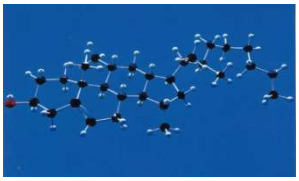
STRUCTURE ELUCIDATION OF BIOACTIVE ORGANIC COMPOUND ISOLATED FROM MYANMAR INDIGENOUS MEDICINAL PLANT, *Vitis latifolia* Roxb.

Khin San Win[1], Thinn Myat Nwe[2], Myint Myint Sein[3]

Abstract

In this research, *Vitis latifolia* Roxb., which is one of the Myanmar indigenous medicinal plants known as Chin taung mwe soke was selected for chemical analysis. Firstly, preliminary phytochemical screening of the tuber of Chin taung mwe soke was carried out, which indicated the presence of alkaloid, flavonoid, glycoside, phenol, polyphenol, sugar, saponin, sterol and terpene. The antimicrobial activity of crude extracts in various solvent systems of the tuber of Chin taung mwe soke was determined by agar well diffusion method on six selected organisms. Furthermore, a pure compound was isolated from the tuber of Chin taung mwe soke as needle shape crystal by Thin Layer and Column Chromatographic methods. The yield percent of this compound was found to be 0.61 % (21 mg) based upon the ethyl acetate crude extract and the melting point was (198-199°C). This pure compound gave positive for sterol test. Moreover, antimicrobial activity of this pure compound was rechecked by using agar well diffusion method. In addition, the molecular formula of pure compound could be determined as applying some spectroscopic methods such as FT IR, ^{1}H NMR, ^{13}C NMR and EI mass spectral data. The complete structure of steriodal derivative compound was elucidated by DQF-COSY, HMQC, HMBC and DEPT spectroscopic method. Finally, the conformational analysis of an organic compound was carried out by using ^{1}H NMR splitting patterns, coupling constant, NOESY spectral data and model studies. The structure of the isolated compound was elucidated as 10, 11, 15-trimethyl-17-(3-methylnona-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta [a] phenanthren-3-ol.



Keywords : *Vitis latifolia* Roxb., antimicrobial activity, agar well diffusion method, spectroscopic techniques

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Introduction

Medicinal plants are integral part of nature. Plants contain natural substances that can promote health. Plants produce a diverse range of bioactive molecules making them rich source of different types of medicine. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products. A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs.

Myanmar is rich in varieties of medicinal plants. Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agent as well as important raw materials for the manufacture of traditional and modern medicine. Natural products are rich source of bioactive compounds and play an important role in the development of new drugs (Sofowara, 1982)

One Myanmar indigenous medicinal plant, *Vitis latifolia* Roxb. is widely distributed in Pyin Oo Lwin Township, Mandalay Region. According to Myanmar traditional medicine, it is medicinally used as tuberculosis, ulcer, breast cancer, bone fractures, antidote for snake poison (Ah Shin Nagathein, 1983).

Vitis latifolia Roxb may be a great natural source for the development of new drugs and may provide a cost-effective mean of treating cancers and other diseases in the developing world. So, *Vitis latifolia* Roxb. was selected for isolation of compound and its structure elucidation. The aim of the present research work is to elucidate the structure of bioactive pure organic compound isolated from the tuber of *Vitis latifotlia* Roxb.

Botanical Description

Botanical name - *Vitis latifolia* Roxb.
Family - Vitaceae
English name - Wild Grape
Myanmar name - Chin taung mwe soke
Habit - a large woody climber
Parts used - Tuber

Figure 1. The tuber of V. *latifolia*

Medicinal Uses

The tuber of V. *latifolia* is used to cure, tuberculosis, ulcers, breast cancer, bone fractures and antidote for snake poison. Root powder is boiled with mustard oil and used for massage in rheumatic pain. Infusion of the whole plant is taken in liver and renal complaints. Juice of crushed root is taken orally to stop excess urination mixed with blood.

Materials And Methods

Instrument

UV-lamp, FT IR Spectrophotometer, ^{1}H NMR Spectrophotometer (500 MHz), ^{13}C NMR Spectrophotometer (125 MHz) and EI-mass Spectrophotometer.

