are shown in Table 2. The peak 3072 cm^{-1} indicates C=C-H stretching vibration of sp^2 hydrocarbons. The bands at 2928 and 2854 cm^{-1} which should be asymmetric and symmetric C-H stretching vibration of sp^3 hydrocarbons. In addition, the band at 1735 cm^{-1} indicates C=O stretching vibration of carbonyl group. The C=C skeletal stretching vibration of aromatic ring could be observed at 1514 cm^{-1}. Moreover, the bands at 1458 cm^{-1} could be presented C-H in plane bending vibration of allylic hydrocarbons. The observed bands at 1273, 1230 and 1091 cm^{-1} which are due to the presence of C-C-O stretching vibration of alcohol and ether groups. The C=C-H out of plane bending vibration was presented in 864 cm^{-1}.

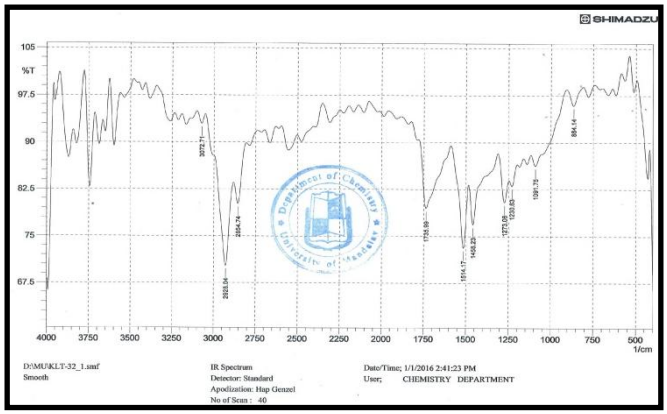


Figure 4 FT IR spectrum of pure compound (KLT-1)

Table 2 Characteristic Absorption Bands and their Assignments of Pure Compound (KLT-1)

No.	Wave number (cm^{-1})	Assignments
1	3072	C=C-H stretching vibration of sp^2 hydrocarbons
2	2928, 2854	Asymmetric or symmetric C-H stretching vibration of sp^3 hydrocarbons
3	1735	C=O stretching vibration of carbonyl group
4	1514	C=C stretching vibration of alkene
5	1458	C-H in plane bending vibration of allylic hydrocarbons
6	1273, 1230, 1091	-C-C-O stretching vibration of ester group
7	864	C=C-H out-of-plane bending vibration

^{1}H NMR Spectrum of Pure Compound (KLT-1)

According to ^{1}H NMR spectrum (Figure 5), this compound contains (30) protons and their assignments are shown in Table 3.

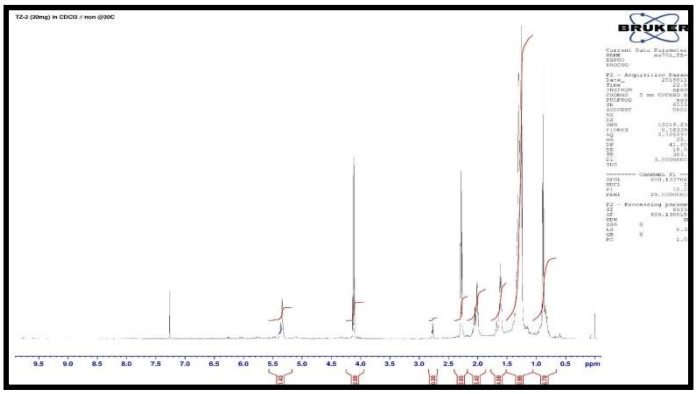


Figure 5 ^{1}H NMR spectrum of pure compound (KLT-1)

Table 3 ^{1}H NMR Spectral Data of Pure Compound (KLT-1)

Proton Assignment	^{1}H (δ/ppm)	J values (Hz)	Splitting Pattern	No. of Proton
sp^3 methyl proton	0.88	6.66, 7.14	t	3H
sp^3 methyl proton	1.25	7.14	t	3H
sp^3 methylene proton	1.2-1.3	-	overlap	12H
sp^3 methylene proton	1.62	6.84, 7.14	t	2H
sp^3 methylene proton	2.03, 2.04	-	m	2H
sp^3 methylene proton	2.28	7.50, 7.62	t	2H
sp^3 methylene proton	2.77	6.78, 6.79	t	2H
sp^3 methylene proton	4.12	7.14	q	2H
sp^2 methine proton	5.33, 5.34	-	m	2H
		Total number of protons		**30**

In this spectrum, there is overlap peaks between δ value 1.2 and 1.3 ppm protons. Thus, this compound should contain at least 30 protons (Figure 5).

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

The ^{13}C NMR spectrum (Figure 6), represents the total number of (18) carbons containing in this compound. The chemical shift values and their assignments are described in Table 4.

