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IDENTIFICATION OF SECONDARY METABOLITE COMPOUNDS USING GC-MS IN METHANOL EXTRACTS OF KEISSI TUBERS (*Stephania venosa* (Blume) Spreng)

IDENTIFIKASI SENYAWA METABOLIT SEKUNDER MENGGUNAKAN GC-MS PADA EKSTRAK METANOL UMBI BELAJANG KEISSI (*Stephania venosa* (Blume) Spreng).

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ABSTRACT

Traditional medicinal plants are an important source of bioactive compounds that have the potential to be developed as raw materials for health products. One species used by the community in Mambi District, West Sulawesi, for cancer treatment is Belajang Keissi' (*Stephania venosa* (Blume) Spreng). This study aims to identify the secondary metabolites contained in the methanol extract of Belajang Keissi' tubers. The extraction process was carried out using the maceration method with methanol as the solvent, followed by qualitative phytochemical testing and compound profile analysis using GC-MS. The results of the phytochemical test showed that the methanol extract of Belajang Keissi' tubers contained alkaloids and flavonoids. Further GC-MS analysis revealed the presence of several dominant compounds, namely 9,12-octadecadienoic acid (Z,Z)-, methyl ester; trans-13-octadecenoic acid, methyl ester; methyl stearate; 6H-dibenzo[a,g]quinolizine, 5,8,13,13a-tetrahydro-2,3,9,10-tetramethoxy-; and (±)-2,3,3′,4′-tetramethoxy-α-methyl-5-(prop-1-enyl)stilbene. These findings indicate that Belajang Keissi tubers have potential as a source of bioactive compounds that can support the development of traditional medicines based on natural ingredients.

Keywords: *Alkaloids; GC-MS; Identification; Secondary metabolites; Stephania venosa (Blume) Spreng*.

ABSTRAK

Tanaman obat tradisional merupakan sumber penting senyawa bioaktif yang berpotensi dikembangkan sebagai bahan baku produk kesehatan. Salah satu spesies yang dimanfaatkan oleh masyarakat di Kecamatan Mambi, Sulawesi Barat, untuk pengobatan kanker adalah Belajang Keissi' (*Stephania venosa* (Blume) Spreng). Penelitian ini bertujuan mengidentifikasi metabolit sekunder yang terkandung dalam ekstrak metanol umbi Belajang Keissi'. Proses ekstraksi dilakukan melalui metode maserasi menggunakan pelarut metanol, diikuti uji fitokimia kualitatif serta analisis profil senyawa menggunakan GC–MS. Hasil uji fitokimia menunjukkan bahwa ekstrak metanol umbi Belajang Keissi' mengandung alkaloid dan flavonoid. Analisis GC–MS lebih lanjut mengungkap keberadaan beberapa senyawa dominan, yaitu 9,12-octadecadienoic acid (Z,Z)-, methyl ester; trans-13-octadecenoic acid, methyl ester; methyl stearate; 6H-dibenzo[a,g]quinolizine, 5,8,13,13a-tetrahydro-2,3,9,10-tetramethoxy-; serta (±)-2,3,3′,4′-tetramethoxy-α-methyl-5-(prop-1-enyl)stilbene. Temuan ini menunjukkan bahwa umbi Belajang Keissi' memiliki potensi sebagai sumber senyawa bioaktif yang dapat mendukung pengembangan obat tradisional berbasis bahan alam.

Kata Kunci: *Alkaloid; GC-MS; Identifikasi; Senyawa metabolit sekunder; Stephania venosa (Blume) Spreng.*

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INTRODUCTION

Indonesia is one of the megabiodiversity countries with enormous floristic diversity, making it an important source for the exploration of medicinal plants and bioactive compounds (Wirasisya et al., 2023). Various ethnobotanical studies and literature reviews in recent years have reported hundreds to thousands of plant species used by traditional communities in Indonesia to treat various ailments such as infections, digestive disorders, and chronic diseases, and many of these species have shown pharmacological activity in in vitro and in vivo tests (Mutiah et al., 2025). This biodiversity potential makes Indonesia an important source for the exploration of bioactive compounds from medicinal plants (Illian et al., 2021).

