MOLECULAR FORMULA DETERMINATION AND STRUCTURE ELUCIDATION OF BIOACTIVE PORPHYRIN DERIVATIVE COMPOUND ISOLATED FROM THE WHOLE PLANT OF *Corallodiscus lanuginosus* (WALL. ex R.Br.) BURTT

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Abstract

In this research paper, *Corallodiscus lanuginosus* (Wall. ex R.Br.) Burtt which is one of the Myanmar indigenous medicinal plants known as Pan ma o` was selected for chemical analysis. The preliminary phytochemical screening of the whole plant of Pan ma o` was carried out, which indicated the presence of alkaloid, steroid, terpenoid, glycoside, lipophilic, polyphenol, saponin, phenolic and tannin, respectively. Antimicrobial activities of the crude extract of the whole plant of Pan ma o` were tested in various solvents system by using agar well diffusion method on six selected organisms. The ethyl acetate crude extract of Pan ma o` gives rise to high activities on all selected organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and E.coli.* Moreover, porphyrin derivative compound was isolated from the whole plant of Pan ma o` as indigo crystal (19.6 mg) by thin layer and column chromatography separation methods. The yield percent was found to be (0.529%) based upon the EtOAc crude extract. Moreover, antimicrobial activities of this compound were rechecked by using agar well diffusion method. The ethyl acetate extract of this compound responds high activities on all selected organisms except *Staphylococcus aureus*. In addition, the molecular formula of this compound could be determined as $C_{30}H_{35}N_9O_4$ (Hydrogen Deficiency Index = 18), applying some spectroscopic methods such as FT IR, ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT and FAB-mass spectral data respectively. The complete structure of this porphyrin derivative compound could be elucidated by

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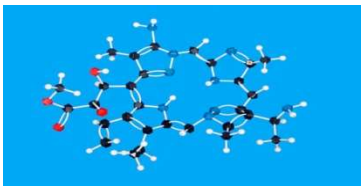
DQF-COSY, HMQC, HMBC and DEPT spectroscopic methods. The elucidated compound could be described as below.



Methyl-3-((1E, 4Z, 6Z, 10Z, 14E, 16E) -18- amino-7- (1-aminoethyl) -12-ethyl-3, 8, 17-trimethyl-13-vinyl-2, 9, 19-triazaporphyrin-15-yl) -3-hydroxy-2-oxopropano-ate

Keywords: Pan ma o`, *Corallodiscus lanuginosus,* porphyrin derivative compound, antimicrobial activities

Introduction

Traditional medicine has existed in Myanmar long long ago (WHO). At present day the image of traditional medicine differs from that of olden day image. Traditional medicine is always taking vital role in the most essential part of humanity for their survival and longevity. Over the years, traditional medicine has been instrumental in maintaining physical and mental strength of people of Myanmar.

Plants have been used in treating human diseases for thousands of years and nowadays many currently used medicines are derived from natural sources. For thousands of years medicine and natural products have been closely linked through the use of traditional medicines and natural poisons (Grabley and Thiericke, 2000). In most areas of the world, herbals medicine has remained the mainstay of the therapy because of the cause and availability and perhaps cultural preference.

Herbal medicine is a major remedy in traditional medicine system, which is largely based on the use of roots, leaves, barks, seeds and flowers of the plants. They are free from side effects, adverse effects and they are economical, easily available and beneficial for the mankind over a century (Genapathy *et al*., 2013). Medicinal plants are rich source of bioactive compounds and thus serve as important raw materials for drug production. Nowadays the potent formulation of traditional medicines are extensively used to complete with that of the western medicines in fight against various dreadful

diseases such as heart diseases, AIDS, tuberculosis, cancer, liver diseases and malaria. In this research work, a porphyrin derivative compound was isolated from the whole plant of *Corallodiscus lanuginosus* (Wall. ex R.Br.) Burtt, locally known as Pan- ma- o` ( Figure 1), which is one of the indigenous medicinal plant. It was chemically analyzed for new source of compound in this field (Harborne, J.B, 1984). The plant of Pan ma o` is widely distributed in Lwe Tan Mountain, Paung Lin, Pa Lè Tint village, Hopone Township, Taunggyi Region. It is medicinally used as liver diseases, disorders of kidney, to cure poisoning, diarrhoea caused by hot diseases.

Botanical Description

Family name	: Gesneriaceae
Botanical name	: *Corallodiscus lanuginosus* (Wall. ex R.Br.) Burtt
Local name	: Pan ma o`
Common name	: Blue stone flower
Part used	: The whole plant
Medicinal uses	: To cure poisoning, diarrhoea caused by hot diseases, liver diseases, kidney problems and wounds

Figure 1 The plant of *Corallodisus lanuginosus* (Wall. ex R.Br.) Burtt

Materials and Methods

The sophisticated instruments used in the isolation and structure elucidation of pure compound were UV-lamp (Lambda-40, Perkin-Elmer Co., England), FT IR spectrophotometer (Shimadzu, Japan), ^{1}H NMR spectrometer (500 MHz),^{13}C NMR spectrometer (125 MHz), FAB-mass spectrometer, electric balance (Shimadzu, Japan) and melting point apparatus.

