Abundance of yellow alkaloid “Berberine” in the medicinal plant of Tali Kuning (*Tinospora dissitiflora Diels*) collected from Manokwari – Papua Barat

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Cross section of Tali kuning

Type: Climbing plant (liana);
Habitat: virgin forest
Taste: Bitter;
Utilization: Decoction of its Stem used for anti-malaria

Cultivated on polybag

- **Previous works (Wahyudi et al. 2011) revealed that major bioactive compound of MeOH extract, chloroform fraction was berberine.**

^{1}H-NMR spectrum of berberine

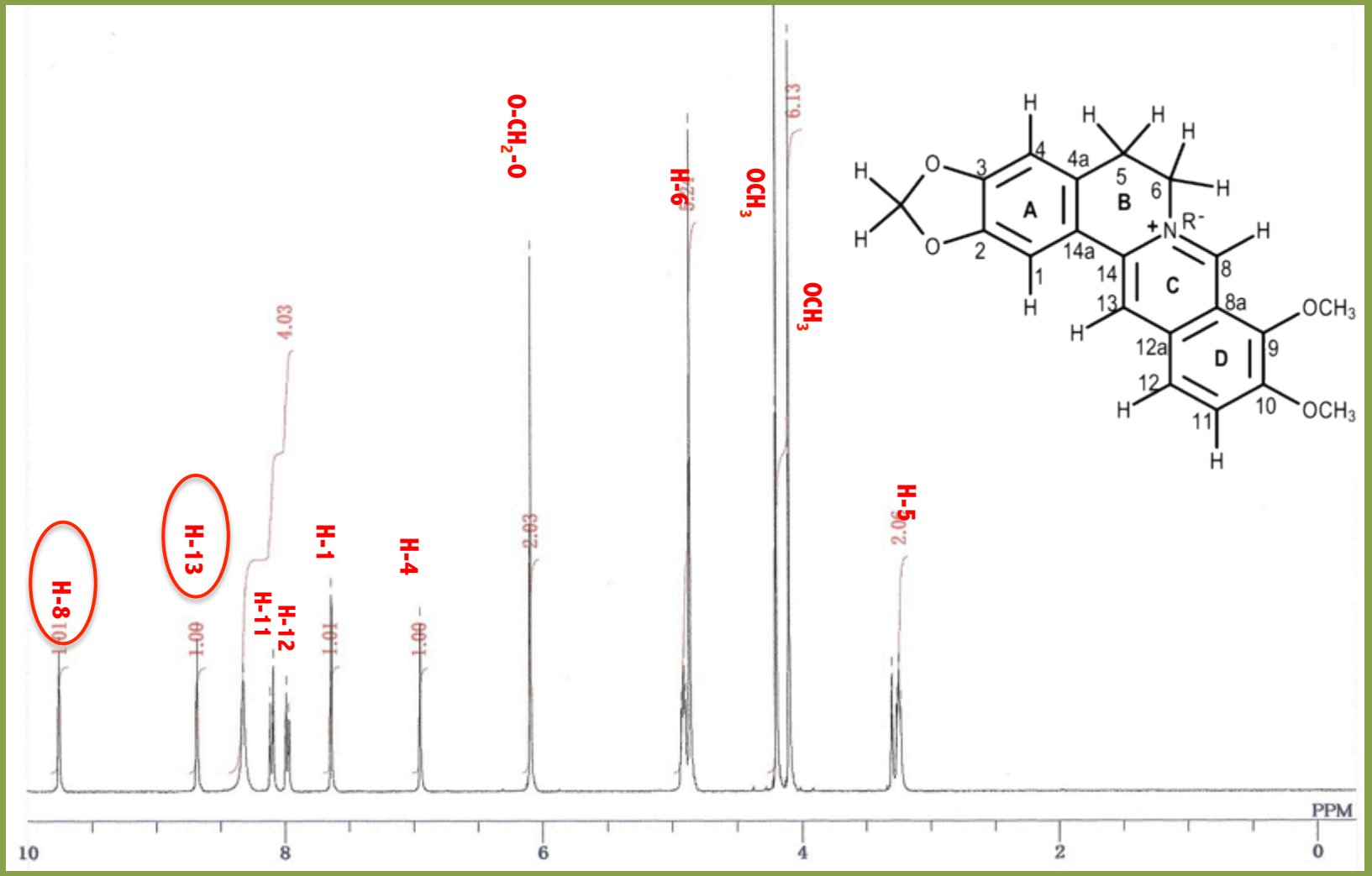


Figure 1. ^{1}H-NMR for berberine from the chloroform fraction of Tali kuning

1. ***The previous experiment*** **highlighted that on ^{1}H-NMR spectrum of berberine, the two proton signal of H-13 and H-8 resonanced at empty region and well separated as single peak, could be used for rapid berberine determination;**

2. **Amur corktree or Kihada (*P. amurense* Rupr) is well reported as good producer of berberine;**

3. **^{1}H-NMR is one of advanced tools in phytochemical analysis, which is capable to do analysis in single step, simplest way, rapid and repeatable, compared to the conventional methods. It has been successfully employed to determine an identity of medicinal herbs and controlling medicinal herb prescriptions.**

Goals

1. To execute the quantitative and qualitative analysis of berberine in the medicinal plants of Tali kuning using ^{1}H-NMR;