Plants produce a variety of secondary metabolites that function as bioactive compounds with specific biological activities. This group of metabolites, such as alkaloids, terpenoids, steroids, phenolics, flavonoids, and saponins, plays an important role in the development of natural-based medicines (Chaachouay & Zidane, 2024). Therefore, the exploration of secondary metabolites is a strategic step in discovering new candidate compounds with pharmacological potential.

One of the most intensively studied families of medicinal plants is Menispermaceae. This family is described as a group of climbing plants (lianas) and several shrubs that are widely distributed in tropical and subtropical regions; various reviews and modern phylogenetic studies report the size of the family to be in the range of ~440–520 species divided into approximately 70–72 genera, making it an important source of pharmacologically potent benzyl-isoquinoline alkaloids. (Becker et al., 2024; Laksana et al., 2025; Shi et al., 2025). The genus *Stephania* (Family Menispermaceae) is known to be rich in various alkaloids, especially isoquinoline types such as aporphine, protoberberine, bis-benzylisoquinoline, and hasubanan. A comprehensive review states that these compounds exhibit diverse pharmacological activities, including antimicrobial, antiviral, antitumor, antioxidant, antidiabetic, and neuroprotective effects (Wang et al., 2022). For example, research on *Stephania yunnanensis* identified two aporphine alkaloids (crebanine and stephanine) that possess potent anti-inflammatory and analgesic effects (Cui et al., 2023). Meanwhile, a comparative cross-species study of Stephania from China revealed significant variation in alkaloid profiles, including palmatine, stephanine, and other stephanine derivatives, suggesting the potential of certain genotypes for pharmaceutical applications (Qi et al., 2023). Additionally, the isolation of hasubanan alkaloids from *Stephania longa*, such as stephalonester A and B, demonstrated anti-inflammatory activity through the suppression of TNF-α and IL-6 production (Liu et al., 2022). Bioactivity studies also show that in *Stephania dielsiana*, aporphine alkaloids (oxostephanine, thailandine, stephanine) and tetrahydroprotoberberine exert cytotoxic effects on cancer cells and antiplasmodial activity (Knockleby et al., 2020).

In Indonesia, the Mambi ethnic group in West Sulawesi uses *Stephania venosa* (Blume) Spreng., known as Belajang Keissi', as a traditional medicine, especially for cancer (Jumadi et al., 2012).

Although various *Stephania* species have been reported to have pharmacological activity, data on the composition of secondary metabolites in *S. venosa* from West Sulawesi is still very limited. To date, there have been few reports on the identification of compounds in methanol extracts of *S. venosa* tubers using GC–MS, so information on their chemical potential has not been fully revealed. Therefore, this study aims to identify secondary metabolites in the methanol extract of *Stephania venosa* (Blume) Spreng. tubers from West Sulawesi as a first step in supporting the utilization and development of natural-based medicines.

MATERIALS AND METHODS

Research Object

The object of this study is Keissi' Belajang Tubers obtained from Mambi District, Mamasa Regency, West Sulawesi Province (2°57'14.8"S 119°10'13.2"E).

Tools and Materials

The tools used in this study were a set of maceration tools, a Buchner funnel, a stirring rod, vials, a chamber, capillary tubes, fractionation tools, vacuum liquid chromatography, pressure chromatography (KKT), a spoid, tweezers, scissors, a vacuum pump, a hot plate (Stuart®), stand and clamp, evaporator (Hahn Shin®HS2005VN), UV lamp VL-4 LC 254-356 nm, analytical balance (Cheetah®FA2204B), *Stuart* type SMP 11 *melting point*, and *Shimadsu QP2010 pyrolysis* GC-MS instrument.

