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Short Communication

Quantification of Berberine Chloride in *Tinospora cordifolia* Collected from Three Different Localities, Through HPLC

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The oldest kind of medicine known to humanity is the usage of herbs, which has been used throughout history in all cultures [1]. The chemical compounds these plants contain, which have the potential to change specific physiological activities in the human body, provide evidence of their therapeutic significance. Terpenes, alkaloids, flavonoids, and phenolic chemicals are the most significant of these plant bioactive components [2]. For primary healthcare, about 80% of the world's population uses traditional medicines, the majority of which employ plant extracts [3]. The chemical components present in the sample can be identified and quantified using a variety of procedures. The desired chemical, which is, in this experiment, an alkaloid-berberine, is identified and quantified using the High-Performance Liquid Chromatograph (HPLC) technique.

The main reasons HPLC has become so good are its dependability (usage of pressure-driven liquid support) and adaptability (ability to change the make-up of each mobile and stationary phase). The final interacting relationships between the stationary half, the mobile half, and additionally the analyte determine the activity mode or separation process. In standard or miniature HPLC, particle-packed columns with either fully porous or the most recent core-shell particles are used, as well as monolithic columns. With HPLC, quantitative analysis is frequently completed. On modern commercial systems, an automatic injector that offers repeatable injection volumes is a standard feature. HPLCs are rather easy to use. The selection of the column and the effectiveness of the entire system determine how well a specific pair of compounds may be separated by High Performance Liquid Chromatograph (HPLC).

A solute-solvent kind of interaction with the column substrate that competes with a solute-solvent interaction with the mobile phase affects the relative position of the different sample components on the chromatogram. Column efficiency refers to how an initially narrow band of solutes spreads out as it moves through the column. The broadening is a product of the operating conditions and column design. It is frequently preferable to use solvent programming, where the mobile phase composition is changed continuously or in stages as the separation takes place, for samples with a wide variety of retention times. A very wide range of retention times are commonly produced by the analysis of mixes with greatly different components. One type of liquid chromatography, HPLC, uses a liquid as the mobile phase. The most used kind of HPLC is reversed-phase HPLC. Reversed-phase refers to a situation in which the stationary phase is relatively non-polar and the movable phase is substantially polar. Therefore, non-polar chemicals will be more maintained than polar compounds (i.e., have longer retention durations). The stationary phase is relatively polar in normal phase HPLC, while the mobile phase is generally non-polar. Partition, adsorption, ion-exchange, size-exclusion, and thin-layer chromatography are further, more generic HPLC techniques [4].

The primary drug, any reaction contaminants, all readily available synthetic intermediates, and any degradation products are all targeted, separated, and quantified using the HPLC method. In modern analytical chemistry, high-performance liquid chromatography is one of the most potent technologies available. Any material that can dissolve in a liquid can have its constituents separated, identified, and quantified using this technique. The most precise analytical technique, HPLC, is frequently used to assess the stability of pharmacological products as well as their quantitative and qualitative composition [5].

An is quinoline alkaloid known as berberine has been linked to antimalarial, antipyretic, antibacterial, antitumor, and antiprotozoal properties [6-7]. The main chemical components that were recovered from the stem of *Tinospora cordifolia* are alkaloids, specifically berberine and magnoflorine [8-9]. The main topic of the current study is the quantification of the essential component utilized to treat diabetes. The main component from *Tinospora cordifolia* gathered from three separate locations was quantified through a comparison analysis. Understanding the amount of the product (berberine) contained in the aforementioned sample is the study's main goal.

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Plant material

The plant material is the stem of *Tinospora cordifolia*, which is collected from three different localities. One sample plant of *Tinospora cordifolia* was collected from the Bhadutala forest of Paschim Medinipur district. The other sample plant of *Tinospora cordifolia* was collected from Digha region of Purba Medinipur district. Another plant sample was collected from Morabadi, Ranchi. The plant sample was authenticated by Scientist E & HOO of Botanical Survey of India, Shibpur, Howrah, West Bengal, India.

Standard berberine chloride

The standard berberine chloride has Formula Weight (FW): 336.4. Batch: B48074. CAS No: 2086-83-1, with purity 98%.

