EJTAS
ISSN 2786-7447
EUROPEAN JOURNAL OF THEORETICAL AND APPLIED SCIENCES
OPEN ACCESS
PEER REVIEWED

Phytochemical Analysis and Toxicity Test of Secondary Metabolite Compounds in Methanol Extract from Tuber of Belajang Keissi' (*Stephania venosa* (Blume) Spreng)

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Suggested Citation

Salempa, P., Salnus, S., Hasri, Abbas, G.H., & Adimuntja, N.P. (2024). Phytochemical Analysis and Toxicity Test of Secondary Metabolite Compounds in Methanol Extract from Tuber of Belajang Keissi' (*Stephania venosa* (Blume) Spreng). *European Journal of Theoretical and Applied Sciences, 2*(6), 575-579.
DOI: 10.59324/ejtas.2024.2(6).52

Abstract:

The tuber of *Stephania venosa* (Blume) Spreng, locally known as Belajang Keissi', belongs to the Menispermaceae family and is traditionally used in Indonesia for treating diabetes mellitus, as a nerve tonic, and as an anti-cancer remedy. This plant is recognized for its bioactive compounds with anti-inflammatory, analgesic, antioxidant, and anti-tumor properties. The objective of this study was to analyze the phytochemical content and evaluate the toxicity of the methanol extract of *Stephania venosa* tubers to explore its potential as an anticancer agent. The study involved sample preparation, extraction by maceration, phytochemical group testing using specific reagents, and toxicity assessment through the Brine Shrimp Lethality Test (BSLT) method. Phytochemical screening revealed the presence of alkaloids and flavonoids as the major secondary metabolites, while the toxicity test showed that the extract exhibited significant lethality to Artemia salina larvae, with an LC_{50} value of 14.92 ppm. This result indicates high toxicity, meeting the threshold of bioactivity for potential therapeutic applications (LC50 ≤ 1000 ppm). The mechanism of toxicity is hypothesized to involve the interference of alkaloids with the neural and digestive systems of the larvae. This study highlights the potential of *Stephania venosa* tubers as a source of bioactive compounds for antibacterial, antifungal, or anticancer development, warranting further isolation and advanced pharmacological testing to confirm its therapeutic applications.

Keywords: *secondary metabolites, phytochemical analysis, alkaloids, flavonoids, anticancer potential.*

Introduction

Secondary metabolites, as naturally occurring chemical compounds in plants, play a crucial role in human health due to their bioactivity in preventing degenerative and infectious diseases. These compounds, including alkaloids, flavonoids, terpenoids, and phenolics, often exhibit pharmacological properties that can be leveraged for therapeutic purposes. Modern phytochemical research, integrating ethnobotanical and ethnopharmacological approaches, has greatly contributed to the exploration of secondary metabolites for drug development. According to Cragg and Newman (2020), natural products remain a significant source for the discovery of new pharmaceuticals, particularly in oncology and antimicrobial therapies.

The Menispermaceae family, consisting of approximately 450 species across 71 genera, has been widely studied for its bioactive constituents. Plants in this family, primarily climbing species with alternate leaves, are abundant in tropical regions worldwide. Members of this family are known for producing alkaloids, which possess diverse bioactivities, including anti-inflammatory, analgesic, antioxidant, and anticancer properties (Pérez et al., 2022).

In Indonesia, traditional knowledge about medicinal plants has been preserved through ethnomedicine practices among various ethnic groups. The Mambi community in Mamasa Regency, West Sulawesi, has long utilized *Stephania venosa* (Blume) Spreng, locally known as Belajang Keissi', for its anticancer properties. Ethnopharmacological surveys suggest that this plant holds significant potential as a natural therapeutic agent due to its phytochemical composition (Jumadi et al., 2020).

Initial studies have shown that secondary metabolites in *Stephania venosa* possess bioactivities that align with its traditional uses. Alkaloids, the primary metabolites found in the genus *Stephania*, are reported to exhibit neuroactive properties, including antifedant effects, which interfere with neural and digestive functions in pests (Luo et al., 2021). Similarly, flavonoids contribute to antioxidant activity and cytoprotective effects, making them potential candidates for anticancer therapies (Singh et al., 2023).