Materials

Commercial grade solvents were used after distillation. Analytical preparative thin layer chromatography was performed by using percolated silica gel (Merk Co. Inc, Kiesel gel 60 F_{254}). Silica gel Merk Co.Inc, Kiesel gel 70-230 Mesh ASTM) was used for column chromatography. Iodine vapor and UV detector were used for visualizing the compound on TLC plates.

Preliminary Phytochemical Screening

Phytochemical analysis for alkaloids, flavonoids, steroids, terpenoid, glycosides, reducing sugars, lipophilic, polyphenols, saponins , phenolic compounds and tannin were carried out according to reported methods (Harborne, 1993; Yadav and Munin, 2011).

Extraction and Isolation of Porphyrin Derivative Compound

Air dried sample (625 g) was percolated with ethanol (4.3 L) for about two months. Percolated solution was filtered and concentrated to yield residue. It was extracted with ethyl acetate (150 mL) and evaporated. The ethyl acetate crude sample (3.7 g) was obtained. It was fractionated by column chromatography over silica gel (70-230 mesh) eluted by various solvent ratio of n-hexane and ethyl acetate from non- polar to polar. Totally (333) fractions were obtained. These fractions were combined according to same R_f values under UV lamp and iodine detector. Ten combined fractions were obtained. The combined fraction (VIII) was checked by TLC for purity amount. It gave one spot on TLC in (R_f = 0.37) with n-hexane : EtOAc (7 : 3 v/v) and UV

active. The pure compound indigo crystal (19.6 mg) was obtained. The yield percent was found to be (0.529%) based upon the EtOAc crude extract.

Investigation of Antimicrobial Activities of the Crude Extract of the Whole plant of *Corallodisus lanuginosus* and Isolated Porphyrin Derivative Compound

Antimicrobial activities of the crude extract of the whole plant of *C. lanuginosus* (Pan ma o`) and isolated porphyrin derivative compound were tested in various solvents system by using agar well diffusion method on six selected organisms, such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and E.coli* at Pharmaceutical Research Department (PRD) in Insein, Yangon.

Structure Elucidation of the Isolated Compound

The molecular formula determination of isolated compound was done by spectroscopic methods such as ^{1}H NMR, ^{13}C NMR, DQF-COSY, HMQC, DEPT, FAB-mass and HMBC respectively (Breitmaier, 2002; Crews *et al*., 1998; Hesse *et al*., 1997; Nakanishi, 1962; Silverstein *et al*., 2005).

Results and Discussion

Preliminary Phytochemical Screening of the Whole Plant of *C. lanuginosus* (Wall. ex R.Br.) Burtt

Phytochemical screening was carried out by general methods to indicate the presence of general classes of phytochemical constituents. Table 1 shows the results of the phytochemical tests.

Table 1 Result of Preliminary Phytochemical Tests

No.	Constituents	Reagents used	Observation	Results
1	Alkaloid	Wagner's reagent Dragendorff's reagent	Reddish brown Orange ppt	+
2	Flavonoid	Mg coil, Conc:HCl	No pink color solution	-
3	Steroid	Pet ether, Conc:H_2SO_4, acetic anhydride	Green color solution	+
4	Terpenoid	Ethanol, $(CH_3CO)_2O$, Conc: H_2SO_4,$CHCl_3$	Red color solution	+
5	Glycoside	10% Lead acetate	White ppt	+
6	Sugar	Benedict's solution	No orange ppt	-
7	Lipophilic	0.5 M KOH, NaOH, distilled water	Deep color solution	+
8	Polyphenol	1% $FeCl_3$, $K_3Fe(CN)_6$	Deep green blue solution	+
9	Saponin	distilled water	Frothing	+
10	Phenolic	10% $FeCl_3$	Green blue solution	+
11	Tannin	10% $FeCl_3$, dil H_2SO_4	Yellowish brown	+

(+) = Present (-) = Absent

According to this Table 1, the sample consists of alkaloid, steroid, terpenoid, glycoside, lipophilic, polyphenol, saponin, phenolic and tannin, respectively.

Antimicrobial Activity of the Whole plant of *C. lanuginosus* (Wall. ex R.Br.) Burtt

The results of the antimicrobial activities of the crude extract of the whole plant of *C. lanuginosus* are shown in Figure 2 and Table 2. According to this table, ethyl acetate crude extract of *C. lanuginosus* showed high activities on all selected organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and E.coli.* Moreover, ethanol extract responded high activity on *Bacillus pumilus*.