Materials

Commercial grade reagents and solvents were used after distillation. Analytical preparative thin layer chromatography was performed by using precoated silica gel (Merck Co. Inc, Kiesel gel 60 F_{254}). Silica gel (Merck 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.

Collection and Preparation of Sample

Chin taung mwe soke, one of the effective medicinal plants was collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar. The tuber of sample was cut into small pieces and allowed to air-dry for one month. The cut-dried pieces were stored in a well-stoppered bottle and used throughout the experiment.

Preliminary Phytochemical Screening of the Tuber of V. *latifolia*

Phytochemical screening on the tuber of V. *latifolia* was performed in order to know the presence of general classes of phytochemical constituents in the plant sample. (Harborne, 1984) The results are shown in Table (1).

Determination of Antimicrobial Activity on the Tuber of V. *latifolia*

Antimicrobial activity of the crude extract of the tuber of V. *latifolia* were tested in various solvent (n-hexane, ethyl acetate and ethanol) by using agar well diffusion method on six selected organisms at PFRD (Pharmaceutical and Food Research Department), Ministry of Industry, Yangon.

Extraction and Isolation of Pure Compound

Air dried sample (700 g) was percolated with ethanol (3 L) for about two months. The ethanol extract was filtered and concentrated. It was extracted with ethyl acetate (125 mL) and evaporated. The ethyl acetate crude sample (3.42 g) was obtained. It was fractionated by column chromatography over silica gel (70-230 mesh) eluting with various volume ratios of n-hexane and ethyl acetate from non-polar to polar. Totally 191 fractions were obtained. Each fraction was checked by TLC and combined the fractions with same R_f value. Finally, nine combined fractions were obtained.

The combined fraction (C) gave only one spot on TLC. The R_f value is 0.42 (7 : 3 v/v, n-hexane : EtOAc). Then this fraction was purified by recrystallization with n-hexane and ethyl acetate solution (4 : 1 v/v, n-hexane : EtOAc). After recrystallization, needle shape crystal (21 mg) was obtained. The yield percent of this pure compound was found to be 0.61 % based upon the ethyl acetate crude extract.

Determination of Melting Point of Pure Compound

The compound was inserted into the capillary tube and melting point was determined by using SMP 30 ADV melting point apparatus (UK) at Department of Chemistry, University of Mandalay.

Determination of Antimicrobial Activity of Pure Compound

Antimicrobial activity of pure compound was tested by using agar well diffusion method on six selected organisms at PFRD (Pharmaceutical and Food Research Department), Ministry of Industry, Yangon.

Results and Discussion

Preliminary Phytochemical Screening of the Tuber of V. *latifolia*

According to the results of phytochemical screening, the tuber of V. *latifolia* contained alkaloid, flavonoid, glycoside, phenol, polyphenol, sugar, saponin, sterol and terpene. (Table 1)

Table 1. Results of Phytochemical Screening of the Tuber of V. *latifolia*

No.	Constituents	Reagent used	Observation	Results
1	Alkaloid	Wagner's reagent	Brown ppt	+
2	Flavonoid	EtOH, Conc: HCl, Mg turning	Pink colour solution	+
3	Glycoside	10 % lead acetate	Yellow ppt	+
4	Phenol	10 % $FeCl_3$ solution	Brown colour solution	+
5	Polyphenol	EtOH, 1 % $FeCl_3$, K_3 [$Fe(CN)_6$]	Greenish blue colour solution	+
6	Sugar	Benedict's solution	Brick red ppt	+
7	Saponin	EtOH, Conc: H_2SO_4	Frothing	+
8	Sterol	Petether, acetic anhydride, Conc: H_2SO_4, $CHCl_3$	Greenish blue colour solution	+
9	Terpene	EtOH, acetic anhydride Conc: H_2SO_4, $CHCl_3$	Reddish brown colour solution	+

(+) = presence

(–) = absence

Antimicrobial Activity of the Tuber of V. *latifolia*

As the results of activity tests, the ethyl acetate crude extract of the tuber of V. *latifolia* responded to high activity on all selected organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli*. Ethanol extract responded high activity on *Candida albicans* and medium activity on *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus* and *E. coli*. (Table 2)

