COMPARATIVE STUDY ON THE TOTAL ALKALOID CONTENT AND ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF *TINOSPORA CORDIFOLIA* AND *TINOSPORA CRISPA* COLLECTED IN AN GIANG PROVINCE

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Abstract: *The morphological characteristics of Tinospora cordifolia and Tinospora crispa were observed and described. The stem powder of both species was analyzed for moisture content, total ash, foreign matter ratio, preliminary phytochemical analysis activity, qualitative and quantitative determination of total alkaloids by thin layer chromatography (TLC) and UV-Visible spectrophotometry method. The α-glucosidase inhibitory effect was evaluated. Research recorded morphological characteristics, moisture content, total ash, and foreign matter ratio of Tinospora cordifolia and Tinospora crispa. Quantitative analysis by spectrophotometry showed significant differences in total alkaloids between the two species. Moreover, the α-glucosidase enzyme inhibition activity of both species was potentially stronger than the positive control Acarbose, in Tinospora crispa has the strongest activity.*

Keywords: *Tinospora cordifolia, Tinospora crispa, Berberin, α-glucosidase.*

1. INTRODUCTION

Tinospora cordifolia and *Tinospora crispa*, both belonging to the Menispermaceae family, have both been demonstrated to possess biological effects including anti-inflammatory, immunomodulatory, antibacterial, antidiabetic, antioxidant, and anticancer properties [1,2]. In traditional medicine, *T. cordifolia* and *T. crispa* are known for their bitter taste and cold nature and are used in remedies for treating diabetes [3]. Preferring sunlight and scattered growth in forests, species of the *Tinospora* genus thrive in the forested mountainous regions of An Giang province, offering an abundant and easily harvested source of raw materials. Furthermore, An Giang is home to a large ethnic population who frequently use these species as safe and effective medicinal herbs for disease treatment and dietary supplements. Currently, there is a lack of comparative studies evaluating both *T. cordifolia* and *T. crispa*, especially with regard to their chemical composition and pharmacological effects. Previous phytochemical studies on *Tinospora* species have shown that alkaloids are the main group of compounds present. Alkaloids have been proven to offer multiple health benefits and therapeutic effects [4].

For these reasons, this study aims to update data on preliminary phytochemical analysis, qualitative and determination of alkaloid compounds present in these plants, and their α-glucosidase inhibitory activity. The results are expected to provide predictions about their antidiabetic efficacy across different stages of the disease from the perspective of modern medicine.

2. MATERIALS AND METHODS

2.1. Research subjects

Tinospora cordifolia and *Tinospora crispa* were collected in An Giang province and identified by Prof. Dr. Tran Cong Luan from Tay Do University. Specimens with codes TT.01 and KN.01 are preserved Unit of Traditional Medicine and Pharmacy - Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City.

2.2. Solvents, Chemicals, and Research Equipments

Solvents and Chemicals: Bromocresol green (BCG), sodium hydroxid, sodium hydrophosphate, citric acid, berberin chloride (Batch no. R04130, provided by the National Institute of Drug Quality Control), chloroform solution, hydrocloric acid, sodium carbonate, *α*-glucosidase

solution, *p*-nitrophenyl-*α*-D-glucopyranoside (*p*NPG) solution, acarbose (Sigma, USA), dichloromethane, methanol, Dragendorff's reagent.

Equipment: Labomed microscope (India), Contherm convection drying oven (New Zealand), desiccator, Mettler Toledo moisture analyzer (Switzerland), Buchii Rotavapor R-220 vacuum rotary evaporator (Switzerland), Labomed dual-beam UV-Vis spectrophotometer (USA), and common laboratory glassware.

2.3. Methods

2.3.1 Morphological Characterization

Plant samples (roots, stems, leaves) of *T. cordifolia* and *T. crispa* were observed and described morphologically in the field, photographed, collected, and made into dried herbarium specimens. Scientific names were identified through morphological comparison against published documents.

2.3.2. Evaluation of Several Quality Indicators of the Medicinal Material

2.3.2.1. Moisture Content

Moisture content of the stems of *T. cordifolia* and *T. crispa* was determined using the loss on drying method, Appendix 9.6, Vietnamese Pharmacopoeia V (VP V).

2.3.2.2. Total Ash Content

Total ash content was determined according to Appendix 9.8, VP V.

2.3.2.3. Foreign Matter

The foreign matter ratio was determined according to Appendix 12.11, VP V.

