STRUCTURE ELUCIDATION AND SOME BIOACTIVITIES OF PURE ORGANIC COMPOUNDS ISOLATED FROM THE STEM BARK OF *Polyalthia cerasoides*(ROXB.) BEDD. (THAYET-THAMON)

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Abstract

In this research work, one of the Myanmar indigenous medicinal plants, *Polyalthia cerasoides* (Roxb.) Bedd. (thayet-thamon) was chosen for chemical analysis and pharmacological investigation of the stem bark. The acute toxicity test of 95 % ethanol extract of the stem bark of *P. cerasoides* was carried out in this study. The ethyl acetate extract was chromatographed by various chromatographic techniques to give pale yellow powder of pure compound (MML-1) and brown oily form of pure compound (MML-2). The antimicrobial activities of three different solvent extracts of crude sample and pure compounds (MML-1) and (MML-2) were tested by agar well diffusion method on six selected organisms; *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli*. Then, the antioxidant activity of ethanol crude extract and pure compounds (MML-1) and (MML-2) was evaluated by using DPPH radical scavenging assay. The molecular structures and conformational analyses of compounds (MML-1) and (MML-2) were determined by high resolution spectrometric techniques such as ^{1}HNMR, ^{13}CNMR, DEPT, DQF-COSY, HMQC, HMBC, EI MS spectral data and molecular modeling. The pure compounds (MML-1) and (MML-2) possess the types of flavonoid and lignan compounds.

Keywords: *Polyalthia cerasoides*(Roxb.) Bedd., the acute toxicity test, antimicrobial activities, antioxidant activity

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Introduction

For thousands of years, natural products have played a very important role in health care and prevention of diseases. The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, lotions and oils with many of these bioactive natural products still being unidentified. According to recent studies conducted by the World Health Organization (WHO), about 80 % of the world's population relies on traditional medicine (Butler, 2004).

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health they have been antiplatelet, anti-inflammatory, reported to have antiviral, anti-allergic, antitumor and antioxidant activities (Marais *et al*., 2006).

The lignans are a group of chemical compounds found in plants. Plant lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols, known as monolignols, to a dibenzylbutane skeleton. Lignans serve an antioxidant role in the plant's defenses against biotic and abiotic factors, and have shown anti-inflammatory and antioxidant activity in basic research models of human diseases. Lignans may also have anticarcinogenic activities. Some epidemiological studies have shown that lignin exposure associates with lower risk of breast cancer.

In this study, one of Myanmar traditional medicinal plants namely, *Polyalthia cerasoides* (Roxb.) Bedd. (Figure1), belonging to the family Annonaceae was used for isolation and structural elucidation of pure organic compounds. Plants belonging to the family Annonaceae have long been used as a major source of medicines for the prevention and treatment of a variety of diseases in India and many Asian countries. To date, ethnopharmacological claims for Annonaceae include the use of its bark to control blood pressure, diabetes and its use as a febrifuge. In this research work, screening of phytochemical constituents, determination of antimicrobial activities were

carried on the crude extracts from the bark of*P. cerasoides*. Moreover, evaluation of the antioxidant activities, determination of acute toxicity of ethanol crude extracts were also carried out. In addition, pure compounds were isolated from the bark of *P.cerasoides* by applying advanced separation techniques such as thin layer, preparative thin layer chromatography and column chromatography. The chemically isolated pure compounds (MML-1 and MML-2) were used for examination of antimicrobial and antioxidant activities, and structural elucidation.

Scientific Classification

Family name	Annonaceae
Botanical name	*Polyalthiacerasoides* (Roxb.) Bedd.
Myanmar name	Thayet-thamon
Common name	Cherry Ashok
Parts of plant used	Bark
Medicinal uses	Hypertension, tonic to combat stress and pain, anti-inflammatory, anti-analgesic

(a) **(b)** **(c)**

Figure 1 (a)Plant, (b) flowers and (c) fruits of *Polyalthia cerasoides* (Roxb.) Bedd.

Materials And Methods

General Experimental Procedures

IR spectra were recorded on FT IR-8400 spectrophotometre. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA-500 (^{1}H: 500 MHz and ^{13}C: 125 MHz). Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to solvent signal (chloroform-*d*: δ_H 7.26 and δ_C 77.0) as internal standard. EI mass was obtained with a JEOL JMS MS-700. The melting point of the pure compound in crystal form was measured by Stuart SMP 30 melting point apparatus. Commercial grade reagents and solvents were purchased from Chemico Co. Ltd, Yangon. Column chromatography was carried out on silica gel (BW – 820 MH, Fuji Silysia, Aichi, Japan). Analytical preparative thin layer chromatography was conducted on Kieselgel 60 (F_{254}, Merck).

Plant Material

Bark of *P. cerasoides* was collected from Ye-kyi-su village, Pa-thein-gyi Township, Mandalay Region.

Solvent Extraction and Partition of *P. cerasoides*

The air dried powder of the stem bark of Thayet-thamon (1000 g) was percolated with methanol (3000 mL) for about two months. Then the percolated solution was filtered and evaporated to concentrate at room temperature. The residue was extracted with ethyl acetate and water. The ethyl acetate fraction was concentrated to dryness in vacuum, to give 3.2 g of dried extract.

