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Development and Validation of Analytical Method for Estimation of Berberine in Herbal Extract of *Momordica Dioica*

Research Article

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Abstract

Background:While several anti-diabetic medications have been found to lower blood glucose levels, there are numerous negative effects connected with the use of currently marketed anti-diabetic medications.However, there are medicinal plants with anti-diabetic effects with low or no side effects. Among the Cucurbitaceae family of plants, *Momordica dioica* is a dioecious climbing herb. It contains many phytoconstituents.One of it is a quaternary ammonium salt of benzyl-isoquinoline alkaloid called as berberine exhibiting a wide variety of pharmaceutical properties.In the current investigation, the activities of berberine for its anti-diabetic potential in the *Momorica Dioica* plant.The goal of the recent effort is to establish and validate an HPTLC method that is quick, accurate, exact, and specific for determining berberine from herbal extract of *Momordica dioica*. Methodology/Conclusions/ significance: For a quick examination to determine the amount of berberine, a (HPTLC) process was created as well as validated.On HPTLC aluminium plate 60 F254,precoated with silica gel chromatographic separation was accomplished using the solvents: methanol ,ethyl acetate, toluene and formic acid (2:4:3:0.5). A wavelength of 348 nm was used for detection. Rf was found to be 0.44±5% for berberine. There was linearity for berberine in the 400ng/ band concentration range.The LOD and LOQ was found to be0.0096ng/band and 0.0293ng/ band for Berberine.The average berberine recovery rate was (0.041).The method's precision, accuracy, linearity, robustness, and specificity were all authenticated in accordance with ICH guidelines. The discovered approach can be used to regularly analyse Berberine in herbal formulations for quality control.

Key Words: Berberine, HPTLC, Herbal Extract, *Momordica Dioica.*

Introduction

As a form of supplementary medicine, medicinal plants and their secondary metabolites are being employed to cure ailments.The herbal plants have rich therapeutically active phytoconstituents. From few decades nutraceutical formulations are in demand and there is a desire to guarantee the effectiveness, safety, and quality of herbal medicines. Phytochemical analysis is one of the tools for evaluating qualitythe chromatographic technic is important tool for quantitative and qualitative evaluation. HPTLC is the sophisticated technic used for estimation of chemical marker and biomarker. The use of synthetic pharmaceuticals increases the risk of developing cancer, diabetes, and neurological diseases.

Because presently there are few viable treatments for type 2 diabetes, this disease may cause a hazardous effect on global health. All presently used oral hypoglycemic medications eventually fail when used for an extended period of time.New oral drugs are therefore required for the long-term management of blood glucose in type 2 diabetic patients.

There is an desperate necessity to generate medicines using natural herbs as a cure for that. Due to their ability to mitigate the detrimental effects of synthetic pharmaceuticals, indigenous medicines provide a promising solution to the global health threat.(1)

Administration of oral hypoglycemic medicines like metformin addresses these antidiabetic activity therapeutic methods.All of these medications have a variety of adverse effects that limit their continued use and complicate treatment options.In order to treat diabetes(type 2), Finding secure and non-toxic materials is essentialanti-diabetic medications derived from plants.

Momordica dioica, perennial, dioecious climbing herb from Cucurbitaceae family. (locally called teasle gourd, kakrol or a spiny gourd.) It is endemic to Asia and has a large population in Bangladesh and India. There are numerous phytoconstituents in it. Alkaloids, steroids, triterpenes, flavonoids, glycosides, saponin, flavonoids, and traces of berberine-specific alkaloids are among the phytoconstituents of Momordica dioica. (1-3)

It has been used for thousands of years as a vegetable with high nutritional content in addition to

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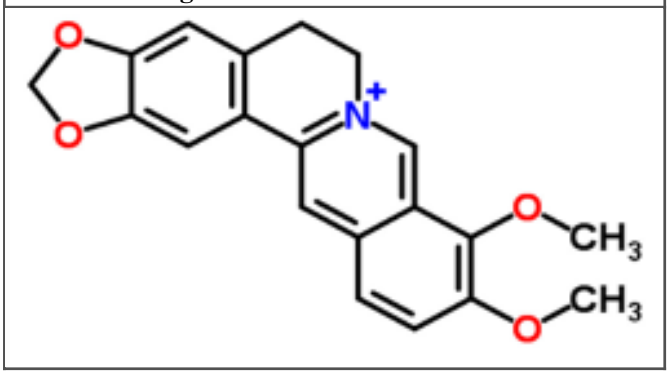
being utilised as a preventive and therapeutic agent for a variety of ailments. (7)

Berberine is a significant lead chemical among the many alkaloids with a wide variety of pharmacological properties, including hypoglycemic,anti-inflammatory, anticancer and anti-obesity effects.(2).

