# Chemical Constituents Of The Bulbs Of *ScadoxusMultiflorus*Mart. And Their Biological Activities

Hnin Yu Win[1], Theint Theint San[2], Kay Thi Moh Moh Win[3], Hartmut Laatsch[4]

## Abstract

Chemical constituents of the bulbs of *Scadoxusmultiflorus* Mart., locally known as ball-lone-ga-mone,belonging to the family Amaryllidaceae were investigated. Two new (1-2) and one known (3) dihydrochalcone derivatives namely 4'-methoxy-2-hydroxy-4-methoxy-dihydrochalcone (1), 4'-methoxy-2-hydroxy-4,6-dimethoxy-dihydrochalcone (2) and loureirin A (3) from dichloromethane extracts and flavonoid derivative, (2S)-4'-hydroxy-7-methoxy-flavan (4) and antitumor alkaloid narciclasine (5) from methanol extracts of the bulbs of *Scadoxusmultiflorus* Mart. were isolated by using normal phase chromatography such as silica gel, size exclusion chromatography such as Sephadex LH-20 and preparative thin layer chromatography. The structures of isolated compounds were assigned on the basis of NMR and mass studies. Moreover, the biological activities of isolated compounds were evaluated.

**Keyword:**chromatography,dihydrochalcone, alkaloid

## Introduction

Traditional knowledge on plants and biodiversity still plays an important role in health care, culture, religion, food security, environment and sustainable development in many parts of the world. Many widely used plant-based medicines are derived from traditional knowledge (DeFilipps&Krupnick., 2018). The majority of the population in Myanmar widely practiced traditional medicines either as an alternate or as a supplement to modern medicine (Swe& Win., 2005).As part of the ongoing studies of bioactive compounds from Myanmar medicinal plants, the chemical constituents of *Scadoxusmultiflorus* Mart.,were investigated. Blood Lily (*Scadoxusmultiflorus* Mart.), belongs to the family Amaryllidaceae is a perennial herb, growing from a large bulb. In Myanmar, it is widely used to treat dropsy, scabies and wounds.

From dichloromethane extracts, two new dihydrochalcone derivatives, namely 4'-methoxy-2-hydroxy-4-methoxy-dihydrochalcone (1) and 4'-methoxy-2-hydroxy-4,6-dimethoxy-dihydrochalcone (2) together with one known dihydrochalcone derivative loureirin A (3) were isolated. From methanol extracts, (2S)-4'-hydroxy-7-methoxy-flavan (4) and antitumor alkaloid narciclasine (5) were also investigated.

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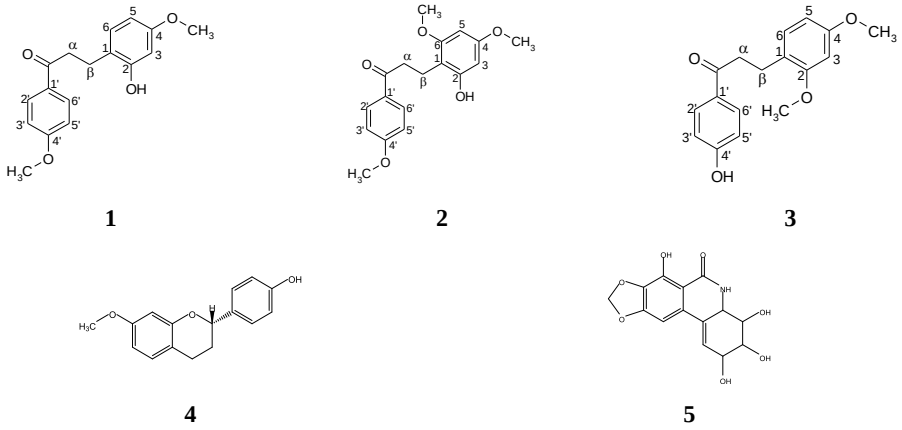


*Figure 1.Isolated compounds (1-3) from Scadoxusmultiflorus Mart.*

## Materials and Methods

### General Experimental Procedures

NMR spectra were measured on a Varian Inova 600 (599.740 MHz) and a Varian Unity 300 (300.145 MHz) spectrometer. ESIMS were measured on a Quattro Triple Quadrupol mass spectrometer with a Finnigan TSQ 7000 with nano-ESI API ion source. EIMS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosene as standard.HRESIMS were measured on a Micromass LCT mass spectrometer coupled with a HP 1100 HPLC and a diode array detector. Column chromatography was carried out on MN silica gel 60, 0.05-0.2 mm; TLC was performed on Polygram SIL G/$UV_{254}$. All silica gel materials were purchased from Macherey-Nagel, Düren, Germany.

### Spray reagents

Anisaldehyde/sulphuric acid: 1 mLanisaldehyde was added to 100 mL of a stock solution containing 85 mL methanol, 14 mL acetic acid and 1 mLsulphuric acid. After spraying, the TLC cards were heated with hot air until colour development.

### Sample Collection

Plant materials were collected from PyinOoLwin Township, Mandalay Region, Myanmar. The samples (1000 g) were cut into small pieces and dried in air for about two weeks.