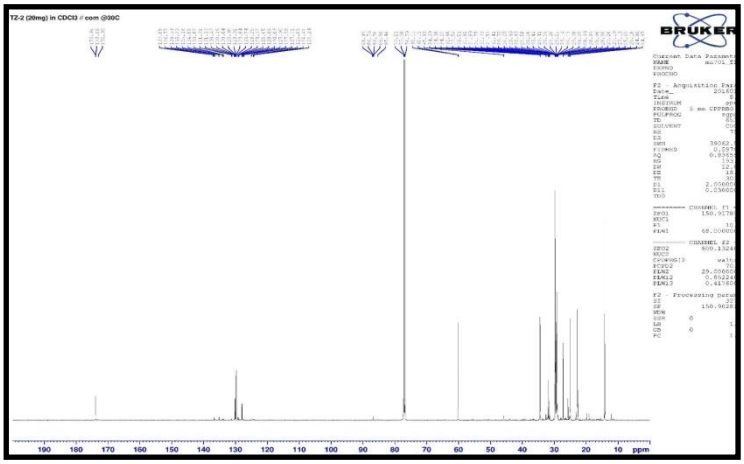


Figure 6 ^{13}C NMR spectrum of pure compound (KLT-1)

Table 4 ^{13}C NMR Spectral Data of Pure Compound (KLT-1)

No	^{1}H (δ/ppm)	Types of Carbon	Remarks
1	14.08	sp^3 methyl carbon	1
2	14.23	sp^3 methyl carbon	1
3	22.67	sp^3 methylene carbon	1
4	24.98	sp^3 methylene carbon	1
5	25.62	sp^3 methylene carbon	1
6	27.15	sp^3 methylene carbon	1
7	27.20	sp^3 methylene carbon	1
8	29.14	sp^3 methylene carbon	1
9	29.34	sp^3 methylene carbon	1
10	29.67	sp^3 methylene carbon	1
11	31.91	sp^3 methylene carbon	1
12	34.39	sp^3 methylene carbon	1
13	60.12	sp^3 methylene carbon	1
14	128.03	sp^2 methine carbon	1
15	129.89	sp^2 methine carbon	1
16	130.03	sp^2 quaternary carbon	1
17	130.19	sp^2 quaternary carbon	1
18	173.19	quaternary carbon	1
Total number of carbons			**18**

According to ^{13}C NMR spectrum (Figure 6), there is overlap proton region between δ 28-30 ppm. Number of carbon was accounted as (18) carbons. Therefore, this compound should contain at least (18) carbons.

HSQC Spectrum of Pure Compound (KLT-1)

HSQC spectrum of pure compound (Figure 7) which gives the proton-carbon direct correlation. The chemical shift values of protons and carbons are shown in Table 5.

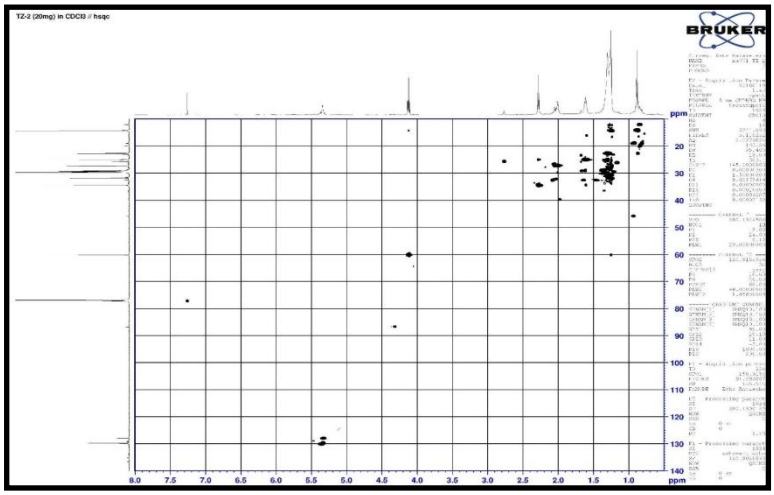


Figure 7 HSQC Spectrum of Pure Compound (KLT-1)

Table 5 ^{1}H-^{13}C Correlation Spectrum of Pure Compound (KLT-1)

No	^{13}C (δ/ppm)	^{1}H (δ/ppm)	Assignments	
1	14.08	0.88	sp^3 methyl carbon	CH_3
2	14.23	1.25	sp^3 methyl carbon	CH_3
3	22.67	1.25	sp^3 methylene carbon	CH_2
4	24.98	1.62	sp^3 methylene carbon	CH_2
5	25.62	2.77	sp^3 methylene carbon	CH_2
6	27.15	2.03	sp^3 methylene carbon	CH_2
7	27.20	2.04	sp^3 methylene carbon	CH_2
8	29.14	1.25	sp^3 methylene carbon	CH_2
9	29.34	1.25	sp^3 methylene carbon	CH_2
10	29.67	1.25	sp^3 methylene carbon	CH_2
11	31.91	1.25	sp^3 methylene carbon	CH_2
12	34.39	2.28	sp^3 methylene carbon	CH_2
13	60.12	4.12	sp^3 methylene carbon	CH_2
14	128.03	5.33	sp^2 methine carbon	=CH
15	129.89	5.34	sp^2 methine carbon	=CH
16	130.03	-	sp^2 quaternary carbon	=C
17	130.19	-	sp^2 quaternary carbon	=C
18	173.19	-	quaternary carbon	$\diagdown\!\!\diagup$C=O

Number of methylene groups that account carbon chemical shift value at about 29 ppm and proton chemical shift value at about 1.25 ppm may be at least 18 carbons and 30 protons as mentioned in the table.

