

## Isolation of Active Organic Compounds from *Phyllanthus Aibizzoides* (Kurz Hook.f.) (Shit-Shar)

and

### *Phyllanthus Maderaspatensis* L. (Taw-Zee-Phyu)

Daw Hla Ngwe<sup>1</sup>, Myint Myint Khin<sup>2</sup>, Khin Chaw Win<sup>3</sup>, Myat Noe Hlaing<sup>4</sup>, Theingi Phy<sup>5</sup>

#### Abstract

This research deals with the investigation of active organic compounds from the whole plant of Taw-zee-phyu, TZP (*Phyllanthus maderaspatensis* L.) and barks of Shit-shar, SS (*Phyllanthus albizzioides* (Kurz) Hook.f.). One compound, quercetin-3-O- $\beta$ -D- glucopyranosyl (1-4)  $\alpha$ -rhamnopyranoside from EtOAc extract of TZP and two compounds : lupeol and (*E*)-pentyl 3-3',4' dihydroxyphenyl acrylate from pet-ether extract of SS bark were isolated by solvent extraction followed by column and thin layer chromatographic separation methods. The structures of isolated compounds were elucidated and identified by modern spectroscopic techniques such as UV, FT IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, 2DNMR such as <sup>1</sup>H-<sup>1</sup>H COSY, NOESY, HSQC and HMBC, and ESI- MS. All of these isolated compounds showed the antioxidant activity in the order of : quercetin-3-O- $\beta$ -D- glucopyranosyl (1-4)  $\alpha$ - rhamnopyranoside (IC<sub>50</sub> = 2.50  $\mu$ g/mL) > lupeol (IC<sub>50</sub> = 4.97  $\mu$ g/mL) > (*E*)-pentyl 3-3',4' dihydroxyphenyl acrylate (IC<sub>50</sub> = 7.78  $\mu$ g/mL), determined by DPPH radical scavenging assay.

**Keywords:** *Phyllanthus maderaspatensis*, *Phyllanthus albizzioides*, Quercetin-3-O- $\beta$ -D- glucopyranosyl (1-4)  $\alpha$ -rhamnopyranoside, lupeol, (*E*)-pentyl 3-(3', 4' dihydroxyphenyl) acrylate, antioxidant activity

#### Introduction

In Myanmar, traditional medicine (TM) has been an important health care service throughout history, and can be regarded as a priceless national asset unique to the Myanmar people. In addition to histological evidence, scientific research has repeatedly proven its effectiveness and usefulness in health care. The most common reasons for using traditional medicine is that it is more affordable and being more closely corresponding to the Myanmar's culture and ideology. Regardless of why an individual uses it, TM undoubtedly provides an important health care service, both with and without geographic or financial access to modern medicine, and in all areas of health care, whether it is promotion, prevention, treatment, or rehabilitation (Thaw Zin *et al.*, 2007).

*Phyllanthus* has been used in traditional medicine to treat chronic liver diseases. *Phyllanthus* appears to be promising in the treatment of patients with chronic hepatitis B virus (HBV) infection. Bioactive compounds like alkaloids, tannins, flavonoids, lignans, phenols and terpenes could be found in various species of *Phyllanthus* showing the antinociceptive activity (Lakshmi *et al.*, 2012). Since the previous work focused on the investigation of some biological activities such as antimicrobial activity, antioxidant activity, brine shrimp cytotoxic effect,  $\alpha$ -glucosidase inhibitory activity and antitumor activity of two locally grown *Phyllanthus* species: Taw-zee-phyu, TZP (*Phyllanthus maderaspatensis* L.) and barks of Shit-shar, SS (*Phyllanthus albizzioides* (Kurz) Hook.f.) based on the plant extracts (Khin Hnin Mon *et al.*, 2015; Tin Shine Aung, 2015; Yadanar Oo, 2015), the present work aimed to isolate and identify some free radical scavengers from the whole plant of Taw-zee-phyu and Shit-shar resulting three secondary metabolites namely quercetin-3-O- $\beta$ -D- glucopyranosyl (1-4)  $\alpha$ -

<sup>1</sup> Professor and Head, Dr, Department of Chemistry, University of Yangon

<sup>2,3</sup> Lecturers, Dr, Department of Chemistry, University of Yangon

<sup>4,5</sup> MRes Students (2015-16), Department of Chemistry, University of Yangon

rhamnopyranoside, and lupeol and (*E*)-pentyl 3-3',4' dihydroxyphenyl acrylate possessing the antioxidant activity.

### Materials and Methods

The whole plant of Taw-zee-phyu was collected from Magwe region and the barks of Shit-shar were collected from Meikhtila Township, Mandalay Region. The taxonomists of the Department of Botany, Magwe University botanically identified and authenticated the plants. The collected samples were cleaned by washing with water and air-dried at room temperature for two weeks. The air-dried samples were ground into powder by means of a grinding mill and stored separately in air-tight containers to prevent the moisture and other contaminations. Various crude extracts were prepared by using different solvents such as pet-ether, ethyl acetate, chloroform, ethanol, methanol and water.

### Separation and Isolation of Organic Compounds from the Samples

The ethyl acetate extract (3.9 g) of the whole plant of Taw-zee-phyu was column chromatographically separated on silica gel by eluting with EtOAc : MeOH (99:1, 19:1, 15:1, 9:1 v/v) and successive fractions obtained were combined on the basis of their behaviours on TLC. After the solvents have been evaporated, five fractions F-I (f<sub>1-18</sub>), F-II (f<sub>19-30</sub>), F-III (f<sub>31-62</sub>), F-IV (f<sub>62-68</sub>) and F-V (f<sub>69-100</sub>) were obtained as a mixture. From fraction F-V (f<sub>69-100</sub>) compound **A** was directly isolated as solid material. This isolated compound **A** was purified by washing with pet-ether and ethyl acetate followed by crystallization from ethyl acetate and methanol. The compound 'A' (0.003%) was obtained as pale yellow crystal.