2.3.2.4. Preliminary Phytochemical Analysis

Dried stem samples of *T. cordifolia* and *T. crispa* were ground into coarse powder and successively extracted with solvents of increasing polarity (diethyl ether, 96% ethanol, and water). The extracts were tested qualitatively for various compound groups using the modified Ciulei method.

2.3.2.5. Qualitative Determination of Alkaloid

Qualitative determination of Alkaloid was performed using thin-layer chromatography (TLC), as described in Appendix 5.4, VP V. Silica gel G plates were used with a mobile phase of chloroform : methanol (95 : 5). Spots were visualized under UV 254 nm, UV 365 nm, and Dragendorff's reagent..

2.3.2.6. Quantitative Determination of Total Alkaloid Content

The total alkaloid content is determined based on the method involving complex formation with bromocresol green (BCG) reagent, producing a yellow-colored product (Patel et al.) [5].

- Step 1: Preparation of bromocresol green (BCG) solution (10^{-4} M): Heat 69.8 mg of BCG with 3 mL of 2 N NaOH and 5 mL of distilled water at 50 - 60°C for 10 - 15 minutes until fully dissolved. Dilute the solution to 1000 mL with distilled water.

- Step 2: Preparation of phosphate buffer solution (pH 4.7): Adjust the pH of 2 M sodium phosphate solution (71.6 g Na_2HPO_4 in 1 liter of distilled water) to 4.7 (within a range of 4.5 - 4.9) using 0.2 M citric acid solution (42.02 g citric acid in 1 liter of distilled water).

- Step 3: Preparation of stock standard berberine chloride solution (100 µg/mL): Accurately weigh approximately 2 mg of berberine standard and dissolve it in methanol in a 20 mL volumetric flask to obtain a stock solution with a concentration of 100 µg/mL.

- Step 4: Calibration curve for berberine chloride (2 - 10 µg/mL): Transfer an appropriate amount of the stock standard solution into a separating funnel, add 5 mL of phosphate buffer (pH 4.7), and 5 mL of BCG solution (10^{-4} M). Shake the mixture, and extract the formed complex with 5 mL of chloroform. Collect the chloroform layer into a 10 mL volumetric flask and make up to volume with chloroform. Measure the absorbance at 415 nm against a blank. The calibration curve is constructed by plotting absorbance versus berberine chloride concentration (µg/mL).

- Step 5: Determination of total alkaloid content in the stem powder: Each 2 g of powdered herbal material is extracted three times with 20 mL of methanol at 50 - 60°C. Combine the methanol extracts and evaporate to dryness to obtain a residue. Dissolve the residue in 2 N hydrochloric acid (HCl) and filter. Transfer the test solution to a separating funnel, add 5 mL of phosphate buffer (pH 4.7), and 5 mL of BCG solution (10^{-4} M). Shake the mixture and extract the formed complex with 5 mL of chloroform. Collect the chloroform layer in a 10 mL volumetric flask and make up to volume with chloroform. Measure the absorbance at 415 nm against a blank. The solutions remain stable for up to 2 hours.

The total alkaloid content is determined using the regression equation. This method is performed using a Labomed double-beam UV-Vis spectrophotometer.

$$M = \frac{C \times V \times K}{m_{dl} \times (1 - a)}$$

Where:

M: Total alkaloid content in the sample (µg/g);

C: Berberine concentration calculated from the regression equation (µg/mL);

V: Volume of the test solution (mL);

k: Dilution factor

m_{dl}: Weight of the powdered herbal material (g).

a: Moisture content of the powdered material (%).

Validation of Analytical Procedure Parameters: Validation parameters and procedures are carried out according to the AOAC guidelines.

2.3.3 Evaluation of Alpha-Glucosidase Inhibitory Activity

This is based on the method described by Fahimeh Moradi Afrapoli et al. (2012) [6] with some modifications: Incubate a mixture containing 60 µL of the sample solution and 50 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase enzyme solution (0.2 U/mL) in the wells of a 96-well microplate at room temperature for 20 minutes. Then add 50 µL of pNPG solution (prepared in 0.1 M phosphate buffer, pH 7.4) to each well. The wells are further incubated for 10 minutes. The reaction is then terminated by adding 160 µL of 0.2 M Na_2CO_3. Measure the absorbance at 405 nm and compare with a control sample that contains 60 µL of buffer solution instead of the test sample. Acarbose is used as the positive control. The *α*-glucosidase inhibitory activity is calculated as follows:

$$\text{Inhibitory effect (\%)} = \frac{Ac - Am}{Ac} \times 100$$

Where:

A_c: Absorbance of the control group

A_m: Absorbance of the sample group

The experimental data are expressed as the mean value of three independent measurements.

