Chemical constituents from the tuber of *Stephania venosa (Blume) spreng*

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ABSTACT

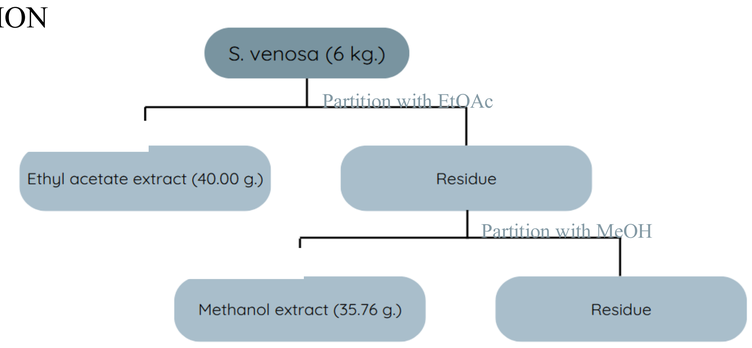
The sabu-lueat (*Stephania venosa* (Blume) *Spreng*) is a traditional medicinal plant using in various diseases treatment. In this study, the dried and finely ground tuber of *Stephania venosa* was extracted using hexane, ethyl acetate, and methanol at room temperature. After removal the solvents, the crude extracts were obtained as follows: 148.58 g. of ethyl acetate extract and 61.89 g. of methanol extract, respectively. The portion of ethyl acetate extract (11.97 g) was further separated using column chromatography, yielding five pure compounds: dehydroisolaureline (**1**), dehydrodicentrine (**2**), tetrahydropalmatine (**3**), crebanine (**4**), and dicentrine (**5**), . The structures of these isolated compounds were identified using spectroscopic techniques and compared with previously reported data. Additionally, this study investigated the application of these five alkaloids as metal ion sensors for Zn^{2+}, Fe^{3+}, Pb^{2+}, Co^{2+}, Cr^{3+}, and Cu^{2+}. The results indicated that dehydroisolaureline (**1**) and dehydrodicentrine (**2**), exhibited specificity in binding with Fe^{3+} ions. The complex formation ratio was determined using the Job's plot method, revealing a 1:1 molar ratio between both compounds dehydroisolaureline (**1**) and dehydrodicentrine (**2**) with Fe^{3+}.

INTRODUCTION

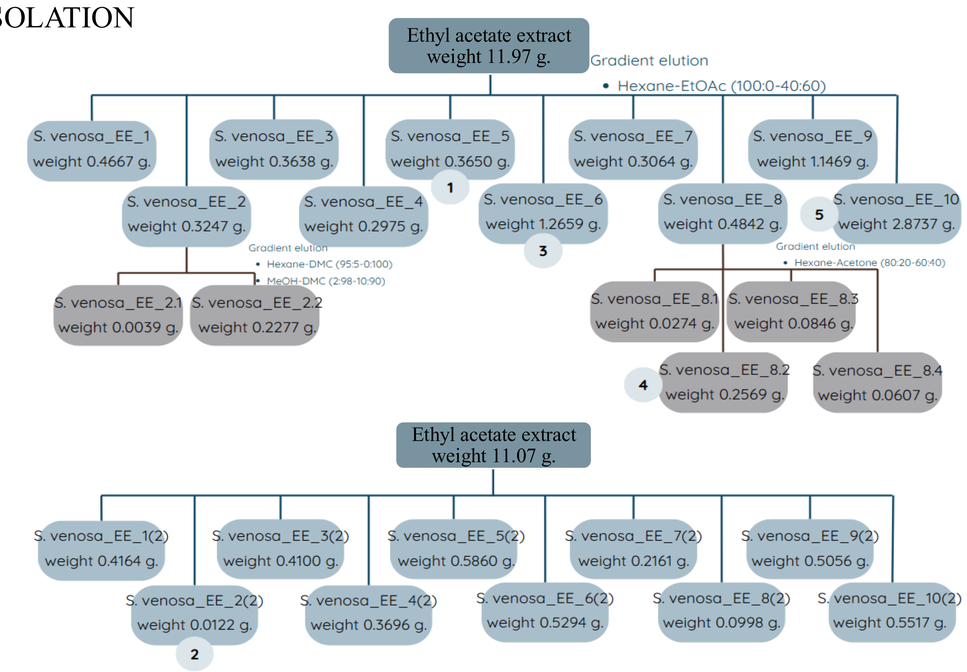
Sabu-lueat (*S. venosa*) belongs to the family Menispermaceae and is classified as a small perennial plant. It is commonly found in mixed deciduous forests, tropical rainforests, and dry evergreen forests on limestone mountains at altitudes ranging from sea level to 1,500 meters Its distribution spans across Asia, including Thailand, Malaysia, Indonesia, Cambodia, Laos, Myanmar, and Vietnam. In Thailand, it is widely found in all regions and is known as sabu-lueat . This plant is a rich source of bioactive alkaloid compounds, which exhibit various biological activities such as antimalarial properties, platelet aggregation inhibition, anticancer effects, antimicrobial activity, and potential benefits in preventing Alzheimer's disease.