Table 2. Results of Antimicrobial Activity of Tuber of V. *latifolia*

	Exliucli	Inhibition Zone Diameters (mm) of Different Exliucli against Six Microorganisms					
		I	II	III	IV	V	VI
	n-hexane	–	–	–	–	–	–
	EtOAc	28 (+++)	25 (+++)	25 (+++)	27 (+++)	26 (+++)	25 (+++)
	EtOH	14 (+)	18 (++)	18 (++)	19 (++)	20 (+++)	18 (++)
Control	n-hexane	–	–	–	–	–	–
	EtOAc	–	–	–	–	–	–
	EtOH	–	–	–	–	–	–

Agar well~10 mm
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*

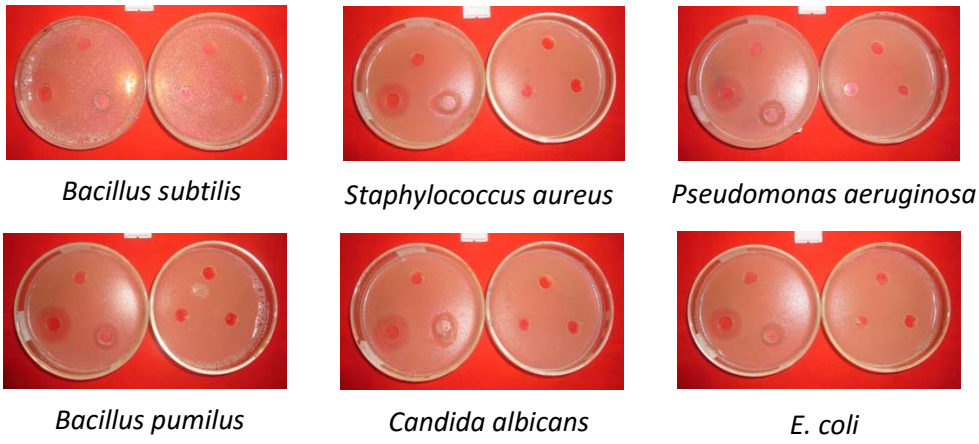


Figure 2. Antimicrobial activity of tuber of V. *latifolia* against six tested Microorganisms

Antimicrobial Activity of Pure Compound

The results in Table 3 and Figure 3 informed that the pure compound responded medium activity on *Candida albicans* and low activity on *Bacillus subtilis, Pseudomonas aeruginosa, Bacillus pumilus* and *E. coli*.

Table 3. Antimicrobial Activity of Pure Compound

Sample	Exliucli	Inhibition Zone Diameters (mm) of Pure Compound against Six Microorganisms					
		I	II	III	IV	V	VI
Compound	EtOH	12 (+)	–	13 (+)	14 (+)	19 (++)	11 (+)
Control	EtOH	–	–	–	–	–	–

Agar-well ~ 10 mm
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*

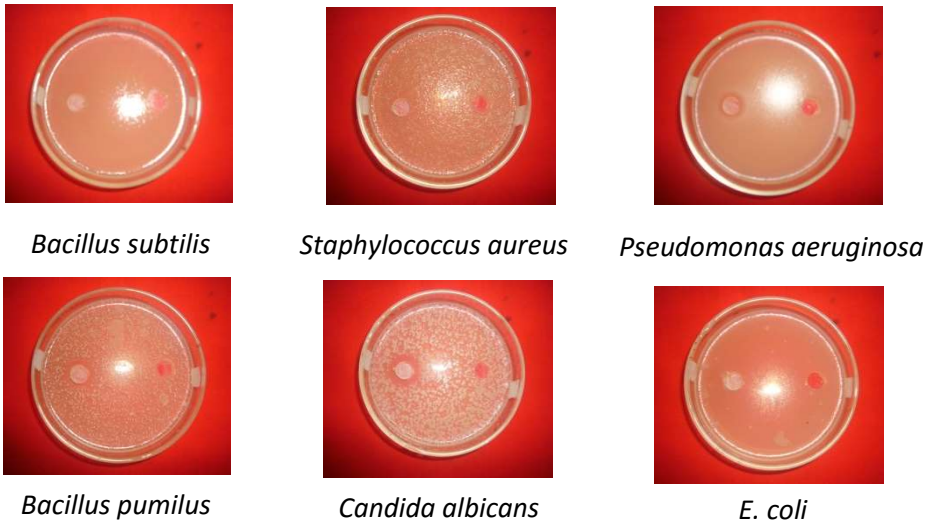


Figure 3. Antimicrobial activity of pure compound against six tested microorganisms