Pet-ether extract (4 g) of the bark of Shit-shar was separated on silica gel by successive column chromatographic separation method using PE: EtOAc (30:1, 15:1, 9:1, 7:1, 5:1, 3:1v/v) solvent systems and 115 fractions were collected. The fractions were monitored by TLC. The fractions gave the similar appearance on TLC were combined and finally six main fractions F<sub>I</sub> to F<sub>VI</sub> were collected. After removal of the solvents, fractions F<sub>III</sub> and F<sub>IV</sub> provided solid substances. Fraction F<sub>III</sub> was purified by washing with PE followed by crystallization from PE and EtOAc to give 0.006 % of compound 'B' as colourless needle shape crystal. Fraction IV was obtained as a mixture and 0.66 g of fraction IV was then column chromatographically separated on silica gel using PE : EtOAc (15:1, 9:1, 7:1, 5:1, 3:1, 1:1 v/v) solvent systems. From this separation, a total of 83 fractions were collected and these were checked by TLC. After combination of similar fractions, six main fractions (F<sub>I</sub> to F<sub>VI</sub>) were collected and fractions I, II, IV, V and VI were obtained as mixture. After removal of the solvent, fraction F<sub>III</sub> provided a solid substance. This solid material was washed with PE, EtOAc and purified by crystallization from PE and EtOAc to provide compound 'C' (0.002 %) as a pale yellow crystal.

### Characterization and Identification of Isolated Compounds

Some physical properties such as  $R_f$  values, melting points, solubility in some organic solvents as well as some chemical properties of the isolated compounds (**A**, **B** and **C**) were determined. The structures of isolated compounds were then elucidated and identified by modern spectroscopic techniques which are UV, FT IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, 2D NMR such as <sup>1</sup>H-<sup>1</sup>H COSY, NOESY, HSQC and HMBC, and ESI- MS. All of the NMR and MS spectroscopic measurements were supported by Graduate School of Agricultural Sciences, Nagoya University, Japan.

### Screening of Antioxidant Activity of Isolated Compounds by DPPH Assay

DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical scavenging assay was chosen for the antioxidant activity of isolated compounds from the selected plant materials. DPPH free radical scavenging activity was determined by UV-visible spectrophotometric method according to the procedure described by Marionova and Bachvarov (2011) at Organic Chemistry Laboratory, Department of Chemistry, University of Yangon. The control solution was prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution in different concentrations of 0.3125, 0.625, 1.25, 2.50, 5.0  $\mu\text{g/mL}$ . These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance of these solutions was measured at 517 nm by using UV-visible spectrophotometer (UV-7504, KWF, China).  $\text{IC}_{50}$  values were calculated by linear regressive excel program from a plot of % radical scavenging activity against concentrations of the isolated compounds (Ashokkumar and Ramaswamy, 2013).

### Results and Discussion

According to the preliminary phytochemicals tests, almost of the second metabolites namely alkaloids,  $\alpha$ -amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids were found to be present in TZP and SS. Although starch was present in TZP, it was not observed in SS. No cyanogenic glycoside was detected in both samples, indicating that both of the plant samples tested might be free from harmful effect.

On the basis of the screening of TLC chromatograms, there were many organic compounds composed in the samples. Among these compounds, only one compound (**A**) could be isolated from TZP and two compounds (**B** & **C**) isolated from SS by silica gel column chromatographic separation technique.

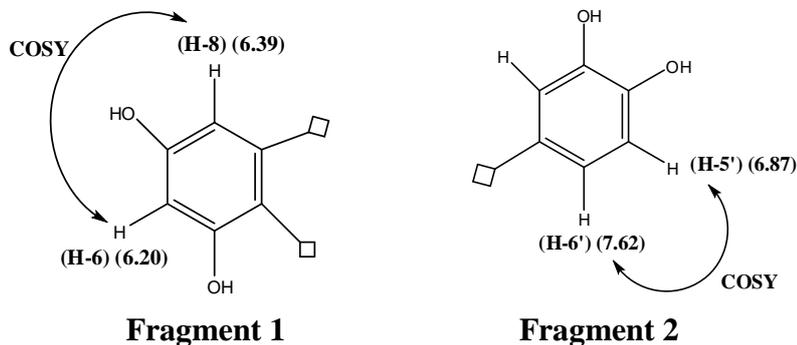
#### Structural Elucidation of Isolated Compound 'A'

Compound 'A' (0.003 % yield, m.pt.186 °C, pale yellow crystal) was isolated from EtOAc extract of the whole plant of TZP. In accordance with FT IR assignments, pure compound should consist of at least one hydroxyl group ( $\text{cm}^{-1}$ ), carbonyl group ( $\text{cm}^{-1}$ ) and ether functional group ( $\text{cm}^{-1}$ ). Since it gave white ppt with 10 % lead acetate solution test and pink colour with magnesium-conc. HCl in ethanol test, isolated compound is a flavonoid glycoside. In the  $^1\text{H}$  NMR spectrum (Figure 2), two doublets were found at  $\delta$  6.39 ppm ( $J = \text{Hz}$ , H-8) and  $\delta$  6.20 ( $J = \text{Hz}$ , H-6) indicating the presence of a tetra-substituted aromatic ring. Other doublets at  $\delta$  7.66 ( $J = \text{Hz}$ , H-2'),  $\delta$  7.62 ( $J = \text{Hz}$ , H-6') and  $\delta$  6.87 ( $J = \text{Hz}$ , H-5') revealed the tri-substituted aromatic ring. The two doublet signals at  $\delta$  5.10 ( $J = \text{Hz}$ , H-1'') and  $\delta$  4.51 ( $J = \text{Hz}$ , H-1''') indicated anomeric signal with other signals in the range of  $\delta$  1.1 ~ 3.8 for a  $\beta$  linked sugar moiety. In the  $^{13}\text{C}$  NMR spectrum (Figure 3), the downfield signal at  $\delta$  179.4 attributed a carbonyl group.