Isolation and Purification of Pure Compounds

The ethyl acetate extract (2.5 g) was chromatographed over a silica gel column, eluted with a gradient solvent system of increasing polarity (n-hexane, 19:1-1:4, then ethyl acetate only) to give 544 fractions (Figure 2),. Each fraction was checked by TLC, iodine vapour and UV lamp. Then, the fractions of the same R_f values were combined and (19) combined fractions were

obtained. The sub-fraction 18-4 was subjected to preparative thin layer chromatography (PTLC) using n-hexane : ethyl acetate (6:4) as eluents to afford brown oily form of pure compound MML-2 (8.2 mg).The combined fraction (19) was rechromatographed by silica gel column chromatography using n-hexane : ethyl acetate as eluents to give nine sub-fractions. The sub fraction 19-8 was also subjected to PTLC using
n-hexane : ethyl acetate (1:1) as eluents to give pale yellow crystals of pure compound MML-1 (6.4 mg).

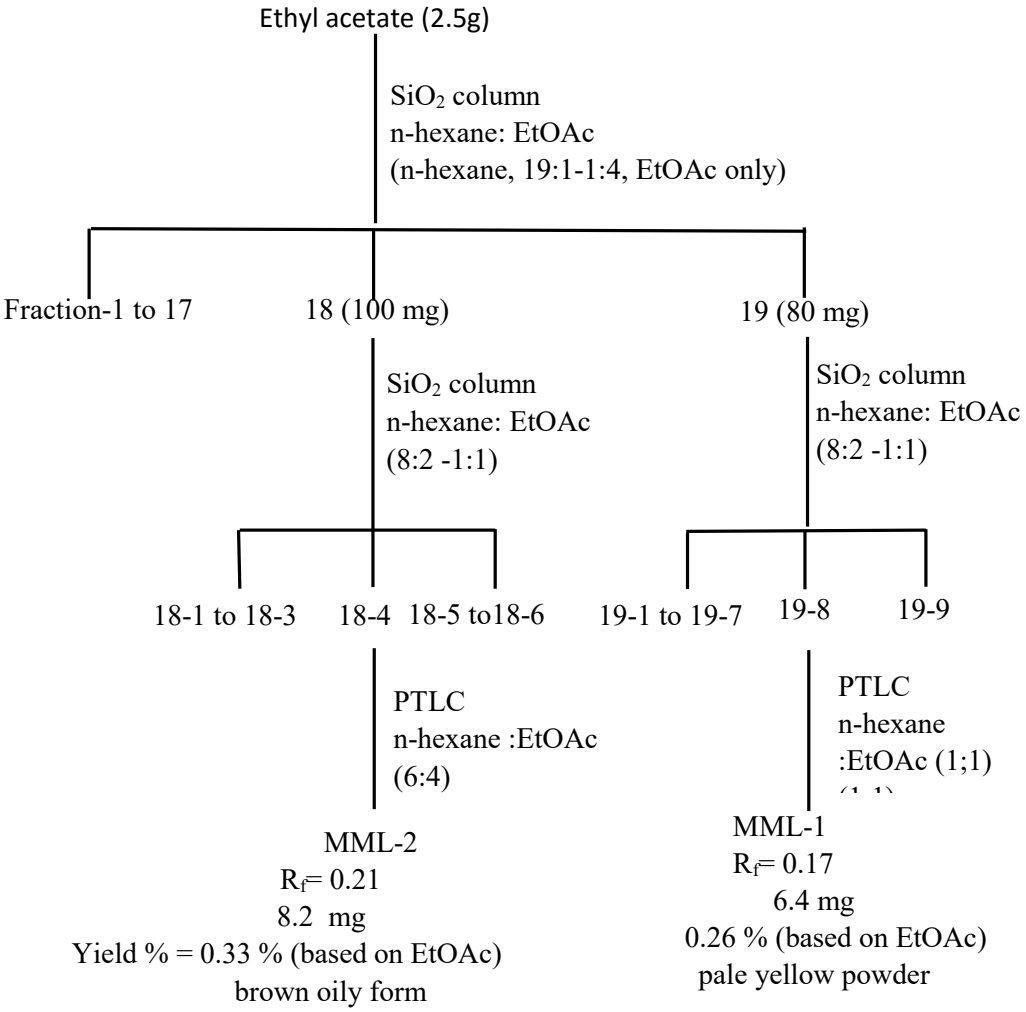


Figure 2 Flow diagram for isolation and purification of pure compounds MML-1 and MML-2 from the bark of *P. cerasoides*

Phytochemical Analysis

Phytochemical analysis for alkaloids, flavonoids, terpenes, steroids, glycosides, reducing sugars, lipophilic, polyphenols, tannins, saponins and phenolic compounds were carried out according to general methods mentioned in phytochemical methods (Harborne, 1993; Yadav and Munin, 2011).

Investigation of Antimicrobial Activities of the Barks of *P. cerasoides*

The bark was extracted with three solvents n-hexane, ethyl acetate and ethanol. Antimicrobial activities of the bark extracts were tested by using agar-well diffusion method on six selected organisms; *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli* at Pharmaceutical Research Department (PRD), Insein, Yangon.

Acute Toxicity Study of Plant Crude Extract of *P. cerasoides*

An acute toxicity study was carried out on 95 % ethanol extract of the stem bark of *P.cerasoides* using mice as the experimental model. Both sexes of healthy Albino ICR (Institute of Cancer Research) Strain mice (25g to 30g) were randomly selected and kept in their cages for at least 5 days prior to the experiment to allow for acclimatization for Laboratory conditions. Before the experiment, the animals were kept fasting overnight for 18 h but were allowed with free access to water. Following period of fasting, mice were weighed and dose was calculated according to the body weight. The doses were selected from the sequence 500mg/kg, 1000 mg/kg, 1500 mg/kg, 2000mg/kg and 2500 mg/kg respectively for specific regulatory needs. Then, the test substance was dissolved in ethanol for required concentration and administered orally in a single dose by using cannula. One group was served as the control and only distilled water was given orally.

