ChinaRxiv

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-202210.00207

Postprint: Study on Alkaloid Constituents from the Tuberous Roots of Stephania dolichopoda

Authors: Sun Guofeng, Chen Fengzheng, Tian Chong, Cheng Ying, Li Shuhua

Date: 2022-10-27T00:00:00+00:00

Abstract

To investigate the alkaloid constituents in the tuberous roots of Stephania macrantha, total alkaloids were extracted from the tuberous roots of S. macrantha using the acid extraction and base precipitation method, and subsequently separated and purified by silica gel column chromatography and preparative liquid chromatography. The structures of the compounds were elucidated by spectroscopic methods. The results demonstrated that: (1) Eleven compounds were isolated from the total alkaloid extract and identified as sinomenine (1), sinoacutine (2), stepharine (3), roemerine (4), isocorydine (5), corydalmine (6), asimilobine (7), sukhodianine (8), dicentrine (9), 7-oxocrebanine (10), and palmatine (11); (2) In vitro cytotoxicity assays showed that the IC50 values of the total alkaloids from S. macrantha and its main component sinomenine against human lung cancer cell line A549 were 7.5$×10^{-4}$ g·mL^{-1} and 6.59$×10^{-9}$ g·mL^{-1}, respectively. Compounds 2, 3, 4, 7, 8, 9, and 10 were isolated from S. macrantha for the first time. The tuberous roots of S. macrantha contain five types of alkaloids: morphinane, protoaporphine, aporphine, benzyltetrahydroisoquinoline, and proberberine.

Full Text

Preamble

Isolation and Identification of the Alkaloids from Rhizomes of *Stephania macrantha*

Guofeng Sun, Fengzheng Chen, Chong Tian, Ying Cheng, Shuhua Li*
(School of New Energy Materials and Chemistry / Leshan Engineering Research Center for Medicinal Components of Characteristic Agro-Products, Leshan Normal University, Leshan 614000, Sichuan, China)

Abstract

To investigate the alkaloid constituents in the rhizomes of *Stephania macrantha*, total alkaloids were extracted using acid extraction and alkali precipitation methods. The crude alkaloid extract was then separated and purified by silica gel column chromatography and preparative high-performance liquid chromatography (HPLC), and compound structures were elucidated using spectroscopic techniques. The results showed that: (1) Eleven compounds were isolated from the total alkaloid extract and identified as sinomenine (1), sinoactine (2), stepharine (3), reticuline (4), isocorydine (5), corydalmine (6), asimilobine (7), sukhodianine (8), dicentrine (9), 7-oxocrebanine (10), and palmatine (11). (2) In vitro cytotoxicity testing revealed that the total alkaloids from *S. macrantha* and its main component sinomenine exhibited IC_{50} values of 7.5$\times$10^{-4}$ g·mL^{-1} and 6.59$\times$10^{-9}$ g·mL^{-1}, respectively, against human lung cancer A549 cells. Compounds 2, 3, 4, 7, 8, 9, and 10 were isolated from *S. macrantha* for the first time. The rhizomes of *S. macrantha* contain five types of alkaloids: morphinane, proaporphine, aporphine, benzyltetrahydroisoquinoline, and protoberberine.

Keywords: *Stephania macrantha*, alkaloids, chemical constituents, isolation and purification, structure identification, cytotoxicity

---

Stephania macrantha is a species first reported in 1988 (Luo, 1988). It belongs to the subgenus *Tuber Stephania* and section *Tuber Stephania* within the genus *Stephania*. The species is primarily distributed in Yunnan Province, where its distinctive floral morphology has led to its cultivation as an ornamental plant in some households in Yangbi County (Xie, 2014). According to *Flora of China*, the genus *Stephania* (Menispermaceae) comprises approximately 60 species distributed across tropical and subtropical regions of Asia and Africa, with a few species found in Oceania. In China, there are 39 species belonging to three subgenera (*Stephania*, *Pseudostephania*, and *Tuber Stephania*). Among these, plants in the subgenus *Tuber Stephania* are characterized by tuberous roots with high alkaloid content and strong biological activity (Zhu et al., 1983).

