

PHYTOCHEMICAL STUDY OF *PHELLODENDRON*
AMURENSE BARK FROM VIETNAM

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Abstract

A phytochemical study of *Phellodendron amurense* bark of Vietnamese origin was carried out to achieve a detailed characterisation of its alkaloid composition. A total of twelve alkaloids were isolated and determined, including four tertiary (canthin-6-one, γ -fagarine, skimmianine, and platydesmine), and eight quaternary alkaloids (berberine, palmatine, jatrorrhizine, columbamine, N-methyltetrahydrocolumbamine, N-methylcanadine, N-methylisocorydine (menisperine), and magnoflorine). Centrifugal partition chromatography operating in pH-zone-refining mode was successfully applied for the separation of quaternary isoquinoline alkaloids. The alkaloid N-methyltetrahydrocolumbamine is reported for the first time in the species *P. amurense*, while N-methylcanadine is newly found for the genus *Phellodendron*.

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The results demonstrate that *P. amurense* with Vietnamese origin retains the characteristic to the species alkaloid profile, dominated by protoberberine-type derivatives.

Key words: canthinone, quinoline and isoquinoline alkaloids, pH-zone-refining centrifugal partition chromatography

Introduction. *Phellodendron amurense* Rupr. (Rutaceae), also known as Amur cork tree, is a medicinal species with a well-established role in traditional East Asian medicine. Its stem bark (*Phellodendri amurensis cortex*) is recognized as principal medicinal raw material and is widely used in the treatment of infectious, inflammatory, and chronic pathological conditions [1, 2]. The species is naturally distributed mainly in northern Asia, including northern China and Siberia but has also been reported in Vietnam as an introduced species [3].

The pharmacological activity attributed to *P. amurense* is largely related to its alkaloid-rich phytochemical profile [4, 5]. A wide range of structurally diverse alkaloids have been identified, mainly represented by tertiary and quaternary isoquinoline derivatives such as berberine, palmatine, magnoflorine, and phellodendrine [6, 7]. In addition, quinoline alkaloids such as skimmianine, γ -fagarine and dictamine have also been reported [8, 9].

Despite the extensive studies of alkaloid composition on plant material originating from China, Korea and Japan, the investigations on bark of Vietnamese origin (Hoàng Bá Bắc) are limited and are mainly focused on several major quaternary protoberberine alkaloids [10, 11]. Therefore, the overall alkaloid profile of Vietnamese plant material remains poorly characterised.

Furthermore, the high polarity and ionic nature of quaternary isoquinoline alkaloids create additional methodological limitations related to their separation in complex plant mixtures. In this context, advanced separation techniques such as centrifugal partition chromatography (CPC), particularly when operated in pH-zone-refining mode, has been confirmed as an efficient approach for the isolation of quaternary isoquinoline alkaloids [12, 13]. To date, this approach has been successfully applied to species of *Coptis*, *Corydalis*, and related genera, however, its application to species of the genus *Phellodendron* has not yet been reported [12–14].

The present study aims to characterise the alkaloid composition of the stem bark of *P. amurense* of Vietnamese origin by separating the crude extract into tertiary and quaternary alkaloid fractions. In addition, centrifugal partition chromatography (CPC) operating in pH-zone-refining mode was applied for the selective isolation of quaternary isoquinoline alkaloids.

Materials and methods. General. ^1H NMR spectra were recorded on Bruker Avance II+ 400 and 600 MHz spectrometers using MeOD and CDCl_3 as solvents, with tetramethylsilane (TMS) as an internal standard.

Column chromatography (CC) was performed on neutra alumina (Merck, Aluminiumoxid 90, activity II-III, Brockmann, 70-230 mesh) using petroleum ether:Me₂CO mixtures with increasing polarity as eluents.

Centrifugal partition chromatography (CPC) was performed in pH-zone-refining mode using a FCPC 200 mL chromatograph (Kromaton, France).

Thin-layer chromatography (TLC) was performed on DC-Alufolien Kieselgel 60 F₂₅₄ plates (Merck), while Preparative TLC (PTLC) was conducted on Kieselgel 60 GF₂₅₄ (Merck).

The mobile phases used for TLC and PTLC included petroleum ether:CHCl₃:Me₂CO:MeOH (8:4:1:1, v/v/v/v) and CHCl₃:Me₂CO:NH₄OH (30:8:0.4, v/v/v).

Plant material. The stem bark of *Phellodendron amurense* Rupr. was supplied by the Institute of Medicinal Materials (Ministry of Health, Vietnam), a national institution specialising in medicinal plant materials and their authentication. The material was provided as an authenticated medicinal raw material of Vietnamese origin.