Each dose of (500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 2500 mg/kg) was administered orally to each of five group of mice. Mice were observed after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h and daily up to 10 days. Signs of toxicity and mortality of the mice were recorded. Observations

included changes in fur, eyes, mucous membranes, respiratory rate, autonomic central nervous systems and behavioral pattern. The time of death if any was recorded. Individual body weights of mice were measured and recorded shortly before the test substance was administered and once weekly thereafter. At the end of the test (i.e. 10 days) the mice were weighed.

Determination of Antioxidant Activities of Crude Extract and Isolated Compounds by DPPH Assay

DPPH radical scavenging activity was determined by using UV-visible spectrophotometer. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm and the percentage of radical scavenging activity (% RSA) was calculated by the following equation.

$$\% \text{ RSA} = [(A_{DPPH} - A_{Sample}) - A_{Blank} / A_{DPPH}] \times 100$$

The antioxidant power is expressed as IC_{50} (50 % inhibitory concentration). It is the test substances concentration (μg/ mL) that result in a 50 percent reduction of initial absorbance of DPPH solution. IC_{50} values were calculated by linear regressive excel program (Marinova and Batchvarov, 2011).

Structure Elucidation of Pure Compounds MML-1 and MML-2

The structures of two pure compounds MML-1 and MML-2 were elucidated by high resolution spectrometric techniques such as ^{1}H NMR, ^{13}C NMR, DEPT, DQF-COSY, NMQC, HMBC, EI MS spectral data and molecular modeling (Silverstein *et al*., 2005).

Results and Discussion

Phytochemicals Present in the Bark of *P. cerasoides*

The results of the phytochemical screening tests from the bark of *P. cerasoides* are shown in Table 1. The presence of alkaloids, flavonoids, terpenes, sterols, glycosides, reducing sugars, lipophilic, polyphenols, saponins and phenolic compounds was detected in the extract of bark of *P. cerasoides.*

Table 1 Results of Phytochemical Analysis of the Stem Bark of *P. cerasoides*

No.	Constituents	Test Reagent	Observation	Results
1	Alkaloids	1%HCl, Dragendorff's reagent	Orange ppt	+
2	Flavonoids	EtOH, Mg turning, Conc:HCl	Pink color solution	+
3	Terpenes	Pet ether, Conc:H_2SO_4, Acetic anhydride	Pink color solution	+
4	Steroids	$CHCl_3$, Conc:H_2SO_4, Acetic anhydride	Blue color solution	+
5	Glycosides	H_2O, 10% Lead acetate	White ppt	+
6	Reducing sugars	H_2O, Benedict's solution	Bricked red color ppt	+
7	Lipophilic	H_2O, 0.5 M KOH	Deep color solution	+
8	Polyphenols	EtOH, 10% $FeCl_3$, 1% $K_3Fe(CN)_6$	Greenish blue color solution	+
9	Saponins	H_2O, Shake	Frothing	+
10	Phenolic compounds	H_2O, 10% $FeCl_3$	Purplish color solution	+
11	Tannins	H_2O, 10% $FeCl_3$, H_2SO_4	No yellowish brown color	-

Plus sign indicates the presence and minus sign indicates the absence.

Antimicrobial Activities of Extracts of *P. cerasoides*

The antimicrobial activities of crude extract in various solvents from the bark of *P. cerasoides* were tested by using agar-well diffusion method on six selected organisms. The results are tabulated in Table 2.

Table 2 Antimicrobial Activities of the Different Extracts of *P. cerasoides*

Samples	Solvents	Diameter of Inhibition Zone(mm)					
		1	**2**	**3**	**4**	**5**	**6**
P. cerasoides	n-hexane	-	-	-	-	-	-
	ethyl acetate	-	-	19 (++)	26 (+++)	22 (+++)	24 (+++)
	ethanol	11(+)	-	12 (+)	-	11 (+)	-
Control	n-hexane	-	-	-	-	-	-
	ethyl acetate	-	-	-	-	-	-
	ethanol	-	-	-	-	-	-

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

1. *Bacillus subtilis* (N.C.T.C-8236)
2. *Staphylococcus aureus* (N.C.P.C-6371)
3. *Pseudomonas aeruginosa* (6749)
4. *Bacillus pumilus* (N.C.I.B-8982)
5. *Candida albicans*
6. *Escherichia coli* (N.C.I.B-8134)

According to Table 2, n-hexane extracts of the bark of *P. cerasoides* did not show any activity on all tested organisms. Ethanol extract showed low activities on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*. Ethyl acetate extract gave rise to medium activity on *Pseudomonas aeruginosa* and high activities against three tested organisms such as *Bacillus pumilus, Candida albicans* and *Escherichia coli.* That is why ethyl acetate extract was selected for further analysis. All of the tested extracts did not show any activity against *Staphylococcus aureus*.

Antimicrobial Activities of Pure Compounds (MML-1 and MML-2)

The antimicrobial activities of pure compounds (MMl-1) were investigated by agar well diffusion method against six selected organisms. The results are shown in Table 3.

Table 3 Antimicrobial Activities of Pure Compounds (MML-1 and MML -2)

Samples	Solvents	Diameter of Inhibition Zone(mm)					
		1	**2**	**3**	**4**	**5**	**6**
Pure Compound MML-1	EtOAc	12(+)	14 (+)	12 (+)	14 (+)	12 (+)	14 (+)
Pure Compound MML-2	EtOAc	-	12 (+)	-	13 (+)	11 (+)	11 (+)

According to Table 3, pure compounds (MML-1 and MML-2) showed low activities on all tested organisms and pure compound (MML-2) showed low activities on four tested organisms such as *Staphylococcus aureus***,** *Bacillus pumilus, Candida albicans* and *Escherichia coli*.