Despite having its roots in the Indo-Malayan region, this species is presently found to flourish in countries such as India, Myanmar,Bangladesh, China,Polynesia,Sri Lanka, Japan,Tropical Africa, South East Asia as well as South America.(9-10)

Fig. 1: Structure of Berberine



Berberine (5,6-dihydro-9,10-dimethoxy-benzo[g]-1, 3-benzodioxolo [5,6-a]quinolizinium) (Fig.1) It is a pentacyclic terpenoid that possesses a variety of medicinal qualities.A secondary plant metabolite called berberine is typically found in the fruit peel, stem bark, and leaves.This chemical has been unwittingly utilised for generations as a component in herbal extracts used in folk medicine because of its health-promoting properties. Recently, scientists who were hunting for naturally occurring physiologically active chemicals turned to this reservoir of information accumulated over many generations.

A pentacyclic triterpenoid carboxylic acid known as berberine is the primary constituent of numerous plants, including oregano, apples, peppermint, cranberries, basil, rosemary as well as prunes. It has been shown to have antioxidant and anti-tumor activities. When compared to other regularly used hypoglycemic drugs like sulphonylureas, biguanides, thiazolidinediones, or acarbose, Berberine and similar isoquinoline alkaloids have a very different chemical structure. As a result, berberine may represent a new class of anti-diabetic drugs if its effectiveness and safety are established. (4-5)

When diabetic individuals received 500 mg of berberine three times a day for 13 weeks, HbA1c levels dramatically fell (from 9.5% to 7.5%), according to a pilot research by Yin J et al.[4], which was compared with metformin. (6)

In addition to other pharmacological characteristics Other pharmacological effects of berberine have included antihypertensive, hepatoprotective antidepressant, anti-inflammatory, cholagogue, antioxidant, anti-cancer, anti-diarrhea, and most importantly, antibacterial effects.(8)

Materials and Methods

Chemicals and reagents

Standard Berberine was obtained from Global Pharma Ltd., Mumbai. All chemical as well as reagents used were of analytical grade.

Collection and preparation of plant material

Momordica dioica fruit was procured at a local market. Selection and slicing of the mature fruits took place. Then, they were ground into a fine powder and dried at room temperature.

Extraction of plant material

It was extracted using the maceration process. Methanol (250 mL) was used to extract 100 g of powdered ground fruit over the course of two days. Herbal extract of *Momordica dioica* was prepared.

Instrumentation

Chromatographic separation was carried out using a Camag HPTLC system that included a WinCAT software version 1.sss254.3.6336, a CamagLinomat-v semiautomatic sample applicator, twin trough development chamber, Camag 100l syringe, and Camag TLC Scanner-3.

Preparation of standard solution

A precisely measured amount of 1 mg of berberine was added to 1 ml of Eppendorf and dissolved in 1000 g/ml of methanol and once more, 0.1 ml of berberine stock solution should be dissolved in 1 ml of methanol. (100µg/ml)

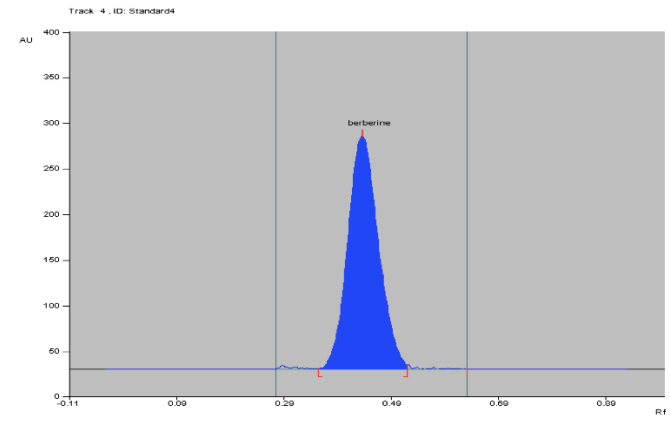
Preparation of sample solution

Momordica Dioica Extracted Powder was taken as sample for analysis Take a sample of powder containing 20 mg of Momordica Dioica that has been precisely weighed and dissolved in 1 ml of Eppendorf (20000 mg/ml). The solution was filtered using Syringe filter paper after being sonicated for one hour.