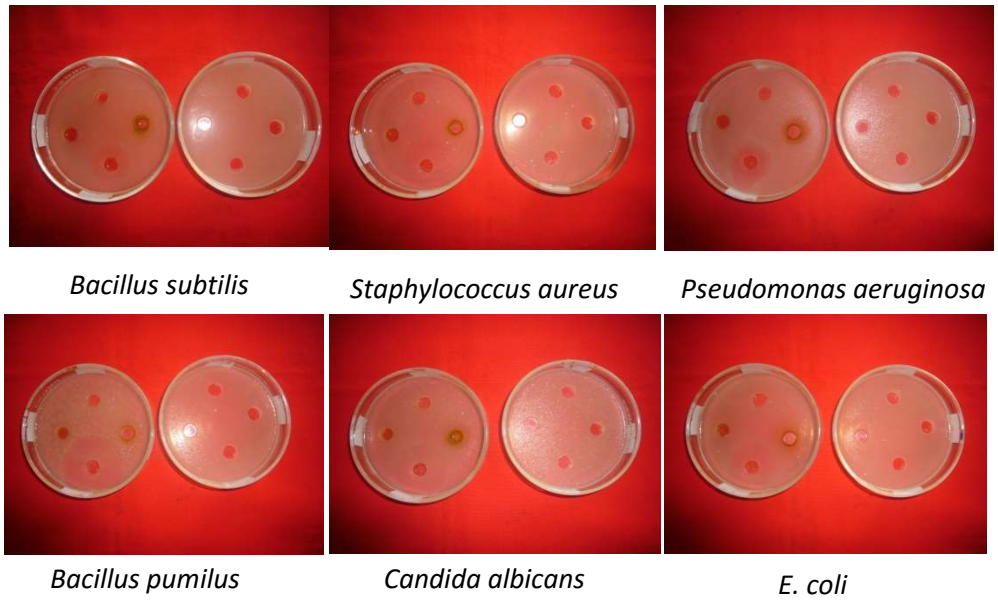


Figure 2 Antimicrobial activities of the whole plant of *C. lanuginosus* (Wall. ex R.Br.) Burtt

Table 2 Results of Antimicrobial Activities of the whole plant of *C. lanuginosus* (Wall. ex R.Br.) Burtt

No	Solvent	Inhibition Zone Diameter (mm)					
		I	II	III	IV	V	VI
1	n-hexane	-	-	-	-	-	-
2	$CHCl_3$	13 (+)	12 (+)	12 (+)	15 (++)	12 (+)	-
3`	EtOAc	22 (+++)	34 (+++)	29 (+++)	40 (+++)	32 (+++)	34 (+++)
4	EtOH	16 (++)	13 (+)	15 (++)	20 (+++)	15 (++)	18 (++)

agar well ~ 10mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
I. *Bacillus subtilis*
II *Staphylococcus aureus*
III *Pseudomonas aeruginosa*
IV *Bacillus pumilus*
V *Candida albicans*
VI *E. coli*

Antimicrobial Activities of the Porphyrin Derivative Compound

Antimicrobial activities of isolated compound were rechecked by using agar well diffusion method on six selected organisms. The results are described in Figure 3 and Table 3.

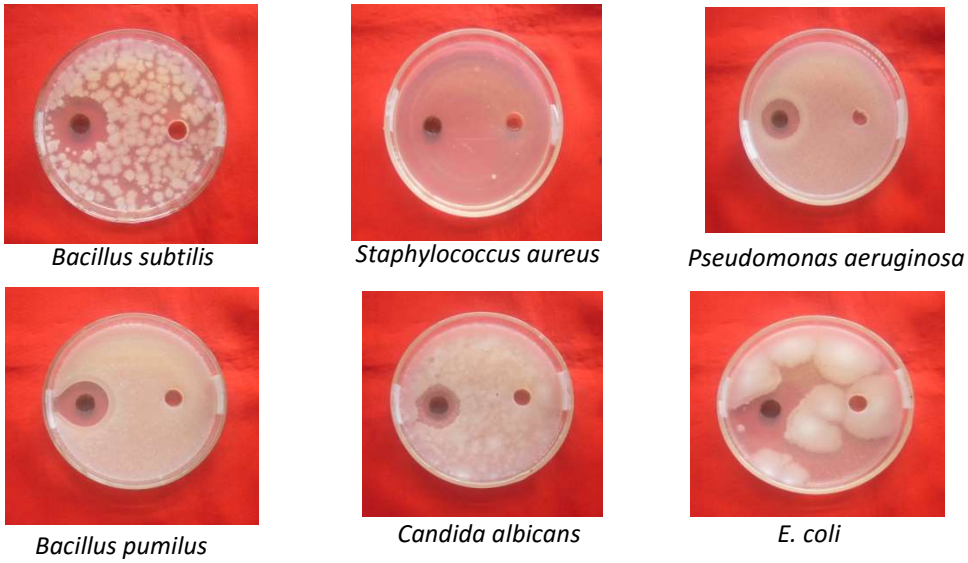


Figure 3 Antimicrobial activities of porphyrin derivative compound

Table 3 Results of Antimicrobial Activities of Porphyrin Derivative Compound

Sample	Solvent	Inhibition Zone Diameter (mm)					
		I	II	III	IV	V	VI
Pure compound	EtOAc	+++	–	+++	+++	+++	+++
Control	EtOAc	–	–	–	–	–	–

agar well ~ 10mm		Organisms	
10 mm ~ 14 mm	(+)	I.	*Bacillus subtilis*
15 mm ~ 19 mm	(++)	II	*Staphylococcus aureus*
20 mm above	(+++)	III	*Pseudomonas aeruginosa*
		IV	*Bacillus pumilus*
		V	*Candida albicans*
		VI	*E. coli*