### Extraction and Isolation

The metabolites from the selected medicinal plant were successively extracted with pet-ether (3.5 L), dichloromethane (3.5 L) and methanol (3.5 L) using Soxhlet apparatus. Pet-ether extracts (4.77 g), dichloromethane extracts (3.57 g) and methanol extracts (20 g) were obtained. The dichloromethane extracts showed yellow and red color spots on TLC with anisaldehyde-sulphuric acid on heating. The dichloromethane extracts were chromatographed on silica gel using stepwise gradient of dichloromethane/methanol. Fraction III afforded three components, which after purification on PTLC resulted in 4'-methoxy-2-hydroxy-4-methoxy-dihydrochalcone (1) (15 mg) and 4'-methoxy-2-hydroxy-4,6-dimethoxy-dihydrochalcone (2) (20 mg) together with one known dihydrochalcone derivative loureirin A (3)(18 mg).

The methanol extract was subjected to silica gel column using step-wise gradient of dichloromethane/methanol. The pure compound (2S)-4'-hydroxy-7-methoxy flavan (4)(10.5 mg) was isolated as pale-yellow solid from fraction II after purification again on Sephadex LH-20 column with MeOH. It shows UV absorbing band at 254 nm and stained to red colour with anisaldehyde/ sulphuric acid on heating.From fraction III, narciclasine (5) (30 mg) was isolated as white powder. It showed strong UV absorbing band at 256 nm and stained to blue with anisaldehyde/sulphuric acid on heating.

## Results and Discussion

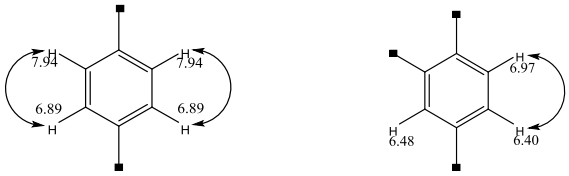
### Structure Elucidation of Isolated Compounds

The structure elucidation of isolated compoundswas determined by using 1D-NMR, different types of 2D-NMR experiments like COSY, HMQC, HMBC and mass spectrometry.

### 4'-Methoxy-2-hydroxy-4-methoxy-dihydrochalcone (1)

Compound (1) was isolated as pale yellow solid and turned to red colour with anisaldehyde-sulphuric acid on heating. In the aromatic region of $^{1}$H NMR spectrum, two doublets at $\delta$6.89 ($J$ = 9.0 Hz) and $\delta$7.94 ($J$ = 9.0 Hz) with the integration of two protons in each signal indicated the presence of 1, 4-disubstituted benzene ring. Moreover, in DQF-COSY spectrum, these two sets of chemical shift equivalence protons showed correlation as expected.

Furthermore, in the $^{1}$H NMR spectrum, doublet of doublet at $\delta$ 6.40 ($J$ = 2.6, 8.4 Hz) showed ortho coupling to the proton at $\delta$ 6.97 ($J$ = 8.4 Hz) and meta coupling to the proton at $\delta$ 6.48 ($J$ = 2.6 Hz). The pattern in the aromatic region was the ABX system of a 1,2,4-trisubstituted benzene ring. Moreover, DQF-COSY spectrum displayed the correlation between two methine protons at $\delta$ 6.40 and $\delta$ 6.97 as expected. From these observations, the substructures (A) and (B) could be drawn.




(A) (B)

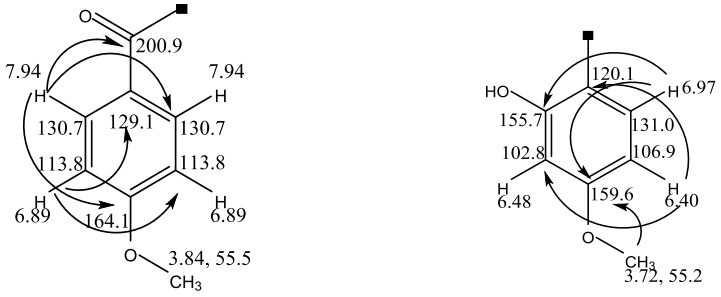
*Figure 2.(↔) DQF COSY correlations in substructures (A) and (B)*

In the aliphatic region of $^{1}$H NMR spectrum, two methoxy signals at $\delta$ 3.72 and 3.84 were observed. In addition, two double doublets at $\delta$2.93 and 3.35 with the integration of two protons were also detected.The $^{13}$C NMR spectrum displayed total of 17 carbon signals which were comprised of carbonyl of ketone, twelve $sp^2$ hybridized carbons (5 quaternary and 7 methine), two methoxy carbons and two $sp^3$ methylene carbons.

In the HMBC spectrum, one methine doublet at $\delta$7.94 ($\delta_c$130.7) from the substructure (A) showed $\beta$-correlations with the signals at $\delta$200.9 (C=O), 164.1 ($sp^2$ quaternary), 130.7 ($sp^2$methine) and $\alpha$-correlation with the methine signal at $\delta$113.8. In addition, the methine doublet at $\delta$6.89 ($\delta_c$113.8) showed $\alpha$ and $\beta$-correlations with the two $sp^2$ quaternary carbons at $\delta$164.1, 129.1 and $sp^2$methine carbon at $\delta$113.8.