DEPT Spectrum of Pure Compound (KLT-1)

DEPT spectrum (Figure 8) which classify the types of carbon and the number of carbons as well as protons containing in this pure compound (KLT-1). The respective data assignments are tabulated in Table 6.

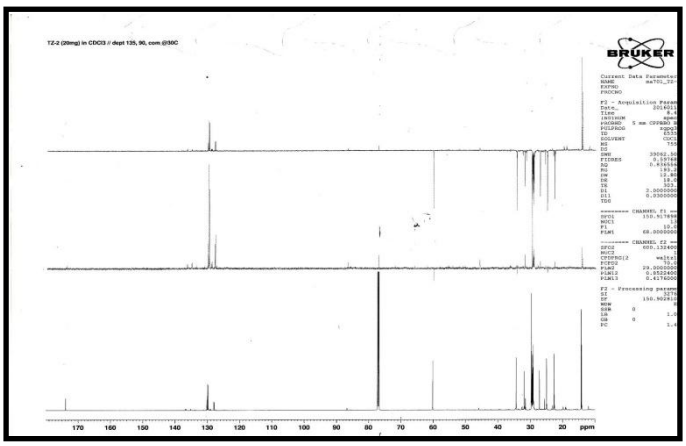


Figure 8 DEPT Spectrum of Pure Compound (KLT-1)

Table 6 DEPT Spectral Data of Pure Compound (KLT-1)

No	^{13}C (δ/ppm)	Assignments	No. of Carbons	No. of Protons
1	14.08	sp^3 methyl carbon	1	3
2	14.23	sp^3 methyl carbon	1	3
3	22.67	sp^3 methylene carbon	1	2
4	24.98	sp^3 methylene carbon	1	2
5	25.62	sp^3 methylene carbon	1	2
6	27.15	sp^3 methylene carbon	1	2
7	27.20	sp^3 methylene carbon	1	2
8	29.14	sp^3 methylene carbon	1	2
9	29.34	sp^3 methylene carbon	1	2
10	29.67	sp^3 methylene carbon	1	2
11	31.91	sp^3 methylene carbon	1	2
12	34.39	sp^3 methylene carbon	1	2
13	60.12	sp^3 methylene carbon	1	2
14	128.03	sp^2 methine carbon	1	1
15	129.89	sp^2 methine carbon	1	1
16	130.3	sp^2quaternary carbon	1	-
17	130.19	sp^2 quaternary carbon	1	-
18	173.91	quaternary carbon	1	-
Total number of protons and carbons			18	30

Confirmation of Molecular Formula of Pure Compound (KLT-1)

The elucidated partial molecular formula could be confirmed by DEPT (Figure 8) and FT IR (Figure 4) spectral evidences. The actual formula mass of this compound could be confirmed by DART MS spectrum (Figure 9). The assignments are represented in Table 7.

Table 7 Results Given by DEPT and FT IR Spectra

Assignment	Carbon	Proton	Oxygen
DEPT Spectral Data			
- Two sp^3 methyl carbon	2	6	-
- Eleven sp^3 methylene carbon	11	22	-
- Two sp^2 methine carbons	2	2	-
- Two sp^2 quaternary carbons	2	-	-
- One quaternary carbon	1	-	-
FT IR spectral data			
- One carbonyl group		-	1
- One ester group	-	-	1
Molecular Formula	C_{18}	H_{30}	O_2

The molecular formula of compound (KLT-1) could be assigned as $C_{18}H_{30}O_2$. The mass spectrum shows the molecular ion peaks either 391.2827 or 279.1580 Da. Among these two possibilities the mass at m/z 279.1580 is assigned as molecular ion peak of this compound. Hence molecular mass of pure compound (KLT-1) is now assigned as 278 by subtracting one unit of proton from the mass at m/z 279.1580 Da.

From the index of hydrogen deficiency index (HDI)

$$= C - \frac{H}{2} + \frac{N}{2} + 1$$

$$= IV - \frac{I}{2} + \frac{III}{2} + 1$$

$$= 18 - \frac{30}{2} + 0 + 1$$

$$= 4$$

According to formula index of hydrogen deficiency, the number of (HDI) is 4. This index number should be, in accordance with FT IR and NMR spectra, one $>C=O$ group, two $>C=C<$ groups and the last one should one cyclic (ring) system. Therefore, it must contain one $-C(=O)-$ group, two $C=C$ group and one ring system.