2.4. Statistical Methods

Data were processed using Microsoft Excel 2016. A p value < 0.05 was considered statistically significant.

3. RESULTS

3.1. Morphological Characteristics

3.1.1. Morphological Characteristics of Tinospora cordifolia

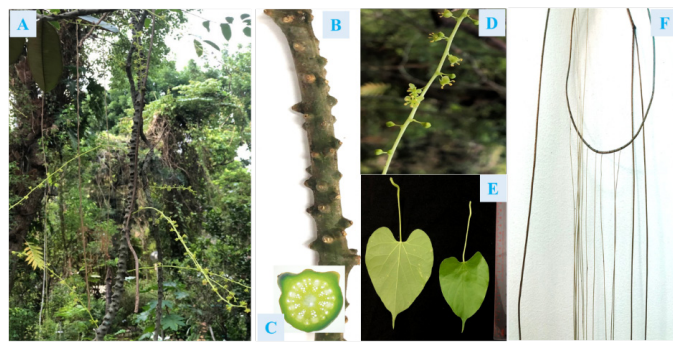
T. cordifolia is a climbing vine species that clings to other plants. The entire plant is smooth and can grow up to about 10 meters long. It has aerial roots that grow downward from the stem and attach to the ground. Its stem is less rough than that of *Tinospora crispa*, with very small, scattered warty nodes. As the plant ages, the stem bark becomes hardened, with longitudinal cracks appearing along the stem at lenticel points, and gradually peels off over time. When fresh, the plant exudes a milky white, slightly ivory sap that is very bitter in taste. The leaves are broader and more rounded than heart-shaped, measuring approximately 9 x 8 cm. They are sinuate at the base, pointed at the tip, and have palmate venation with 5 to 7 veins arising from the base. The leaf petiole is flat and measures 3 - 6 cm in length. The inflorescences are racemes that grow from the leaf axils or on leafless stems. The flower clusters are typically 5 - 15 cm long, and the flowers have a greenish-yellow color. The drupes are spherical, red when ripe, and about 2 - 3 cm in diameter (Figure 1).

Hình 1. *3.1.1. Characteristics of Tinospora cordifolia*

Note: A-T. cordifolia in the field, B-Stem, C-Cross-section of the stem, D-Aerial root, E-Front and back of the leaf, F-Flower cluster

3.1.2. Morphological Characteristics of Tinospora crispa

T. crispa is a climbing vine that clings to other plants, can grow up to 5 - 6 meters long or more. The young stems are smooth, dark green, and have few warty spots. In contrast, the mature stems are very rough, prominently covered with warty outgrowths resembling toad skin, and are grayish-brown in color. The plant grows vigorously and has aerial roots that grow downward from the stem and attach to the ground. It contains a milky white sap with an intensely bitter taste. The leaves are petiolate, smooth, and alternate. They are heart-shaped with a deep base, tapering to a pointed tip, with entire margins. The leaf blade is somewhat thick, measuring 9 - 11 cm in length and 5 - 7 cm in width. The slender petioles are shorter than the blades, measuring 5 - 8 cm in length. The leaf venation is palmate, with 5 - 7 main veins originating from the base. The flowers are arranged in 1 - 2 clusters that grow from the leafless axils of old stems, and 9 - 25 cm in length. The flowers are yellowish-green. The bracts are ovate, slightly thickened, and 2 - 3 mm long. The fruits are ovoid, approximately 10 - 12 mm in length. When ripe, they turn from yellow-orange to red. Each fruit contains a single flattened, black seed. The thick, irregular pulp of the fruit encroaches deeply into the seed cavity (Figure 2).