Previous research on the subgenus *Tuber Stephania* has focused primarily on *S. kwangsiensis*, *S. epigaea*, and *S. succifera*. Studies on *S. kwangsiensis* have been particularly comprehensive, covering its alkaloid constituents (Min & Zhong, 1980; Cheng et al., 1981; Huang et al., 2018), endophytic fungi (Qing et al., 2016; Zhou et al., 2016; Liu et al., 2017; Tang et al., 2019), and germplasm resources (Qin, 2006). In contrast, fundamental research on *S. macrantha* remains scarce, with only one study on its alkaloid components published in the 1990s (Chen et al., 1994). To further investigate the chemical constituents and biological activities of this plant, we conducted a detailed study on the alkaloids from its rhizomes, resulting in the isolation of eleven compounds: sinomenine (1), sinoactine (2), stepharine (3), reticuline (4), isocorydine (5), corydalmine (6), asimilobine (7), sukhodianine (8), dicentrine (9), 7-oxocrebanine (10), and

palmatine (11). The structures of compounds 1–11 are shown in Figure 1 [Figure 1: see original paper]. Among these, compounds 2, 3, 4, 7, 8, 9, and 10 were isolated from *S. macrantha* for the first time.

1. Instruments and Materials

A rotary evaporator RE212BW-B (Yamato, Japan), cooling water circulation unit CF810C (Yamato, Japan), diaphragm vacuum pump LVS302Z (Welch, USA), preparative HPLC SACID 100 mm (Chengdu Gelai Precision Instruments Co., Ltd.), and Varian INOVA 400 NMR spectrometer were used.

Tuberous roots of *Stephania macrantha* were purchased in March 2019 from Anguo Lengbei Medicinal Materials Co., Ltd. The material was authenticated as the tuberous roots of *Stephania macrantha* H. S. Lo et M. Yang (Menispermaceae) by Associate Professor Ying Cheng of Leshan Normal University. A voucher specimen (No. 20190301) is deposited at the Leshan Engineering Research Center for Medicinal Components of Characteristic Agro-Products, Leshan Normal University.

2. Extraction and Separation

2.1 Extraction of Total Alkaloids

Three kilograms of *S. macrantha* rhizomes were dried at 60 °C, pulverized, and passed through a 10-mesh sieve. The powdered material was placed in a 100 L extraction tank, and 30 L of 3.65$×10^{-3}$ g · mL^{-1} hydrochloric acid solution was added. The mixture was stirred for 6 h at 20 r · min^{-1}, after which the extract was discharged. An additional 20 L of 3.65$×10^{-3}$ g · mL^{-1} hydrochloric acid solution was added to the tank, stirred for 3 h at 20 r · min^{-1}, and discharged. The combined extracts were adjusted to pH 9 with sodium hydroxide solution, then extracted twice with 30 L of dichloromethane. The combined dichloromethane extracts were washed with 20 L of distilled water and concentrated under reduced pressure at 50 °C to yield 95 g of total alkaloids.

2.2.1 Silica Gel Column Chromatography of Total Alkaloids

Eight hundred grams of 200-mesh silica gel was packed into a column using dichloromethane. Eighty grams of total alkaloids was loaded onto the column. Elution was performed with dichloromethane:methanol mixtures of increasing polarity (50:1 → 5:1). Fractions were collected and monitored by TLC, resulting in eight combined fractions.

2.2.2 Preparative HPLC Purification of Alkaloids

Each of the eight fractions obtained from silica gel column chromatography was further purified by preparative HPLC. The sample was dissolved in acidic

water, loaded onto the preparative column, and eluted with an acetonitrile-water gradient (starting at 20% acetonitrile and increasing to 80%). Detection wavelength: 254 nm. Column: C18, 50 mm × 450 mm, 5 μm.

Eluates were collected according to peaks, neutralized with ammonia solution, concentrated to remove acetonitrile, basified with ammonia solution, and extracted with dichloromethane. Following repeated preparative HPLC purification, eleven compounds were obtained in order of elution: 1 (3,100 mg), 2 (850 mg), 3 (81 mg), 4 (296 mg), 5 (126 mg), 6 (109 mg), 7 (225 mg), 8 (76 mg), 9 (168 mg), 10 (527 mg), and 11 (237 mg).