2. To evaluate the medicinal value of Tali kuning (*T. dissitiflora* Diels) by comparing with well known berberine's supplier of Amur corktree (*P. amurense* Rupr).

Experiments

Material, Chemicals and tools

- Wood powders from stem of Tali kuning and bark of Amur corktree were used.
- MeOH, anthracene, and berberine chloride standard were purchased from Wako Inc.
- Ultrasonic cleaner, vacuum evaporator and analytical balance etc were also used.

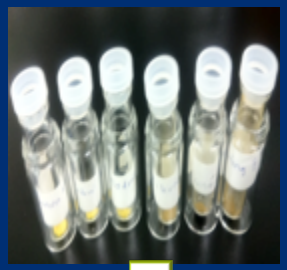
Extraction and sample preparation

- 10 mg wood powders of the two medicinal plants were extracted with MeOH using ultrasonic at room temperature during 30 min.

- The methanolic extracts were evacuated and transferred to the microtubes. These procedure were repeated three times consecutively.

- The extracts were combined and dried up.

- The dried extracts were dissolved in 0.5 ml methanol-*d4* (containing 84.4μg anthracene), and transferred to a NMR tube for analysis.



Evaporated to give dryness and dissolved in MeOH-d_4, and transferred to NMR tubes

- Three replicates were employed
- 500 μg berberine chloride standard were dissolved in methanol-*d4* (containing 84.4μg anthracene) used for berberine standard (control).

NMR variables

- ^{1}H-NMR 500 MHz, scan for 100 times, using the following parameters: 0.187 Hz/point, relaxation delay: 2 Second; pulse width: 4.0 μs, spectra width: 14400 Hz.

- Berberine : δ 8.69 ppm(H-13, s); δ 9.79 ppm (H-8, s); and δ 8.43 ppm (anthracene, s) .