Hình 2. *Characteristics of Tinospora crispa*

Note: A-T. crispa in the field, B-Stem, C-Cross-section of the stem, D-Flower cluster, E-Front and back of the leaf, F-Aerial root

3.2 Evaluation of Selected Quality Parameters of Medicinal Materials

The results of the evaluation of selected quality parameters of the medicinal materials are presented in Table 1. Moisture content is one of the key factors affecting the preservation of medicinal herbs. Each medicinal material has a safe moisture threshold. For optimal preservation, the moisture content must be at or below this safe threshold. The measured moisture content of *T. cordifolia* and *T. crispa* powders was 6.17 ± 0.09% and 5.66 ± 0.08%, respectively, both meeting the requirements of the Vietnamese Pharmacopoeia V. The total ash content determined from *T. cordifolia* and *T. crispa* powders was 6.04 ± 0.12% and 8.08 ± 0.14%, respectively. After removing foreign matter and examining the samples, the foreign matter ratio in *T. cordifolia* and *T. crispa* was found to be 3.92 ± 0.09% and 3.73 ± 0.07%, respectively.

Table 1. *Results of selected quality parameters of medicinal materials*

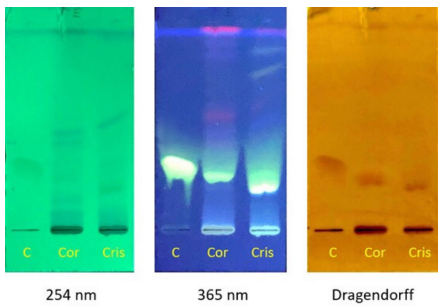
Parameters	*Tinospora cordifolia* (%)	*Tinospora crispa* (%)
Moisture content (n=3)	6.17 ± 0.09	5.66 ± 0.08
Total ash content (n=3)	6.04 ± 0.12	8.08 ± 0.14
Foreign matter ratio (n=3)	3.92 ± 0.09	3.73 ± 0.07

3.3. Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of the stems of *T. cordifolia* and *T. crispa* showed the presence of several compound groups including alkaloids, terpenoids, flavonoids, reducing substances, and organic acids. Among these, the chemical reactions for alkaloids and flavonoids were the most pronounced. Alkaloids are considered the characteristic chemical compounds for species in the *Tinospora* genus. Qualitative methods for detecting alkaloids will contribute to establishing pharmacognostic standards for the medicinal materials derived from *T. cordifolia* and *T. crispa* through chemical reaction-based assays.

3.4. Qualitative determination of Alkaloids

The chromatography results indicated that both extract samples from the stems of *T. cordifolia* and *T. crispa* showed orange spots when treated with Dragendorff's reagent, a spot was observed with an Rf value of 0.25 (Cor sample), and two spots were observed with Rf values of 0.21 (Cris sample). These results provide preliminary evidence that alkaloid compounds are present in both samples. Based on this finding, the total alkaloid content can be further quantified using spectrophotometric methods (Figure 3).



***Hình 3**. TLC chromatogram of Berberin (C), stem extract of T. cordifolia (Cor), and T. crispa (Cris)*

3.5. Quantitative Determination of Total Alkaloids

3.5.1. Determination of Linear Range and Calibration Curve

The calibration curve was constructed using a berberine chloride standard solution. Accurately weigh approximately 2 mg of berberine reference substance and dissolve it in methanol in a 20 mL volumetric flask to obtain a standard solution with a concentration of 100 µg/mL. Transfer an appropriate volume to a separating funnel, then add 5 mL of phosphate buffer (pH 4.7) and 5 mL of BCG solution (10^{-4} M). Shake the mixture, and the resulting complex is extracted with 5 mL of chloroform. The chloroform layer is collected into a 10 mL volumetric flask and made up to volume with chloroform. Then, prepare a dilution series with concentrations ranging from 0.5 to 12 µg/mL. The absorbance was measured at a wavelength of 415 nm, using a blank solution as reference. Absorbance values were plotted against berberine chloride concentrations (µg/mL) to generate the calibration curve.

The optical density (A) of these solutions was measured. A calibration curve was established with the y-axis representing the measured absorbance and the x-axis representing the concentration in µg/mL of the corresponding alkaloid derivatives. The resulting linear regression equation was: y = 0.0985x – 0.043 with R^2 = 0.9994. The measurement results are presented in Table 2.

***Table 2**. Concentration Range and Optical Density*

C_{Ber} (µg/mL)	0.5	1	2	4	6	8	10	12
A	0.012	0.052	0.163	0.343	0.533	0.749	0.958	1.132
C_{Alk} (%)	0.55	0.96	2.09	3.92	5.85	8.04	10.16	11.93

3.5.2. Quantitative Determination of Total Alkaloid Content in Medicinal Materials and Evaluation of Method Repeatability

To assess repeatability, the quantification procedure was performed six times within the previously established concentration range. The mean value, standard deviation (SD), and relative standard deviation (RSD) were then calculated and compared with the acceptable criteria set by AOAC for method validation. The results are presented in Table 3.