Moreover, the methoxy signal at $\delta$3.84 showed correlation to the oxygenated $sp^2$ quaternary carbon at $\delta$164.1. Therefore, the partial structure (I) could be elucidated.

In the HMBC spectrum, one doublet methine proton at $\delta$ 6.97 ($\delta_c$ 131.0) showed $\beta$-correlations with the two oxygenated quaternary $sp^2$ carbons at $\delta$ 159.6 and 155.7. In addition, one doublet methine proton at $\delta$ 6.48 ($\delta_c$ 102.8) showed correlations with the two oxygenated $sp^2$quaternary carbons at $\delta$ 159.6 and 155.7, one $sp^2$quaternary carbon at $\delta$ 120.1, one $sp^2$methine carbon at $\delta$ 106.9. Moreover, the methine proton at $\delta$ 6.40 ($\delta_c$ 106.9) showed HMBC correlations to two $sp^2$ quaternary carbons at $\delta$ 159.6, 120.1 and $sp^2$methine carbon at $\delta$ 102.8. Furthermore, the methoxy signal at $\delta$3.72 showed correlation to oxygenated quaternary $sp^2$carbon at $\delta$ 159.6. According to the chemical shift, the $sp^2$ quaternary carbon at $\delta$ 155.7 could be connected to hydroxyl group. Thus, the partial structure (II) could be assigned.




Partial Structure (I) Partial Structure (II)

Figure 3. ($\rightarrow$) HMBC correlations in partial structures (I and II)

In DQF COSY spectrum, the correlations between two $CH_2$ groups at $\delta$ 2.93 and 3.35 were observed. These two $CH_2$ groups showed HMBC correlation to carbonyl of ketone at $\delta$ 200.9. One of these $CH_2$ groups at $\delta$ 2.93 showed HMBC cross signals to $\delta$120.1, 131.0 and 155.7.The high-resolution (-)-ESI mass spectrum gave a *pseudo*molecular ion peak at *m/z* 285.1126 $[M - H]^-$ corresponding to a molecular formula $C_{17}H_{18}O_4$. From all these information, 4'-methoxy-2-hydroxy-4-methoxy-dihydrochalcone (1)was derived.

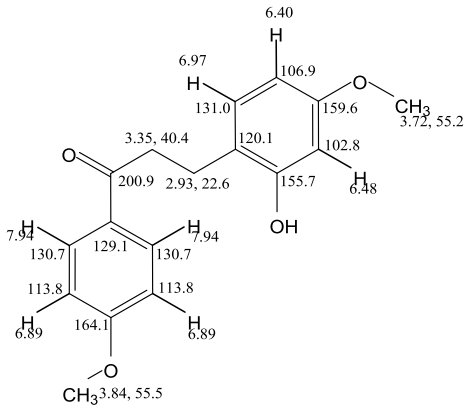



Figure 4. Selected ($\rightarrow$) HMBC correlations in compound (1)

**4'-Methoxy-2-hydroxy-4,6-dimethoxy-dihydrochalcone (2)**

The compound (2) was isolated as yellow solid. The $^1$H NMR spectrum of this compound was very similar to that of compound (1). The $^1$H NMR spectrum displayed two doublets at $\delta$6.88 ($J$ = 8.9 Hz) and $\delta$7.95 ($J$ = 8.9 Hz) with the

integration of two protons in each signal and indicated the presence of 1, 4-disubstituted benzene ring as in compound (1). Furthermore, in the upfield aromatic region of $^1$H NMR spectrum, the two doublets at $\delta$6.16 and 6.02 showed meta coupling with each other. In the aliphatic region of $^1$H NMR spectrum, three methoxy signals and two methylene signals were detected. The molecular weight of compound (2) was found to be 316 Daltons by ESI MS. The HR ESI mass spectrum gave the molecular formula $C_{18}H_{20}O_5$.

In the HMBC spectrum, the methine doublet at $\delta$7.95 ($\delta_c$130.8) showed cross signals with carbonyl of ketone at $\delta$201.8, one $sp^2$methine carbon at $\delta$130.8 and one $sp^2$ quaternary carbon at $\delta$164.0. Moreover, the methoxy signal at $\delta$3.84 showed correlation to $sp^2$ quaternary carbon at $\delta$164.0. In addition, the methine doublet at $\delta$6.88 ($\delta_c$113.7) showed HMBC correlations with $sp^2$ quaternary carbon at $\delta$129.3 and $sp^2$methine carbon at $\delta$113.7.Furthermore, in the HMBC spectrum, one $sp^2$methine doublets at $\delta$6.02 ($\delta_c$91.3) showed cross peaks with the two oxygenated $sp^2$ quaternary carbons at $\delta$158.9 and 159.8, one $sp^2$methine carbon at $\delta$94.7 and one $sp^2$ quaternary carbon at $\delta$108.8. By analysis of other HMBC correlations, the proton at $\delta$6.16 showed a coupling with the carbons at $\delta$156.4, 159.8, 108.8 and 91.3.Moreover, the two $CH_2$ groups at $\delta$3.33 and 2.92 showed HMBC correlation to carbonyl of ketone at $\delta$ 201.8. One of these $CH_2$ groups at $\delta$ 2.92 showed HMBC cross signals to $\delta$156.4 and 158.9. Based on these data, compound (2) could be elucidated.