3. Structure Elucidation of Compounds

Compound 1 was obtained as a white amorphous powder that showed an orange-red color with Dragendorff' s reagent, suggesting it is an alkaloid. ESI-MS m/z 330 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.63 (1H, d, J = 8 Hz, H-2), 6.54 (1H, d, J = 8 Hz, H-1), 6.07 (1H, brs, HO-4), 5.47 (1H, s, H-8), 4.35 (1H, d, H-5e), 3.80 (3H, s, OCH_3-3), 3.49 (3H, s, OCH_3-7), 3.17 (1H, m, H-9), 2.43 (3H, s, NCH_3-17). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 194.1 (C-6), 152.3 (C-7), 145.0 (C-3), 144.7 (C-4), 130.4 (C-11), 122.6 (C-12), 118.2 (C-1), 115.1 (C-8), 108.9 (C-2), 56.7 (C-9), 56.1 (3-OCH_3), 54.8 (7-OCH_3), 49.2 (C-5), 47.1 (C-16), 45.9 (C-14), 42.8 (-NCH_3), 40.5 (C-13), 36.0 (C-15), 24.2 (C-10). These NMR data are consistent with those reported for sinomenine in the literature (Zeng & Yin, 2010), identifying compound 1 as sinomenine.

Compound 2 was obtained as a white amorphous powder that showed an orange-red color with Dragendorff' s reagent, suggesting it is an alkaloid. ESI-MS m/z 328 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.55 (1H, s, H-5), 6.75 (1H, d, J = 8 Hz, H-2), 6.66 (1H, d, J = 8 Hz, H-1), 6.33 (1H, s, H-8), 3.89 (3H, s, OCH_3-3), 3.75 (3H, s, OCH_3-6), 3.69 (1H, d, J = 4 Hz, H-9), 3.33 (1H, d, J = 16 Hz, H-10b), 2.98 (1H, m, H-10a), 2.60 (1H, m, H-16b), 2.47 (1H, m, H-16a), 2.45 (3H, s, NCH_3), 2.37 (1H, m, H-15b), 1.75 (1H, m, H-15a). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 181.5 (C-7), 161.7 (C-14), 151.0 (C-6), 145.4 (C-3), 143.3 (C-4), 129.8 (C-11), 124.0 (C-12), 120.5 (C-5), 118.8 (C-1), 109.5 (C-2), 61.0 (C-9), 56.3 (3-OCH_3), 54.9 (6-OCH_3), 47.0 (C-16), 43.7 (C-13), 41.7 (-NCH_3), 37.8 (C-15), 32.6 (C-10). These NMR data are consistent with those reported for sinoactine in the literature (Shen et al., 2016), identifying compound 2 as sinoactine.

Compound 3 was obtained as a brown amorphous powder that showed an orange-red color with Dragendorff' s reagent, suggesting it is an alkaloid. ESI-MS m/z 298 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.03 (1H, d, J = 8 Hz, H-12), 6.89 (1H, d, J = 8 Hz, H-8), 6.64 (1H, s, H-3), 6.41 (1H, d, J = 8 Hz, H-9), 6.29 (1H, d, J = 8 Hz, H-11), 4.29 (1H, m, H-6a), 3.81 (3H, s, OCH_3-1), 3.60 (3H, s, OCH_3-2), 3.45 (1H, m, H-5), 3.15 (1H, m, H-5), 2.77 (2H, m, H-4), 2.40 (1H, m, H-7), 2.30 (1H, m, H-7). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 186.2 (C-10), 153.5 (C-1), 150.0 (C-8, 12), 144.3 (C-2), 135.5 (C-7b), 132.5 (C-3b),

128.2 (C-9, 11), 127.4 (C-3a), 112.2 (C-3), 61.0 (2-OCH_3), 57.7 (C-6a), 56.3 (1-OCH_3), 51.2 (C-7a), 48.2 (C-5), 45.0 (C-4), 26.3 (C-7). These NMR data are consistent with those reported for stepharine in the literature (Gong & Ding, 2006; Zhang, 2009), identifying compound 3 as stepharine.

Compound 4 was obtained as a light yellow amorphous powder. ESI-MS m/z 330 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.77 (1H, s, H-2), 6.73 (1H, d, J = 8 Hz, H-5), 6.59 (1H, d, J = 8 Hz, H-6), 6.54 (1H, s, H-5), 6.37 (1H, s, H-8), 3.85 (6H, s, 6-OCH_3 and 4 -OCH_3), 3.71 (1H, m, H-1), 3.19 (1H, m, H-3a), 3.05 (1H, m, H-9a), 2.80 (3H, m, H-3b, H-9b, H-4a), 2.60 (1H, m, H-4b), 2.40 (3H, s, NCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 145.3 (C-6), 145.2 (C-4), 145.0 (C-7), 143.4 (C-3), 133.0 (C-8a), 130.1 (C-1), 125.0 (C-4a), 120.9 (C-6), 115.6 (C-2), 113.7 (C-5), 110.6 (C-8), 110.4 (C-5), 64.5 (C-1), 55.9 (6-OCH_3), 55.8 (4 -OCH_3), 46.6 (C-3), 42.2 (-NCH_3), 40.9 (C-9), 24.8 (C-4). These NMR data are consistent with those reported for reticuline in the literature (Wang et al., 2008), identifying compound 4 as reticuline.