To evaluate the bioactivity of *Stephania venosa*, toxicity screening using the Brine Shrimp Lethality Test (BSLT) provides a rapid and cost-effective method to determine its potential pharmacological relevance. The BSLT method, first introduced by Meyer et al. (1982), has since been refined and widely adopted for preliminary cytotoxicity testing of plant extracts and natural products. Recent advancements in bioassay techniques have validated its reliability in correlating larval mortality with cytotoxic properties, establishing LC50 as a critical indicator of toxicity (Zuraida et al., 2023).

Given its ethnopharmacological significance and promising preliminary results, this study focuses on the phytochemical analysis and toxicity evaluation of the methanol extract of *Stephania venosa* tubers. The findings aim to contribute to the development of natural anticancer agents and expand the scientific understanding of bioactive secondary metabolites in tropical medicinal plants.

Materials and Methods

Sample Preparation and Extraction

Tubers of *Stephania venosa* (Blume) Spreng collected from Mambi District, Mamasa Regency, West Sulawesi, Indonesia. Identification of the plant species was conducted and verified by a botanical expert. The tubers were washed thoroughly, sliced into smaller pieces, air-dried, and then ground into a coarse powder using a blender. A total of 2 kg of powdered tubers was macerated in methanol (solvent-to-sample ratio 1:10 w/v) at room temperature for 72 hours. The solvent was replaced every 24 hours to maximize extraction.

The resulting macerates were filtered through Whatman No. 42 filter paper using a Buchner funnel and concentrated using a rotary evaporator under reduced pressure at 45°C until a thick methanol extract was obtained.

Phytochemical Screening

Phytochemical analysis was conducted to identify major secondary metabolite groups in the methanol extract. The procedures were as follows:

Alkaloids: Tested using Mayer's and Wagner's reagents. A positive reaction was indicated by the formation of precipitates (white for Mayer and brown for Wagner).

Flavonoids: Identified using 1% ferric chloride ($FeCl_3$) solution, with a positive result indicated by a color change from yellow to green.

Triterpenoids and Steroids: Determined using the Lieberman-Burchard test, with a positive result indicated by a greenish-yellow color change.

The results were recorded based on visual observations of the color changes or precipitate formation.

Toxicity Test (Brine Shrimp Lethality Test - BSLT)

The BSLT method was utilized to assess the toxicity of the methanol extract. The steps involved were:

Preparation of Test Solutions: The extract was prepared at concentrations of 1000 ppm, 100 ppm, and 10 ppm in seawater.

Hatching of Artemia salina Larvae: A. salina eggs were hatched in artificial seawater under constant aeration and light for 24–48 hours until the nauplii (larvae) reached the second instar stage.

Exposure of Larvae: Ten nauplii were transferred into vials containing 5 mL of each test solution. Control vials containing seawater without extract were prepared for comparison. Each concentration was tested in triplicate.

Incubation and Observation: The vials were incubated at room temperature under light for 24 hours. Larval mortality was recorded, and the percentage mortality was calculated using the equation:

$$\text{Mortality } (\%) = \frac{\text{total of dead larva}}{\text{total of larva}} \times 100 \quad (1)$$

LC50 Determination: Probit analysis was conducted using the percentage mortality data. Probit values were plotted against the logarithm of extract concentrations, and the LC_{50} value was derived from the regression equation.

Data Analysis

The results of the phytochemical screening were analyzed descriptively based on the visual outcomes. For the BSLT, probit regression analysis was performed to determine the LC_{50} value, which served as an indicator of toxicity. Extracts with $LC_{50} \leq 1000$ ppm were categorized as toxic based on Meyer et al. (1982).

Results

Extraction

Maceration of 2 kg of *Stephania venosa* powder yielded methanol extract, which was concentrated to one-quarter of its original volume.

Phytochemical Analysis

The methanol extract of *Stephania venosa* tubers tested positive for alkaloids and flavonoids but negative for triterpenoids and steroids.

Table 1. Phytochemical Analysis

Reagent	Observed Results	Interpretation
Wagner	Yellow → Brown precipitate	(+) Alkaloids
Lieberman-Burchard	Yellow → Yellow-green	(-) Terpenoids and Steroids
Ferric Chloride 1%	Yellow → Green	(+) Flavonoids
Mayer	Yellow → White precipitate	(+) Alkaloids

Toxicity Test (BSLT Method)

The LC_{50} value of 14.92 ppm was obtained, confirming the high toxicity of the methanol extract, which aligns with active compound thresholds ($LC_{50} \leq 1000$ ppm). The relationship between extract concentration and *Artemia salina* larval mortality was linear, as evidenced by probit regression analysis.

