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Chemical Analysis of Heartwood of Bijayasal (*Pterocarpus marsupium* Roxb.)

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Abstract
Metlaline (**1**), piyaline (**2**), piyaline methyl ester (**3**), a pair of diasteriomeric epimers of marsuposide (**4a** and **4b**), and pterostilbene (**5**) were isolated from the water soluble fraction of 70% MeOH extract of *Pterocarpus marsupium* (Roxb.) heartwood. Among them compound **3** was isolated for the first time from natural source. Pterostilbene has been found as antioxidant, anti-diabetic and anti-cancer agent.

Key words: marsuposide, phenolic, piyaline, pterostilbene.

Introduction
Pterocarpus marsupium Roxb. (Fabaceae) is commonly known as Bijayasal in Nepal. The wooden tumbler made up of heartwood of *P. marsupium* is used for drinking water as traditional remedy because of its medicinal property (Gairola *et al.* 2010). Heartwood juice of this plant is used for the treatment of diabetics, jaundice, ulcer, gastric, etc. Regarding its traditional uses, it may contain compounds with strong antioxidative, anti-inflammatory, antidiabetic, antimicrobial and anticancer activities. The plant is reported to be rich in poly-phenolic compounds. The known constituents represent various flavonoids, diphenylpropane derivatives, and sesquiterpenes. Since flavone glycosides and plant extracts rich in this class of compounds are suggested to be useful for therapy and prophylaxis of diabetes (Achari *et al.* 2012), similar constituents of this plant have been under intense investigation.

To determine the chemical basis for traditional uses, we carried out the chemical analysis on the heartwood of *P. marsupium* and isolated metlaline (**1**), piyaline (**2**), piyaline methyl ester (**3**), a pair of diasteriomeric epimers of marsuposide (**4a** and **4b**) (2α/2β-hydroxy-2-*p*-hydroxybenzyl-3(2*H*)-benzofuranone-7-*C*-β-D-glucopyranoside), and pterostilbene (**5**), (Figure 1) from the water soluble fraction of 70% MeOH extract.

Pterostilbene was found to be active constituents in extracts of the heartwood of *P. marsupium*, used in Ayurvedic medicine for the treatment of diabetes. When administered to streptozotocin-induced hyperglycemic rats, pterostilbene significantly decreased plasma glucose level by 42%. It was also found to be more effective than Trolox as free radical scavenger. Pterostilbene also showed potent cancer chemopreventive activity on mouse mammary gland culture model of carcinogenesis (Rimando *et al.* 2002).

Methodology
General experimental procedures
Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. ^{1}H-, ^{13}C-NMR spectra were measured on a JOEL α-500 spectrometer. Chemical shifts are given in ppm with reference to tetramethylsilane (TMS). Mass spectra were recorded

on JEOL JMS 700 MStation mass spectrometer. Column chromatography was carried out with silica gel 60 (0.040-0.063 mm, Merck), MCI gel CHP20P (75-150 µm, Mitsubishi Chemical Industries Co., Ltd.), Sephadex LH20 (Amersham Pharmacia Biotech) and Chromatorex ODS (30-50 µm, Fuji Silysia Chemical Co., Ltd.). TLC was performed on a precoated silica gel 60 F254 (0.2 mm, aluminum sheet, Merck).

Plant material

Fresh heartwood of *P. marsupium* was collected in August 2010 from Mahendranagar, Kanchanpur, Mahakali, Nepal and shade dried for 3 months at room temperature. The voucher specimen has been deposited at the School of Health and Allied Sciences, Pokhara University, Nepal and Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

Extraction and isolation

The shade dried heartwood of *P. marsupium* (250 g) was extracted twice with 70% MeOH (1 l) and evaporated under reduced pressure to afford extract (25.2 g) which was then dissolved in water and separated into water soluble and insoluble fractions. The water soluble fraction (19.3 g) was subjected to MCI gel CHP20P column (volume 260ml) eluting with water, 40%, 60%, 80% and 100% MeOH to give 8 fractions (1~8). The water eluted fraction 1 (8.7 g) was further subjected to MCI gel CHP20P column (volume 180 ml) eluting with water, 10%, 20%, 30%, 40% and 100% MeOH to give 7 subfractions (1-1~1-7). Fraction 1-2 (1.0 g, water eluent) was applied to Sephadex LH20 (volume 230 ml) afforded 7 fractions (1-2-1~1-2-7). Similarly fraction 1-2-4 (250 mg, 15% MeOH) was subjected to ODS column (volume 230 ml) to give **1** (7 mg), **2** (76 mg) and **3** (8 mg). Fraction 1-4 (399 mg, MeOH eluent) was applied to Sephadex LH20 (volume 210 ml), which afforded 2 fractions (1-4-1~1-4-2). Subfration 1-4-2 (324 mg, 15% MeOH) was also subjected with ODS column (volume 230 ml) to give diastereoisomeric epimers **4a** and **4b** (79 mg). Fraction 8 (94 mg) was applied on silica gel column (Hexane:EtOAc = 1:5, volume 36 ml) to obtain **5** (74 mg).