Compound 5 was obtained as a light yellow amorphous powder. ESI-MS m/z 342 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.84 (1H, s, 11-OH), 6.86 (1H, d, J = 8 Hz, H-8), 6.84 (1H, d, J = 8 Hz, H-9), 6.70 (1H, s, H-3), 3.92 (3H, s, 10-OCH_3), 3.91 (3H, s, 2-OCH_3), 3.71 (3H, s, 1-OCH_3), 3.18 (1H, m, H-6a), 3.05 (2H, m, H-4), 2.88 (1H, d, J = 16 Hz, H-7a), 2.71 (1H, d, J = 16 Hz, H-7b), 2.54 (3H, s, NCH_3), 2.45 (2H, m, H-5). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 151.3 (C-2), 149.5 (C-10), 144.0 (C-11), 142.2 (C-1), 130.2 (C-3a), 130.0 (C-7a), 129.2 (C-11b), 126.0 (C-3b), 120.2 (C-11a), 119.0 (C-8), 111.1 (C-3), 110.0 (C-9), 62.9 (C-6a), 62.1 (1-OCH_3), 56.2 (10-OCH_3), 55.9 (2-OCH_3), 52.7 (C-5), 43.9 (-NCH_3), 35.9 (C-7), 29.3 (C-4). These NMR data are consistent with those reported for isocorydine in the literature (Zhang, 2009), identifying compound 5 as isocorydine.

Compound 6 was obtained as an off-white amorphous powder. ESI-MS m/z 342 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.80 (1H, d, J = 8 Hz, H-11), 6.76 (1H, d, J = 8 Hz, H-12), 6.73 (1H, s, H-1), 6.62 (1H, s, H-1), 4.22 (1H, d, J = 16 Hz, H-8a), 3.89 (3H, s, 2-OCH_3), 3.87 (3H, s, 3-OCH_3), 3.81 (3H, s, 9-OCH_3), 3.59 (1H, m, H-13a), 3.57 (1H, m, H-8b), 3.24 (1H, m, H-6a), 3.20 (1H, m, H-13), 3.16 (1H, m, H-5a), 2.83 (1H, m, H-13), 2.69 (1H, m, H-5b), 2.66 (1H, m, H-6b). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 147.5 (C-3), 147.4 (C-2), 146.6 (C-10), 143.3 (C-9), 129.6 (C-4b), 127.9 (C-12a), 127.2 (C-4a), 126.6 (C-8a), 124.9 (C-12), 114.4 (C-11), 111.3 (C-4), 108.5 (C-1), 60.6 (9-OCH_3), 59.5 (C-13a), 56.1 (2-OCH_3), 55.9 (3-OCH_3), 53.9 (C-8), 51.6 (C-6), 36.2 (C-13), 29.0 (C-5). These NMR data are consistent with those reported for corydalmine in the literature (Yang, 2010), identifying compound 6 as corydalmine.

Compound 7 was obtained as a brown amorphous powder. ESI-MS m/z 268 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.29 (1H, d, J = 8 Hz, H-11), 7.21-7.33 (3H, m, H-8, 9, 10), 6.71 (1H, s, H-3), 3.80 (1H, dd, J = 16, 4 Hz, H-8), 3.59 (3H, s, 1-OCH_3), 3.35 (1H, m, H-6a), 2.99 (2H, m, H-5), 2.77 (2H, m, H-4), 2.70 (2H, m, H-7). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 148.2 (C-2), 142.8 (C-1),

136.2 (C-7a), 132.0 (C-11a), 130.0 (C-3b), 128.7 (C-8), 128.0 (C-9), 127.6 (C-10), 127.3 (C-3a), 127.2 (C-11), 125.3 (C-11b), 114.6 (C-3), 60.4 (1-OCH_3), 53.6 (C-6a), 43.3 (C-5), 37.4 (C-7), 29.0 (C-4). These NMR data are consistent with those reported for asimilobine in the literature (Gong & Ding, 2006; Zhong et al., 2016), identifying compound 7 as asimilobine.

