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

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Abstract

Tali kuning (*Tinospora dissitiflora* Diels) is liana or climbing plant growth naturally on the virgin forest of Papuasia. Traditionally, Tali kuning has been using to prevent and treat malaria by indigenous community. Main stem of this plant are produced yellowish compound alkaloid, which is supposed representing major bioactive compound namely as Berberine. ^{1}H-NMR spectra of berberine has special manner particularly coupling proton of H-13 and H-8. Using both proton signals, the abundance of major bioactive compound could be determined. This research was conducting to quantify the berberine concentration. Abundance of alkaloid in this medicinal plant is crucial to be determined first, before further research is taking place or Tali Kuning is being nominated for berberine producer.

Rapid method using ^{1}H-NMR was used to determine berberine quantitative and qualitatively from the crude extract. Tali kuning was collected from Manokwari Papua Barat and powdered with hammer mill. Sonication was employed to extract using methanol at room temperature. Three replicates were used. A comparison was made from worldwide known berberine producer of Amur Corktree (*Phellodendron amurense* Rupr). Moisture content (MC) of wood powders was 12 and 8 percent, for Tali kuning and Amur corktree, respectively. 500 µg authentic berberine chloride were dissolved in 5.4 mL methanol-*d4* (containing 84.4 µg anthracene). ^{1}H-NMR spectra were recorded in methanol-*d4* (99,9%) using JEOL JNM-ECX 500. Each sample was scanned for 100 using the following parameter 0.18 Hz/point, spectral width 14400 Hz, pulse with 4.0 US, relaxation delay 2 sc. Peak areas were used for qualitative analysis and integration of each peach were employed for quantitative analysis.

The results demonstrated that ^{1}H-NMR signal pattern of H-13 and H-8 recorded from Tali Kuning, and Amur corktree were well recorded, and in accordance to the berberine chloride standard. Using peak integration of H-13 and H-8, the berberine quantity in Tali kuning is 18.06 mg/g of dried powder, and 22.78 mg//gr for Amur corktree. Berberine percentage based on the weight of oven-dried-extracts was 8.34% (MC 7.54%) and 12.04% (11,54%) for Amur corktree and Tali kuning. In conclusion, concentration of berberine in medicinal plant of Tali kuning (*T. dissitiflora* Diels) is 12.04%, and it could be recommended for berberine producer from Papua.

Keywords: Berberine, Tali kuning, Malaria, and Papuasia

Introduction

Several methods for quantitative and qualitative analysis of bioactive compounds in medicinal plants have been reported by several researchers, ranging from conventional methods, such as column chromatography (CC), high performance liquid chromatography (HPLC), high performance displacement liquid chromatography (HPDC) to advanced methods, like ^{1}H-NMR, and liquid chromatography (LC) with electron impact or electrospray ionization mass spectrometry (EI/ESI-MS). Advanced analytical instruments, such as ^{1}H-NMR and EI/ESI-MS etc, to determine quantities and qualities of QPAs in medicinal plants directly with extracts have been reported and employed by several researchers. Li et al. (2006; 2009) reported that rapid and simple determinations of protoberberines in the two most famous Chinese medicinal herbals, *Cortex phellodendri* (Huangbai) and *Rhizoma coptidis* (Hunglian), using ^{1}H-NMR were successfully achieved.

The ^{1}H-NMR spectra of berberine, mainly proton signals of H-13 and H-8 at ring C appeared as singlet on the specific spectrum regions (non crowded region) at δ 8.69 – 9.79 ppm, where is no interference from other signals occurred (Li et al., 2006; 2009). A suitable internal standard should be a stable compound with signal in non-crowded region of ^{1}H-NMR spectrum. For this purposes, they used anthracene ($C_{14}H_{10}$), because this compound has a stable signal at δ 8.44 ppm and its integral value is remaining constant within 48 hours.

Protoberberines are very high polar alkaloids and highly soluble in MeOH. In ^{1}H-NMR analysis, methanol-d_4 was used to avoid the interference of the phenolic hydroxyl signals (Li et al., 2006). A distinctive description of the ^{1}H-NMR for berberine and palmatine in methanol-d_4 are illustrated in Fig. 4-1, adopted from Li et al. (2006). This figure illustrates that proton signals of H-13 of berberine and palmatine appear at δ 8.69 and δ 8.79 ppm, respectively, where both resonate at non crowded-region. These clue signals could be used as key information determining protoberberine alkaloids qualification and quantification, mainly for berberine and palmatine, respectively (Li et al., 2006; 2009). The proton H-13 signals of both berberine and palmatine are well separated as singlet, and they could be used as targeted peak signals in identifying these two protoberberine alkaloids. The intensity of the signal peak is proportional to the amount of the compound (Li et al., 2006; 2009).