Acute Toxicity of 95% Ethanol Extract of the Bark of *P. cerasoides*

The mice administered with 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 2500 mg/kg doses of ethanol extract of *P. cerasoides* were kept under observation for 10 days. The observation parameters used in this experiment were cage side observations, daily body weight and mortality record. The results obtained from this experiment are shown in the following Table 4 .

Table 4 Acute Toxicity Study on the Ethanol Extract of the Bark of *P. cerasoides* Based on Cage Side Observations

No.	Parameters	Observations
1.	Condition of the fur	Normal
2.	Skin	Normal
3.	Subcutaneous swellings	Nil
4.	Abdominal distension	Nil
5.	Eyes-dullness	Nil
6.	Eyes-opacities	Nil
7.	Pupil diameter	Normal
8.	Ptosis	Nil
9.	Color and consistency of the feces	Normal
10.	Wetness or soiling of the perineum	Nil
11.	Condition of teeth	Normal
12.	Breathing abnormalities	Nil
13.	Gait	Normal

In this experiment, five mice were used for each group. At the end of observation period, all the mice were alive, did not show any toxic symptoms such as diarrhea, inactivity, restlessness, aggressiveness, eye-dullness, breathing, abnormalities, etc. and did not exhibit loss and obvious changes of body weight. Thus, the lethal dose LD_{50} was more than 2500 mg/kg body weight. Hence, the ethanol extract of this plant is practically nontoxic and may be relatively harmless.

Antioxidant Activity of Crude Extract, MML-1 and MML-2by Using DPPH Assay

In the present work, investigation of radical scavenging activity of pure compounds, MML-1,MML-2 and ethanol extract from the stem bark of*P. cerasoides* was performed by using DPPH assay. Figure 3 shows the percent radical scavenging activities of pure compounds, MML-1, MML-2 and ethanol extract together with that of standard ascorbic acid versus concentrations (µg/mL). The antioxidant power is expressed as IC_{50} (50% inhibitory concentration).

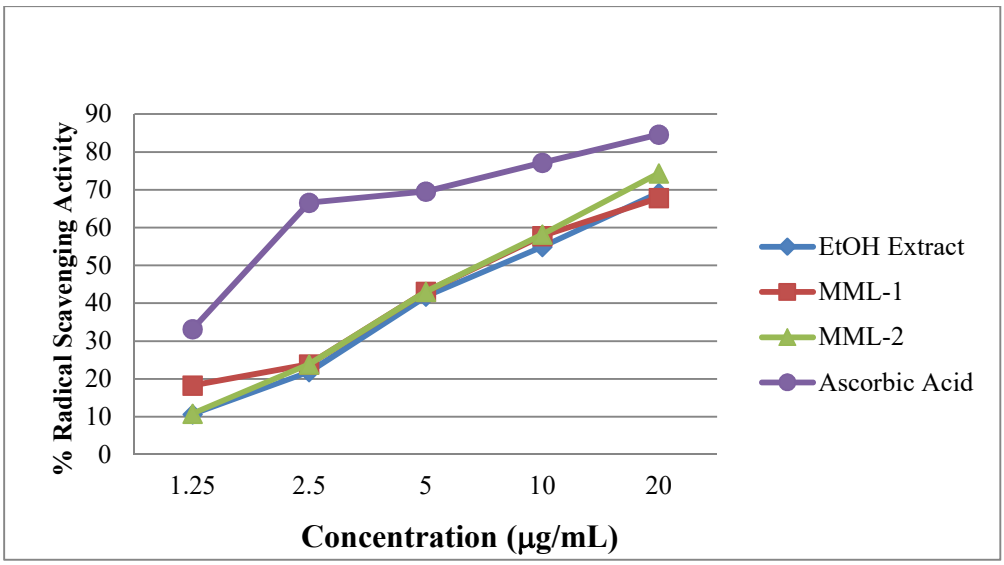


Figure 3 Percent radical scavenging activities versus concentration (µg/mL) of ascorbic acid, ethanol extract, MML-1 and MML-2

The antioxidant activity of the ethanol extract, pure compounds MML-1 and MML-2 exhibited IC_{50} value of 8.14 µg/mL, 7.29 µg/mL and 7.29 µg/mL respectively (Figure 4).

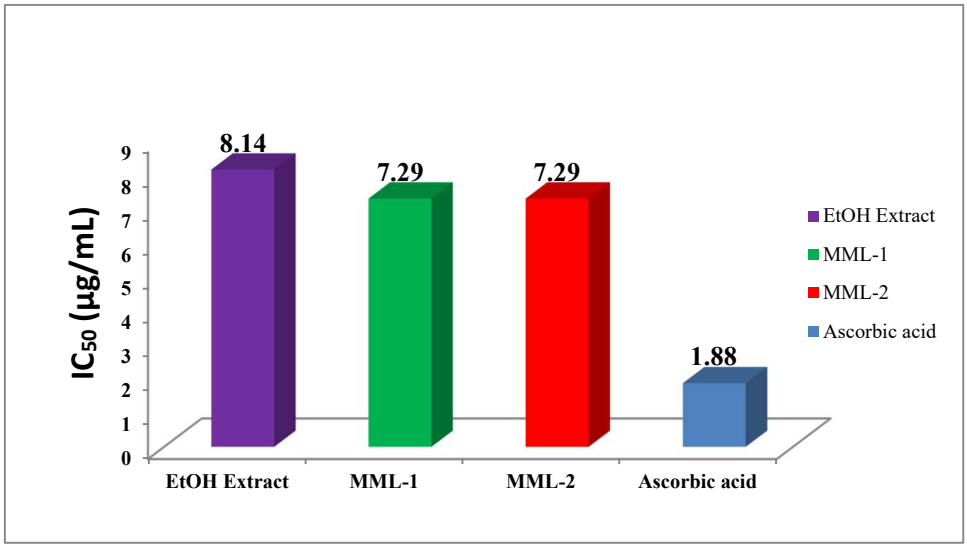


Figure 4 IC_{50} values of ethanol extract, MML-1, MML-2 and ascorbic acid

Isolation of Some Bioactive Compounds from the Bark of *Polyalthia cerasoides* (Roxb.) Bedd.