Compound 8 was obtained as an off-white amorphous powder. ESI-MS m/z 355 $[M]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.91 (1H, d, J = 8 Hz, H-11), 7.08 (1H, d, J = 8 Hz, H-10), 6.57 (1H, s, H-3), 6.08 (1H, s, -OCH_2-), 5.95 (1H, s, -OCH_2-), 5.43 (1H, brs, H-7), 3.92 (6H, s, 8-OCH_3 and 9-OCH_3), 3.21 (1H, m, H-6a), 3.16 (2H, m, H-5), 2.71 (1H, m, H-4), 2.70 (3H, s, NCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 153.3 (C-8), 148.4 (C-9), 147.6 (C-2), 143.5 (C-1), 130.8 (C-7a), 129.4 (C-3a), 124.7 (C-10), 124.6 (C-3b), 122.7 (C-11a), 116.7 (C-11b), 113.6 (C-11), 107.8 (C-3), 101.9 (-OCH_2-), 67.7 (C-7), 61.8 (8-OCH_3), 60.3 (9-OCH_3), 56.1 (C-6a), 54.4 (C-5), 43.3 (-NCH_3), 29.3 (C-4). These NMR data are consistent with those reported for sukhodianine in the literature (Shi, 2013), identifying compound 8 as sukhodianine.

Compound 9 was obtained as an off-white amorphous powder. ESI-MS m/z 340 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.67 (1H, s, H-11), 6.78 (1H, s, H-8), 6.52 (1H, s, H-3), 6.08 (1H, s, -OCH_2-), 5.93 (1H, s, -OCH_2-), 3.92 (6H, s, 9-OCH_3 and 10-OCH_3), 3.14 (1H, m, H-6a), 3.07 (2H, m, H-5), 2.65 (2H, m, H-4), 2.61 (1H, m, H-7), 2.60 (3H, s, NCH_3), 2.52 (1H, m, H-7). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 148.2 (C-10), 147.7 (C-9), 146.6 (C-2), 141.8 (C-1), 128.4 (C-7a), 126.7 (C-3a), 126.4 (C-3b), 123.6 (C-11a), 116.6 (C-11b), 111.3 (C-11), 110.5 (C-8), 106.8 (C-3), 100.6 (-OCH_2-), 62.4 (C-6a), 56.1 (9-OCH_3), 55.9 (10-OCH_3), 53.6 (C-5), 43.9 (-NCH_3), 34.2 (C-7), 29.2 (C-4). These NMR data are consistent with those reported for dicentrine in the literature (Peng, 2014), identifying compound 9 as dicentrine.

Compound 10 was obtained as an off-white amorphous powder. ESI-MS m/z 335 $[M]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.78 (1H, d, J = 4 Hz, H-5), 8.27 (1H, d, J = 8 Hz, H-11), 7.63 (1H, d, J = 4 Hz, H-4), 7.14 (1H, d, J = 8 Hz, H-10), 7.01 (1H, s, H-3), 6.31 (2H, s, -OCH_2-), 4.01 (3H, s, 8-OCH_3), 3.95 (3H, s, 9-OCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 181.7 (C-7), 153.6 (C-1), 151.7 (C-2), 151.2 (C-6a), 146.4 (C-8), 146.2 (C-9), 144.6 (C-5), 135.3 (C-7a), 126.1 (C-3a), 125.6 (C-11a), 123.8 (C-11), 123.5 (C-10), 120.0 (C-11b), 117.0 (C-4), 108.4 (C-11b), 102.2 (C-3), 102.0 (-OCH_2-), 62.5 (8-OCH_3), 56.2 (9-OCH_3). These NMR data are consistent with those reported for 7-oxocrebanine in the literature (Peng, 2014), identifying compound 10 as 7-oxocrebanine.