Results and discussion

Qualitative analysis

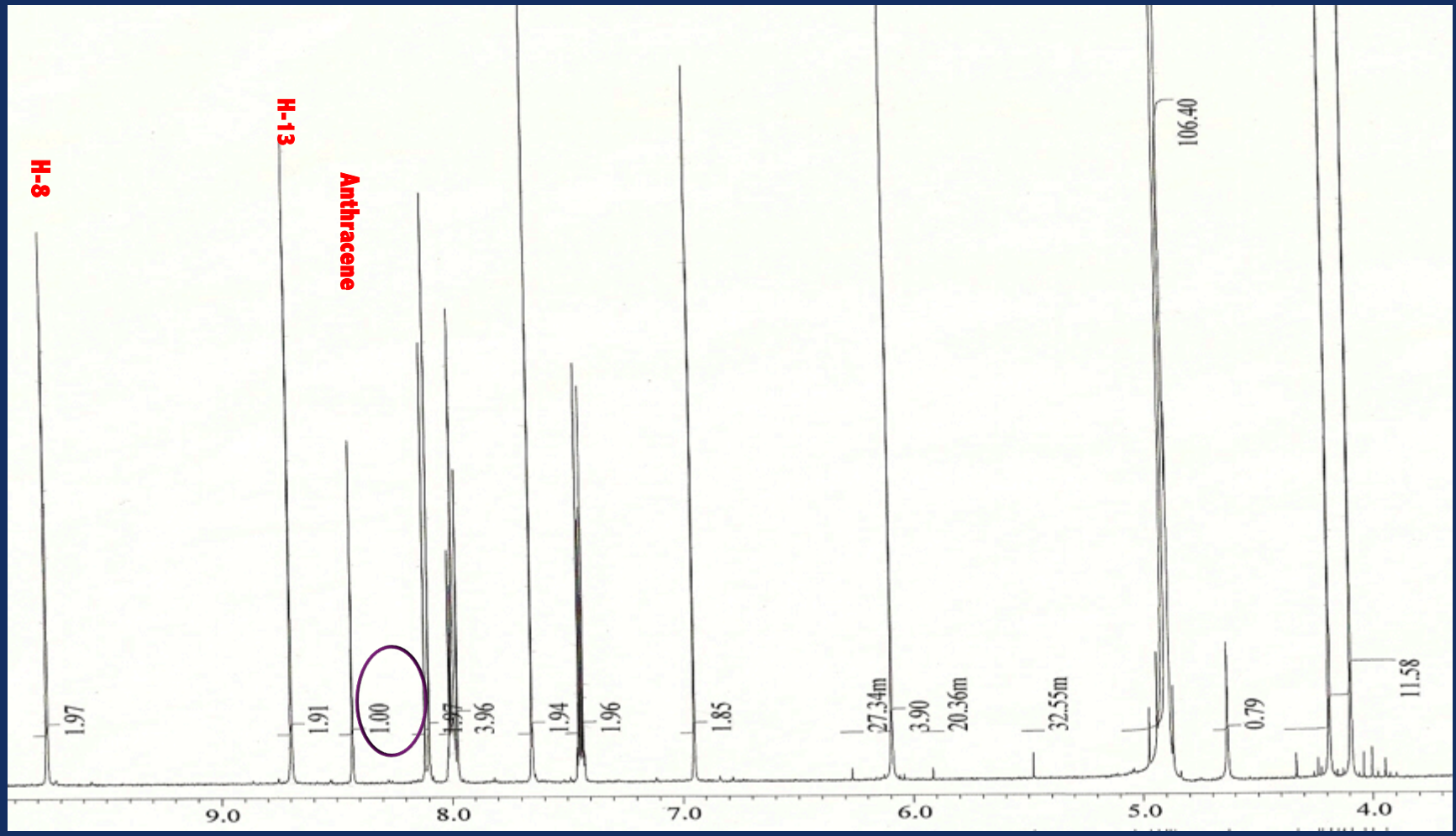


Figure 2. ^{1}H-NMR for berberine chloride standard (control)