EXPERIMENTAL

- EXTRACTION



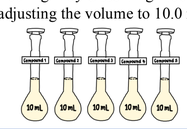
- ISOLATION



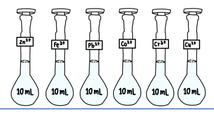
- SPECIFICITY TESTING WITH METALS

1. The application of natural compounds to test for specificity with metals.
   - Preparation of alkaloid and heavy metal solutions.

Alkaloid Solution: The alkaloid solution of 1-5 were prepared at a concentration of 100 mg/L by dissolving 1.00 mg of each compound in methanol and adjusting the volume to 10.0 mL with methanol.



→ Heavy Metal Solutions: 100 mg/L of heavy metal solution were prepared by dissolving 1.00 mg of each metal salt in deionized water and adjusting the final volume to 10.0 mL.



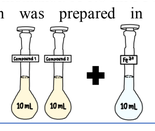
   - Specificity test of alkaloid with heavy metal.

Testing Procedure: 0.50 mL of alkaloid solution was pipetted into a test tube, followed by 0.50 mL of heavy metal solution. 1.00 mL of methanol was added to the mixture.

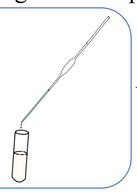
→ Observation: The color change of the solution was observed, and the absorbance shift was measured using a UV-Vis spectrophotometer.

2. Determination of the complex formation ratio using the Job's plot method.

Sample Preparation: Solutions of compounds 1 and 2 were prepared at 1.0 mM in methanol. A 1.0 mM $FeCl_3 \cdot 6H_2O$ solution was prepared in deionized water.



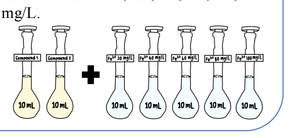
→ Mixing Ratios: The alkaloid solutions were mixed with Fe^{3+} in different volume ratios (e.g., 1.00:0.00, 0.90:0.20, ..., 0.00:1.00).



→ Measurement: The mixtures were incubated for 10 minutes, and the absorbance was measured at 400 nm using a UV-Vis spectrophotometer to establish the binding ratio of alkaloids to Fe^{3+}.

3. Determine the lowest detectable concentration (LOD).

Prepare solution of compounds 1 and 2 at a concentration of 100 mg/L in methanol. Additionally, prepare $FeCl_3 \cdot 6H_2O$ solutions at concentrations of 0, 20, 40, 60, 80, and 100 mg/L.



→ Pipette 0.50 mL of the prepared alkaloid solution into a test tube and mix it with 0.50 mL of Fe^{3+} solution at each concentration. Then, add 1.00 mL of methanol to the mixture. After thorough mixing, measure the absorbance at 400 nm. a spectrophotometer.

→ The limit of detection (LOD) is determined from the calibration curve, which represents the relationship between the Fe^{3+} concentration (x-axis) and absorbance (y-axis).
The LOD is calculated using the formula:
$LOD = S_{x/y}/b$

RESULTS AND DISCUSSION

The investigation of the chemical constituents from EtOAc extract of the tuber of *S. venosa* has led to isolation of dehydroisolaureline (**1**), dehydrodicentrine (**2**), tetrahydropalmatine (**3**), crebanine (**4**), and dicentrine (**5**). Their structures were elucidated by spectroscopic methods.

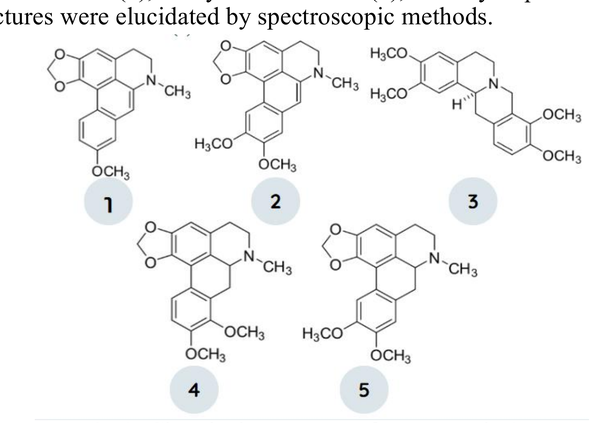


Fig 1: Chemical structures of compounds **1-5.**

- SPECIFICITY TESTING WITH METALS

1. The application of natural compounds to test for specificity with metals.

Compounds	Metal solution											
	Zn^{2+}		Fe^{3+}		Pb^{2+}		Co^{2+}		Cr^{3+}		Cu^{2+}	
1	+	pH 6	-	pH 2	+	pH 6	-	pH 6	+	pH 6	+	pH 5
2	-	pH 6	+	pH 3	-	pH 6	+	pH 6	-	pH 6	+	pH 4
3	-	pH 6	+	pH 4	-	pH 6	-	pH 6	-	pH 6	-	pH 5
4	-	pH 6	+	pH 3	-	pH 6	-	pH 6	-	pH 6	-	pH 5
5	-	pH 6	+	pH 3	-	pH 6	-	pH 6	-	pH 6	-	pH 5

Table 1: Investigation of the selectivity of alkaloid compounds for individual metals.