Compound 11 was obtained as a light yellow amorphous powder. ESI-MS m/z 353 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.79 (1H, s, H-8), 8.82 (1H, s, H-13), 8.14 (1H, d, J = 8 Hz, H-11), 8.03 (1H, d, J = 8 Hz, H-12), 7.68 (1H, s, H-1), 7.07 (1H, s, H-4), 4.96 (2H, m, H-6), 4.23 (3H, s, 10-OCH_3), 4.13 (3H, s, 9-OCH_3), 4.01 (3H, s, 2-OCH_3), 3.96 (3H, s, 3-OCH_3), 3.31 (2H, m, H-5). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 153.6 (C-9), 151.6 (C-3), 150.6 (C-2), 146.1 (C-8), 145.5 (C-10), 139.5 (C-13a), 135.0 (C-12a), 130.0 (C-4a), 127.8 (C-12), 124.1 (C-

11), 123.0 (C-8a), 121.0 (C-4b), 111.9 (C-4), 109.6 (C-1), 62.2 (9-OCH_3), 57.3 (3-OCH_3), 57.0 (C-6), 56.7 (2-OCH_3), 56.4 (10-OCH_3), 27.2 (C-5). These NMR data are consistent with those reported for palmatine in the literature (Wang et al., 2008), identifying compound 11 as palmatine.

4. Biological Activity Testing

The cytotoxic activities of the total alkaloids from *S. macrantha* rhizomes and its main component sinomenine were evaluated. The CCK-8 assay was performed according to the literature method (Li et al., 2021). The total alkaloids exhibited an IC_{50} value of 7.5$\times$10^{-4}$ g·mL^{-1} against A549 human lung cancer cells, while sinomenine showed an IC_{50} value of 6.59$\times$10^{-9}$ g·mL^{-1}. These results indicate that both the total alkaloids and sinomenine possess cytotoxic activity against A549 lung cancer cells.

5. Discussion and Conclusion

Previous studies on *S. yunnanensis* (subgenus *Tuber Stephania*) have identified sinoactine as the main alkaloid component, followed by crebanine, which belong to the morphinane and aporphine types, respectively (Peng, 2014). *S. kwangsiensis* primarily contains L-tetrahydropalmatine, a protoberberine-type alkaloid (Min & Zhong, 1980). *S. succifera* mainly contains sinoactine (Zuo et al., 2013) and L-tetrahydropalmatine (He et al., 2017). In the present study, the main alkaloid component of *S. macrantha* was found to be sinomenine, a morphinane-type alkaloid. From a phytochemical taxonomy perspective, the principal alkaloids of several *Stephania* species from the subgenus *Tuber Stephania* in China differ significantly, belonging to morphinane, aporphine, and protoberberine types, which aligns with the findings of Yang & Chen (1994).

This study provides a detailed investigation of the alkaloid constituents from *S. macrantha* rhizomes, leading to the identification of eleven alkaloids, all of which are isoquinoline derivatives. Compound 1 is the major alkaloid component of the plant. Compounds 1 and 2 belong to the morphinane type, compound 3 to the proaporphine type, compound 4 to the benzyltetrahydroisoquinoline type, compounds 5, 7, 8, 9, and 10 to the aporphine type, and compounds 6 and 11 to the protoberberine type. Compounds 2, 3, 4, 7, 8, 9, and 10 were isolated from *S. macrantha* for the first time. Furthermore, cytotoxicity testing of the total alkaloids and the main component sinomenine demonstrated that both exhibit cytotoxic activity against A549 lung cancer cells.

References

Chen, Y., Yuan, T.S., Xu, Y.M., et al., 1994. Studies on the alkaloids of *Stephania macrantha* H. S. Lo et M. Yang. *Acta Botanica Sinica* 36(6), 486–488.
Cheng, G.R., Wang, G.Q., Wen, Y.X., 1981. Studies on non-phenolic alkaloids in Diburong (*Stephania kwangsiensis*). *Acta Pharmaceutica Sinica* 16(2), 49–50.