Extraction. Air-dried and powdered bark of *Phellodendron amurense* Rupr. (2.0 kg) was exhaustively extracted with methanol (MeOH) by ultrasonication (3 × 30 L, 30 min per cycle) at 40 °C. The combined extracts were concentrated under reduced pressure to yield a crude MeOH extract (200 g).

A portion of the crude MeOH extract (100.45 g) was dissolved in 3% aqueous HCl (pH 1–2), then left overnight at room temperature. Insoluble non-alkaloid material was removed by filtration, and the acidic filtrate was extracted three times with petroleum ether to eliminate the rest of the non-alkaloid substances.

The purified acidic solution was subsequently basified to pH 9–10 using 25% aqueous NH₄OH and extracted six times with CH₂Cl₂. The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a crude tertiary alkaloid fraction (Ph_III, 1.56 g).

For the isolation of quaternary alkaloids, the remaining aqueous phase was adjusted to pH 5–6 with concentrated HCl, followed by the addition of saturated aqueous KI solution (1:1, v/v). The resulting mixture was extracted three times with CH₂Cl₂, and the combined organic phases were concentrated under reduced pressure to yield a crude quaternary alkaloid fraction (Ph_IV, 1.04 g).

Fractionation and purification of tertiary alkaloids. The tertiary alkaloid fraction (Ph_III) was subjected to column chromatography on neutra alumina, using petroleum ether:Me₂CO mixtures with gradually increasing polarity as eluents, affording six fractions (Ph_III_1–Ph_III_6).

Fraction Ph_III_2 yielded the alkaloid canthin-6-one (**1**) (20.80 mg). Further purification of fractions Ph_III_3 and Ph_III_4 by PTLC, eluting with petroleum ether:CHCl₃:Me₂CO:MeOH (8:4:1:1, v/v/v/v) as a mobile phase, resulted in the isolation of γ-fagarine (**2**) (1.41 mg) and skimmianine (**3**) (1.02 mg), respectively. Platydesmine (**4**) (8.83 mg) was obtained from fraction Ph_III_5 after purification by PTLC using the same mobile phase.

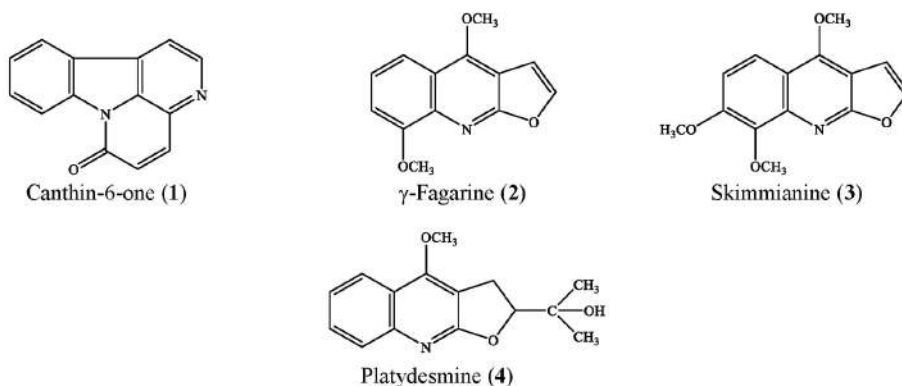


Fig. 1. Structures of the identified tertiary alkaloids

The structures of compounds **1–4** were elucidated by comparison of their ^1H NMR spectroscopic data with those published in the literature [9,15,16] (Fig. 1).

Fractionation and purification of quaternary alkaloids by Centrifugal Partition Chromatography. The CPC fractionation protocol employed in this study was adapted from previously reported methods [12,13]. A biphasic solvent system consisting of $\text{CHCl}_3:\text{MeOH}:\text{H}_2\text{O}$ (4:3:3, v/v/v) was equilibrated in a separatory funnel, and the phases were separated prior to use. The upper aqueous phase, serving as the stationary phase, was acidified with HCl to a final concentration of 60 mM, whereas the lower organic phase, used as the mobile phase, was rendered basic by the addition of triethylamine (TEA) to a final concentration of 5 mM [13].

An aliquot of the crude quaternary alkaloid fraction (Ph_IV, 0.5 g) was dissolved in a 1:1 (v/v) mixture of the acidified upper phase and the neutral lower phase. The separation was carried out on a hydrostatic CPC instrument operated in descending mode. The column was initially filled with the acidified aqueous stationary phase at a flow rate of 30 mL min^{-1} and a rotor speed of 800 rpm. The sample was then injected, and elution was performed with the alkalized organic phase at a flow rate of 5 mL min^{-1} and a rotor speed of 1050 rpm.