Optimization of mobile phase

Because mobile phase is key to the chromatographic process, optimising the solvent system for significant efficiency of extraction by optimising solvent system is the first step in creating a successful method. a technique that provides a compact spot of significant significance for the measurement of berberine in a formulation. Different mobile phase combinations and ratios were examined in order to optimise the mobile phase. Using toluene The Berberine peak was produced by the reaction of methanol ,ethyl acetate, toluene and formic acid (2:4:3:0.5)as shown in (fig 2-3).

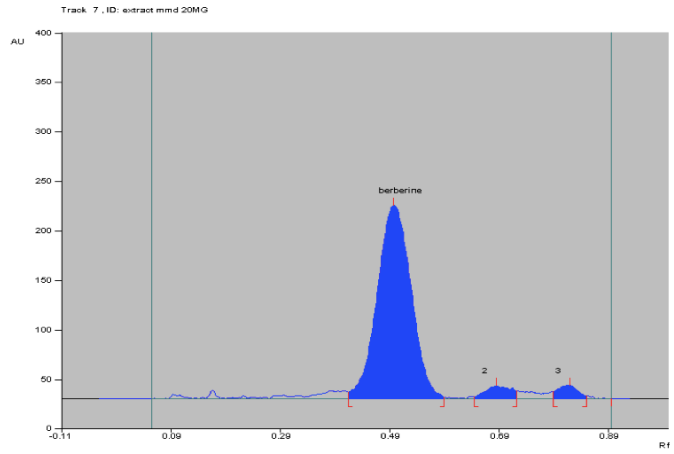
Fig. 2 : Densitogram of Berberine



Track 4, ID: Standard4

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.35 Rf	0.5 AU	0.44 Rf	255.5 AU	100.00 %	0.52 Rf	4.5 AU	12173.7 AU	100.00 %	berberine

Fig. 3 : Densitogram of Momordica dioica extract



Track 7, ID: extract mmd 20MG

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.41 Rf	6.7 AU	0.50 Rf	195.9 AU	87.95 %	0.59 Rf	1.5 AU	10101.9 AU	91.50 %	berberine
2	0.64 Rf	2.1 AU	0.68 Rf	13.3 AU	5.95 %	0.72 Rf	7.9 AU	514.7 AU	4.66 %	unknown *
3	0.79 Rf	7.2 AU	0.82 Rf	13.6 AU	6.10 %	0.85 Rf	3.1 AU	424.0 AU	3.84 %	unknown *

Fig. 4 : Selection of wavelength

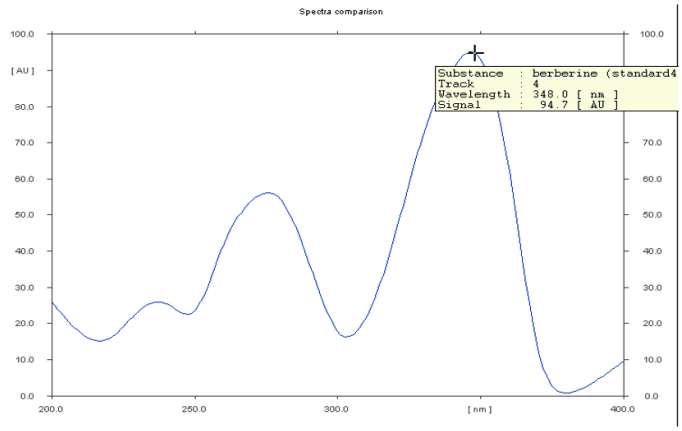
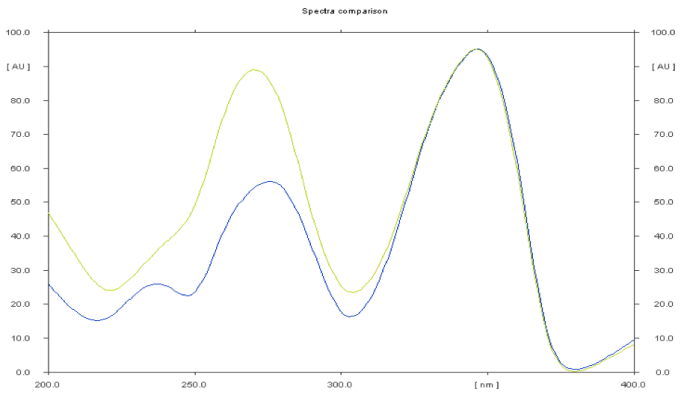