Results and Discussion

P. marsupium is found in Far-Western and Mid-Western plane areas of Nepal, India and Sri Lanka. Wooden tumbler made up of heartwood of *P. marsupium* is used for drinking water as traditional remedy because of its medicinal property. The wood and bark of *P. marsupium* are known for their anti-diabetic activity (Badkhane *et al.* 2010).

Water soluble fraction of 70% MeOH extract of heartwood of *P. marsupium* afforded five compounds. Compound **1** was obtained as pale yellow amorphous powder, $[\alpha]_D^{21}$ –89.1° (*c* 0.65, H_2O). Comparing ^{1}H-NMR, ^{13}C-NMR (Table 1 and 2) and physiochemical data, it was identified to be metlaline, which was previously isolated from the same plant by Ulises *et al*. (2009). Similarly compound **2** was obtained as pale yellow amorphous powder $[\alpha]_D^{21}$ +208° (*c* 0.74, CH_3OH). Comparison of spectroscopic and physiochemical data it was identified as piyaline (Achari *et al*. 2012). There were some distinct peaks which differentiated between compound **1** and **2**. The *á* proton of the compound **1** appeared at 4.42 ppm while compound **2** at 4.22 ppm in the ^{1}H-NMR. Similarly β protons of compound **1** appeared at 2.17 and 2.20 ppm but these protons of compound **2** appeared at 2.12 and 1.98 ppm. The ^{13}C-NMR of the C-4 of compound **2** seemed 194.1 ppm and same carbon of compound **1** at 193.0 ppm. The *á* and β carbon of compound **2** appeared at 70.0 and 35.8 ppm while that of compound **1** are 68.9 and 36.2 ppm respectively. The carbon of COOH has also two different values, in compound **2** it appeared at 177.4 ppm and in compound **1** at 173.2 ppm. Compound **3**, pale yellow amorphous powder, $[\alpha]_D^{21}$ +167.6° (*c* 0.63, CH_3OH), piyaline methyl ester, was isolated for the first time from natural sources. Achari *et al*. (2012) chemically synthesized piyaline methyl ester previously by suspending **2** with MeOH and TFA and mixture was stirred at ambient temperature overnight.

Compounds **4a** and **4b**, obtained as a diastereomeric mixture, white yellow amorphous powder, $[\alpha]_D^{21}$ +119.2° (*c* 0.84,CH_3OH). The ^{1}H-NMR indicated the presence of two anomeric protons at 4.71 and 4.67 ppm along with other sugar and aliphatic resonances between 3.02 and 4.68 ppm. In the aromatic region two sets of ortho coupled aromatic protons at 6.64, 7.10 and 6.65, 7.03 ppm and pair of A_2B_2 system at 6.51, 7.29 and 6.49, 7.31 ppm respectively. Grover *et al*. (2004) had isolated same diastereomeric pair of marsuposide from *P. marsupium*. Compound **4a** and **4b** gave quasi-molecular ion peak at m/z: 435 $[M+H]^+$ (positive- FAB-MS) and m/z: 433 $[M-H]^+$ (negative-FAB-MS), which

suggested the molecular formula $C_{21}H_{22}O_{10}$. The integral ratio of two distinct peaks at δ_H 7.10 and 7.03 ppm suggested the presence of **4a** and **4b** in the almost equal ration.

Compound **5**, white amorphous powder, pterostilbene (Mallavadhani & Sahu 2003) has been found as a potent antioxidant, anti-diabetic and cancer chemopreventive agent (Rimando *et al*. 2002). The structures of all these compounds are given in Figure 1. The ^{1}H-NMR and ^{13}C-NMR data of isolated compounds are given in Table 1 and Table 2 respectively. All these compounds were identified by comparing spectral and physiochemical data with literature.

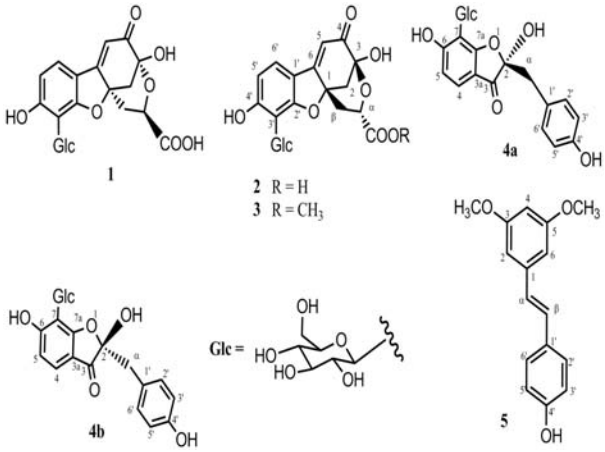

Fig. 1. Structures of isolated compounds

Table 1. ^{1}H-NMR spectroscopic data of isolated compounds (1-5), (*J* values in parentheses)