According to this table, the ethyl acetate extract of isolated compound gives rise to high activities on all selected organisms except *Staphylococcus aureus*.

Molecular Formula Determination of Isolated Compound

The molecular formula of isolated compound could be determined by spectral data of some spectroscopic methods, such as FT IR (Figure 4), ^{1}H NMR (500 MHz)(Figure 5), ^{13}C NMR(125 MHz) (Figure 6), HMQC (Figure 7), DEPT(Figure 8) and FAB-mass (Figure 9).

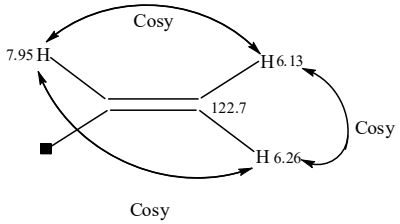
According to ^{1}H NMR and ^{13}C NMR spectral data, the partial molecular formula could be assigned as $C_{30}H_{28}$. In ^{13}C NMR spectrum, down field chemical shift of sp^2 quaternary carbons (δ 169.57 ppm, and δ 189.89 ppm) should be 2 carbonyl carbons. Therefore, the partial molecular formula is $C_{30}H_{28}O_2$. According to the FT IR spectrum, this compound should consist of at least one –OH and one $–NH_2$ groups due to the presence of the bands at 3392.99 cm^{-1} and 3291.05 cm^{-1}. On the other hand, 1735.99 cm^{-1} and 1219.05 cm^{-1} indicate the presence of one ester group. Therefore, the partial

molecular formula and partial molecular mass are $C_{30}H_{31}NO_4$ and 469 respectively.

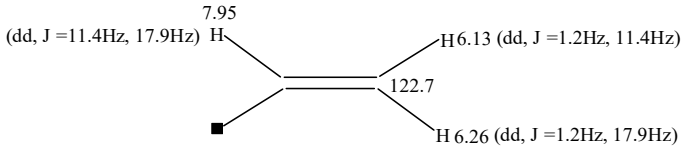
Moreover, in FAB mass spectrum, the $[M^+ + Na]$ ion peak shows at m/z 608. Therefore, the molecular ion peak of isolated compound is m/z 585 which indicates the molecular mass of this compound. According to the nitrogen rule, the odd number of molecular mass indicates the containing of odd number of nitrogen atoms. Hence, the partial molecular formula becomes $C_{30}H_{31}N_3O_4$. And so, the partial molecular mass is 497. Therefore, the remaining partial molecular mass is 585 - 497 = 88. The remaining partial molecular mass (88) may be one $–NH_2$ group, 2 –NH groups and 3 nitrogen atoms. Consequently, the real molecular formula of this compound could be assigned as $C_{30}H_{35}N_9O_4$. The hydrogen deficiency index of isolated compound is 18.

Structure Elucidation of Isolated Compound

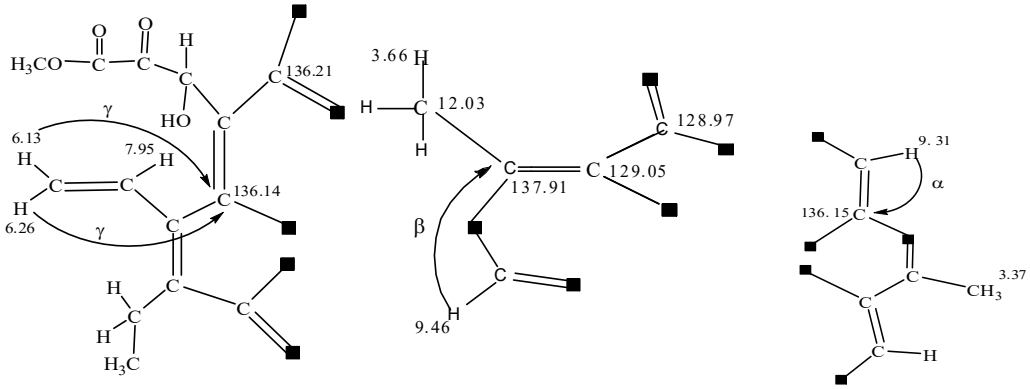
According to the DEPT, HMQC and DQF-COSY (Figure 10) spectral data, the exomethylene fragment could be assigned.