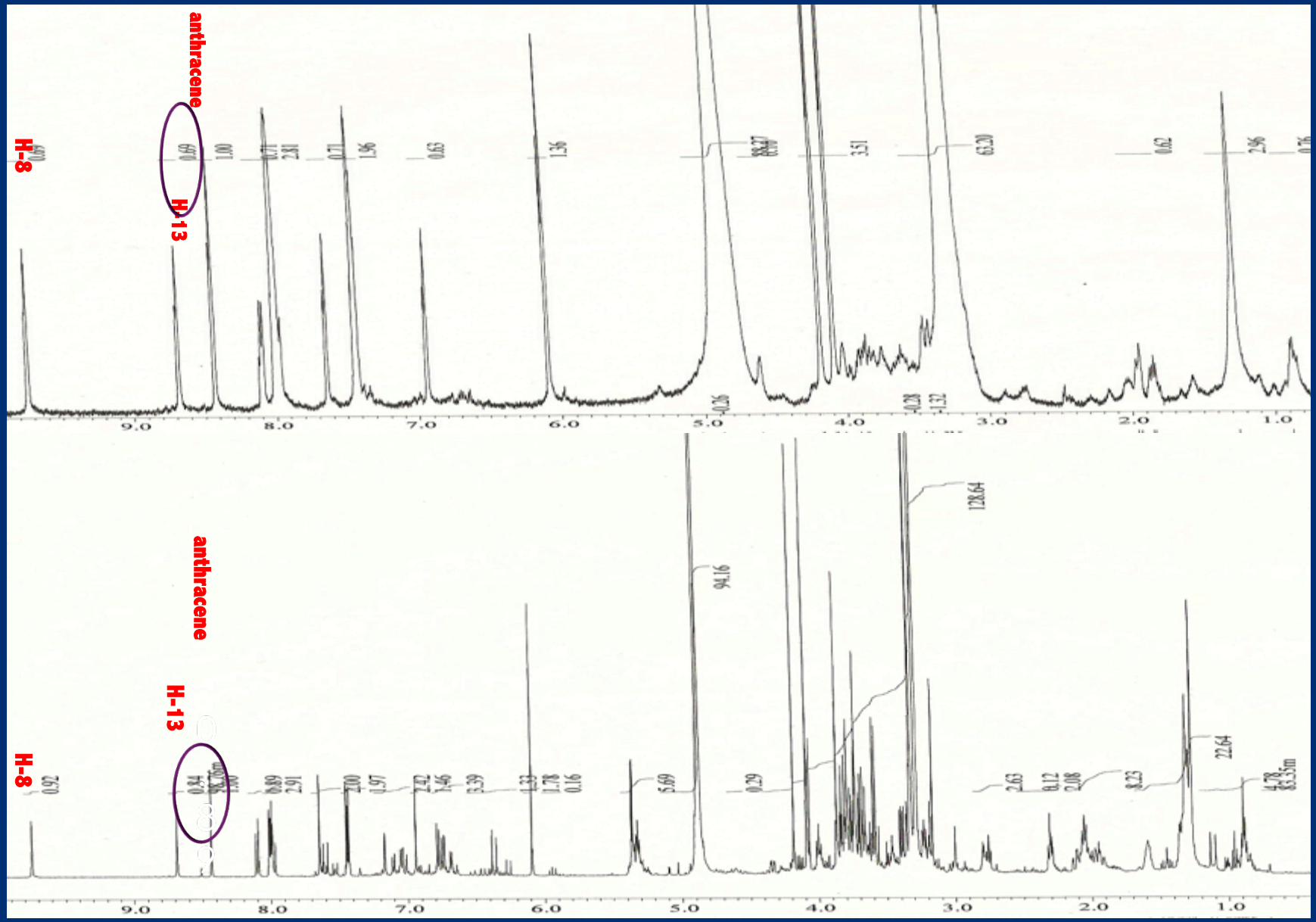


Figure 3. ^{1}H-NMR spectra of berberine recorded from Tali kuning (*above*), and Amur corktree (*below*)

- As been shown in Fig. 2 and 3, the chemical shifts of three targeted protons, H-8, H-13, and anthracene on ^{1}H-NMR spectra at three different conditions, were appeared consistently, at δ 9.79 ppm for H-8, δ 8.69 ppm for H-13, and δ 8.43 ppm for anthracene,.
- The two proton signals of H-8 and H-13 of berberine were appeared as single peak, well separated, and without interferences from the other peaks.
- They were appeared similarly and consistently both on ^{1}H-NMR spectra of Tali kuning and Amur corktree.

Quantitative analysis

- Fig. 2 and 3 also indicate that the integration peaks of internal standard (anthracene) of the control, Tali kuning, and Amur corktree were always constant of 1.

- However, the integration peaks of proton signal of H-13 of berberine at three different conditions were significantly different. For example, the integration peak of H-13 of berberine chloride standard was of 1.91, which was equal to 500 μ g, 0.84 and 0.69 for Amur corktree and Tali Kuning, respectively

- Using those data, therefore, berberine quantities in the two medicinal plants were summarized in Figure 6.