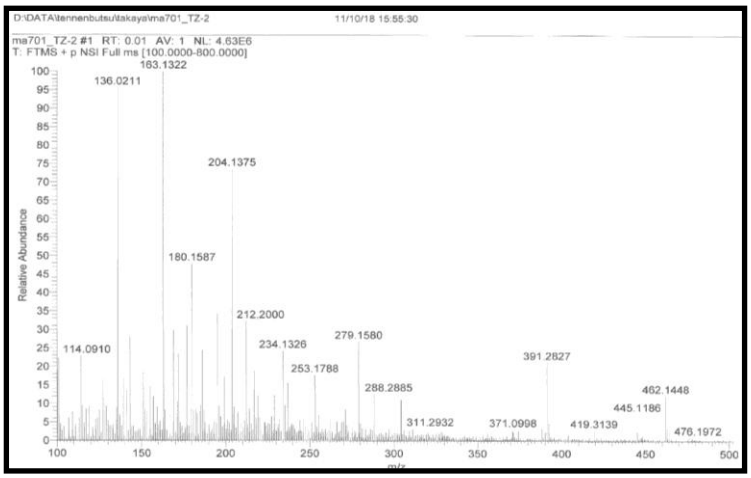


Figure 9 DART MS spectrum of pure compound (KLT-1)

Structural Elucidation of Pure Compound (KLT-1)

DQF COSY spectrum (Figure 10) shows the large square peaks that account the proton signal quartet at δ 4.12 ppm is coupling with protons at 1.25 ppm. From HSQC spectrum (Figure 7) direct attachment of these protons to carbons can be assigned as follows. In the ^{1}HNMR spectrum (Figure 5), first order coupling of signal, J = 7.14 Hz, quartet pattern clearly informs that the neighbouring carbon atom must bear three protons, that is methyl group.

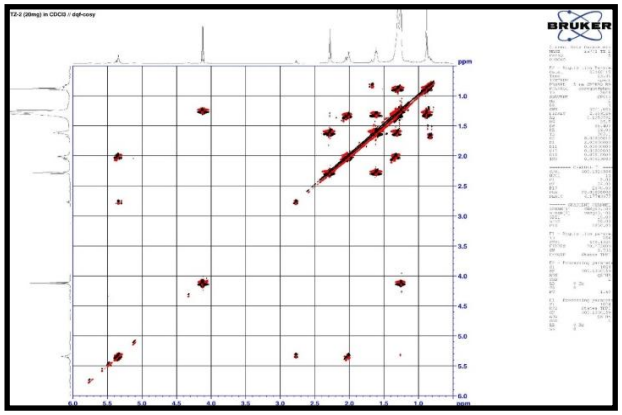


Figure 10 DQF COSY spectrum of pure compound (KLT-1)

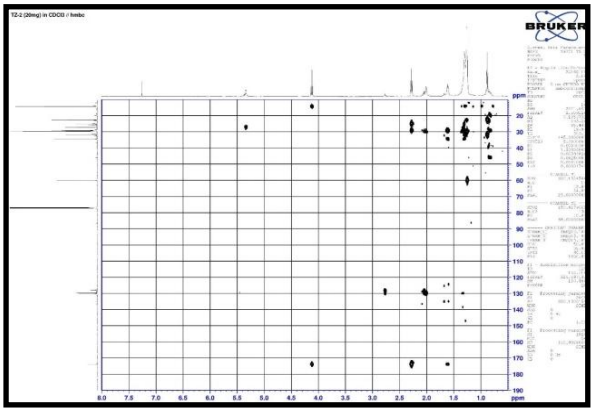
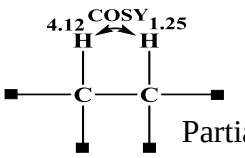
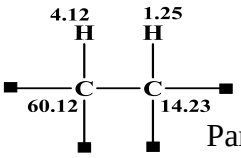


Figure 11 HMBC spectrum of pure compound (KLT-1)



Partial (2)



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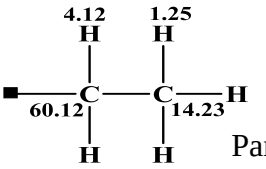
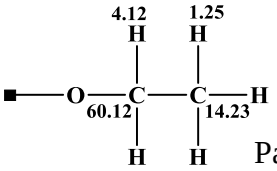
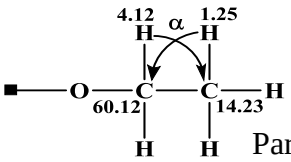


Partial (3)

In accordance with DEPT spectrum (Figure 8), above two carbons are assigned as methylene (60.12 ppm) and methyl (14.23 ppm) respectively. Again, based on lower field chemical shift values of both protons and carbon, sp^3 methylene carbon is considered to attach directly with oxygen atom. Thus following partial structure (A), ethoxy group is assigned. Presence of partial structure (A) can be confirmed by occurrence of proton-carbon correlation cross peak corresponding to δ 4.12 to 14.23 ppm and δ 1.25 to 60.12 ppm in HMBC spectrum (Figure 11).