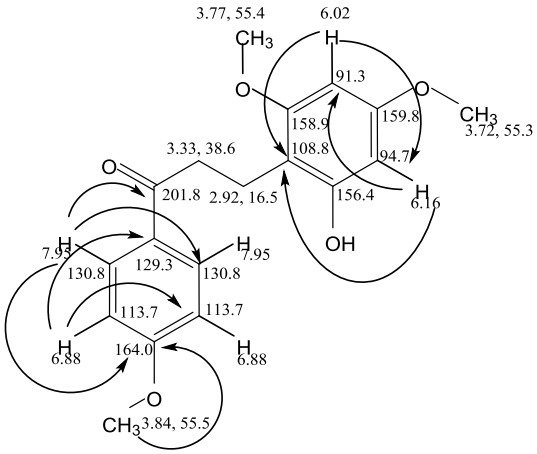



*Figure 5. Selected (→) HMBC correlations in compound (2)*

## Loureirin A (3)

Compound (3) was isolated as red oil. From HR ESI MS, the molecular formula was determined as $C_{17}H_{18}O_4$. The H,H COSY, HSQC and HMBC correlations of (3) showed similar structural feature as (1 and 2) and indicated again a dihydrochalcone skeleton. In compound (3), the hydroxyl group on C-4', two methoxy groups on C-2 and C-4 were observed. Loureirin A (3) displayed marginal antibacterial or cytotoxic activity. It was the constituent of *Dracaena loureiri*. It also shows antifungal activity (Meksuriyen, et al., 1988).

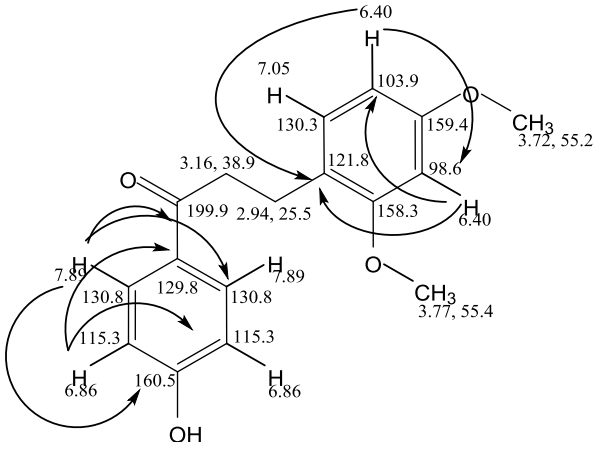



*Figure 6. (→) Selected HMBC correlations in compound (3)*

**(2S)-4'-hydroxy-7-methoxy flavan(4)**

In the aromatic region of $^1$H NMR spectrum, two doublets at $\delta$ 6.77 ($J$ = 8.6 Hz) and $\delta$ 7.22 ($J$ = 8.5 Hz) with the integration of two protons in each signal indicated the presence of 1, 4-disubstituted benzene ring. Moreover, in DQF-COSY spectrum, these two sets of chemical shift equivalence protons at $\delta$ 6.77 and $\delta$ 7.22 ppm showed correlation as expected. In HMBC spectrum, one methine doublet proton at $\delta$ 6.77 ppm showed β coupling with $sp^2$ quaternary carbon at $\delta$134.2 ppm and $sp^2$methine carbon at $\delta$ 116.1 ppm and α coupling with one quaternary carbon at $\delta$ 158.2 ppm.In addition, the proton at $\delta$7.22 ppm which is attached to carbon at $\delta$ 128.5 ppm showed β coupling with one $sp^2$ quaternary carbon at $\delta$ 158.2 ppm, one $sp^2$methine carbon at $\delta$ 128.5 ppm and αcoupling with one $sp^2$methine carbon at $\delta$116.1 ppm. According to these correlations, the carbons in the benzene ring could be assigned as shown in fragment (a).


*Figure 7. (→) HMBC correlations in fragment (a)*

Moreover, in $^1$H NMR spectrum, doublet of doublet at $\delta$ 6.41 ($J$ = 8.7, 2.6 Hz) showed ortho coupling to the proton at $\delta$ 6.93 ($J$ = 8.4 Hz) and meta coupling with the proton at $\delta$ 6.35 ($J$ = 2.6 Hz). The pattern in the aromatic region was the ABX system of a 1,2,4-trisubstituted benzene ring. Furthermore, DQF-COSY spectrum displayed the correlation between two methine protons at $\delta$ 6.41 and $\delta$ 6.93 ppm as expected and they were attached to carbons at $\delta$108.0 and $\delta$ 130.9 ppm respectively.In HMBC spectrum, one $sp^2$methine proton at $\delta$6.41 ppm from fragment (b) showed two cross peaks to one $sp^2$ quaternary carbon at $\delta$ 115.4 ppm and one $sp^2$methine carbon at $\delta$ 102.6 ppm. Furthermore, the proton at $\delta$6.93 ppm correlated to two $sp^2$ quaternary carbons at $\delta$ 160.5 and $\delta$157.3 ppm.