Molecular Formula Determination of Pure Compound

In Figure 4, the FT IR spectrum of pure compound informed that alcohol group (3409.9 cm–^{1}), sp^{2} hydrocarbon (3024.5 cm^{-1}), sp^{3} hydrocarbon (2931.6 cm^{-1}, 2858.3 cm^{-1}), alkenic group (1643.2 cm^{-1}), allylic hydrocarbon (1461.9 cm^{-1}) and methyl group (1373.2 cm^{-1}) could be assigned (Silverstein, Webster and Kiemle, 2005).

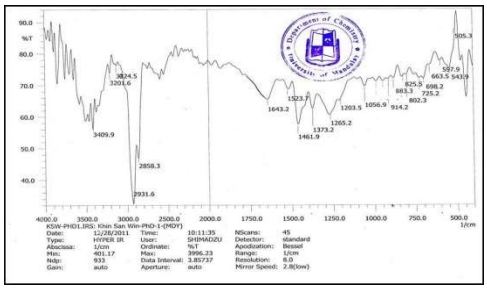


Figure 4. FT IR spectrum of pure compound isolated from V. *latifolia*

The ^{1}H NMR spectrum represents the chemical shift, splitting pattern and J value of the protons. According to the spectrum as shown in Figure 5, the isolated compound contained 51 protons (John, 2003).

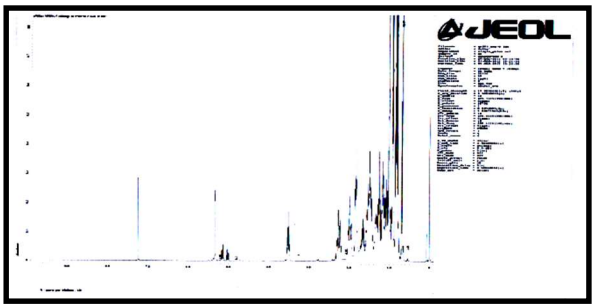


Figure 5. ^{1}H NMR spectrum of pure compound isolated from V. *latifolia*

The ^{13}C NMR spectrum in Figure 6 represents the totally 30 carbons in this compound (Le Roy and William, 1972).

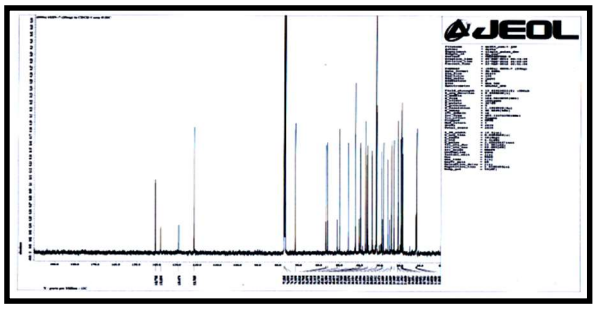


Figure 6. ^{13}C NMR spectrum of pure compound isolated from V. *latifolia*

The DEPT spectrum (Figure 7) confirms the number and kinds of carbon as well as protons (Silverstein, Webster and Kiemle, 2005).

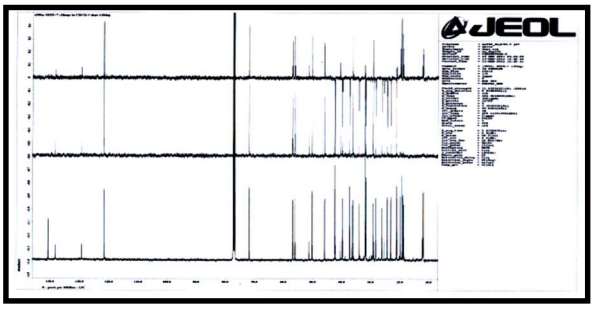


Figure 7. DEPT spectrum of pure compound isolated from V. *latifolia*

HMQC spectrum in Figure 8 of compound indicates the proton carbon direct correlation.