Carbon no.	**1**[a]	**2**[b]	**3**[b]	**4a**[b] *	**4b**[b] *	**5**[c]
2	2.54, d (11.3) 2.40, dd (1.52, 11.3)	2.55, d (11.3) 2.48, d (11.3)	2.54, d (11.3) 2.40, dd (2.4, 11.3)			7.36, d (8.5)
3						6.80, d (8.5)
4				7.31, d (8.5)	7.29, d (8.5)	
5	6.36, s	6.41, s	6.35, s	6.49, d (8.5)	6.51, d (8.5)	6.80, d (8.5)
6						7.36, d (8.5)
2`				7.03, d (8.5)	7.10, d (8.5)	6.63, d (2.1)
3`				6.65, d (8.5)	6.64, d (8.5)	
4`						6.37, t (2.1)
5`	6.62, d (8.5)	6.69, d (8.8)	6.63, d (8.5)	6.65, d (8.5)	6.64, d (8.5)	
6`	7.52, d (8.5)	7.59, d (8.8)	7.53, d (8.5)	7.03, d (8.5)	7.10, d (8.5)	6.63, d (2.1)
Glc 1	4.71, d (10.0)	4.71, d (10.0)	4.70, d (10.0)	4.71, d (9.8)	4.67, d (9.5)	
Glc 2	4.01, dd (8.8, 10.0)	4.01, dd (8.8, 10.0)	4.01, dd (8.8, 10.0)	4.10, dd (8.5, 9.8)	4.10, dd (8.5, 9.5)	
Glc 3	3.30-3.49, m	3.51-3.70, m	3.30-3.45,m	3.53~3.56, m	3.53~3.56, m	
Glc 4	3.30-3.49, m	3.51-3.70, m	3.30-3.45,m	3.53~3.56, m	3.53~3.56, m	
Glc 5	3.30-3.49, m	3.51-3.70, m	3.30-3.45,m	3.53~3.56, m	3.53~3.56, m	
Glc 6	3.87, d (10.4)	3.83, d (12.4)	3.87, d (12.4)	3.91, dd (12.2, 3.7)	3.91, dd (12.2, 3.7)	
α	4.42, dd (3.4, 12.5)	4.22, dd (3.4, 12.5)	4.47, dd (3.6, 12.2)	4.21, d (8.8), 4.19, d, (8.8)	4.21, d (8.8), 4.19, d, (8.8)	6.99, d (16.4)
β	2.17, t (12.5); 2.20, d (12.5)	2.12, t (12.5); 1.98, d (12.5)	2.18, t (12.2); 1.98, br d (12.8)			6.86, d (16.4)
OCH_3			3.71, s			3.80, s

[a]CD_3OD, [b]D_2O, [c]$CDCl_3$, *assignments may be reversed in the same row.

Table 2. ^{13}C-NMR spectroscopic data of isolated compounds (1-5)

Carbon no.	1[a]	2[b]	3[b]	4a[b] *	4b[b] *	5[c]
1	89.2	89.5	89.0			126.6
2	45.8	45.0	48.5	106.9	107.1	128.0
3	95.8	95.2	95.5	199.8	199.8	155.7
3a				113.3	113.4	
4	193.0	194.1	192.9	127.6	127.7	160.9
5	112.0	112.0	112.0	112.1	112.2	155.7
6	167.3	167.7	167.3	167.4	167.6	128.0
7				107.8	108.1	
7a				172.2	172.6	
1`	114.6	114.8	114.5	125.4	125.5	128.8
2`	166.7	167.7	166.7	132.4	132.8	104.6
3`	110.2	108.4	110.3	115.7	115.8	155.5
4`	165.2	165.8	165.5	155.3	155.3	99.7
5`	113.6	113.1	113.6	115.7	115.8	155.5
6`	126.6	127.6	126.6	132.4	132.8	104.6
Glc 1	75.8	74.2	75.9	73.7	73.8	
Glc 2	72.9	71.5	72.9	71.2	71.6	
Glc 3	79.8	78.6	79.9	78.5	78.6	
Glc 4	71.7	70.5	71.8	70.4	70.9	
Glc 5	82.4	81.4	82.6	81.2	81.3	
Glc 6	62.9	61.6	62.9	61.6	62.1	
α	68.9	70.0	69.2	40.6	40.9	130.0
β	36.2	35.8	36.1			139.8
COO	173.2	177.4	171.8			
OCH_3			52.9			55.4

[a]CD_3OD, [b]D_2O, [c]$CDCl_3$, *assignments may be reversed in the same row.

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