The positions of substituted groups were confirmed by  $^1\text{H}$ - $^{13}\text{C}$  correlation in HSQC (Figure 4) and HMBC (Figure 5). The correlation of H-8 ( $\delta$  6.39 ppm) to C-6 ( $\delta$  99.97 ppm) and C-10 ( $\delta$  105.6 ppm); H-6 ( $\delta$  6.20 ppm) to C-8 ( $\delta$  94.88 ppm) and C-10 ( $\delta$  105.5 ppm); H-1'' ( $\delta$  5.10) to C-3 ( $\delta$  135.6) and H-1''' ( $\delta$  4.51) to C-4'' ( $\delta$  72.1 ppm) and C-5'' ( $\delta$  78.1) revealed the substitution of hydroxyl groups at C-5, C-7, C-3 and C-4. In addition, the correlation between an anomeric proton H-1'' ( $\delta$  5.10) and C-3 ( $\delta$  135.6 ppm) indicated the position of sugar at C-3. Moreover the correlation between another anomeric proton H-1''' ( $\delta$  4.51 ppm) and C-4'' ( $\delta$  72.1 ppm) revealed the position of rhamnose sugar at C-4''.

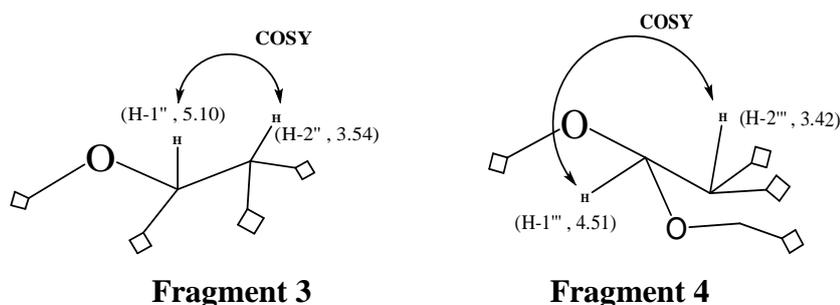
The complete structure elucidation of a pure bioactive organic compound could be done by applying DQF-COSY spectral data.

In the DQF-COSY spectrum (Figure 6), the observation of medium graphic area between the two aromatic protons ( $\delta$  6.39 ppm and  $\delta$  6.20 ppm) indicates the following Fragment 1.



Moreover, in the DQF-COSY spectrum (Figure 6), the determination of small graphic area between the two aromatic protons ( $\delta$  7.62 ppm and  $\delta$  6.87 ppm) indicates the above figure Fragment 2.

In addition, the anomeric proton at  $\delta$  5.10 ppm is coupling with the methine proton at  $\delta$  3.54 ppm, and another anomeric proton at  $\delta$  4.51 ppm is coupling with the methine proton at  $\delta$  3.42 ppm. Therefore Fragments 3 and 4 can be confirmed. On the other hand, in HMBC spectrum (Figure 5), the existence of two anomeric protons are directly attached to C-3 and C-4''.

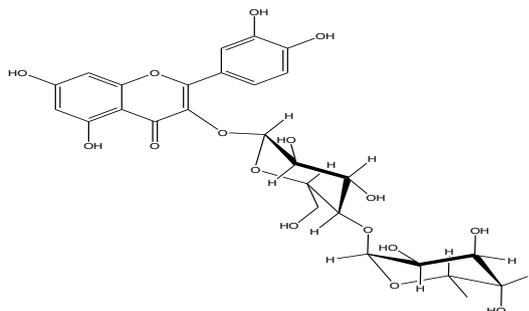


The NOESY spectrum of isolated compound 'A' is presented in Figure 7. The molecular weight of the isolated compound 'A' was observed by ESI MS ( $m/z$  610.3) calculated for  $C_{27}H_{30}O_{16}$  as depicted in Figure 8. Finally, the molecular structure of the isolated compound 'A' could be assigned as quercetin-3-O- $\beta$ -D-glucopyranosyl (1-4)  $\alpha$ -rhamnopyranoside. The resulting spectroscopic data of 'A' were also found to be identical with that of the reported data (Ni Ni Than, 2005)

### Structural Elucidation of Isolated Compound 'B'

Compound 'B' (0.006 % yield, m.pt. 215 °C, colourless needle shape,) was isolated from PE extract of bark of Shit-shar. The melting point of compound 'B' found to be 215 °C. It was soluble in ethyl acetate, chloroform, ethanol and methanol but insoluble in pet-ether and water. Its  $R_f$  value was found to be 0.45 (PE: EtOAc = 9:1 v/v) and UV inactive. According to chemical tests, it was observed that carbonyl group and phenolic OH (or) enolic OH group were absent in compound 'B'. It can also discharge 10%  $KMnO_4$  solution. These observation showed the presence of C=C bond in compound 'B'. Reaction with Liebermann Burchard reagent (glacial acetic acid & acetic anhydride) giving pink colour ( $CHCl_3$  used as solvent) and it also gave a purple spot on TLC after spraying and heating with 5 %  $H_2SO_4$  reagent

confirmed that the isolated compound 'B' was a terpenoid compound (Mya Bwin and Sein Gwan, 1973). The melting point of compound 'B' is coincident with that of melting point of lupeol (215 °C) (Merck index, 2001).



### quercetin-3-O- $\beta$ -D- glucopyranosyl (1-4) $\alpha$ - rhamnopyranoside.

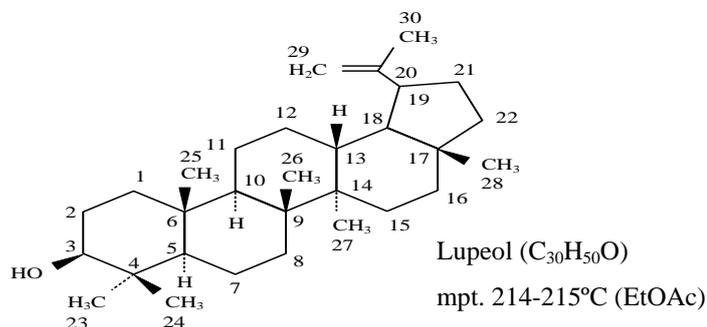
The functional group present in compound 'B' was studied by FT IR spectrum (Figure 9). The presence of OH stretching of hydroxyl group that appeared at  $3307\text{ cm}^{-1}$ . The alkenic group of  $=\text{CH}$  stretching vibration was observed at  $3068\text{ cm}^{-1}$  in weak intensity. Asymmetrical and symmetrical stretching vibration of methyl and methylene appeared at  $2917\text{ cm}^{-1}$  and  $2849\text{ cm}^{-1}$ . The  $\text{C}=\text{C}$  stretching vibration was observed at  $1639\text{ cm}^{-1}$ . The CH bending of  $\text{CH}_2$  and  $\text{CH}_3$  was assigned by absorption at  $1462\text{ cm}^{-1}$ . The CH bending of gem dimethyl group appeared at  $1379\text{ cm}^{-1}$ . The band at  $1042\text{ cm}^{-1}$  confirmed that  $-\text{C}-\text{O}-$  stretching for  $2^\circ$  alcohol ( $-\text{CHOH}$ ) in cyclic compound. The aromatic ring of CH in plane bending group was assigned by absorption at  $1014\text{ cm}^{-1}$  and the CH out of plane wagging vibration was found at  $878\text{ cm}^{-1}$ , respectively present in this compound.