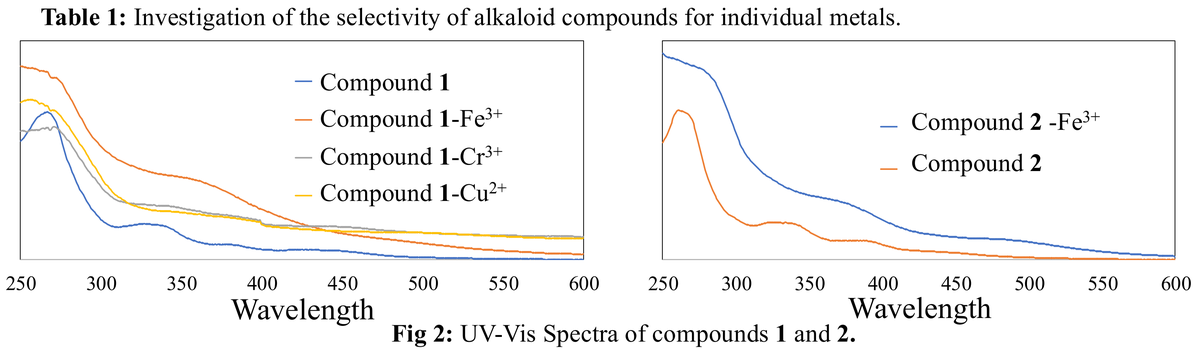


Fig 2: UV-Vis Spectra of compounds **1** and **2.**

2. Determination of the complex formation ratio using the Job's plot method.

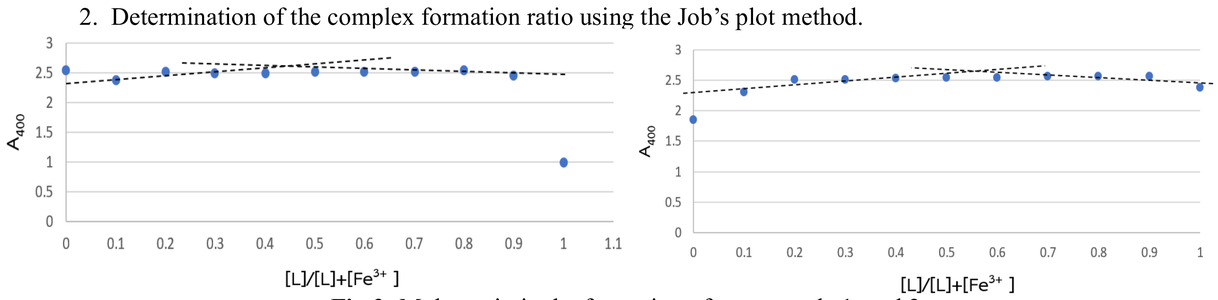


Fig 3: Molar ratio in the formation of compounds **1** and **2.**

3. Determine the lowest detectable concentration (LOD).

Substituting the value

$$S_{y/x} = \sqrt{\frac{1.158}{5-2}} = 0.621$$

From the equation y= 0.0057x+1.521, the slope is 0.3769
From the formula $LOD = 3 \times \frac{S_{y/x}}{b}$
Substituting the values, we get:
$LOD = 3 \times \frac{0.621}{0.3769} = 4.943$ mg/L

Substituting the value

$$S_{y/x} = \sqrt{\frac{0.056}{5-2}} = 0.137$$

From the equation y= 0.0107x+1.396, the slope is 0.8893
From the formula $LOD = 3 \times \frac{S_{y/x}}{b}$
Substituting the values, we get:
$LOD = 3 \times \frac{0.137}{0.8893} = 38.52$ mg/L

CONCLUSION

Four aporphine and one protoberberine alkaloids were isolated from the ethyl acetate extract of dried tuber of *S. venosa*. Their stuctures were identified as dehydroisolaureline (**1**), dehydrodicentrine (**2**), tetrahydropalmatine (**3**), crebanine (**4**), and dicentrine (**5**). These all isolated compounds were tested for their specificity toward heavy metal ions (Zn^{2+}, Fe^{3+}, Pb^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}). Dehydroisolaureline (**1**) and dehydrodicentrine (**2**) showed interaction for Fe^{3+}. Upon interaction with Fe^{3+}, **1** changed from clear yellow to turbid, while **2** shifted from yellow to orange-red, confirmed by UV-vis spectroscopy, indicating a bathochromic shift due to alkaloid-Fe^{3+} complex formation. Job's plot analysis determined a 1:1 molar ratio for these complexes. The LOD for Fe^{3+} detection showed dehydroisolaureline (**1**) as the most sensitive, detecting Fe^{3+} at 4.943 mg/L, whereas dehydrodicentrine (**2**) had an LOD of 38.52 mg/L.

REFERENCES

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