Fig. 5 : Overlay spectra of Berberine (0.4ul)+mmd extract (4ul)



Track	Rf	Assigned Substance	Max. Signal	Display	r(s,m)	r(m,e)
1	0.41	berberine	42 AU @ 200 nm	☐	0.999999	0.999970
2	0.41	berberine	114 AU @ 346 nm	☐	0.999973	0.999954
3	0.42	berberine	203 AU @ 346 nm	☐	0.999981	0.999924
4	0.43	berberine	270 AU @ 347 nm	☑	0.999930	0.999917
5	0.45	berberine	293 AU @ 347 nm	☐	0.999925	0.999893
6	0.45	berberine	357 AU @ 347 nm	☐	0.999466	0.997366
7	0.49	berberine	211 AU @ 346 nm	☑	0.999631	0.998688

Chromatographic conditions:

On a pre-coated silica gel aluminium plate, the sample was spotted in the shape of a 6mm band using a camag microlite syringe. The mobile phase, designated 60 F254, was made up of methanol ,ethyl acetate, toluene and formic acid (2:4:3:0.5v/v).The optimal mobile phase chamber saturation time was found to be 15 minutes at room temperature. The generated TLC plate was dried with the use of an air dryer. The UV light was continually emitted between 400 and 200 nm by the deuterium lamp that served as the radiation source.

Method validation

Linearity

Calibration curves were generated to ascertain the linearity. A concentration of standard stock solution ranging from 10-60ng/band. The calibration curve was obtained using peak areas against each band concentration. (16)

Precision

Six identical samples of the same Berberine content(40ng/band) wasusedby performing intra-day and inter-day measurements, the repeatability and

instrumental precision of methanol were assessed. PrecisionSD as well as % RSD values were calculated.

Accuracy

Samples that had already been tested were spiked with 80, 100, and 120% of standard. Berberine and resulting solution were examined by the improved method using three replicates of each level SD and %RSD values were calculated.

LOD and LOQ

Detection limit (LOD and limit qualification (LOQ) was calculated using the equationY= 923.542+ 239.386X.

LODand LOQ: 0.0096 and 0.0293 respectively.

Robustness

Robustness was evaluated using 40ng/band by making small deliberate change in saturation time (±5%) of mobile phase, the amount of phase (±1). The effect of various deliberate changes was studied on retention factor and peak area, the SD and % RSD was calculated.

Results and Discussion

A wavelength of 348nm was chosen for quantification. The average berberine recovery rate was (0.041).The Rf value of Berberine after development with mobile phase methanol, ethyl acetate, toluene and formic acid (2:4:3:0.5 v/v)was 0.44 ± 5%.

Fig. 6: TLC plate of Berberine + Momordica dioica extract

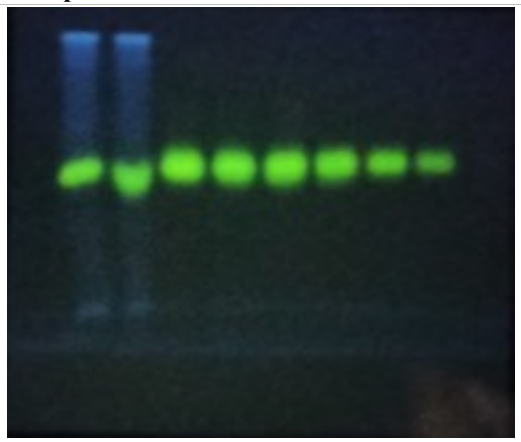


Fig. 7: TLC plate of Berberine + Momordica dioica extract

Linearity:

The capacity of an analytical method to produce test findings that are either directly or indirectly proportional to the analyte concentration within a specified range is known as linearity.