From the observation of  $^1\text{H}$  NMR spectrum shown in Figure 10, it was observed that compound 'B' has 50 protons involving  $\text{sp}^3$  methylene protons appear as doublets at the chemical shift of 4.57 ppm and 4.64 ppm, the 7 sharp signals for 7 methyl groups singlet can also be clearly observed at  $\delta$  1.69, 1.08, 0.98, 0.94, 0.85, 0.78 and 0.76 ppm. The hydrogen on the ring carbon directly bonded to the hydroxyl group can also be seen as a doublet of doublet centered at  $\delta$  3.30 ppm. The rest of the protons i.e., 26 protons give signals between  $\delta$  0.8-2.8 ppm. According to all information obtained from spectral data and melting point, the isolated compound 'B' can be assigned as lupeol (triterpenoid compound) and all of the observed data are consistent with the reported ones (Shashi *et al.*, 1994 and Chaturvedula *et al.*, 2012).

According  $^{13}\text{C}$  NMR (100MHz, Acetone- $d_6$ ) spectral data, compound 'B' contained 30 carbon signals, showed in Figure 11. The  $^{13}\text{C}$  NMR of the compound 'B' showed 30 signals for the terpenoid of lupane skeleton with includes a compound bonded to the hydroxyl group at C-3 position appeared at  $\delta$  79.3, while the olefinic carbon of the exocyclic double bond appeared at  $\delta$  152.2 and 110.7. The above spectral data suggested compound 'B' is also a lupane triterpene having a secondary hydroxyl group. The carbon signals from 7 methyl groups appeared at 15.0, 15.6, 16.8, 17.2, 17.3, 19.8 and 29.0 ppm. Ten methylene carbons can be assigned by the peaks, at  $\delta$  20.2, 26.4, 26.8, 28.9, 29.3, 34.9, 35.9, 38.7, 40.3 and 41.3 ppm and five methines carbons at  $\delta$  37.0, 40.3, 44.4, 49.5 and 57.0 ppm. The remaining signals found at  $\delta$  19.0, 39.7, 42.4, 44.3 and 49.7 ppm indicated the presence of five quaternary carbons. These spectral data were generally found to be same. The slight deviations are due to the solvent effect (Shashi *et al.*, 1994 and Chaturvedula *et al.*, 2012).

HMBC spectrum of compound 'B' shows the following key correlations: H-24 ( $\delta$  0.76 ppm) to C-23 ( $\delta$  28 ppm), C-5 ( $\delta$  55.7) and C-3 ( $\delta$  78 ppm), H-28 ( $\delta$  0.78 ppm) to C-17 ( $\delta$  43 ppm), H-25 ( $\delta$  0.85 ppm) to C-10 ( $\delta$  37 ppm) and C-1 ( $\delta$  38.7 ppm), H-23 ( $\delta$  0.94 ppm) to C-3

( $\delta$  78 ppm), C-4 ( $\delta$  39.7 ppm) and C-5 ( $\delta$  55.7 ppm), H-27 ( $\delta$  0.98 ppm) to C-14 ( $\delta$  42 ppm) and C-15 ( $\delta$  28 ppm), H-26 ( $\delta$  1.08 ppm) to C-8 ( $\delta$  41 ppm) and C-9 ( $\delta$  49 ppm), H-30 ( $\delta$  1.69 ppm) to C-20 ( $\delta$  151 ppm) and C-29 ( $\delta$  111 ppm), H-29 ( $\delta$  4.7 ppm) to C-30 ( $\delta$  19 ppm), C-20 ( $\delta$  151 ppm) and C-19 ( $\delta$  48 ppm). HMBC spectrum data are shown in Figure 12. According to all information obtained from spectral data, the isolated compound 'B' assigned as lupeol (triterpenoid compound).



### Structural Elucidation of Isolated Compound 'C'

Compound 'C' (0.002 % yield, m.pt. 102 °C, pale yellow crystal) was isolated from column chromatographic separation of PE crude extract of bark of *Shit-shar* using silica gel GF<sub>254</sub> as adsorbent and PE : EtOAc (7 : 1 v/v) as eluent. Its  $R_f$  value was found to be 0.51 in PE: EtOAc (3:1 v/v) solvent system. It has the melting point of 102 °C. It was soluble in ethyl acetate, chloroform, ethanol, and methanol, but insoluble in pet-ether. Compound 'C' gave deep blue colouration with 1% FeCl<sub>3</sub> showing that it contains phenolic -OH group. It did not give pink colour under treatment with conc: HCl and Mg ribbon.

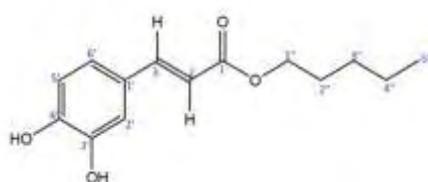
UV-visible spectra of compound 'C' were also studied in MeOH solvent as well as in the presence of flavonoid detecting agents such as NaOH, AlCl<sub>3</sub>, AlCl<sub>3</sub>/HCl reagents. Two absorption peaks at 287 nm (Band II) and 315 nm (Band I) in UV-visible spectrum (MeOH) of compound 'C' were respectively shifted to 394 nm and 455 nm in the presence of NaOH, indicating the presence of phenolic group. It caused a (61 nm) bathochromic shift of Band I with increase in peak intensity, confirmed the presence of free OH at 4' position [Figures 13 (a and b)]. In addition, Band II was observed not to change in the presence of AlCl<sub>3</sub> as well as in AlCl<sub>3</sub>/HCl shift reagents.