Moreover, one $sp^2$methine proton at $\delta$ 6.35 ppm showed α coupling with two $sp^2$ quaternary carbons at $\delta$ 160.5 and $\delta$ 157.3 ppm and β coupling with $sp^2$methine

carbon at $\delta$108.0 ppm and $sp^2$ quaternary carbon at $\delta$ 115.4 ppm. From these data, the carbon in the benzene ring could be assigned as shown in fragment (b).

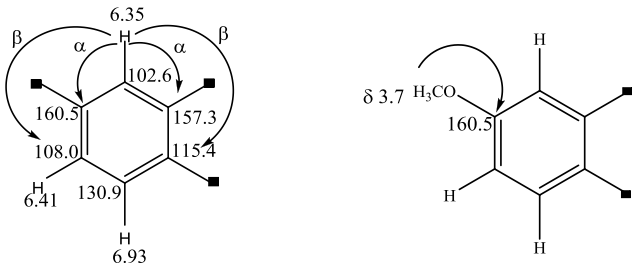



Figure 8. (→) HMBC correlations infragment(b)

In HMBC spectrum, the observation of β-coupling between singlet methoxy protons at $\delta$ 3.71 ppm and aromatic $sp^2$ quaternary carbon at $\delta$ 160.5 ppm. In addition, $^1$H NMR spectrum displayed one doublet at $\delta$4.92 ppm which is probably attached to oxygen and two sets of diastereotopic methylene protons at $\delta$ 2.86 and 2.67 ($\delta_C$25.5) and $\delta$2.09 and 1.99 ppm ($\delta_C$ 31.2).In the aliphatic region of $^{13}$C NMR spectrum, one oxygenated methine carbon at $\delta$ 79.1 ppm and two methylene signals at $\delta$ 31.2 and $\delta$ 25.5 ppm were detected.

Furthermore, in DQF-COSY spectrum, two diastereotopic methylene protons at $\delta$ 2.67 and $\delta$ 2.86 ppm showed correlation with another diastereotopic methylene protons at $\delta$ 1.99 and $\delta$ 2.09 ppm, which in turn showed correlation with one oxygenated methine proton at $\delta$ 4.92 ppm. According to these correlations, the fragment (c) could be drawn.

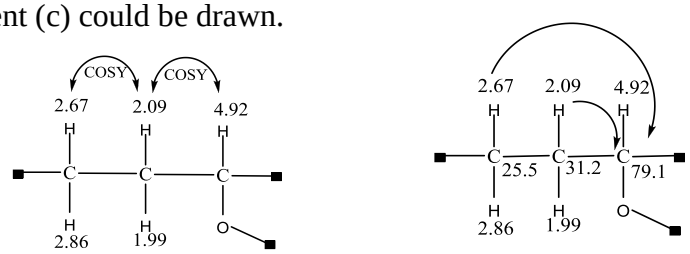
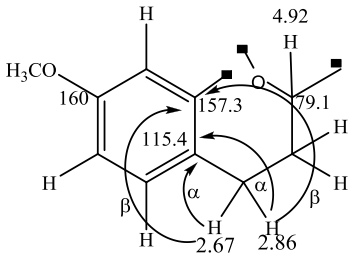


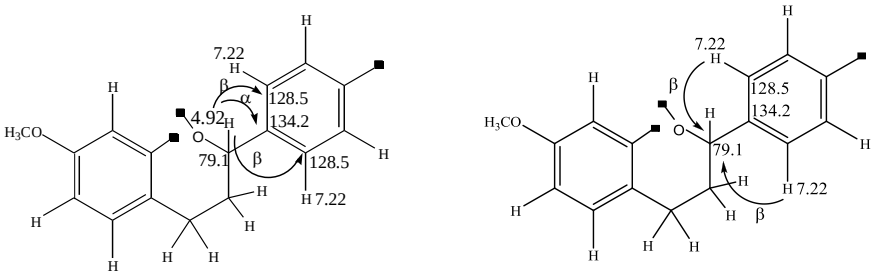

*Figure 9. (↔) H-H COSY and (→) HMBC correlations in fragment (c)*

Furthermore, two sets of diastereotopicmetylene protons at$\delta$ 2.67, 2.86, 2.09, 1.99 ppm showed HMBC correlation to one oxygenated methine carbon at $\delta$79.1 ppm. These correlations confirmed fragment (c).Fragment (b) and (c) could be connected by further HMBC correlation between $sp^3$ methylene protons at $\delta$ 2.67 ppm and 2.86 ppm with $sp^2$ quaternary carbons at $\delta$ 115.4 ppm and $\delta$ 157.3 ppm and these correlation indicated the fragment (d).