Deng, Y.C., Xu, H.H., 2005. Studies on insecticidal activities and active ingredients of *Stephania kwangsiensis* Lo. *Scientia Agricultura Sinica* 38(3), 523–527.
Gong, Y.H., Ding, L.S., 2006. ^{13}*C-NMR Analysis of Natural Products*. Yunnan Science and Technology Press, Kunming.
He, J.Y., Dou, X.T., Fang, Y., et al., 2017. Chemical constituents from the tuberous roots of *Stephania succifera*. *Journal of Chinese Medicinal Materials* 40(6), 1335–1338.
Huang, Q.J., Wang, J., Ye, Y., et al., 2018. Determination of alkaloids in Diburong (*Stephania epigaea* Lo) by HPLC. *Guiding Journal of Traditional Chinese Medicine and Pharmacy* 24(19), 81–84.
Li, S.H., Chen, F.Z., Tian, C., et al., 2021. A new monoterpenoid from *Gynura japonica* (Thunb.) Juel. *Chinese Journal of Medicinal Chemistry* 31(12), 994–1000.
Liu, X.Y., Sun, W.B., Chen, J.P., et al., 2017. Inhibitory activity of metabolites of endophytic fungi DBR-12 and DBR-23 from *Stephania kwangsiensis* Lo. *Journal of Henan Agricultural Sciences* 46(3), 75–80.
Luo, R.F., 1988. Four new species and one new variety of the genus *Stephania* (Menispermaceae). *Guihaia* 8(4), 309–314.
Min, Z.D., Zhong, S.M., 1980. Studies on the alkaloids of *Stephania kwangsiensis* H. S. Lo. *Acta Pharmaceutica Sinica* 15(9), 532–537.
Peng, F.Y., 2014. *Chemical Constituents from Stephania yunnanensis*. Master' s thesis, Yunnan University of Chinese Medicine, Kunming.
Qin, Y., 2006. *Genetic Diversity of Stephania kwangsiensis* Lo. Germplasm Resources by ISSR and RAPD Markers. Master' s thesis, Guangxi Normal University, Guilin.
Qing, Z., Zhou, Y.Q., Sun, W.B., et al., 2016. Antimicrobial activity of endophytic fungus *Myrothecium verrucaria* DBR-11 from *Stephania kwangsiensis*. *Journal of Henan Agricultural Sciences* 45(5), 77–81.
Shen, X.J., Dong, J.W., Yang, R.F., et al., 2016. Study on alkaloids of *Stephania epigaea*. *Chemical Research and Application* 28(12), 1730–1734.
Shi, X.L., 2013. *Studies on the Chemical Constituents of Stephania viridiflavens and Bergenia purpurascens*. Master' s thesis, Yunnan University of Chinese Medicine, Kunming.
Tang, X.A., Liu, C.L., Deng, Y.C., et al., 2019. Optimization of fermentation conditions for the production of citrinin from endophytic fungus DBR-9 of *Stephania kwangsiensis*. *Journal of Henan Agricultural Sciences* 48(7), 81–87.
Wang, E.J., Ma, Y.B., Zhang, X.M., et al., 2008. Five alkaloids from vine stems of *Diploclisia affinis*. *China Journal of Chinese Materia Medica* 33(21), 2503–2505.
Xie, D.T., 2014. *A Taxonomic Study of the Genus Stephania Lour. from China*. PhD dissertation, Fudan University, Shanghai.
Yang, D.L., 2010. *Study on the Bioactive Chemical Constituents from the Tubers of Stephania succifera*. Master' s thesis, Hainan University, Haikou.
Yang, H.M., Chen, Y., 1994. Chemosystematic notes on genus *Stephania* (Menispermaceae) in China. *Acta Botanica Sinica* 36 (Supplement), 14–23.
Zeng, L., Yin, W.Q., 2010. Study on chemical constituents of *Mappianthus*

iodoies. *Pharmaceutical Care and Research* 10(6), 418–421.
Zhang, M.S., 2009. *Extract and Separation, Structure Modification and Activity Evaluation of the Alkaloids of Stephania viridiflavens* H. S. Lo et M. Yang. PhD dissertation, Guizhou University, Guiyang.
Zhong, S.H., Fu, Y.H., Zhou, X.M., et al., 2016. Studies on alkaloids of *Stephania epigaea*. *China Journal of Chinese Materia Medica* 41(15), 2838–2842.
Zhou, Y.Q., Qing, Z., Luo, H.Y., et al., 2016. Study on the inhibitory activity of two endophytic fungi of *Stephania kwangsiensis* Lo. *Guangdong Agricultural Sciences* 43(4), 111–116.
Zhu, Z.Y., Feng, Y.X., He, L.Y., et al., 1983. Utilization of medicinal plant resources of the genus *Stephania* of the family Menispermaceae in China. *Acta Pharmaceutica Sinica* 18(6), 460–467.
Zuo, A.X., Zhang, Q.L., Li, L., et al., 2013. Studies on alkaloids of *Stephania micrantha*. *Chinese Journal of Experimental Traditional Medical Formulae* 19(6), 134–137.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.