From the FT- IR spectral data assignment, it can be inferred that compound 'C' consists of phenolic OH groups assigned by the broad bands at 3446 cm<sup>-1</sup> and 3269 cm<sup>-1</sup> due to OH stretch and the band at 1601 cm<sup>-1</sup> due to aromatic C=C stretch. Asymmetric and symmetric CH stretching vibrations of -CH<sub>3</sub> and -CH<sub>2</sub> groups were observed at 2916 cm<sup>-1</sup> and 2849 cm<sup>-1</sup>. The band at 1687 cm<sup>-1</sup> confirmed the presence of  $\alpha$ ,  $\beta$ - unsaturated carbonyl group, that appeared due to C=O stretch. The band at 1272 cm<sup>-1</sup> appeared due to C-O stretching of ester group. The absorption bands at 1472 cm<sup>-1</sup> and 1363 cm<sup>-1</sup> confirmed the presence of aromatic ring appeared due to aromatic C-H bending vibration modes. The C-H out of plane bending vibrations of phenyl ring attributed by the absorption bands at 979 cm<sup>-1</sup>, 853 cm<sup>-1</sup> and 718 cm<sup>-1</sup>.

By studying the <sup>1</sup>H NMR spectrum (400 MHz, Acetone-d<sub>6</sub>) (Figure 15), 18 protons were found to be present in compound 'C'. Two doublet signals appeared at  $\delta$  6.28 and 7.53 ppm with coupling constants ( $J=15.6$  and 16 Hz) indicated the presence of two protons in the *trans* positions. Three protons occurred at down fields:  $\delta$  6.86, 7.04 and 7.15 ppm with the coupling constants ( $J=8$  Hz,  $J=2$ , 8 Hz,  $J=2$  Hz) are corresponding to the aromatic protons. One proton (H-6') at  $\delta$  7.04 ppm showed doublet of doublet with coupling constants ( $J=2$ , 8 Hz).

The H-5' proton *ortho*-related to H-6' appeared at 6.86 ppm ( $J=8$  Hz) as doublet. Another one doublet peak at  $\delta$  7.15 ppm with coupling constant of  $J=2$  Hz was assigned as an aromatic proton (H-2') *meta*-related to H-6'. In addition, two triplet peaks revealed at  $\delta$  0.88 and 4.14 ppm having the coupling constants ( $J=7.1$  and 6.8 Hz).

A total of 14 carbons were also observed in the  $^{13}\text{C}$  NMR spectrum (100 MHz, Acetone- $d_6$ ) of isolated compound 'C' (Figure 16) and a carbonyl carbon peak was found at  $\delta$  168.1 ppm (C-1). Three quaternary carbons at  $\delta$  128.4, 147.0 and 146.2 ppm correspond to C-1', C-3' and C-4'. Five methine carbons were observed at  $\delta$  123.1, 149.4, 115.9, 116.5 and 117.1 ppm corresponding to C-2, C-3, C-2', C5' and C-6'. Four methylene carbons can be assigned by the peaks, at  $\delta$  65.4, 33.3, 27.9 and 23.9 ppm. The remaining signal found at  $\delta$  14.0 ppm indicated the methyl carbon. According to all information obtained from spectral data, the isolated compound 'C' might be assigned at (*E*)-pentyl 3-(3',4'-dihydroxyphenyl) acrylate (phenolic compound).



$\text{C}_{14}\text{H}_{18}\text{O}_4$   
(*E*)- pentyl 3-(3',4'- dihydroxyphenyl) acrylate