This monosubstituted alkenic fragment could be confirmed by their splitting patterns and coupling constant (J values) of these protons in ^{1}HNMR spectrum. In which the sp^2 methine proton (δ 7.95 ppm, dd, J=11.4Hz and 17.9Hz) one of the sp^2 exomethylene protons (δ 6.13 ppm, dd, J=1.2Hz and 11.4Hz) and one of the sp^2 exomethylene protons (δ 6.26 ppm, dd, J=1.2Hz and 17.9Hz) are shown below.

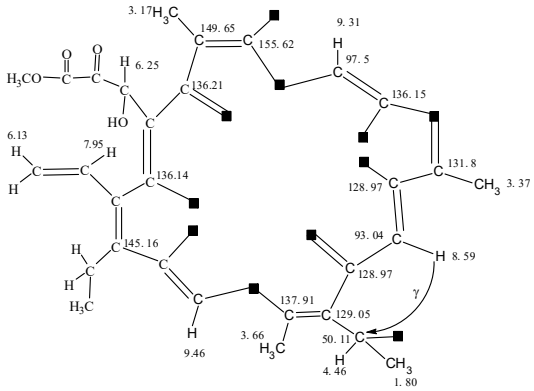


In addition, the observation of α, β and γ ^{1}H-C long range signal in HMBC spectrum (Figure 11) produces the following fragments **a**, **b** and **c**.



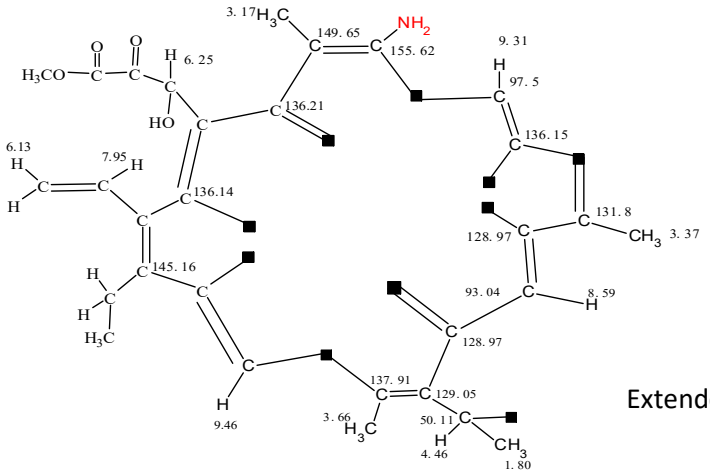
fragment **a** fragment **b** fragment **c**

These fragments could be connected due to the existence of ^{1}H-C long range signal in HMBC spectrum. It gives rise to the following fragment **d.**



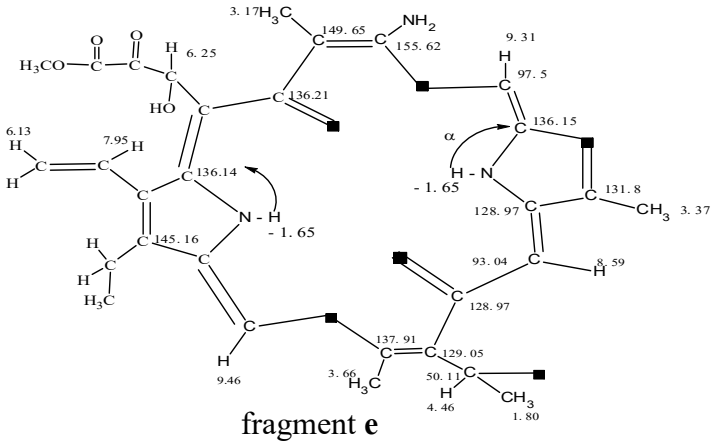
fragment **d**

According to the FT IR spectrum, this compound should consist of at least one $–NH_2$ group due to the presence of the absorption band at 3291.05 cm^{-1}. Hence, this $–NH_2$ group must be connected to the down field chemical shift of sp^2 quaternary carbon (δ 155.62 ppm) due to the logical connection. Therefore, the following extended fragment could be observed.



Extended fragment

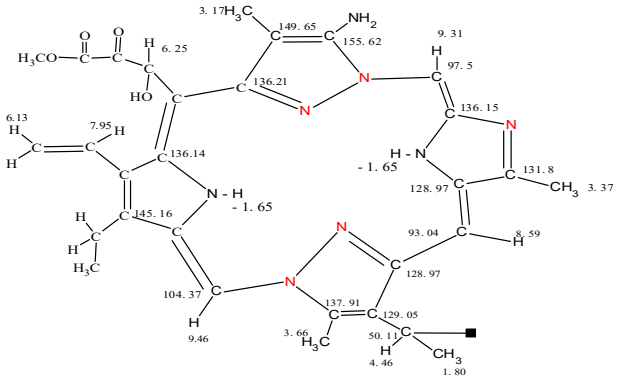
Furthermore, the existence of α ^{1}H – C long range signal between more high field chemical shift two –NH protons (δ – 1.65 ppm) and two sp^2 quaternary carbons (δ 136.14 ppm, δ 136.15 ppm) in HMBC spectrum, informs the following fragment **e**.