The materials used were fine powder of *S. venosa* (Blume) Spreng tubers, n-hexane, chloroform, ethyl acetate, acetone, methanol, Lieberman-Buchard reagent, Wagner and Mayer reagent, and iron(III) chloride ($FeCl_3$ 1%) reagent. Merck 60 silica gel (0.2-0.5 mm), Merck 60 GF_{245}silica gel, Merck 60 silica gel (0.063-0.200 mm), KLT plates, aluminum foil, and Whatman filter paper.

Method

Sample Preparation and Extraction

Samples of *Stephania venosa* (Blume) Spreng tubers that had been collected were washed thoroughly, cut into small pieces and dried, then ground using a blender to obtain a coarse powder. A total of 2 kg of *Stephania venosa* (Blume) Spreng tuber powder was macerated using methanol for 3 x 24 hours, where every 1 x 24 hours the extract was stored and stirred occasionally. The macerate was then filtered using a Buchner funnel lined with Whatman No. 42 filter paper. The methanol extract was then concentrated using an evaporator at 45°C until a thick methanol extract was obtained.

Preliminary Test

Preliminary tests (group tests) were conducted on the concentrated methanol extract obtained with various reagents, including Mayer and Wagner ($HgCl_2$ 0.05 M and KI 0.30 M) for qualitative alkaloid testing, iron (III) chloride reagent ($FeCl_3$) 1% for qualitative testing of phenolics/flavonoids, and Lieberman-Burchard ($(CH_3CO)_2O$ anhydrous and H_2SO_4 concentrated) for qualitative testing of triterpenoids and steroids.

Fractionation

The fractionation method is based on the polarity of the eluent used as the mobile phase, which attracts compounds with similar polarity and passes through silica, which functions as the stationary phase, thereby separating the compounds into several fractions (Aziz et al., 2022).

The concentrated methanol extract was first identified using thin-layer chromatography (TLC) by applying the diluted extract to a silica gel 60 GF154 coated aluminum TLC plate with various eluents at different ratios to determine the appropriate solvent and ratio for vacuum liquid chromatography. It was then detected under a 254 nm and 365 nm UV lamp. Based on the TLC results, good separation was obtained at an ethyl acetate:n-hexane eluent ratio of 8:2.

The fractionation process was carried out using vacuum liquid chromatography. This was done prior to identification so that there would not be too many peaks appearing during chromatogram reading, and the compounds obtained would be purer. A total of 30 g of methanol extract of *Stephania venosa* (Blume) Spreng tubers in paste form was first impregnated with silica gel 60 (0.2-0.5 mm) until it was evenly mixed ly and sand-like in form. The impregnated silica was placed in a vacuum column. This fractionation stage used silica gel 60 GF_{254} as the stationary phase and eluent as the mobile phase using various solvents with increased polarity in a gradient *(Step Gradient Polarity)*. The 39 main fractions obtained were identified using TLC with a ratio of ethyl acetate: n-hexane (8:2) to determine the combined fractions based on the same spot profile, and 9 combined fractions were obtained.

Fraction C was selected for identification because it had good separation spots, crystals were present on the vial walls during evaporation, and it had the heaviest weight (Kowalska & Sajewicz, 2022).

Identification

The fractions were tested using 1% $FeCl_3$, Mayer, Wagner, and Liebermann-Buchard reagents to determine the class of secondary metabolites contained in the

isolates, followed by quantitative identification using a GC-MS instrument. GC-MS analysis was performed using a Shimadzu GCMS-QP2010 Ultra instrument equipped with an Rtx-5 column measuring 30 meters in length, 0.25 mm in inner diameter, and 0.25 µm in film thickness, consisting of diphenyl dimethyl polysiloxane. The temperature protocol was set as follows: starting at 60°C, maintained stable for 2 minutes, then increased to 280°C at a rate of 3°C per minute. The injection temperature was configured to 260°C using split mode. Helium was used as the carrier gas, with a constant flow rate of 1.0 mL/minute. The entire process lasted 50 minutes. Data collection and analysis were performed using LabSolutions DB/CS software. Quality control steps included baseline correction, peak deconvolution, and signal-to-noise ratio filtering to ensure reliable peak detection. Peak identification was performed by comparing the obtained mass spectrum with reference spectra from the NIST or Wiley library, using a match quality threshold typically above 85% to confirm the identity of the compound.