Li et al. (2009) made the quantitative analysis of four compounds belonging to protoberberine alkaloids in *Rhizoma coptidis*, namely berberine, palmatine, coptisine, and jatorrhizine. The chemical structures of these compounds are shown in Fig. 4-2. On the ^{1}H-NMR analyses, the proton H-13 signals were the targeted ones for identification (Li et al., 2009). These workers suggested that these proton signals could be used as fingerprint for the identification of protoberberine in *Rhizoma coptidis* species as well. The ^{1}H-NMR spectra of berberine highlights that proton signal for H-13 of berberine was quite well separated from the others and at this region between δ 8.6 and 8.9 ppm, no interference by the other signals was occurred. The practitioners or traditional healers rarely use the pure compounds or single herbal drug to treat diseases, but they use a combination of several herbal drugs (Tang et al., 2009). Rapid determination of bioactive

compounds in wood powder or its extract of the medicinal plants using advanced instruments, such as ^{1}H-NMR, is highly desirable and recommended in identifying the herbal medicines and quantifying the main chemical constituents (Li et al., 2006; 2009).

This research outlined in this paper is designed: to execute the quantitative and qualitative analysis of berberine in two medicinal plants using ^{1}H-NMR, and To evaluate the medicinal value of Tali kuning (*T. dissitiflora* Diels) by comparing with well-known berberine supplier of Amur corktree (*P. amurense* Rupr.

Material and Method

Two medicinal plants namely Tali kuning (*Tinospora dissitiflora* Diels) and Amur corktree (*Phelodendron Amurense* Rupr) collected from Manokwari, West Papua, and Nankoku, Kochi, Japan, respectively, were selected in this study. A quantitative and qualitative determination of berberine employed in this study was conducted using the procedure developed by Li et al. (2006; 2009). 10 mg of each powder (moisture content of 7, and 11 % respectively) were extracted with MeOH using sonication at room temperature at duration of 30 minutes for three times. The solvent extracts were combined and dried up using vacuum evaporated to give 2.73 mg dried extract in average for Amur corktree and 1.5 mg in average for Tali kuning. Then, the dried extracts were dissolved in methanol-d_4 (0.5 ml containing 84.4 μg anthracene) and transfered into the NMR test tube directly. Three replicates were used for each medicinal plant. Berberine chloride (500 μg) dissolved in methanol-d_4 (0.5 ml containing 84.4 μg anthracene) was used as reference standard for calculating berberine content in each extract. All chemicals used in this experiment were analytical grade (MeOH, anthracene $C_{14}H_{10}$, methanol-d_4, Wako Ltd, Osaka, Japan), and berberine chloride standard ($C_{20}H_{18}NO_4$ x H_2O) was purchased from Tokyo Chemical Industries, Tokyo. Ultrasonic single frequency (As One Co, Osaka), NMR sample test tube 7 mm in long x 5 mm in diameter x 0.43 mm in wall thickness (Sigma-Aldrich, USA) were used, and ^{1}H-NMR was measured by JEOL JNM-ECX500 (JEOL Ltd., Japan). ^{1}H-NMR spectra were recorded in methanol-d4 (99.9%) using JEOL JNM-ECX500 at 500 MHz. For each sample, 100 scans were conducted with the following resolution: 0.286 Hz/point; spectral width, 32768 Hz; pulse width: 5.4 μs; relaxation delay: 2s.

Results and discussion

The results of rapid determination of berberine in two medicinal plants using ^{1}H-NMR are shown in Fig. 1(A, B, and C). Fig.1(A) demonstrates ^{1}H-NMR spectrum of the authentic berberine chloride. Whereas the ^{1}H-NMR spectra of berberine recorded for the crude extracts of bark powders of Amur corktree and stem wood powders from Tali kuning are shown in Fig. 1 (B), and Fig. (C), respectively. These figures show that the typical berberine protons of H-13 and H-8 on the ^{1}H-NMR spectra are appeared on the downfield regions, at δ 8.69 (1H,s) and δ 9.79 (1H,s) ppm for H-13 and H-8, respectively. More importantly, these two proton signals are detected at the same chemical shifts consistently as illustrated in Fig. 4-3 (A, B, and C), respectively. These results are in well

agreement and similar to those in the ^{1}H-NMR of berberine from *Cortex phellodendri* reported by Li et al. (2006)