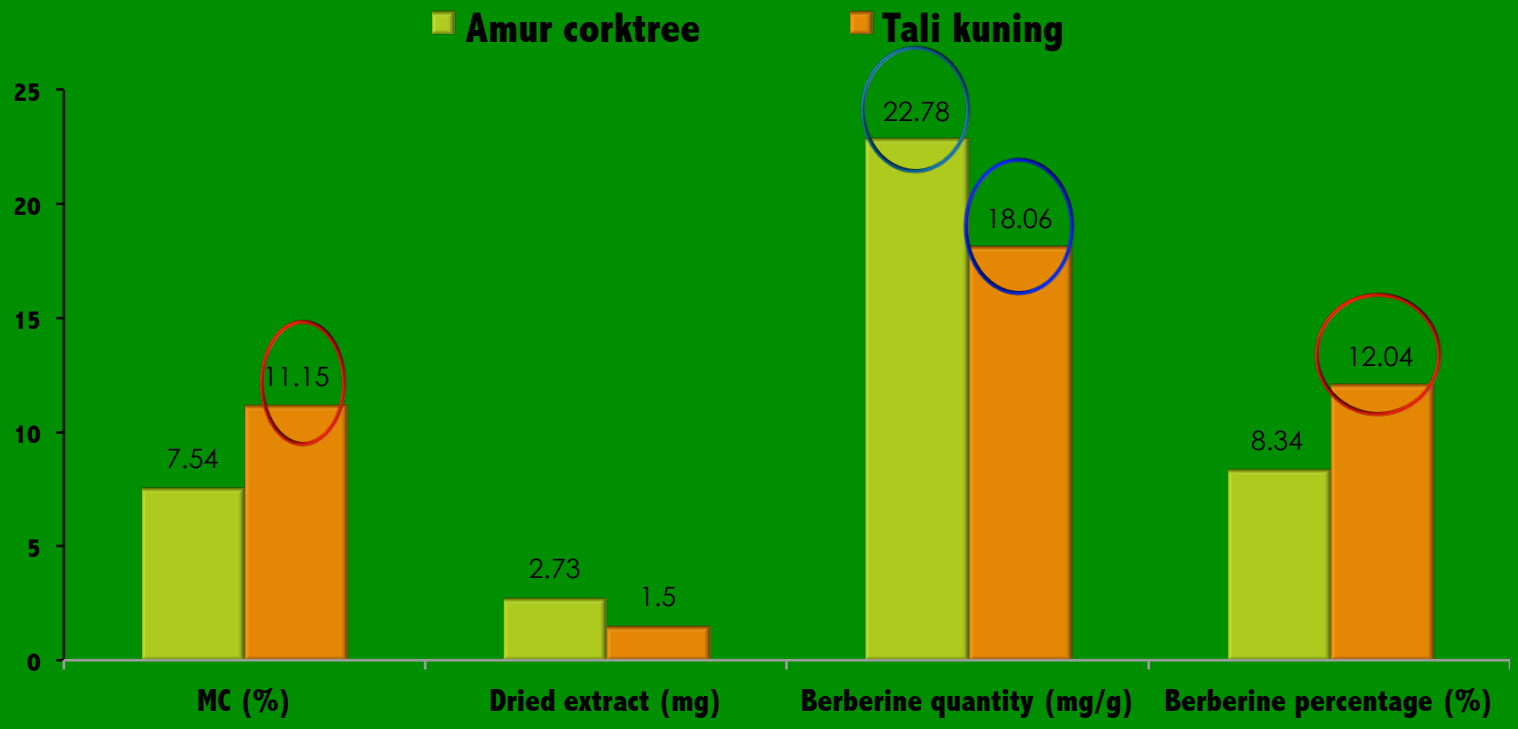


Figure 6. Quantitative analysis of berberine in two medicinal plant determined using integrated intensities of H-13 on ^{1}H-NMR spectra

Conclusion

- Using integrated intensities of H-13 on ^{1}H-NMR spectra, berberine in the medicinal plant of Tali kuning (*T. dissitiflora* Diels) was qualitatively and quantitatively determined, and its quantity is comparable to well-known supplier of berberine, Amur corktree (*P. amurense* Rupr).

- It is suggested that the medicinal plant of Tali kuning could be recommended as a new berberine supplier.