The ethyl acetate extract (2.5 g) was chromatographed over a silica gel column, eluted with a gradient solvent system of increasing polarity (n-hexane, 19:1-1:4, then ethyl acetate only) to give two pure compounds of MML-1 and MML-2.

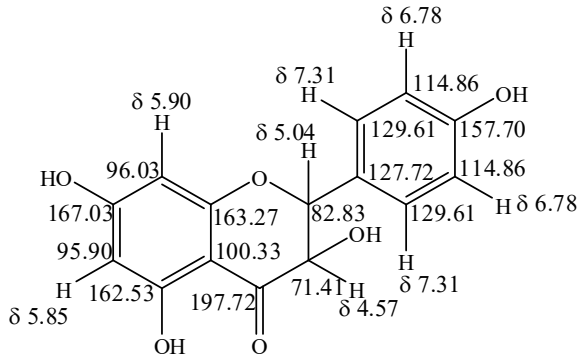
Structure Elucidation of Pure Compounds MML-1 and MML-2

Pure compound MML-1 was identified (Silverstein *et al*., 2005) as [(2R, 3R)-3,5,7-trihydroxy-2-(4-hydroxyphenyl)chroman-4-one] by using FT IR (Figure 5a), ^{1}HNMR (Figure 5 b), ^{13}C NMR (Figure 5 c), DEPT (Figure 5 d), DQF COSY (Figure 5 e), HMQC (Figure 5 f), HMBC (Figure 5 g), NOESY (Figure 5 h) and EI Mass (Figure 5 i) spectra and then MML-2 was identified as [4, 4' (1R,3aS,4R, 6aS) –hexahydrofuro [3,4-c] furan-1,4-diyl) bis (2,6-dimethoxy phenol)]by using FT IR (Figure 6 a), ^{1}HNMR (Figure 6 b), ^{13}C NMR (Figure 6 c), DEPT (Figure 6 d), DQF COSY (Figure 6 e), HMQC

(Figure 6 f), HMBC (Figure 6 g), NOESY (Figure 6 h) and EI Mass (Figure 6 i) spectra. .

The molecular formula of pure compounds (MML-1) and (MML-2) were found to be $C_{15}H_{12}O_6$ and $C_{22}H_{26}O_8$. The molecular mass of pure compound (MML-1) and (MML-2) are 288 and 418, in good agreement with molecular ion peak at (m/z 288) and (m/z 418) in EI mass spectrum. They were also confirmed by the mass fragmentation peaks at (259, 153, 134 and 107 for MML-1) and (193, 181, 167 and 154 for MML-2) in EI mass spectrum.

(2R, 3R)-3,5,7-trihydroxy-2-(4-hydroxyphenyl) chroman-4-one (MML-1)



Complete structure of pure compound MML-1

molecular weight: 288(m/z = 288 in EI mass spectrum)

melting point : 177 - 179˚C

physical form :pale yellow powder

R_f value : 0.17 (n-hexane :EtOAc(1:1)

molecular formula : $C_{15}H_{12}O_6$

FT IR : (KBr), $\nu_{max}(cm^{-1})$

3433.41and 3383.26(ν_{OH}), 3039.91($\nu_{C=CH2}$),2924.18 and 2834.31(ν_{asym} and ν_{sym} of CH), 1639.55($\nu_{C=O}$), 1518.03($\nu_{C=C}$),

1458.23(δ_{C-H} of CH_2 and CH_3), 1359.68(ν_{OH} of phenol),

1273.06(ν_{C-O} of phenol), 1166.97and1035.81(ν_{C-O-C} of ether),

819.77(δ_{C-H} out of plane bending)

^{1}H NMR: 600MHz,DMSO,δ_H(ppm)

4.57(dd, J=4.8Hz, 11.34Hz, 1H), 5.04(d, J=11.34Hz, 1H),

5.85(d,J=2.62Hz, 1H), 5.90(d, J=2.39Hz, 1H),

6.78(d,J=9.08Hz,2H),7.31(d, J=9.0Hz, 2H)