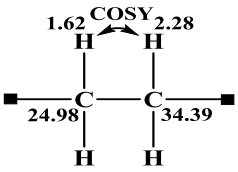


Partial structure (A)

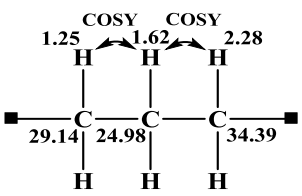


Partial structure (A)

Next, there are square cross peaks in DQF-COSY spectrum (Figure 10), that correspond to the two methylene groups at δ 2.28 ppm and 1.62 ppm respectively. This is an indication for vicinal arrangement of these two groups. Again δ 1.62 methylene protons show further vicinal coupling with respect to methylene protons appeared at δ 1.25 ppm. Therefore, following extended partial (4) can be deduced.

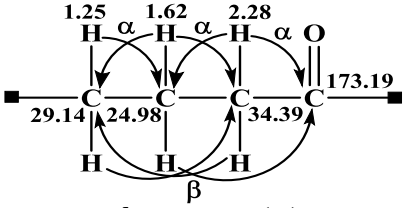


Partial (4)

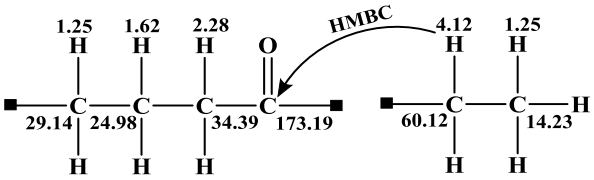


Partial (4)

Existence of partial (4) can be confirmed by HMBC spectrum (Figure 11). There are correlation cross peaks in HMBC spectrum that correspond to (i) δ1.25 ppm protons with 24.98 ppm carbon and 34.39 ppm carbon, (ii) δ 1.62 ppm protons with 29.14 ppm carbon and 34.39 ppm carbon and (iii) δ 2.28 ppm protons with 24.98 ppm carbon and 29.14 ppm carbon respectively. Moreover, there is an important long range coupling cross peak between δ 2.28 methylene protons as well as δ 1.62 ppm proton and carbonyl carbon at 173.19 ppm. This observation provides a good clue to connect carbonyl group with δ 2.28 methylene group. Thus, following partial structure (B) can be assigned. The partial structures (A) and (B) can be connected in accordance with HMBC (Figure 11).

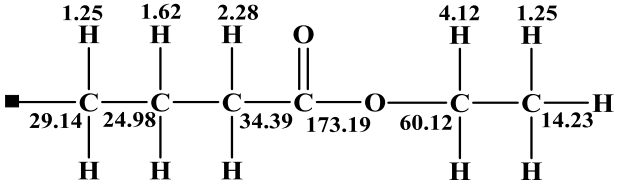


Partial structure (B)



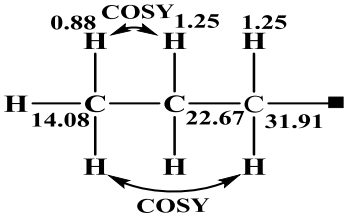
Partial structure (B) Partial structure (A)

In HMBC spectrum (Figure 11), there is a correlation cross peak between the methylene protons at δ 4.12 ppm from partial structure (A) and carbonyl carbon at δ 173.19 ppm from the partial structure (B). This cross peak shows connection sites between the two partial structures (A) and (B). Thus, the following partial structure (C) can be written.

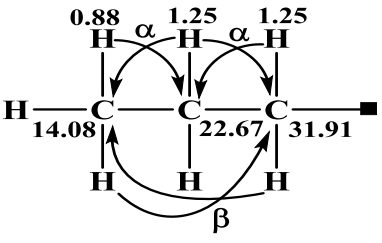


Partial structure (C)

On the other hand, the methyl signal (0.88, triplets) is assigned to coupling with its adjacent methylene protons at 1.25 ppm. This assignment is due to the clear triplet pattern appearance of methyl signals. On the basis of ^{1}H NMR (Figure 5) and DQF-COSY (Figure 10) spectral evidence, following propyl group is deduced. The presence of propyl group is confirmed by proton-carbon long range coupling cross peaks observed in HMBC spectrum (Figure 11). This propyl terminal group is assigned as partial structure (D).