RESULTS AND DISCUSSION

1. Preliminary Testing

The methanol extract obtained was subjected to preliminary testing to determine the class of secondary metabolites contained in the methanol extract of *Stephania venosa* (Blume) Spreng tubers. The testing was performed using several reagents, namely Wagner, Liebarman-Burchard, Iron (III) chloride ($FeCl_3$) 1%, and Mayer. The test results are shown in Table 1. These findings are consistent with previous reports that the *Stephania* genus is known to be rich in isoquinoline alkaloids, especially berberine derivatives such as palmatine and tetrahydropalmatine. The presence of alkaloids and flavonoids at this early stage validates the biological potential of the sample, including antioxidant, antibacterial, or anticancer activities as reported in other *Stephania* species. Thus, the preliminary test results provide a strong basis for further fractionation and compound identification (Qi et al., 2023).

Table 1. Results of Methanol Extract Group Testing of *Stephania venosa* (Blume) Spreng Tubers

Reagent	Results	Notes
Wagner	Yellow → Brown Precipitate	(+) Alkaloid
Lieberman-Burchard	Yellow → greenish yellow	(-) Terpenoids & Steroids
$FeCl_3$ 1%	Yellow → Green	(+) Flavonoids
Mayer	Yellow → White precipitate	(+) Alkaloids

Fractionation

The fractionation process performed on the methanol extract of *Stephania venosa* (Blume) Spreng tubers was the Vacuum Liquid Column Chromatography (VLCC) method. Fractionation was performed prior to identification so that the compounds identified were more Before fractionation with VLCC, thin-layer chromatography (TLC) analysis was first performed to determine the appropriate eluent for VLCC. Based on TLC, it was found that the n-hexane:ethyl acetate (2:8) eluent showed a good spot separation pattern.

Based on separation using silica gel 60 and a gradient eluent of n-hexane to methanol, 39 initial fractions were obtained, which were then tested with TLC to obtain 9 combined fractions (Table 2). Fraction C was selected for further analysis because it showed the most dominant and consistent spot pattern and indicated the presence of a major component with potential as a bioactive compound.

In terms of methodology, VLCC was quite effective for initial separation, but this technique is still less precise than modern methods such as *preparative HPLC*, which can provide higher resolution and sharper separation. Nevertheless, VLCC remains relevant as an efficient and economical method for the initial exploration stage, especially in research on local biological resources.

Table 2. Results of VLCC fraction combination

Fraction	Combined	Weight (g)
1	A	0.1191
6	B	0.1446
7-10	**C**	**1.7636**
11-14	D	3.2876
15-20	E	3.9144
21-22	F	0.3736
23-30	G	1.6471
31-37	H	9.2611
38-39	I	6.2600
Total		**26.7711**

Identification

The VLCC fraction C results were then selected for identification using GC-MS in order to determine more specifically the compounds contained in the methanol extract of *Stephania venosa* (Blume) Spreng tubers. The results of the identification of secondary metabolites in the methanol extract of *Stephania venosa* (Blume) Spreng tubers can be seen in Table 3.