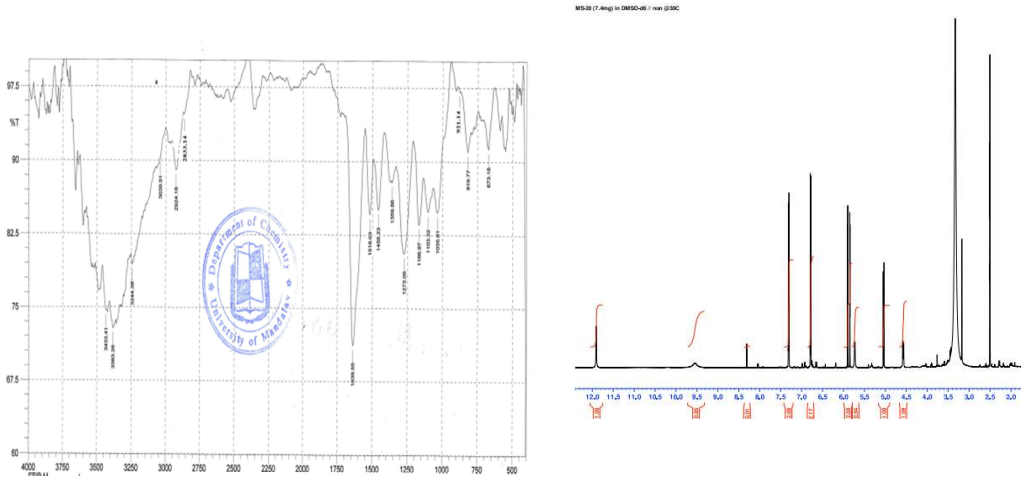
^{13}C NMR : 150MHz, DMSO, δ_C(ppm)

71.41(CH-3), 82.83(CH-2), 95.90(CH-6), 96.03(CH-8),

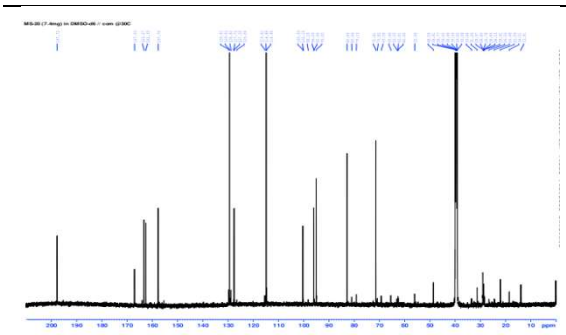
100.33(Cq-10), 114.86(CH-3'), 127.72(Cq-1'), 129.61

(CH-2'),157.70(Cq-4'),162.53(Cq- 6), 163.27(Cq-9),167.03

(Cq-7), 197.72 (Cq- C=O)



(a) (b)



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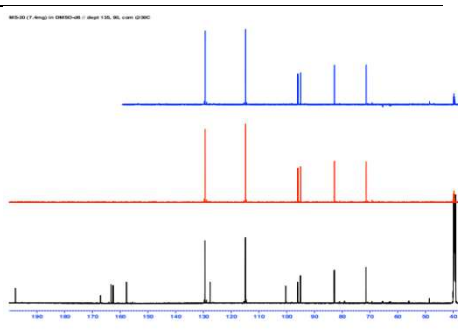

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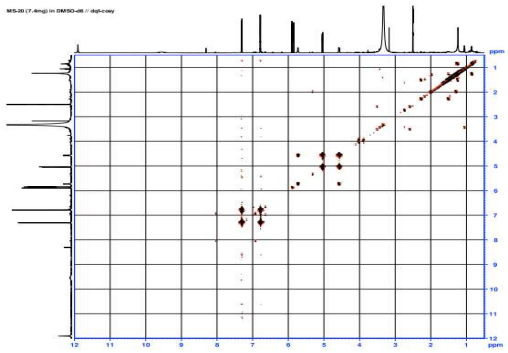
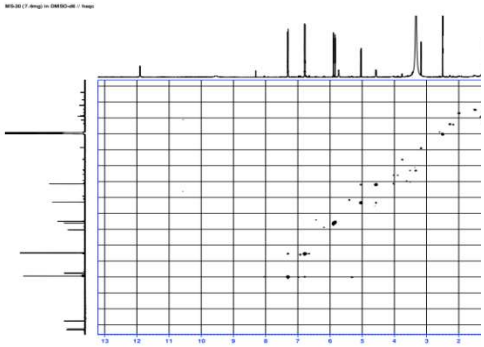


(e)



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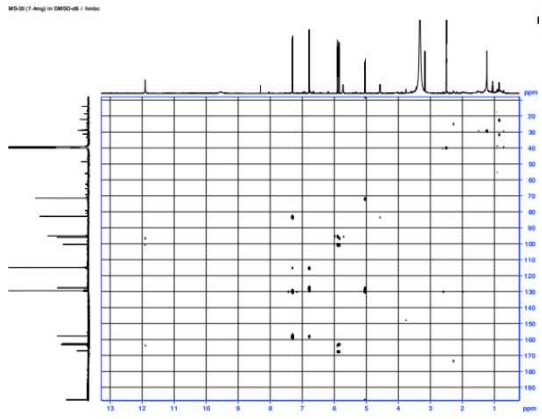
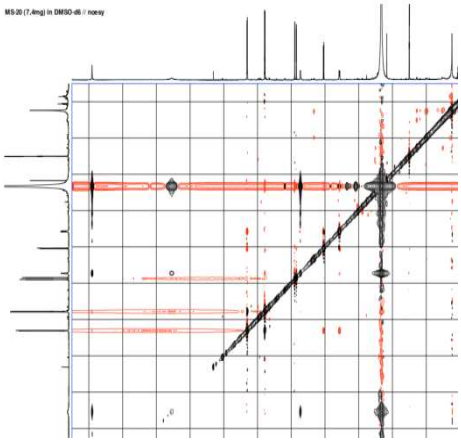
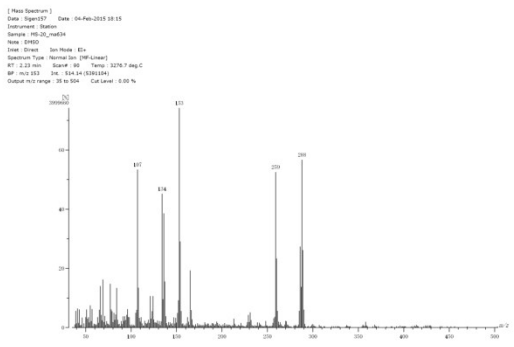