fragment **e**

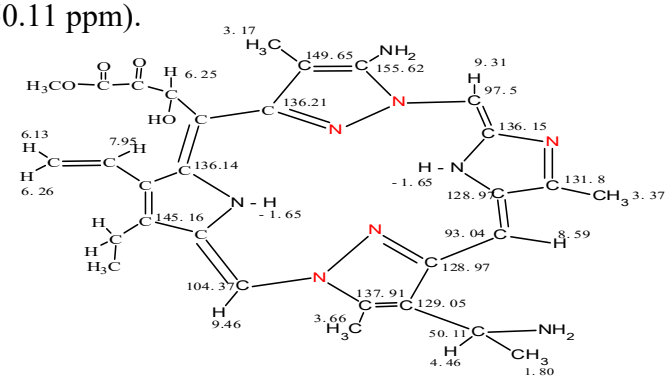
Consequently, the partial molecular formula of fragment **e** could be assigned as $C_{30}H_{33}N_3O_4$. The real molecular formula is $C_{30}H_{35}N_9O_4$. Therefore, the remaining partial molecular formula is H_2N_6. It should be one $–NH_2$ group and five N atoms.

The five trivalent nitrogen atoms are logically connected to the three sets of down field chemical shift of sp^2 quaternary carbons (δ 104.37 ppm, δ 137.91 ppm, δ 128.97 ppm), (δ 131.8 ppm, δ 136.15 ppm) and (δ 136.21 ppm, δ 155.62 ppm, δ 97.5 ppm). Therefore, the following fragment **f** is displayed.

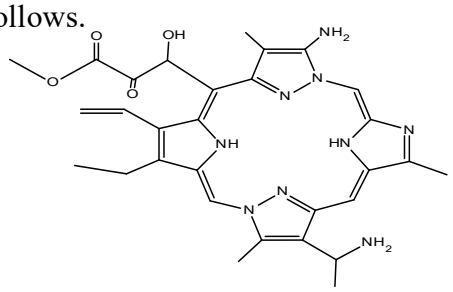


fragment **f**

Finally, the complete structure of porphyrin derivative compound could be assigned by the connection of the remaining $–NH_2$ group to the sp^3 methine carbon (δ 50.11 ppm).



The planar structure of elucidated porphyrin derivative compound could be described as follows.