Fragment (d)

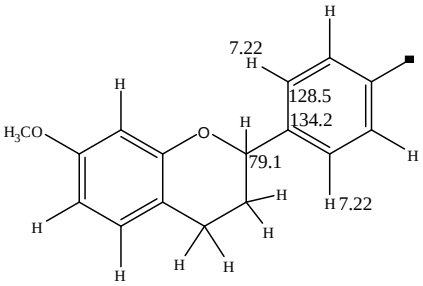
Furthermore, the oxygenated methine proton at $\delta$ 4.92 ppm showed α coupling with one $sp^2$ quaternary carbon at $\delta$ 134.2 ppm and β coupling with two $sp^2$methine carbons at 128.5 ppm from fragment (a). According to these correlations, fragments (a) and (d) could be connected as in fragment (e).




Fragment (e)

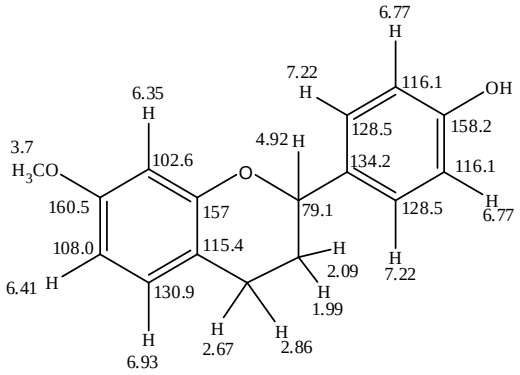
Fragment (e) could be further confirmed by the correlation between two methine protons at $\delta$ 7.22 ppm and oxygenated methine carbon at $\delta$ 79.1 ppm.The ESI mass spectrum gave the pseudo molecular ion peak at *m/z* 279 $[M + Na]^+$. Thus, the molecular mass was deduced as 256 Daltons.According to $^1H$ NMR and $^{13}C$ NMRspectroscopic data, partial molecular formula of isolated compound was assigned as $C_{16}H_{15}O_2$ (one $OCH_3$, one oxygenated methine proton). So, partial molecular mass was 239.Therefore, the remaining molecular mass was 17. According to nitrogen rule, the isolated compound must contain no nitrogen (or) even-numbered of nitrogen. Therefore, the remaining molecular mass 17 must be one oxygen atom and one hydrogen atom. Hence, the real molecular formula is $C_{16}H_{16}O_3$ and hydrogen deficiency index was 9.

According to fragment (e), the hydrogen deficiency index for two benzene ring is 8 and the remaining hydrogen deficiency index is 1. For remaining hydrogen deficiency index, the middle ring could be closed as in partial structure I, which is agreement with the down field chemical shift of $sp^2$ quaternary carbon at $\delta$ 157.2 ppm.

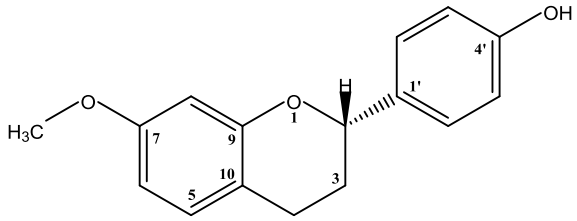



Partial Structure I

In this stage, the elucidated partial molecular formula is $C_{16}H_{15}O_2$. Hence, the remaining partial molecular formula is OH.The remaining hydroxyl group attached to down field chemical shift carbon at $\delta$ 158.2 established the following structure. So the complete structure of compound (4) could be elucidated.




The value of $[\alpha]_D$ is – 20.81 ˚C ($c$ = 0.865). According to the value of optical rotation, the isolated compound was elucidated as (2S)-4'-hydroxy 7-methoxy flavan.



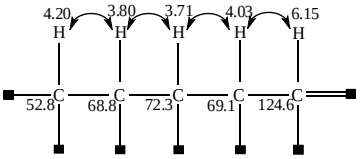

Narciclasine (5)

According to the $^1$H NMR and HMQC spectra, one chelating OH signal at $\delta$ 13.22 ppm and OH or NH signal at $\delta$ 7.82 ppm, one singlet methine proton at $\delta$ 6.84 ppm were observed.Furthermore, one methine proton at $\delta$ 6.15 ppm and methylene protons at $\delta$ 6.08 ppm, two overlapped OH or NH signals at $\delta$ 5.15 ppm and one OH or NH signal at $\delta$ 4.97 ppm were observed.In the aliphatic region of $^1$H NMR spectrum, one doublet at $\delta$ 4.20 ppm, one singlet at $\delta$ 4.03 ppm, one doublet at $\delta$ 3.80 ppm and one singlet at $\delta$ 3.71 ppm with the integration of one proton in each signal were observed and probably bonded by heteroatom.