(g)



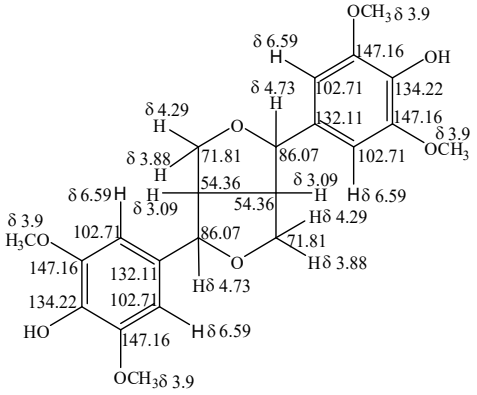
(h)



(i)

Figure 5 (a) FT IR, (b) ^{1}HNMR, (c) ^{13}C NMR, (d) DEPT, (e) DQF COSY, (f) HMQC, (g) HMBC, (h) NOESY and (i) EI Mass Spectra of Pure Organic Compound (MML-1)

4, 4' (1R,3aS,4R, 6aS) –hexahydrofuro [3,4-c] furan-1,4-diyl) bis (2,6-dimethoxy phenol)(MML-2)



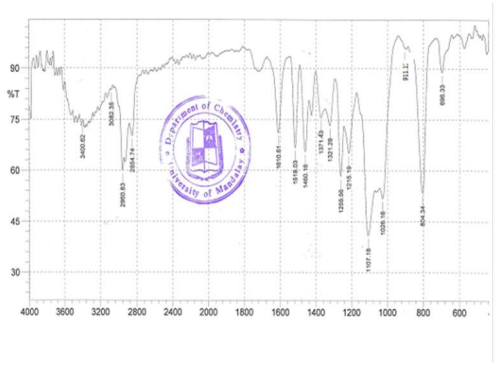
Complete structure of pure compound MML-2

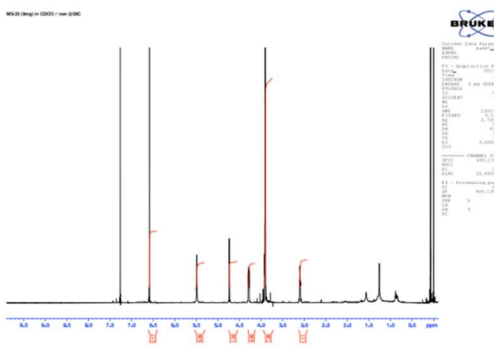
molecular weight	:	418 (m/z = 418 in EI mass spectrum)
physical form	:	brown oily form
R_f value	:	0.12 [n-hexane :EtOAc (6:4)]
molecular formula	:	$C_{22}H_{26}O_8$

FT IR : (KBr), $\nu_{max}(cm^{-1})$
3400.62(ν_{OH}), 3082.35($\nu_{C=CH2}$), 2960.83 and 2854.74(ν_{asym} and ν_{sym} of CH), 1610.61 and 1518.03($\nu_{C=C}$), 1460.16(δ_{C-H} of CH_2 and CH_3), 1371.43(ν_{OH} of phenol), 1259.56 and 1026.16(ν_{C-O-C} of ether), 1215.19(ν_{C-O} of phenol), 804.34(δ_{C-H} out of planebending)

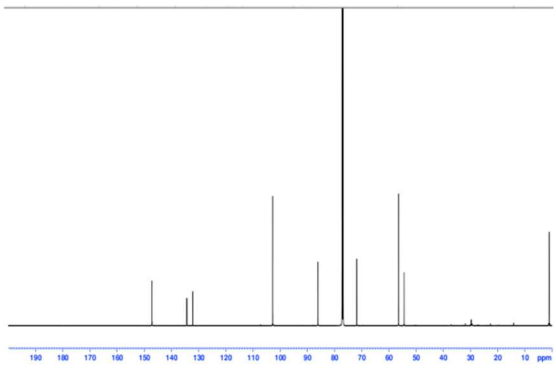
^{1}H NMR : 600MHz, $CDCl_3$,δ_H(ppm)
3.09(ddd, J=4.8Hz, 5.28Hz, 6.39Hz, 1H), 3.90(s,6H) ,
3.88(dd,J=5.28Hz, 9.16Hz, 1H), 4.29(dd, J=6.9Hz, 9.16Hz,
1H), 4.73(d, J=4.8Hz, 1H), 6.59(s,2H)

^{13}C NMR : 150MHz, $CDCl_3$,δ_C(ppm)
54.36(CH-3a), 56.38(CH_3-7'), 71.81(CH_2-2), 86.07(CH-1), 102.71(CH-5'), 132.11 (Cq-4'), 134.22(Cq-1'), 147.16(Cq-2')

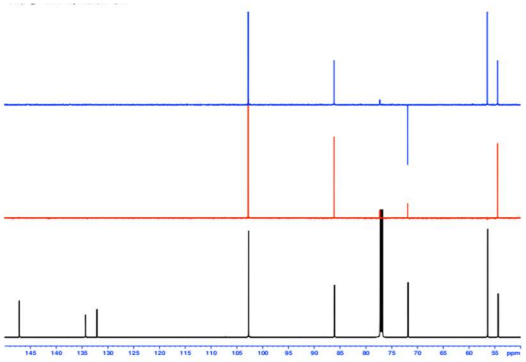




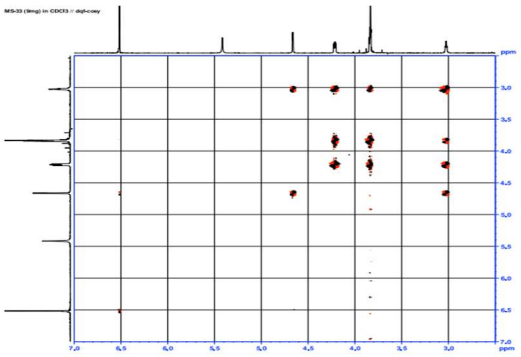
(a) (b)