Extract and stock solution preparation of Tinospora cordifolia

The stems of the sample plant were dried in shade. The dried stems were grinded in to fine powder. The powder of the stem was passed through a sieve of size 20 mesh to obtain fine dust of the stem. The stem powder was then exhausted with the solvent methanol. 5 grams of the stem powder was dissolved in 50ml of ethanol. The solution was stirred on a magnetic stirrer for 24 hours. The solution was then filtered through Whatman filter paper no. 41. The solvent methanol was removed to get the solid mass for analysis [10].

Standard solution preparation

Gradient solution of 100µg, 50µg, 25µg, 12.5µg, 6.25µg of berberine chloride was prepared to obtain the standard curve. The regression and the standard equation of the standard curve was also obtained.

HPLC machine specifications

Agilent 1260 infinity HPLC; Agilent Zorbax SB-C18, 5µ (4.6 × 150 mm)

Chromatographic conditions

Injection volume: 30µl
Flow rate: 1ml/min.
Mobile phase: Acetonitrile : water (70:30)
TFA: 0.1%
Wavelength detection: 350 nm

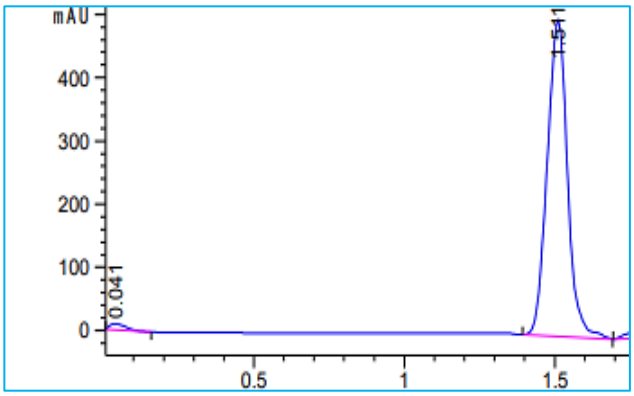

Fig 1 The various concentrations of the crude berberine chloride were prepare and the graph plotted area vs time showed the retention time (RT) of berberine chloride at 1.51 minutes

The calibration curve was found to be linear between the range 6.25µg/ml to 100 µg/ml. The concentration of berberine chloride was found al highest concentration in the region of Digha, followed by Morabadi and least concentration at Bhadutala region. The conclusion of the following interpretation can be drawn as such that the amount of secondary metabolite in the plant increases with the level of stress experienced by the plant. The concentration of berberine which is an alkaloid present in *Tinospora cordifolia* is found maximum in the plant sample collected from the stress full arid region (Digha) whereas its concentration is least in the non-stressed region (Bhadutala).