Discussion

The findings of this study demonstrate the phytochemical composition and high toxicity of the methanol extract of *Stephania venosa* (Blume) Spreng tubers, supporting its potential as a source of bioactive compounds for therapeutic applications.

Phytochemical Composition

Phytochemical analysis revealed the presence of alkaloids and flavonoids as the primary secondary metabolites in the methanol extract of *Stephania venosa* Alkaloids, well-documented for their pharmacological effects, play significant roles in neuroprotection, antimicrobial activities, and anticancer properties. Recent studies highlight the ability of alkaloids to inhibit tumor cell growth and induce apoptosis through diverse mechanisms, including disruption of DNA synthesis and inhibition of cell signaling pathways (Liu et al., 2022).

Similarly, flavonoids are widely recognized for their antioxidant and anti-inflammatory activities. These compounds neutralize free radicals and enhance cellular defense mechanisms, making them valuable for preventing degenerative diseases and mitigating cancer progression (Singh et al., 2023). The coexistence of alkaloids and flavonoids in *Stephania venosa* could account for its traditional medicinal uses, particularly as an anticancer agent by the Mambi community.

Toxicity Assessment

The toxicity evaluation using the Brine Shrimp Lethality Test (BSLT) showed that the methanol extract exhibited an LC50 value of 14.92 ppm. This value is significantly below the threshold of 1000 ppm, indicating high toxicity. Toxicity levels of plant extracts are often linked to their bioactive compounds, particularly alkaloids, which can interact with biological systems at low concentrations (Kumar et al., 2021). The alkaloids in *Stephania venosa* likely act as neurotoxins, disrupting the nervous system and digestive functions in *Artemia salina* larvae, leading to their mortality.

The linear relationship observed between extract concentration and larval mortality further supports the dose-dependent toxicity of the extract. Such findings align with other studies reporting the potent toxicity of alkaloid-rich plant extracts used in traditional medicine (Pérez et al., 2022).

Potential Applications and Implications

The high toxicity of *Stephania venosa* extract, as indicated by its LC_{50} value, suggests its potential for pharmacological applications, including as an anticancer or antimicrobial agent. Alkaloid-rich extracts are being increasingly studied for their cytotoxic effects on cancer cell lines. For instance, berberine, an alkaloid from the same Menispermaceae family, has shown efficacy in inhibiting cancer cell proliferation and inducing apoptosis (Zhao et al., 2023).

Furthermore, the flavonoid content may synergize with alkaloids, enhancing the extract's bioactivity. Flavonoids have been shown to modulate immune responses and potentiate the effects of chemotherapeutic agents, offering a combinatorial approach to cancer treatment (Gupta et al., 2023). However, the toxicity also necessitates cautious handling and further evaluation to identify safe therapeutic dosages.

Mechanisms of Toxicity

The larvicidal effect observed in this study can be attributed to the alkaloids acting as feeding deterrents or neurotoxins. Alkaloids interfere with the nervous system by inhibiting neurotransmitter function, leading to impaired feeding behavior and eventual starvation of the larvae (Luo et al., 2021). This mode of action supports the potential use of *Stephania venosa* extract not only in medicine but also as a biopesticide.

Limitations and Future Directions

Although the findings are promising, further studies are required to isolate and characterize the active compounds responsible for the observed toxicity. Advanced pharmacological studies, including cytotoxicity assays on cancer cell lines and in vivo evaluations, are needed to validate the therapeutic potential of *Stephania venosa*. Additionally, understanding the molecular mechanisms underlying its bioactivity will provide insights into its mode of action.

Conclusion

The high toxicity and phytochemical richness of *Stephania venosa* tubers underscore its potential as a source of bioactive compounds for therapeutic applications. The findings provide a scientific basis for its traditional use and highlight the need for further research to harness its pharmacological potential.

Funding

No funding.

Conflict of Interests

The authors of the study reported no potential conflict of interest.

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