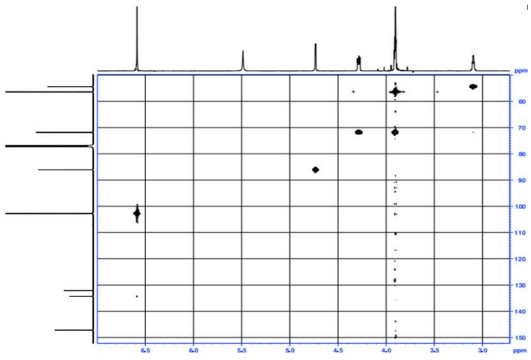
(c)



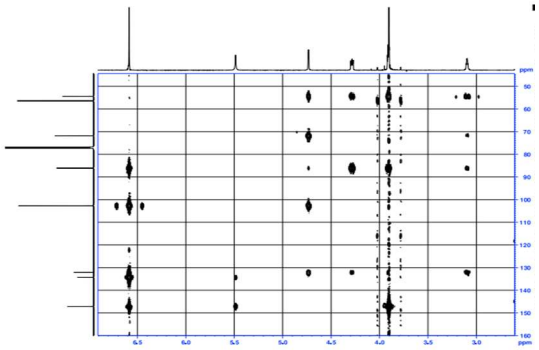
(d)



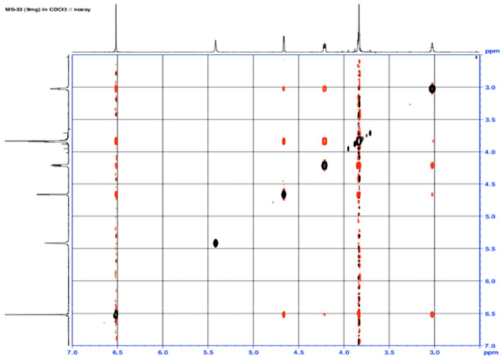
(e)



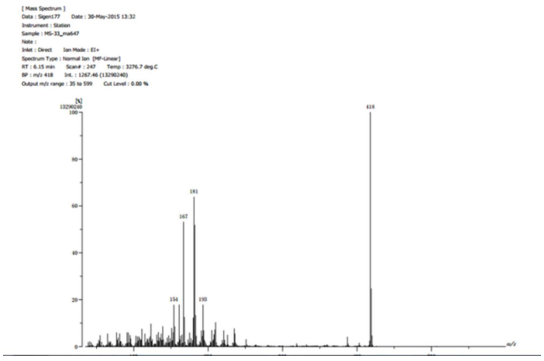
(f)



(g)



(h)



(i)

Figure 6 **(**a) FT IR, (b)[1]HNMR, (c) [13]C NMR, (d) DEPT, (e) DQF COSY, (f) HMQC, (g) HMBC, (h) NOESY and (i) EI Mass Spectra of Pure Organic Compound (MML-2)

Conclusion

In this study, one of Myanmar medicinal plants, *P. cerasoides* was used for isolation and structural elucidation of pure organic compounds. The results of the phytochemical analysis revealed that glycosides, phenolic compounds, reducing sugars, saponins, steroids, flavonoids, alkaloids, terpenes and polyphenols were present in the extract of *P. cerasoides*. The ethyl acetate extract was chromatographed by various chromatographic techniques to give pale yellow powder of pure compound(MML-1) and brown oily form of pure compound (MML-2). The melting point of pure compound (MML-1) was found to be 177-179°C. The Structurer of those pure compounds were elucidated by modern technique

According to the results of the antimicrobial activity, the ethyl acetate extract of *P. cerasoides* showed the high activity on all tested microorganisms except on *Bacillus subtilis* and *Staphylococcus aureus*. The isolated pure

compound (MML-1) showed low antimicrobial activity on all tested organisms. The isolated pure compound (MML-2) showed low antimicrobial activity on four tested organisms such as *Staphylococcus aureus***,** *Bacillus pumilus, Candida albicans* and *Escherichia coli*.

The, antioxidant activity of the crude ethanol extract and pure compounds (MML-1) and (MML-2) were evaluated using DPPH radical scavenging assay. MML-1 andMML-2 showed the antioxidant activity with the IC_{50} values of 8.14 µg/mL and 7.29 µg/ml respectively.

Moreover, the acute toxicity study on 95% ethanol extract of the stem bark of *P. cerasoides* was observed to show no toxic effect up to dose level of 2500 mg/kg. Therefore, the medium lethal dose (LD_{50}) was expected to be more than 2500 mg/kg. The molecular structures and conformational analysis of compounds (MML-1) and (MML-2) were determined by high resolution spectrometric techniques and molecular modeling. The pure compounds (MML-1) and (MML-2) possess the types of flavonoid and lignan compounds. The IUPAC names of the pure compounds (MML-1 and MML-2) are **(**2R, 3R)-3,5,7-trihydroxy-2-(4-hydroxyphenyl) chroman-4-one and 4, 4' (1R,3aS,4R, 6aS) –hexahydrofuro [3,4-c] furan-1,4-diyl) bis (2,6-dimethoxy phenol) respectively.

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