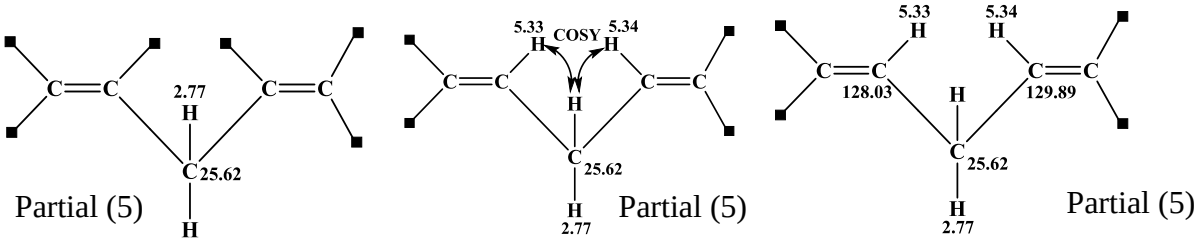
Partial structure (D)



Partial structure (D)

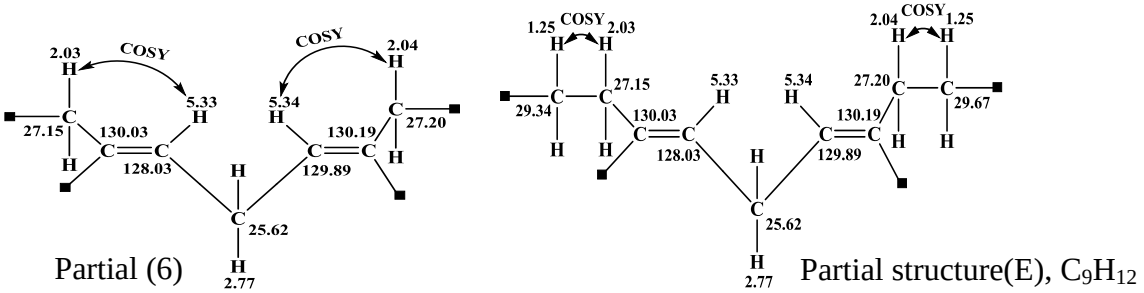
In the ^{1}H NMR spectrum (Figure 5), the triplet proton signal at δ 2.77 ppm is assigned double allylic methylene protons. Proton-carbon assignment is taken by appearance of cross peak in HSQC spectrum (Figure 7). Downward signal in DEPT spectrum (Figure 8) supports the presence of methylene group. Proton chemical shift value δ 2.77 ppm gives a good evidence for the existence of double allylic one. In the DQF-COSY spectrum (Figure 10), these methylene protons reveal the vicinal coupling with two alkenic protons at δ 5.33 ppm and 5.34 ppm respectively. Thus, the following partial (5) can be deduced. Proton-carbon direct attachment of

alkenic protons is assigned by the correlation cross peaks observed in HSQC spectrum (Figure 8).



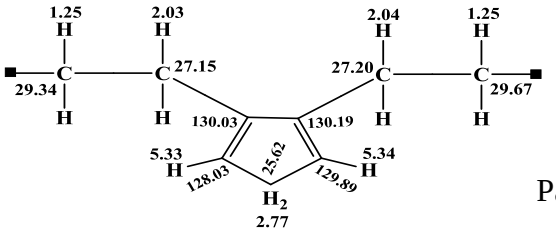
Partial (5) Partial (5) Partial (5)

Again in DQF-COSY spectrum (Figure 10), there are square peaks related to these two alkenic protons and two allylic methylene protons appeared at δ 2.03 ppm and 2.04 ppm. In accordance with this spectral evidence partial (5) is extended to following partial (6). Carbon assignment for the two allylic methylene groups is accomplished by correlation cross peak observed in HSQC spectrum (Figure 8), as well as the downward appearance in DEPT spectrum (Figure 8). In addition, DQF-COSY (Figure 10) represents that these two allylic methylene protons are further coupling with methylene groups appeared at δ 1.25 ppm. The partial (6) that has seen now assigned as partial structure (E).



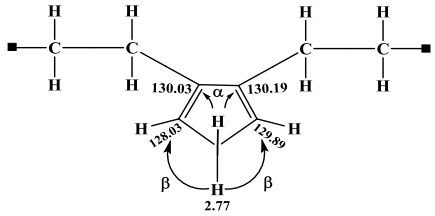
Partial (6) Partial structure(E), C_9H_{12}

Since, the compound showed UV absorption on TLC (black spot). This supported a good information that there should be conjugated system within the compound. This spectrum can have only two missing parts of alkenic quaternary carbons in the partial structure combine to form conjugated five membered cyclic ring. On the basis of UV-absorption phenomenon, partial structure (E) has changed in to following partial structure containing conjugated cyclic ring.