Fig. 1 (A) also shows that the proton signal of the internal standard (anthracene) in authentic berberine chloride on the ^{1}H-NMR spectrum is detected at δ 8.44 ppm, and its integrated intensity is 1, which is equal to 84.4 μg of anthracene. Interestingly, the integrated intensities of the internal standard are completely constant at two different situations, which is 1, and appear at δ 8.44 ppm, as demonstrated in Fig. 1 (B) and (C) for Amur corktree and Tali kuning, respectively. As demonstrated in Fig. 1(A), the integrated intensity of proton H-13 of authentic berberine chloride is 1.91, which is equal to 500 μg of berberine. On the other hand, the integrated intensities of protons H-13 of berberine from the crude extracts of bark of Amur corktree shown in Fig. 1 (B) and stem wood powders of Tali kuning in Fig. 1(C) are obviously different. They are 0.84 and 0.69 for Amur corktree and Tali kuning, respectively.

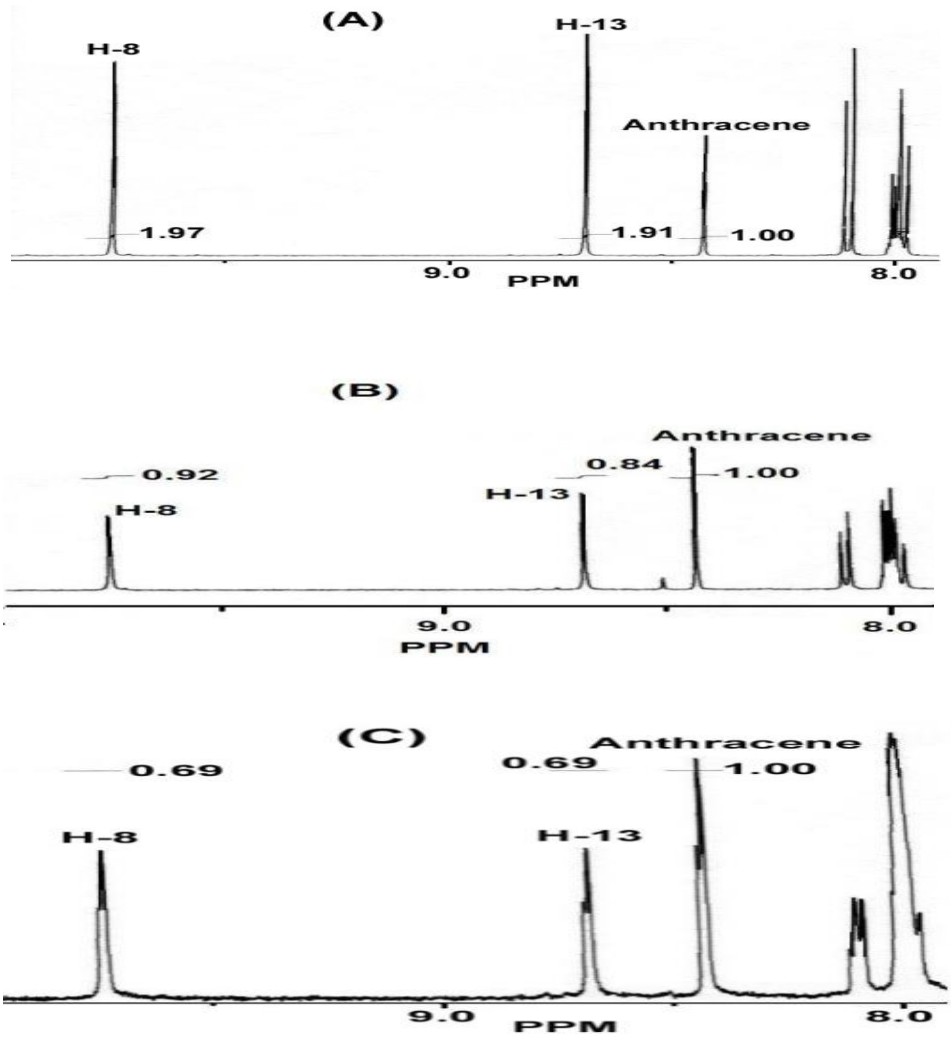

Fig. 1 ^{1}H-NMR of the samples with internal standard (anthracene) in methanol-d_4

(A): Berberine chloride, (B): Extract from Amur corktree, (C): Extract from Tali kuning

From the data mentioned in the previous paragraphs, the berberine contents in the crude extracts from the bark and stem wood powders of Amur corktree and Tali kuning can be summarized in Table 1.