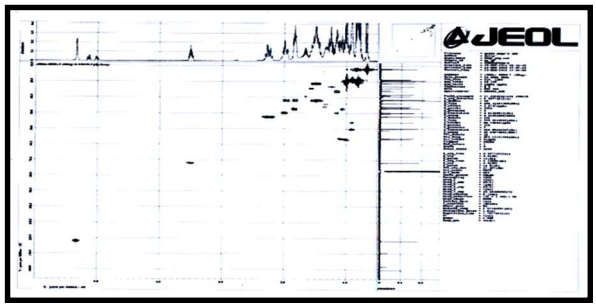


Figure 8. HMQC spectrum of pure compound isolated from V. *latifolia*

In EI-mass spectrum (Figure 9) of this pure compound, the molecular ion peak was m/z 428 which indicated the molecular mass of compound (Silverstein, Webster and Kiemle, 2005, Porter and Baldas, 1971).

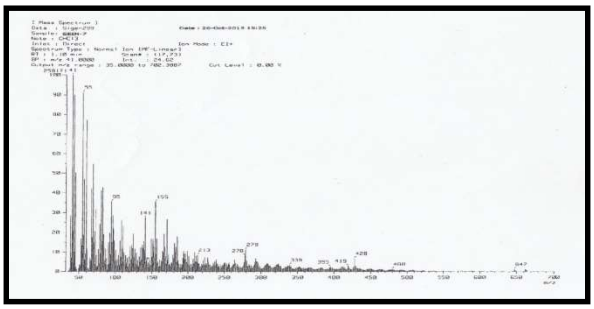


Figure 9. EI-Mass spectrum of pure compound isolated from V. *latifolia*

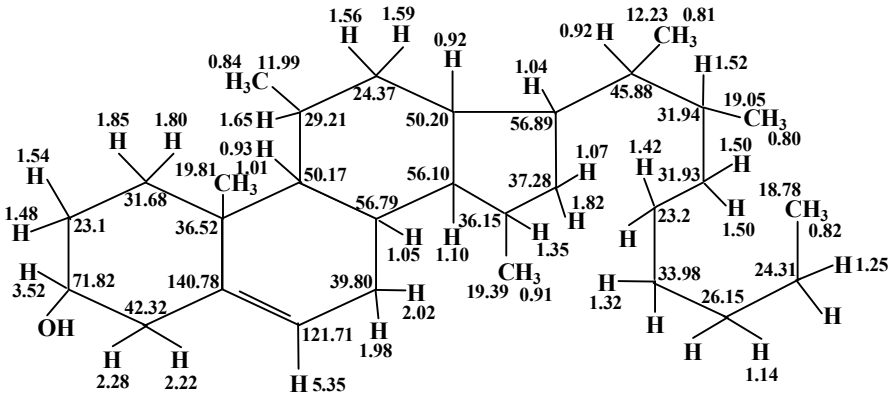
According to above spectrum, the molecular formula of pure compound could be assigned as $C_{30}H_{52}O$.

$$\text{Hydrogen deficiency index, HDI} = 30 - \frac{52}{2} + 1$$

$$= 5$$

Structure Elucidation of Pure Organic Compound

The structure of pure compound was elucidated by applying FT-IR, ^{1}H NNR, ^{13}C NMR, DEPT, DQF-COSY, HMBC, HMQC and EI-mass spectral data. According to these data, the following complete structure could be elucidated as 10, 11, 15-trimethyl-17-(3-methylnona-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta [a] phenanthren-3-ol.



This structure was confirmed by EI-mass fragmentation behaviour. Calculated HDI 5 agreed with that values from the structure of this compound.