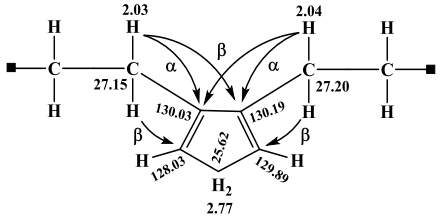


Partial structure (E), $[C_9H_{12}]$

The presence of cyclopentadienyl ring system could be further confirmed by proton-carbon correlation cross peaks found in HMBC spectrum (Figure 11). Double allylic methylene protons δ 2.77 ppm show α- and β- correlation with respect to all of alkenic carbons at δ 130.03ppm, 130.19 ppm, 128.03 ppm and 129.89 ppm. Again methylene protons at δ 2.03 ppm and 2.04 ppm also showed α- and β- correlation to these four alkenic carbons. These correlations can show only if all of alkenic carbons are the part of cyclic ring.

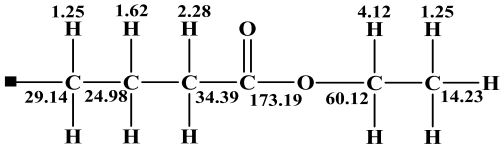


Partial structure (E), [C_9H_{12}]

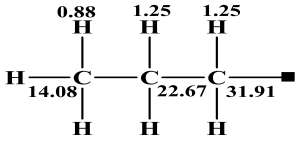


Partial structure (E), [C_9H_{12}]

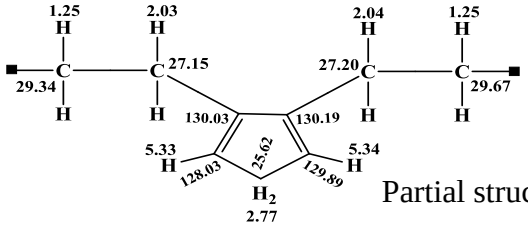
Now, three partial structures could have been elucidated on the basis of spectral evidences.



Partial structure (C), [$C_6H_{11}O_2$]

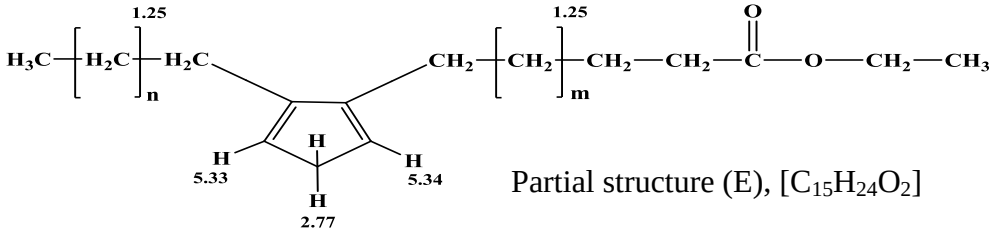


Partial structure (D), [C_3H_7]



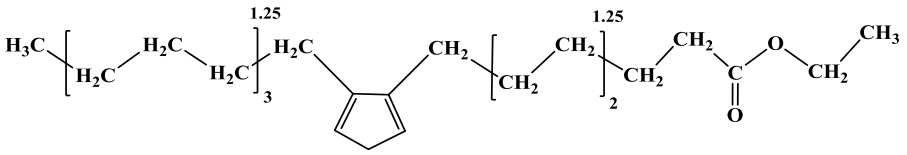
Partial structure (E), [C_9H_{12}]

Among three structures, partial structure (E) is common for linear combination of them this means that the partial structure (C) and partial structure (D) must connect with partial structure (E). But the connection sites on all partial structures show methylene groups at about δ 1.25 ppm. This gives ambiguous combination for following combine structure.



Partial structure (E), [$C_{15}H_{24}O_2$]