***Table 3.** Repeatability of the Quantification Method on Sample Matrix*

Number of analyses (n = 6)	Total alkaloid content in *T. cordifolia* (µg/g)	Total alkaloid content in *T. crispa* (µg/g)
Mean	11.499	24.414
SD	0.312	0.151
RSD (%)	2.72	0.62

The results showed that the total alkaloid content in the samples, along with their corresponding RSD values, were as follows: *T. cordifolia*: 11.499 µg/g with RSD = 2.72%, *T. crispa*: 24.414 µg/g with RSD = 0.62%. The RSD values obtained for both sample matrices were lower than the AOAC reference RSD value of 7.3%.

3.6. Evaluation of Alpha-Glucosidase Inhibitory Activity

The in vitro alpha-glucosidase inhibitory activity of *T. cordifolia*, *T. crispa*, and the positive control Acarbose was assessed and presented in Table 4.

Table 4. *IC_{50} Values of T. cordifolia, T. crispa, and Acarbose Samples*

Sample	IC_{50} (µg/mL)
Tinospora cordifolia	1.25 ± 0.01
Tinospora crispa	0.46 ± 0.03
Acarbose	122.12 ± 1.81

Comment: T. crispa exhibited α-glucosidase inhibitory activity that was 2.7 times stronger than *T. cordifolia* and 265 times stronger than the positive control, Acarbose.

4. DISCUSSION

Tinospora cordifolia and *Tinospora crispa* both belong to the *Tinospora* genus, share relatively similar botanical morphology, and are often not clearly distinguished in traditional medicine. The research results showed that both species exhibit strong α-glucosidase inhibitory activity, significantly surpassing the positive control, Acarbose. This highlights their great potential for the development of therapeutic products derived from local medicinal resources. Previous studies have isolated several groups of bioactive compounds from these two species, particularly alkaloids, flavonoids, terpenoids, and phenolic compounds [1, 2]. These compounds have been proven to inhibit α-glucosidase through mechanisms that slow down the hydrolysis and absorption of carbohydrates, thereby contributing to the stabilization of postprandial blood glucose levels [7], which is consistent with the management of type 2 diabetes – a common health concern in Vietnam as well as globally.

A comparative analysis showed that *T. crispa* exhibited stronger α-glucosidase inhibitory activity (0.46 ± 0.03 µg/mL) than *T. cordifolia* (1.25 ± 0.01 µg/mL). Quantitative analysis of total alkaloids revealed that the alkaloid content in *T. crispa* was approximately twice that of *T. cordifolia*, which may be one of the primary reasons for the difference in their biological activity. In addition, preliminary phytochemical analysis revealed the presence of groups such as flavonoids, terpenoids, phenolics, and alkaloids - compounds that have been proven to contribute to the enzyme inhibitory activity.

Therefore, further research on the isolation, qualitative, and quantitative characterization of bioactive compound groups from both species is essential. These studies not only help clarify the mechanisms of action but also provide a foundation for extract standardization, paving the way for the development of therapeutic products such as capsules, liquid extracts, or dietary supplements to support diabetes treatment. In addition to berberine, attention should also be directed toward discovering other alkaloids that may affect the pathogenesis of diabetes. In summary, the results of the current study affirm the potential of *T. cordifolia* and *T. crispa* in supporting diabetes treatment, while also opening up prospects for developing local medicinal plants into practical, applicable products - contributing to improved public healthcare.

5. CONCLUSION

The botanical characteristics and quality indicators of the medicinal materials, such as moisture content, total ash, and impurity ratio of the stem samples of *Tinospora cordifolia* and *Tinospora crispa* were recorded in detail. Using the UV-Vis method, the total alkaloid content in *T. crispa* was found to be higher than that in *T. cordifolia* collected from An Giang province. The study evaluated the α-glucosidase inhibitory activity of both species, showing that both exhibited strong activity that was notably more potent than the positive control Acarbose. In particular, *T. crispa* showed 2.7 times stronger inhibition than *T. cordifolia*, and 265 times stronger than Acarbose.

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