Conformational Analysis of Pure Compound

Conformational analysis of this compound was assigned by splitting patterns, J-values of some prominent protons in ^{1}H NMR, NOESY spectral data and model studies. (Figure 10)

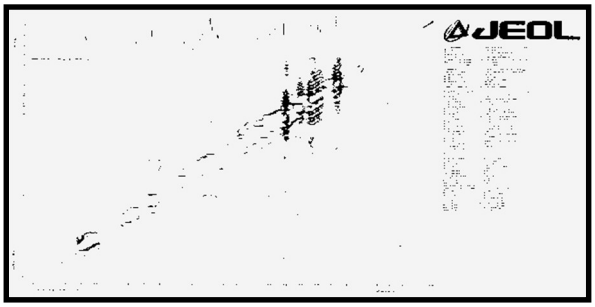
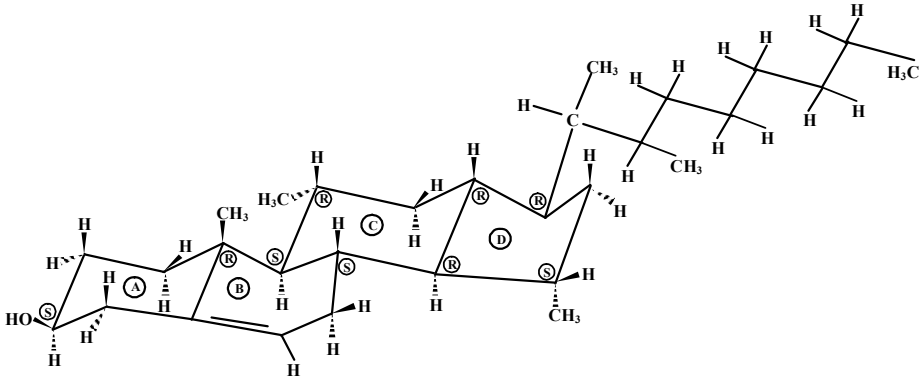


Figure 10. NOESY spectrum of pure compound isolated from V. *latifolia*

Conclusion

In this paper, the tuber of V. *latifolia* was selected for phytochemical screening, antimicrobial activity, isolation of organic compound and structure elucidation. Phytochemical screening of tuber of V. *latifolia* indicated the presence of alkaloid, flavonoid, glycoside, phenol, polyphenol, sugar, saponin, sterol and terpene. The yield percent of pure compound was found to be 0.61 % (21 mg) based upon the ethyl acetate crude extract and its melting point could be measured at (198-199°C). The ethyl acetate extract of the tuber of V. latifolia showed high potent activity (25-28mm) against six tested microorganisms. The antimicrobial activity of pure compound showed medium activity on *Candida albicans* and low activity on *Bacillus subtilis, Pseudomonas aeruginosa, Bacillus pumilus* and *E. coli*. The molecular formula as $C_{30}H_{52}O$ and the structure of this compound were determined by FTIR, ^{1}H NMR, ^{13}C NMR, DEPT, DQF-COSY, HMBC, HMQC and EI-mass spectral data. According to splitting patterns, coupling constant, NOESY spectral data and model studies, ring A and ring B were chair like and boat like conformers and ring C and ring D were chair and envelope conformers. Consequently, the absolute configuration of nine chiral carbons could be determined as C_3(S), C_8(S), C_9(S), C_{10}(R), C_{11}(R), C_{13}(R), C_{14}(R), C_{15}(S) and C_{17}(R) respectively. The isolated pure compound was elucidated as 10, 11, 15-trimethyl-17-(3-methylnona-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta [a] phenanthren-3-ol.



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References

Ah Shin Nagathein, (1983). *Pon Pya Say Abidan*, Yangon. 3rd Edition, Mingala Printing Press.

Harborne, J.B. (1984). *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*. New York. 2nd Edition. Chapman and Hall Ltd.

John, N.H. (2003). *Nuclear Magnetic Resonance Spectroscopy*, New Jersey. Pearson Education Inc., Upper Saddle River.

LeRoy F.J. and William, C.J. (1972). *Carbon 13 NMR Spectra*: A Collection of Assigned, Coded, and Indexed Spectra. A Wiley Interscience Publication.

Porter, Q.N. and Baldas, J. (1971). *Mass Spectrometry of Heterocyclic Compound*, New York. Wiley-Inter Science, A Division of John Wiley & Sons, Inc.

Silverstein, R.M., Webster, F.X. and Kiemle, D.J. (2005). *Spectrometric Identification of Organic Compound*, New York. 7th Edition, John Wiley & Sons, Inc.

Sofowara, E.A. (1982). *Medicinal Plant and Traditional Medicine in Africa*. Nigeria: John Wiley and Sons, Ltd.

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