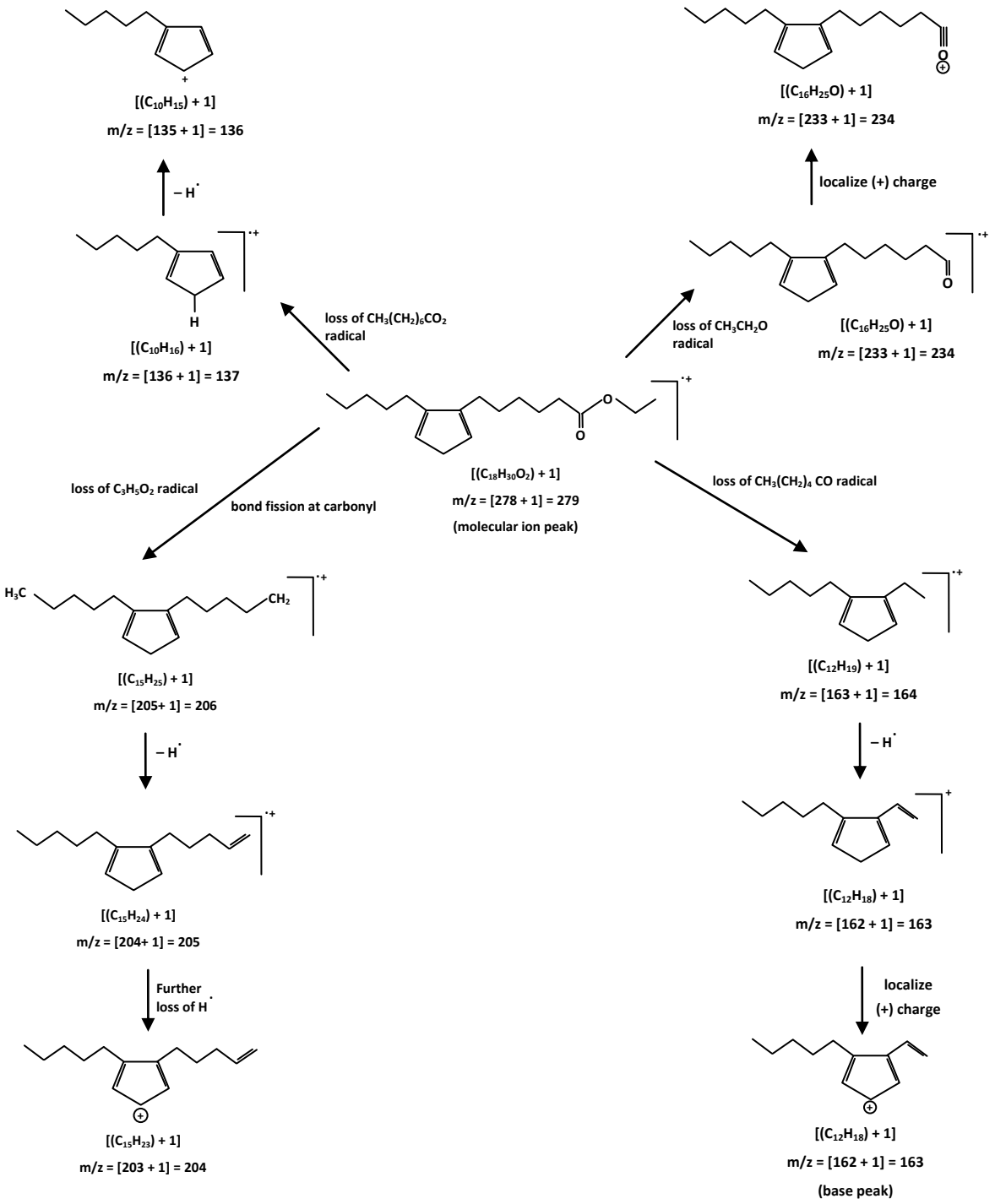
Mass spectrum (Figure 9) of compound (KLT-1) showed the molecular ion peak at m/z 279.1580 Da. Actual mass is therefore obtained by subtracting one unit from the molecular ion peak. Thus, molecular mass is assigned as m/z 278.1501 Da. When 'n' is taken as 3 and 'm' is 2, the resulting structure is in agreement with observed molecular mass m/z 278. The IUPAC name of pure compound (KLT-1) is assigned as ethyl-6-(5-pentyl) cyclopentadienyl hexanoate.



Molecular Formula = $C_{18}H_{30}O_2$ Molecular mass = 278 Da

Assignments of Some Prominent Peaks of Pure Compound (KLT-1)

In DART (Direct Analysis of Real Time) mass spectrum (Figure 9), generally the more stable and the higher the intensity (relative abundance) of the peaks are observed due to the fragmentation behavior. Some prominent peaks m/z 234, 163,136 and m/z 204 can be interpreted by following mechanistic fragmentation pathway.



Conclusion

A biologically active wild plant, *Stephania glabra* (Roxb.) Miers (*S. glabra*), Myanmar named as *Taung-kya* was selected for this research. The tuber part (16 kg) of this plant was selected and done extraction, isolation, antimicrobial activity and structural elucidation. The crude extract from the tuber of *Stephania glabra* was performed by solvent extraction method. Furthermore, the pure compound (KLT-1), ethyl-6-(5-pentyl) cyclopentadienyl hexanoate could has been isolated by using Thin Layer and Column Chromatographic separations.

Antimicrobial activities of this isolated ester compound (KLT-1), was tested on six selected organisms by agar well diffusion method. As the results, It was observed to have good

antimicrobial activities against on testing all organisms. In addition, it showed excellent activity on three bacteria such as *Bacillus subtilis* (which causes food poison, skin and respiratory tract infection), *Bacillus pumilus* (which causes eye and food poisoning) and *E. coli* (which causes diarrhea, dysentery and urinary tract infection). According to these high activities, isolated pure compound (KLT-1) was found to have medicinal effects on stomach disorders, skin, eye, respiratory tract and urinary tract diseases, etc. Finally, the molecular formula and its structure of pure compound (KLT-1), ethyl-6-(5-pentyl) cyclopentadienyl hexanoate from the tuber extract of *S. glabra* was assigned as $C_{18}H_{30}O_2$ by applying advance spectroscopic techniques such as FT IR, ^{1}H NMR (600MHz), ^{13}C NMR (150 MHz), DEPT, HSQC, DQF COSY, HMBC, and DART MS spectral evidences.

Acknowledgements

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