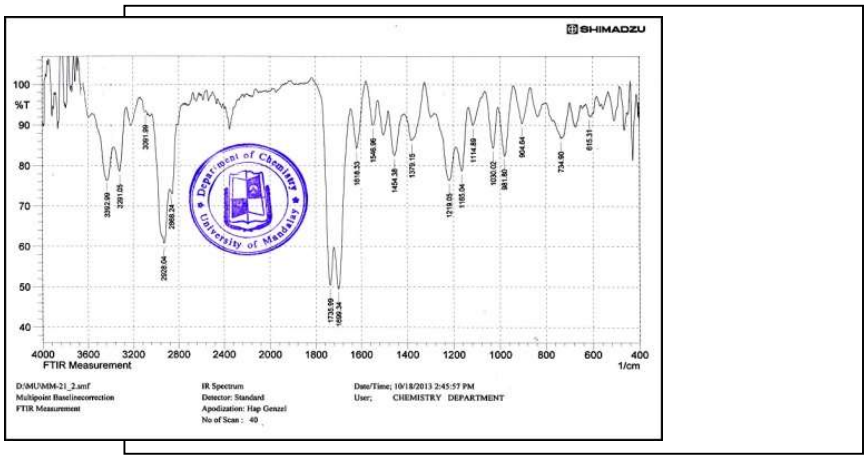


Figure 4 FT IR spectrum of isolated compound

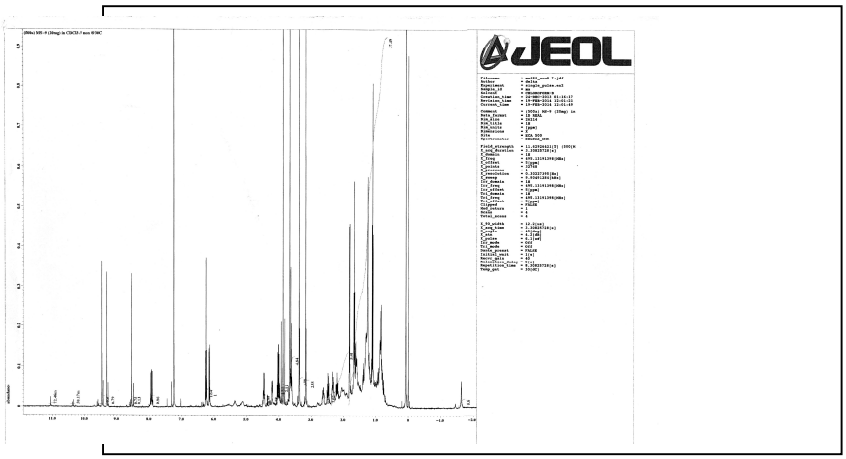


Figure 5 ^{1}H NMR spectrum of isolated compound

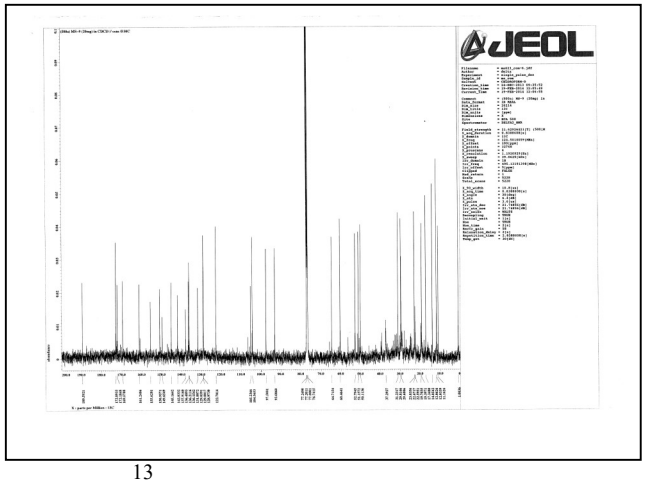


Figure 6 ^{13}C NMR spectrum of isolated compound

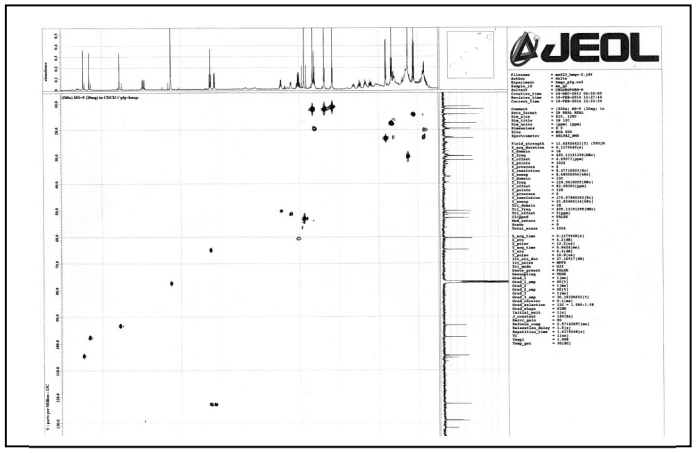
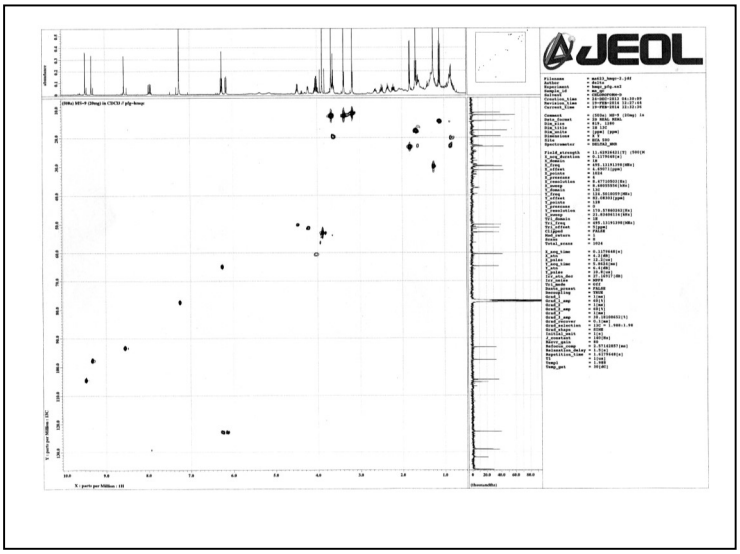