Table 1. Quantitative determination of berberine content in the methanol extractives from bark and stem wood powders of Amur corktree and Tali kuning using integrated intensities of H-13 signals on ^{1}H-NMR spectra

Medicinal plants	Moisture content (%)	Weight of dried extracts (mg) ± SD from 10 mg of air-dried wood meal	Berberine	
			Quantity (μg) ± SD in 10 mg of air-dried wood meal	Percentage based on the weight of oven-dried extract (%) ± SD
Amur corktree	7.54	2.73 ± 0.24	227.8 ± 5.7	8.34 ± 0.96
Tali kuning	11.15	1.50 ± 0.22	180.6 ± 6.4	12.04±1.90

Each value represents the value from three replicates; the following letters are standard deviation (SD)

Table 1 indicates that berberine content of the crude extracts from the bark powders of Amur corktree is 22.78 mg / g of air-dried powders, and it is higher than that (18.06 mg / g) of the crude extracts from the stem wood powders of Tali kuning. However, the percentage of berberine content in Tali kuning based on the weight of dried extract is higher (12.04 %) than that (8.34 %) in Amur corktree (Table 4-1). It is because Tali kuning wood powder has higher moisture content (11.15 %) and less extract content (1.50 mg) than the Amur corktree, 7.5 % and 2.73 mg, respectively.

Berberine quantity in the extracts of the stem powders of Tali kuning was comparable to that from the bark powders in Amur corktree, which is widely known for good producer of berberine (Tang et al., 2009). Based on the weight of oven-dried extracts, berberine concentration in the medicinal plants of Tali kuning (*T. dissitiflora* Diels) was higher (12.04 %) than that (8.3 %) in Amur corktree (*P. amurense* Rupr).

The quantities of berberine in the crude extracts from the two medicinal plants, Tali kuning and Amur corktree, respectively, are comparable to that (14.07 mg / g of air-dried wood meal) in *P. amurense* Rupr collected from Taiwan determined using the similar method of ^{1}H-NMR (Li et al., 2006). It should be considered that the whole stem of Tali kuning was used for determination of berberine content, while only the bark of Amur corktree was used for it. It means that abundance of berberine in Tali kuning is totally considerable. Quantitative differences in berberine contents of the plants are affected by several factors, such as climate, environment, and soil composition, and the occurrence of berberine in some plants is restricted to a specific part of the plant body, but in the other plants is dispersed through the whole body with varying rates in different plant tissues (Grycova et al., 2007).

It is generally claimed that the diverse geographical origin of the plant make the content of alkaloids quite different one to another (Li et al. 2006; 2009). Therefore, a rapid and simple determination of bioactive compounds in the plants using ^{1}H-NMR is necessary to estimate the medicinal values of it. These results support the arguments that ^{1}H-NMR is an effective tool superior to HPLC in detecting and determining the chemical constituents in herbal medicine, and has been utilized for quality control of commercial traditional Chinese one.

Conclusions

It is highly advised that Tali kuning (*T. dissitiflora* Diels) can be recommended for new producer of berberine, because the quantity of berberine in this plant is comparable to that of the Amur corktree (*P. amurense* Rupr). Importantly, ^{1}H-NMR can be employed to detect the bioactive compounds in the medicinal herbs, determine the medical values of it, and maintain the quality control of medicinal plants, as they are rarely sold in single herbal medicine but are sold in mixed ones.

References

Li CY, HJ Lu, CH Lin, TS Wu. 2006. A Rapid and Simple Determination of Protoberberine Alkaloids in *Cortex phellodendri* by 1H-NMR and Its application for Quality Control of Commercial Traditional Chinese Medicine Prescriptions. Journal of Pharmacheutical and Biomedical Analysis 40: 173-178.

Li CY, SI Tsai, AG Damu, TS Wu. 2009. A Rapid and Simple Determination of Protoberberine Alkaloids in *Rhizoma coptidis* by 1H-NMR and Its application for Quality Control of Commercial Prescriptions. Journal of Pharmacheutical and Biomedical Analysis 49: 1272-1276.

Wahyudi, Y Ohtani, H Ichiura. 2011. Berberine in the Medical plant of Tali Kuning (*Tinospora dissitiflora* Diels). Journal of Wood Science 2 (2):100-104.