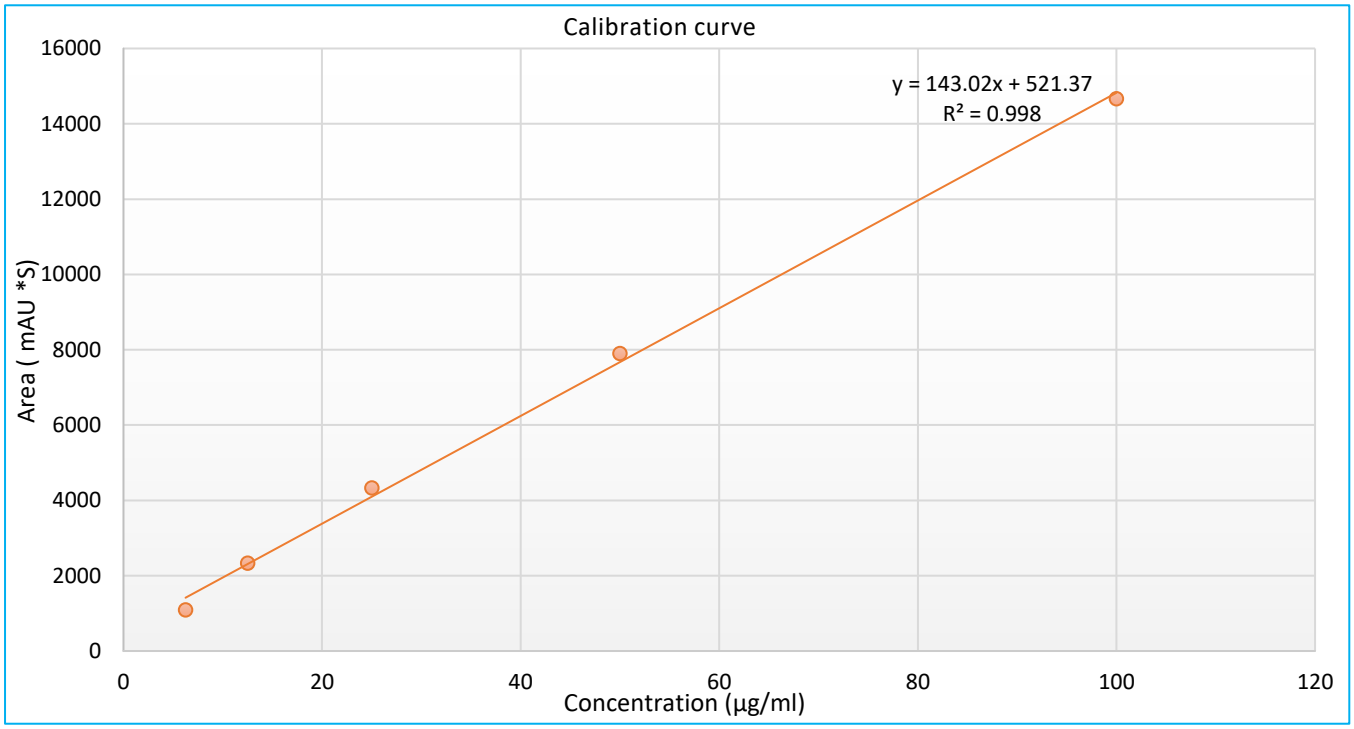

Fig 2 Gradient solution of 100µg, 50µg, 25µg, 12.5µg, 6.25µg of berberine chloride was prepared and the following standard curve was obtained. The regression obtained was 0.998 and the linear regression equation for the calibration curve obtained was y=143.02x+521.37

Using the linear regression equation for the calibration curve, the concentration of berberine chloride was calculated among the plant specimens of the two different localities and the interpretation was made as follows:

Table 1 Concentration of berberine chloride in *Tinospora cordifolia* collected from three different localities

S. No.	Region of plant collection	Concentration of berberine chloride (%)
1	Digha, Purba Medinipur	2.41
2	Bhadutala, Paschim Medinipur	1.34
3	Morabadi, Ranchi	2.38

SUMMARY

High Performance Liquid Chromatograph (HPLC) was used for the estimation of berberine chloride. Berberine is an is quinoline alkaloid. It is found in the liana *Tinospora cordifolia*, belonging to the family Menispermaceae. It is commonly known as Giloy, Amrita, Gulancha etc. The sample specimens of the same liana species (*Tinospora cordifolia*) were collected from three different localities namely Purba Medinipur, Paschim Medinipur and Ranchi. The stems of the plants were turned in to fine powder through grinding and sieving, and was extracted using methanol, which was used for the analysis of the concentration of berberine chloride. Different concentration gradients of the standard chemical were prepared to obtain the calibration curve and the linear regression equation. The concentration level of the alkaloid berberine was found highest in the plant belonging to the stressed region compared to the other two plant samples belonging to the non-stressed regions. The quality and quantity of the desired products from plant sources, which should be the focus in agriculture, have become increasingly important in the modern world due to the rapidly declining amount of agricultural land and rising human population. The current method of our study could help in developing a harvest strategy or taking corrective measures to obtain higher potential bioactive chemicals, especially by cultivating them in a particular climatic condition, which would also help in decision-making in field management and cultivation strategies. Berberine is used in the pharmaceutical industries to treat diabetes, high blood pressure, skin, etc.

Conflict of interest

The authors declare no conflict of interest in any form while fulfilling the objectives of this research work.

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