Figure 7 HMQC spectrum of isolated compound



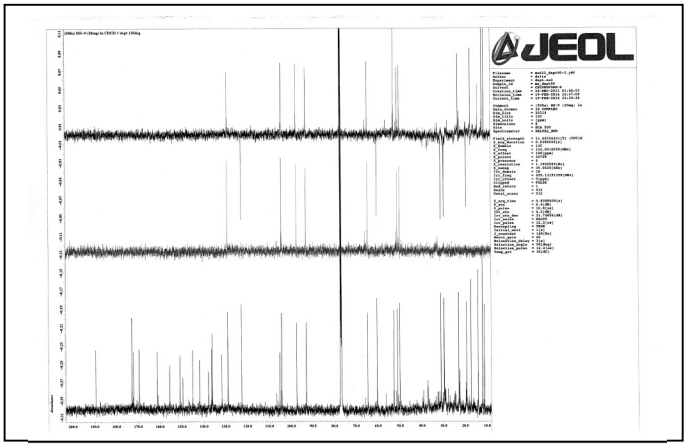


Figure 8 DEPT spectrum of isolated compound

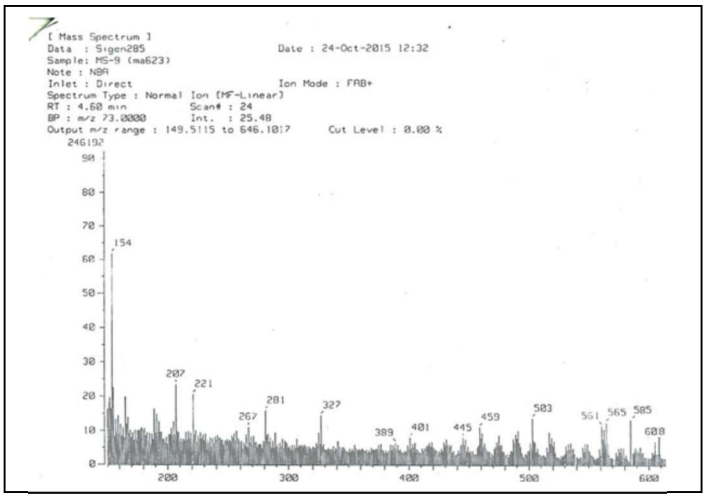


Figure 9 FAB –mass spectrum of isolated compound

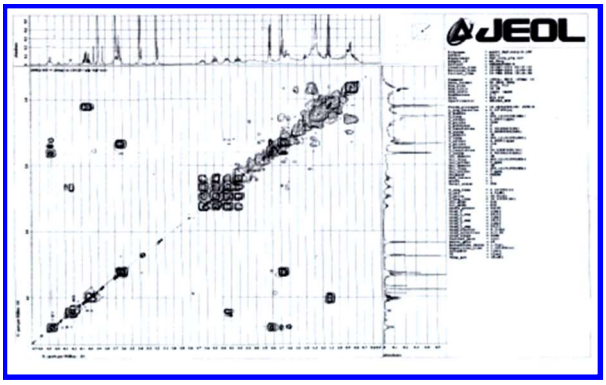


Figure 10 DQF-COSY spectrum of isolated compound

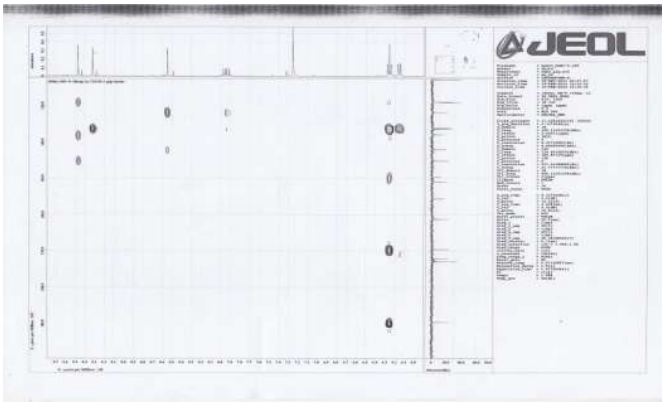


Figure 11 HMBC spectrum of isolated

Conclusion

In this research work, one of Myanmar indigenous medicinal plants, *C. lanuginosus*, locally known as Pan ma o` was selected for preliminary phytochemical screening, antimicrobial activities and isolation of organic compound were carried out. The research systematically focused on the complete structure elucidation of porphyrin derivative compound.

The preliminary phytochemical screening of the whole plant of *C. lanuginosus* was done by usual method which responded a variety of constituents, such as alkaloid, steroid, terpenoid, glycoside, lipophilic, polyphenol, saponin, phenolic and tannin, respectively. Moreover, antimicrobial activities of crude extracts in four solvent systems were tested by agar well diffusion method on six selected organisms. Among four solvents systems, the ethyl acetate extract responded high activity on all selected organisms. In addition, the antimicrobial activities of isolated compound were rechecked by agar well diffusion method on the same microorganisms. The ethyl acetate extract of isolated compound showed high antimicrobial activities on all selected organisms except *Staphylococcus aureus*.

The molecular formula of this isolated compound could be determined as $C_{30}H_{35}N_{92}O_4$. by using some sophisticated spectroscopic methods such as FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and FAB-mass spectral data. The complete structure of this isolated compound was elucidated by DQF-COSY, HMQC and HMBC spectral data. The IUPAC name of this compound is methyl-3-((1E, 4Z, 6Z, 10Z, 14E, 16E)-18-amino-7-(1-aminoethyl)-12-ethyl-3, 8,17-trimethyl-13-vinyl-2,9,19-triazaporphyrin-15-yl)-3-hydroxy-2-oxopropanoate.

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