Fractions of 10 mL were collected during the initial 65 min, followed by 15 mL fractions, giving a total run time of 231 min. The elution profile was monitored by UV detection at 225, 254, 280, and 290 nm.

Upon completion of the CPC separation, six combined fractions were obtained (Fig. 2).

Fractions Ph_IV_1 and Ph_IV_2 afforded berberine (**5**) (46.5 mg) and palmatine (**6**) (6.80 mg), respectively. Further purification of fraction Ph_IV_3 by PTLC, using $\text{CHCl}_3:\text{Me}_2\text{CO}:\text{NH}_4\text{OH}$ (30:8:0.4, v/v/v) as a mobile phase, yielded jatrorrhizine (**7**) (4.4 mg) and columbamine (**8**) (5.4 mg). PTLC purification of fractions Ph_IV_4–Ph_IV_6 under the same conditions resulted in the isola-

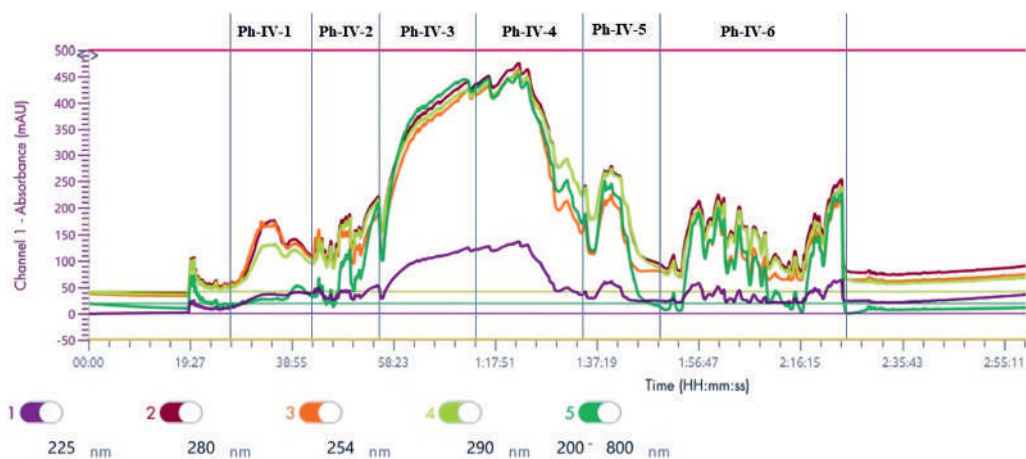


Fig. 2. CPC chromatogram of the quaternary alkaloid fraction (Ph_IV) from *Phellodendron amurense* obtained in pH-zone-refining mode and monitored at 225, 254, 280, and 290 nm