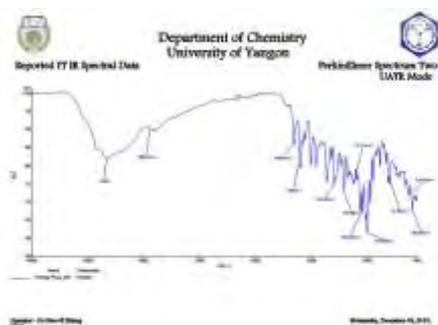


Figure1. FT IR spectrum isolated of isolated compound A

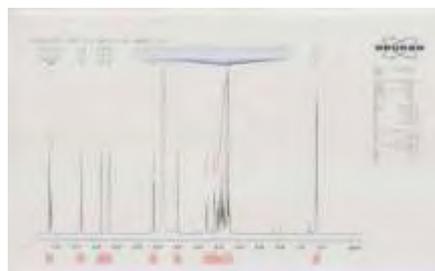


Figure 2.  $^1\text{H}$  NMR spectrum (400 MHz, MeOD) of isolated compound A

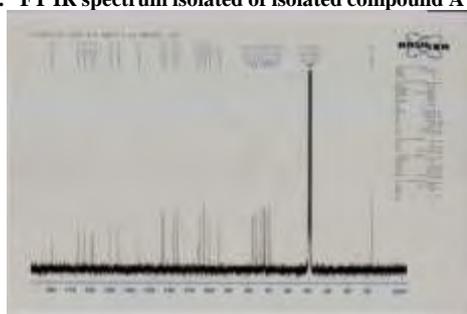


Figure 3.  $^{13}\text{C}$  NMR spectrum (100MHz, MeOD) of isolated compound A

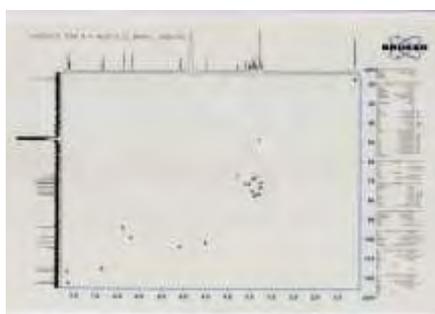


Figure 4. HSQC spectrum (MeOD, 400MHz) of isolated compound A

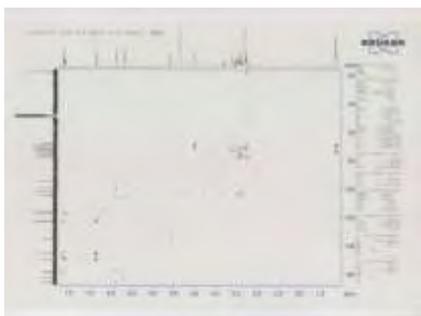


Figure 5. HMBC spectrum (MeOD, 100MHz) of isolated compound A

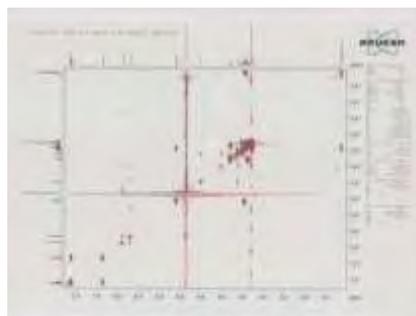


Figure 6. DQF-COSY spectrum (400 MHz, MeOD) of isolated compound A

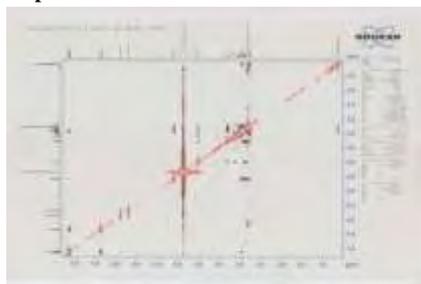


Figure 7. NOESY spectrum (400 MHz, MeOD) of isolated compound A

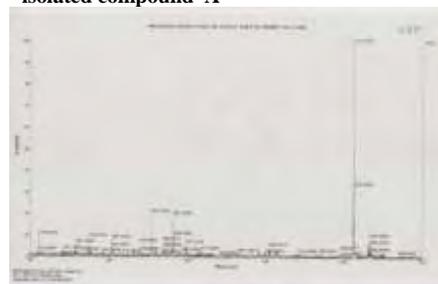


Figure 8. ESI MS spectrum (400 MHz, MeOD) of isolated compound A

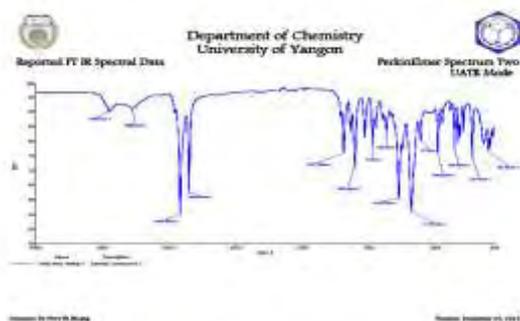


Figure 9. FT IR spectrum of isolated compound 'B'

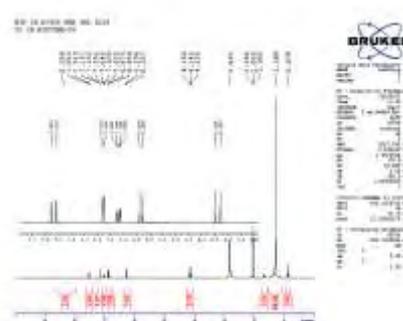


Figure 10.  $^1\text{H}$  NMR spectrum (400 MHz, Acetone- $d_6$ ) of isolated compound "B"

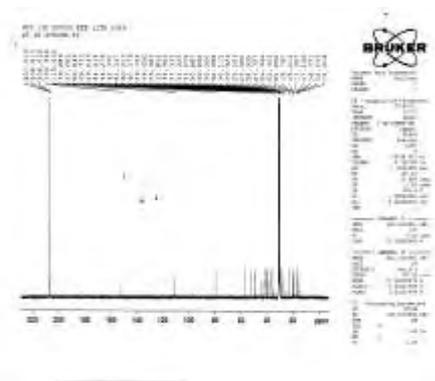


Figure 11.  $^{13}\text{C}$  NMR spectrum (100 MHz, Acetone- $d_6$ ) of isolated compound B

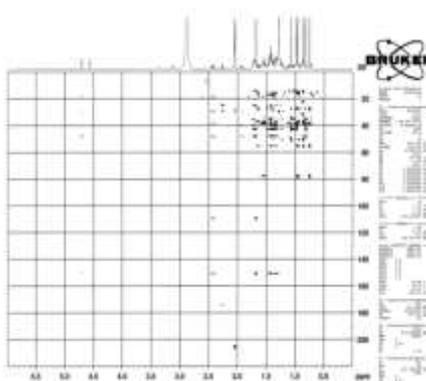


Figure 12. HMBC spectrum (125 MHz,  $\text{CDCl}_3$ ) of isolated compound 'B'

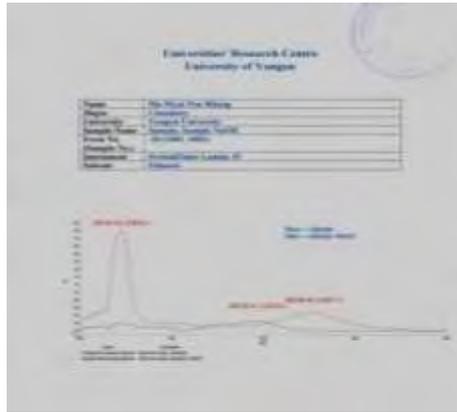


Figure 13 (a) UV-visible spectra of isolated compound 'C' in MeOH and MeOH+NaOH

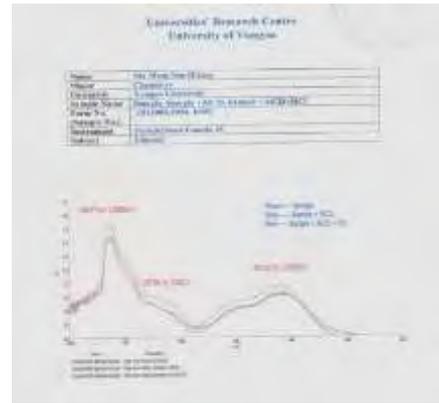


Figure 13 (b) UV-visible spectra isolated compound 'C' in MeOH, in MeOH+ AlCl<sub>3</sub> and MeOH+ AlCl<sub>3</sub>+ HCl