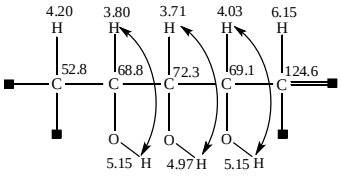
According to $^{13}$C NMR and APT spectra, total of 14 carbon signals were detected which comprised of carbonyl of acid, ester or amide at $\delta$ 168.7 ppm, five $sp^2$ quaternary carbons at $\delta$ 152.1, 144.6, 133.2, 131.9 and 129.1 ppm, one $sp^2$methine carbon at $\delta$ 124.6 ppm, another $sp^2$ quaternary carbon at $\delta$ 105.4 ppm, one methylene carbon at $\delta$ 101.9 ppm and another $sp^2$methine carbon at $\delta$ 95.7 ppm. Moreover, in the aliphatic region of $^{13}$C NMR spectrum, four $sp^3$methine carbons at $\delta$ 72.3, 69.1, 68.8 and 52.8 ppm were observed. Among them, three $sp^3$methine carbons at $\delta$ 72.3, 69.1 and 68.8 ppm were probably attached to oxygen and another $sp^3$methine carbon at $\delta$ 52.8 ppm could be connected to nitrogen.

In COSY spectrum, the methine proton at $\delta$ 3.80 ppm which is attached to carbon at $\delta$ 68.8 ppm showed correlations with another two methine protons at $\delta$ 4.20 and $\delta$ 3.71 ppm. The latter showed correlation again with methine proton at $\delta$ 4.03 ppm which in turn showed correlation with another $sp^2$methine proton at $\delta$ 6.15 ppm. Therefore, fragment (a) could be drawn.

The two overlap OH signals at $\delta$ 5.15 ppm showed correlation with the one methine proton at $\delta$ 4.03 ppm and another methine proton at $\delta$ 3.80. Another OH signal at $\delta$ 4.97 ppm showed correlation with the one methine proton at $\delta$ 3.71 ppm. According to these correlations, the three OH groups at $\delta$ 5.15 and $\delta$ 4.97 ppm could be connected to the carbons as shown in fragment (b).




Fragment (a)



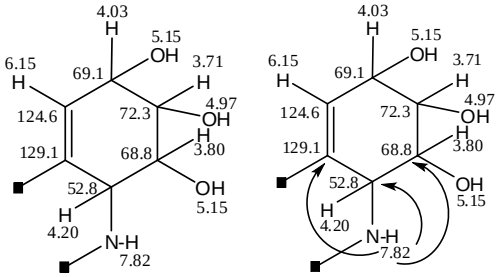

Fragment (b)

Moreover, in COSY spectrum, the methine proton at $\delta$ 4.20 ppm which is attached to carbon at $\delta$ 52.8 ppm from fragment (a) exhibited correlation with $sp^2$methine proton at $\delta$ 6.15 ppm which is attached to carbon at $\delta$ 124.6 ppm from fragment (a). From this spectroscopic data, the fragment (c) could be elucidated.


Fragment (c)

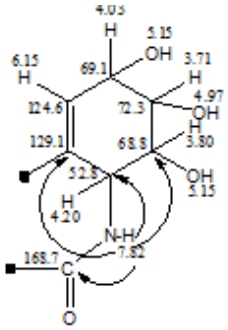
In addition, from the HMBC spectrum, the methine proton at $\delta$ 4.20 ppm ($\delta_c$ 52.8) showed three correlations with the $sp^2$ quaternary carbon at $\delta$ 129.1 ppm, the $sp^2$methine carbon at $\delta$ 124.6 ppm and $sp^3$methine carbon at $\delta$ 68.8 ppm. These spectroscopic data confirmed the fragment (c).

According to the chemical shift, the methine proton at $\delta$ 4.20 ppm from fragment (c) which is attached to carbon at $\delta$ 52.8 ppm could be connected to nitrogen. Therefore, the fragment (d) could be assigned.

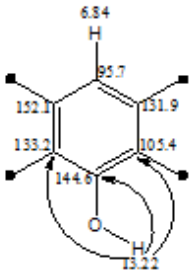



Fragment (d)

Moreover, in HMBC spectrum, NH signal at $\delta$ 7.82 ppm showed cross peaks to carbon at $\delta$ 129.1, 68.8 and 52.8 ppm. Therefore, fragment (d) could be confirmed.Furthermore, in HMBC spectrum, -NH signal showed couplings with carbonyl carbon at $\delta$ 168.7. Therefore, fragment (e) could be assigned.




Fragment (e)

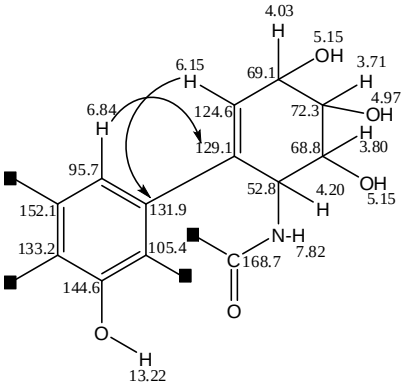



Fragment (f)

Moreover, methine proton at $\delta$ 6.84 ppm which is attached to carbon at δ 95.7 ppm showed α-couplings with two $sp^2$ quaternary carbons at $\delta$ 152.1 and 131.9 ppm, β-coupling with another three $sp^2$ quaternary carbons at $\delta$ 133.2 and 105.4 ppm.