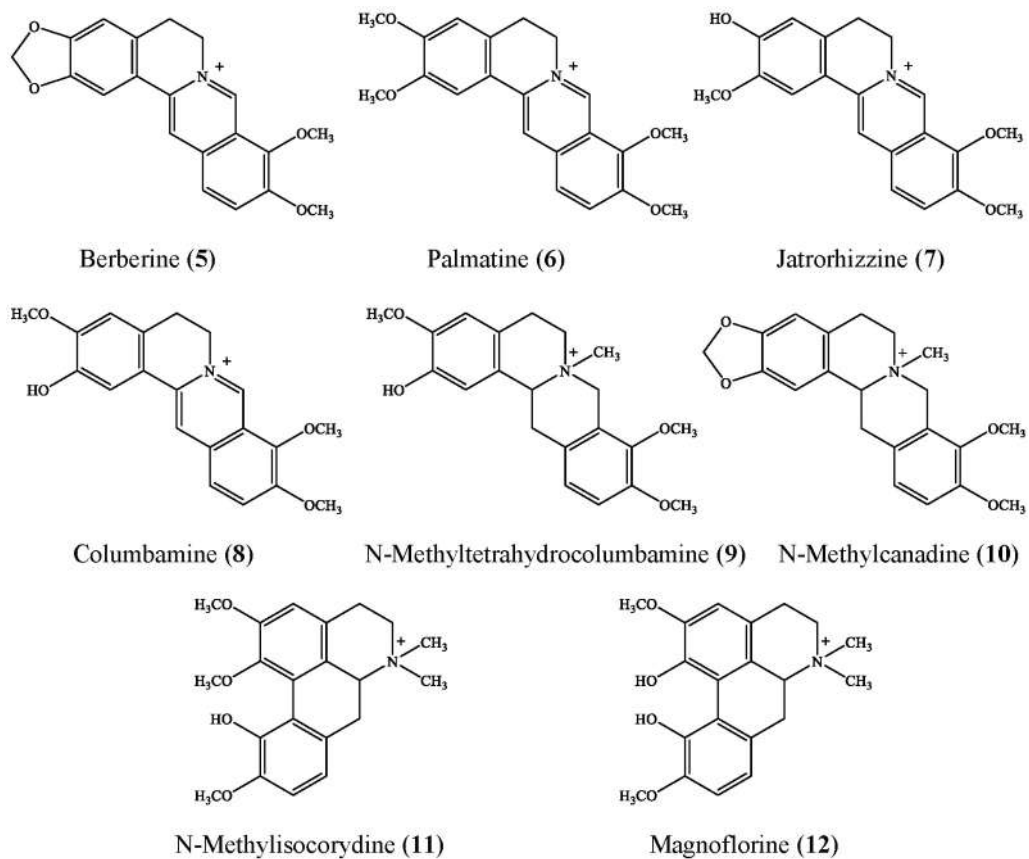


Fig. 3. Structures of the identified quaternary alkaloids

tion of N-methyltetrahydrocolumbamine (**9**) (8.50 mg), N-methylcanadine (**10**) (4.30 mg), N-methylisocorydine (**11**) (7.50 mg) and magnoflorine (**12**) (5.70 mg).

The structures of compounds **5–12** were elucidated by comparison of their ^1H NMR spectral data with those published in the literature [17–20] (Fig. 3).

Results and discussion. The phytochemical study of the bark of *Phellodendron amurense* of Vietnamese origin revealed a structurally diverse alkaloid profile comprising tertiary canthinone and quinoline alkaloids, as well as quaternary protoberberine and aporphine derivatives. To the best of our knowledge, this study provides the first comprehensive characterisation of the alkaloid composition of *P. amurense* bark of Vietnamese origin.

Fractionation of the tertiary alkaloid fraction by column chromatography, followed by PTLC, afforded one canthinone and three quinoline alkaloids: canthin-6-one (**1**), γ -fagarine (**2**), skimmianine (**3**), and platydesmine (**4**). These compounds represent characteristic members commonly reported in Rutaceae species and confirm the expected biosynthetic capacity of the genus *Phellodendron*.

The isolation of quaternary isoquinoline alkaloids requires a distinct separation strategy due to their high polarity and ionic character. In this context, centrifugal separation chromatography (CPC) operating in pH-zone-refining mode has proven to be highly effective for their isolation. The two-phase solvent system $\text{CHCl}_3\text{:MeOH:H}_2\text{O}$ (4:3:3, v/v/v), combined with an acidified stationary phase and an alkaline mobile phase and previously applied for the separation of alkaloids from *Coptis chinensis*, allows for a clear zone separation of some major protoberberine alkaloids [12, 13]. Given the structural similarity of the alkaloids identified in *P. amurense*, the same chromatographic conditions were successfully applied in the present study.

The CPC separation yielded six combined fractions (Fig. 2), from which berberine (**5**) and palmatine (**6**) were obtained directly. The chromatographic profile exhibited clear zone-based separation, with early fractions dominated by these major components, followed by intermediate fractions containing jatrorrhizine (**7**) and columbamine (**8**). Subsequent purification by PTLC afforded these compounds, along with N-methyltetrahydrocolumbamine (**9**), N-methylcanadine (**10**), N-methylisocorydine (**11**), and magnoflorine (**12**) from later, more polar fractions. The successful isolation of these alkaloids confirms the suitability of CPC for the separation of complex mixtures of quaternary isoquinoline alkaloids from *P. amurense*.

The alkaloid composition of the studied material *P. amurense* of Vietnamese origin largely corresponds to previously reported data for the species from its native range, where protoberberine alkaloids such as berberine and palmatine predominate [4, 5]. However, differences were observed in the relative abundance of individual constituents, as well as in the occurrence of certain minor alkaloids as N-methylated tetrahydroprotoberberine derivatives. To the best of our knowledge, the alkaloid **9** is newly found for the species *P. amurense* and **10** for the genus *Phellodendron*.

It should also be noted that phellodendrine, an alkaloid commonly found as a characteristic constituent of *P. amurense*, was not isolated from the studied material of Vietnamese origin. This may be attributed to both analytical limitations related to the efficiency of alkaloid detection and isolation under the applied experimental conditions and factors associated with material origin and handling. Since environmental conditions and habitat specificities strongly influence the accumulation of specific alkaloids, further studies are needed to clarify the phytochemical variability of the species.

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