Table 3. Results of the identification of secondary metabolite compounds in methanol extracts *of Stephania venosa* (Blume) Spreng *tubers* using GC-MS

No	Retention Time	Chemical Component	Area
1	12.336	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	3.76
2	12,370	trans-13-Octadecenoic acid, methyl ester	6.03
3	12,407	trans-13-Octadecenoic acid, methyl ester	7.68
4	12,530	Methyl stearate	4.25
5	19,706	6H-Dibenzo[a,g]quinolizine, 5,8,13,13a-tetrahydro-2,3,9,10-tetramethoxy-, (±)-	43.77
6	20,920	2,3,3',4'-Tetramethoxy-a-methyl-5-(prop-1-enyl)stilbene	0.33

Based on the GC-MS analysis results on fraction C, there were 6 secondary metabolite compounds such as fatty acids, alkaloids, and stilbenes that were successfully determined. The major compounds were 6H-Dibenzo[a,g]quinolizine,5,8,13,13a-tetrahydro-2,3,9,10 tetramethoxy-,(±)- with the trivial name Tetrahidropalmatin, which has an area of 43.77%. This compound belongs to the berberine alkaloid group, isochinoline alkaloid class, alkaloid derivative (PubChem CID: 5417). Tetrahydropalmatine is a berberine derivative isoquinoline alkaloid known to have important pharmacological activities, such as analgesic, sedative, antibacterial, and anticancer potential through the mechanism of proliferation inhibition and apoptosis induction. The dominance of THP in fraction C reinforces the hypothesis that *S. venosa* tubers have therapeutic potential, particularly in the context of the traditional use of this plant as an herbal medicine related to the treatment of pain, infections, or degenerative diseases.

The presence of tetrahydropalmatine in *S. venosa* extract is consistent with previous reports on the *Stephania* genus, which is known to be rich in isoquinoline alkaloids. These alkaloids have biological activities as antibacterial, antimalarial, analgesic, and antitumor agents (Bhagya & Chandrashekar, 2018) . Alkaloids found in the genus Stephania include 11-hydroxypalmatine, glabradine, gindarudin, cepharatine, and fangchinoline (Semwal et al., 2010 ; et al., 2012; Semwal et al.,2015; Gulcin et al., 2010). Additionally, from the genus *Stephania yunnanensis*, the alkaloid compounds Tetrahidropalmatin and palmatin were discovered, which are used

as antibiotics to inhibit the growth of *methicillin-resistant Staphylococcus aureus* bacteria (Shi et al., 2015) . Thus, the results of this study support the traditional use of *S. venosa* as a cancer treatment by the Mamasa community in West Sulawesi, as the dominant compounds found do indeed have relevant biological activity.

This study successfully identified secondary metabolite components in *Stephania venosa* tuber methanol extract using a fractionation and GC–MS approach. The main finding, namely the dominance of tetrahydropalmatin, contributes significantly to the phytochemical literature on the *Stephania* genus and can serve as a basis for further development in the field of pharmacy, particularly as a candidate for active ingredients in herbal medicines. This study is the first report to evaluate the GC–MS profile of *Stephania venosa* (Belajang Keissi') tubers originating from Sulawesi. Information on its chemical composition was previously unavailable, so these results enrich the phytochemical data on local Indonesian plants and can serve as a reference for further exploration.

This study is still limited to initial identification based on GC–MS and has not been accompanied by the purification of individual compounds. This study also did not conduct *in vitro* or *in vivo* bioactivity tests to confirm the hypothesized pharmacological effects. These limitations open up opportunities for further research, including the isolation of pure compounds, structure validation using NMR, and specific bioactivity tests.

CONCLUSION

Based on the results obtained, it can be concluded that qualitative testing of *Stephania venosa* tuber methanol extract revealed the presence of alkaloids and flavonoids, while quantitative testing of fractions analyzed using GC-MS revealed the presence of secondary metabolites such as fatty acids, alkaloids, and stilbenes. The fraction contained the compound 6H-Dibenzo[a,g]quinolizine, 5,8,13,13a-tetrahydro-2,3,9,10-tetramethoxy-,(±)- with the trivial name Tetrahidropalmatin, which has an area of 43.77% and is the most abundant component of the fraction.

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