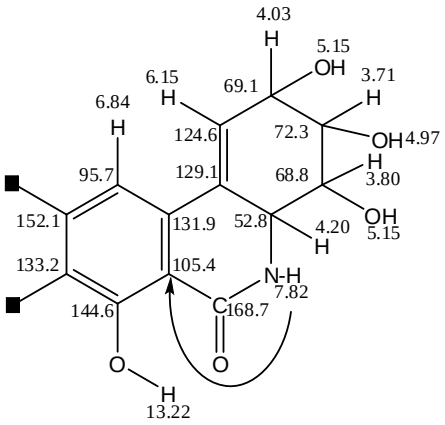
In addition, chelating OH signal at $\delta$ 13.22 ppm showed three HMBC correlations to three $sp^2$ quaternary at $\delta$ 144.6, 133.2 and 105.4 ppm.According to these correlations, the atoms in the benzene ring could be assigned as shown in fragment (f).

Moreover, in HMBC spectrum, one $sp^2$methine proton at $\delta$ 6.84 from fragment (f) showed β-coupling with another $sp^2$ quaternary carbon at $\delta$ 129.1 from fragment (d). According to this correlation fragment (g) could be elucidated.



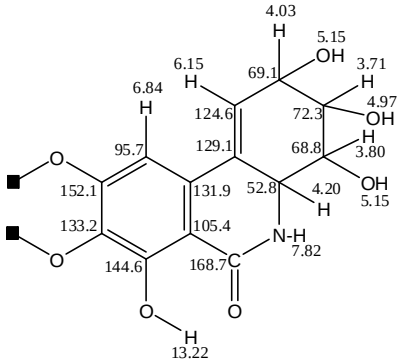

Fragment (g)

In addition, one $sp^2$methine proton at $\delta$ 6.15 which is attached to carbon at $\delta$ 124.6 showed β-coupling with one $sp^2$ quaternary carbon at $\delta$ 131.9. This correlation further confirmed fragment (g).Moreover, in HMBC spectrum, the -NH proton at $\delta$ 7.82 showed β-coupling with $sp^2$ quaternary carbon at $\delta$ 105.4. According to the correlation, the ring could be closed as shown in fragment (h).



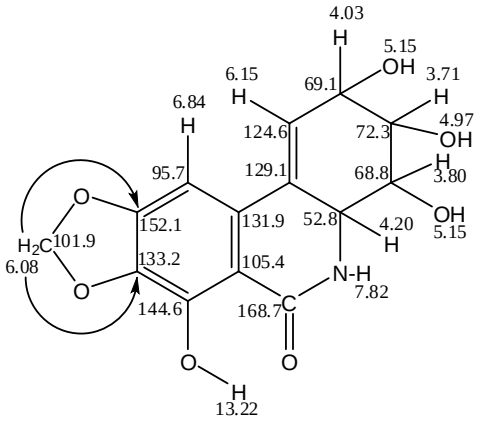

Fragment (h)

According to chemical shift, two quaternary carbons at $\delta$ 152.1 and 133.2 could be connected to oxygen. Therefore, the fragment (i) could be assigned.




Fragment (i)

Moreover, in HMBC, the methylene proton at $\delta$ 6.08 which is attached to carbon at $\delta$ 101.9 showed β-coupling with two $sp^2$ quaternary carbons at $\delta$ 152.1 and 133.2. Therefore, the complete structure of compound could be elucidated as antitumor alkaloid narciclasine.




Antimicrobial activities of pure compound (5) were tested by agar-well diffusion method on six selected organisms such as *Bacilliussubtilis*, *Staphylococcus aureus*, *Pseudomonusaeruginosa*, *Bacilliuspumilus*, *Candida albican* and *E-coli*. It showed weak activities against *Bacilliuspumalis*, *Candida albican*and *E-Coli* by causing inhibition zones of 12 mm. The antioxidant activity of compound (5) was tested by DPPH assay and $IC_{50}$ value was found to be 19.05 μg/mL.

Narciclasine, also known as lycoricidinol, is an isocarbostyril alkaloid discovered in the Amaryllidaceae family. In 1967, Gazzaniga and co-workers isolated narciclasine from Narcissus varieties and it was known to inhibit cell division. In the early 2010, narciclasine was established as an anticancer agent. In 2016, Robert Fürst from Institute of Pharmaceutical Biology at Goethe University, Frankfurt am Main, Germany reviewed the antitumor and anti-inflammatory activities of narciclasine (Fürst, 2016).

## Conclusion

In the present study, dichloromethane and methanol extracts of *Scadoxusmultiflorus* Mart. were analyzed. Two new and one known dihydrochalcone derivatives from dichloromethane extracts and antitumor alkaloid and flavonoid derivative from methanol extracts were characterized.

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