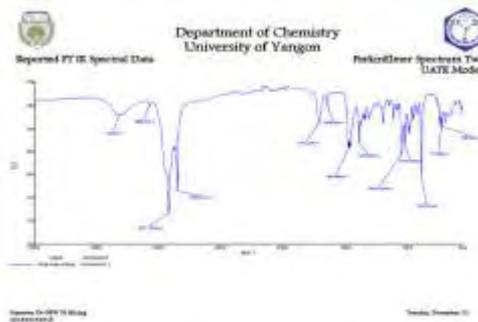


Figure 14 FT IR spectrum of isolated compound 'C'

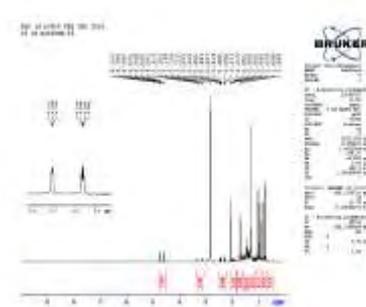


Figure 15 <sup>1</sup>H NMR spectrum (400 MHz, Acetone-d<sub>6</sub>) of isolated compound 'C'

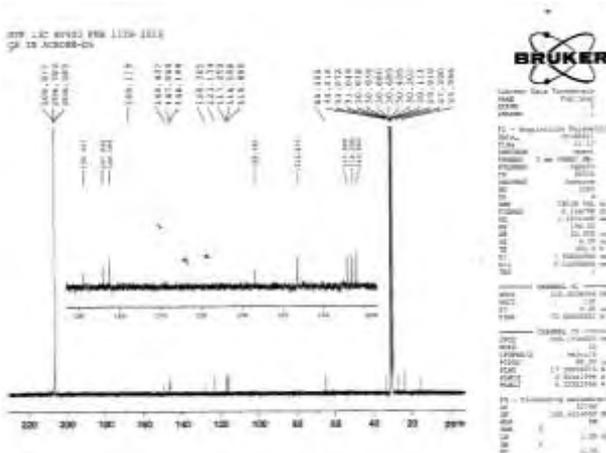


Figure 16 <sup>13</sup>C NMR spectrum (100 MHz, Acetone-d<sub>6</sub>) of isolated compound 'C'

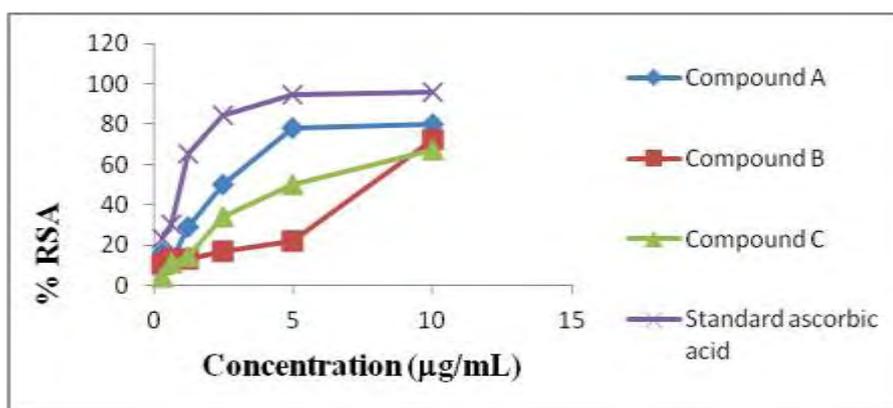
### Antioxidant Activity of Plant Extract and Isolated Compounds

The radical scavenging activity of isolated compounds were expressed in term of % inhibition and IC<sub>50</sub> (50 % inhibition concentration) values were calculated by linear regressive excel program. The IC<sub>50</sub> values of isolated compounds **A**, **B** and **C** were found to be 2.5, 7.78 and 4.97 µg/mL respectively,. It can be inferred that isolated compounds have the antioxidant activity. However standard ascorbic acid was slightly more potent than isolated compounds in antioxidant property. The results were summarized in Table 2. From the experimental results, isolated compound **A** was found to be more potent than compound **B** and **C** in antioxidant activity. However, it was observed that these three isolated compounds have the lower antioxidant potency than standard ascorbic acid (IC<sub>50</sub> = 0.75 µg/mL). The radical scavenging

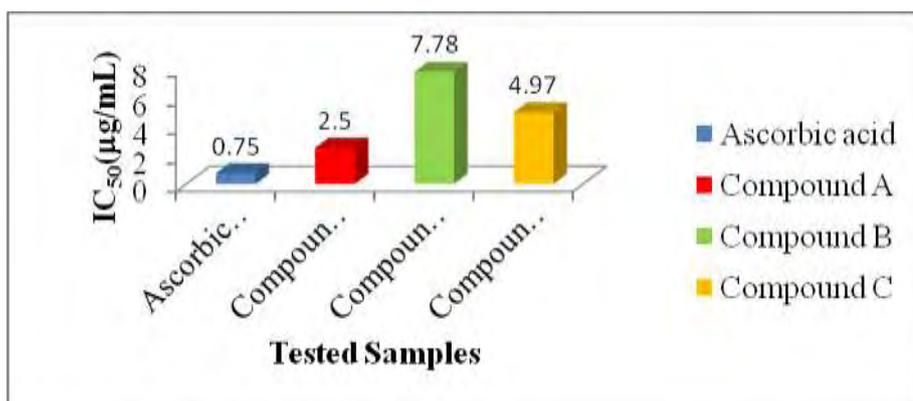
activity (% RSA) and  $IC_{50}$  values of compound **A**, **B**, **C** and standard ascorbic acid are shown in Figure 17 and Figure 18.

**Table 2 Radical Scavenging Activity (% RSA) and  $IC_{50}$  Values of Isolated Compounds and Ascorbic Acid**

Compounds	% RSA $\pm$ SD of different concentration ( $\mu\text{g/mL}$ )						$IC_{50}$ ( $\mu\text{g/mL}$ )
	0.3125	0.625	1.25	2.5	5	10	
St. ascorbic acid	23.08 $\pm$ 0.75	30.37 $\pm$ 0.56	65.52 $\pm$ 2.88	84.75 $\pm$ 2.06	94.56 $\pm$ 0.19	96.15 $\pm$ 0.19	0.75
A	16.67 $\pm$ 3.19	12.61 $\pm$ 14.65	28.83 $\pm$ 7.64	50 $\pm$ 10.83	77.93 $\pm$ 0.64	80.13 $\pm$ 0.00	2.5
B	10.42 $\pm$ 3.75	13.43 $\pm$ 0.50	13.25 $\pm$ 0.75	16.78 $\pm$ 2.75	22.26 $\pm$ 2.00	72.12 $\pm$ 4.75	7.78
C	4.45 $\pm$ 2.10	11.23 $\pm$ 2.70	14.19 $\pm$ 3.30	34.32 $\pm$ 10.79	50.21 $\pm$ 7.49	67.22 $\pm$ 11.09	4.97



**Figure 17 Plot of % RSA of ascorbic acid and isolated compounds (A, B & C)**



**Figure 18 Bar-graph of  $IC_{50}$  values of standard ascorbic acid and isolated compounds (A, B and C)**

#### 4. Conclusion

From the present research, on silica gel column chromatographic separation, a flavonoid glycoside: Quercetin-3-O- $\beta$ -D-glucopyranosyl (1-4)  $\alpha$ -rhamnopyranoside (**A**, 0.003 %, 186 °C, pale yellow crystal) was isolated from ethyl acetate extract of the whole plant of Taw-zee-phyu. Whereas a terpenoid compound: lupeol (**B**, 0.006 %, m.pt. 215 °C, colourless needle shape), and a phenolic compound: (*E*)-pentyl 3-(3', 4'-dihydroxyphenyl) acrylate (**C**, 0.002 %, m.pt.102 °C, pale yellow crystal) were isolated from pet-ether extract of bark of Shit-shar. These compounds are not previously reported in the title plants. This could be reported as new finding on these three isolated compounds from the respective plant sample.

According to DPPH free radical scavenging assay, the isolated compounds were found to possess the antioxidant properties with the  $IC_{50}$  values of 2.5 (A), 7.78 (B) and 4.97 (C)  $\mu\text{g/mL}$ . Among three isolated compounds, Quercetin-3-O- $\beta$ -D-glucopyranosyl (1-4)  $\alpha$ -rhamnopyranoside (A) was found to be more potent than lupeol (B) and phenolic compound (C) in antioxidant activity. It may be due to the flavonoid type of compound A. The finding from the present work will contribute to the scientific development of Myanmar traditional medicine, specifically in the areas concerned with the treatment of diseases related to the oxidative stress.

### Acknowledgements

Authors would like to thank to Department of Higher Education (Yangon Office), Ministry of Education, Yangon, Rector Dr Pho Kaung, Pro-rectors Dr Ohmar Kyaw and Dr Aung Kyaw University of Yangon for giving us the research grant and research facilities, and also allowing us to publish the research paper in this research journal.

### References

- Ashokkumar, R. and M. Ranaswamy. (2013). "Determination of DPPH Free Radical Scavenging Oxidation Effects of Methanolic Leaf Extracts of some Indian Medicinal Plant Species". *Journal of Chemical, Biological and Physical Sciences*, **3** (2), 1273-1278
- Chaturvedula, V. S. P. and P. Indra. (2012). "Isolation and Structural Characterization of Lupane Triterpenes from *Polypodium Vulgare*". *Research Journal of Pharmaceutical Sciences*, **1** (1), 24.
- Khin Hnin Mon, Yadanar Oo, Khing Zar Win Lae and Daw Hla Ngwe, (2015). "Screening of Phytochemical Constituents and some Biological Activities of the whole plant of Taw-ziphyu (*Phyllanthus maderaspatensis* L.) and Barks of Shit-shar (*Phyllanthus albizioides* (Kurz) Hook . f.)". *Universities Research Journal* , **8**, 1-12.
- Lakshmi Narasimhudu, C. and R. R., Venkata Raju, (2012). "Phytochemical Constituents of *Phyllanthus* Species (Euphorbiaceae) from Eastern Ghats of Andhra Pradesh, India". *International Research journal*, **3** (5), 184-200.
- Merck Index. (2001). *An Encyclopedia of Chemical Drugs and Biological*. USA: 13<sup>th</sup> Ed., Merck & Co. Inc, 322.
- Marinova, G and V. Batchvarov. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH". *Bulgarian Journal of Agricultural Science*, **17**(1), 11 – 24.
- Mya Bwin and Sein Gwan. (1973). "Burmese Indigenous Medicinal Plants". *Medical Research Institute*, Yangon, Myanmar.
- Shashi, B. M and R. K. Asish. (1994). "<sup>13</sup>C NMR Spectra of Pentacyclic Terpenoids-A Compilation and some Salient Features". *Phytochemistry*, India: Elsevier Science Ltd, **37**(6), 1517-1575.

- Thaw-Zin, Sein-Win, Khin-Chit. (2007). "Influence of Cultural Characteristics on the Utilization of Traditional Medicine and its Impact upon Health Care in Myanmar", Myanmar Health Research congress 2006, Abstracts of paper and poster, 24-25.
- Tin Shine Aung. (2015). *Determination of Total Phenol Content and Study on Antioxidant Activity of Phyllanthus maderaspatensis L. (Taw-ziphyu)*, MSc Thesis, Department of Chemistry, University of Yangon, 10-12
- Yadanar Oo. (2015). *Screening of Phytochemical Constituents and Some Biological Activities of the Whole Plant of Taw-ziphyu (Phyllanthus maderaspatensis L.) and Barks of Shit-sha (Phyllanthus albizzioides (Kurz) Hook.f.)*, MRes Thesis, Department of Chemistry, University of Yangon, 19-21.