A strong linear relationship exists between the response (peak area) and quantity was acquired between 10-60ng/band. Data from linear regression are used as the correction coefficients in the calibration graphic(r) to be discovered was 0.973. (Fig. no.8) It was discovered that theBerberine regression equation was resulted to be Y= 923.542+ 239.386X.

Fig. 8: Linearity graph of Berberine

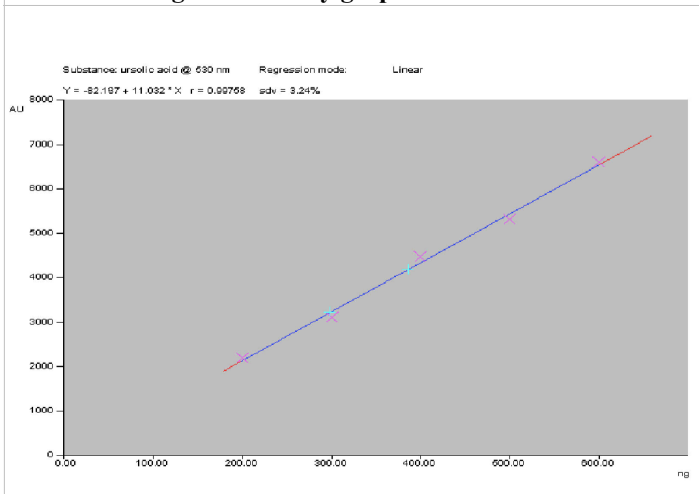
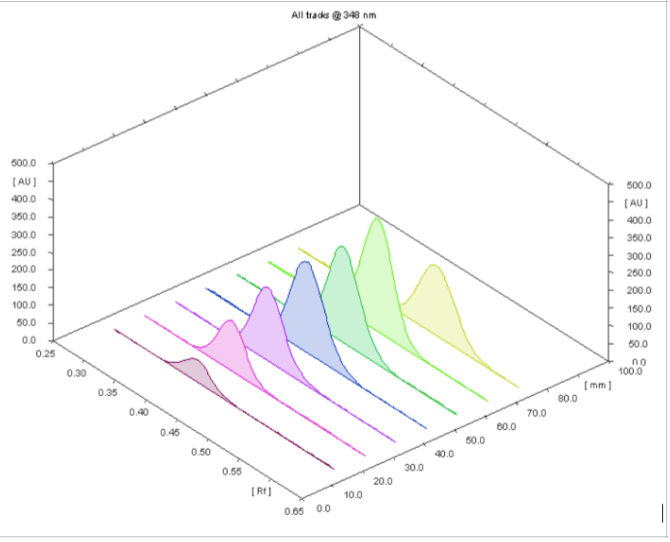


Fig. 9: 3D spectrafor Berberine + Momordica dioica extract



Precision

For the purpose of managing the scanner's settings, namely the repeatability of peak area measurements and repeated scanning (n = 6) of the same high-precision device was used to verify band of Berberine (40ng/band), and was it was determined to be

less than 3% when reported as %RSD, as shown table no(2).

Six replicate injections of standard sample solutions were made for the intraday precision studies, and the response factor of the standard compounds and the percent RSD were computed. The result are shown in table no (1).

Table no.1: Statistical evaluation for system precision and method precision

Sr. No	System precision
	Peak area
Mean	12132.33
S.D	35.57
RSD.	0.0029

Accuracy:

It is measured by how closely test results match the true value of the analyte. Applying analytical technique investigations allowed for its determination.The marketed formulation was spiked with 80, 100, and 120% of Berberinestandards.The mixtures underwent triple analysis. Results is shown in Table no (2).

Table no: 2 Statistical validation for recovery study

Level of recovery	% Recovery	S. D (±)	R. S. D
80%	56.16%	6.90	0.00071
100%	95.2%	15.87	0.0013
120%	105%	15.22	0.0010

Limits of detection and quantification

By injecting progressively lower concentrations of the standard solutions while utilising the devised HPTLC method, the LOD and LOQ of the method was established. The calculations for Berberine's LOD and LOQ yielded values of 0.0096(ng/Band) and 0.0293(ng/Band), respectively.

Robustness

Through the minor changes in the conditions of chromatography, robustness of the method was found. (mobile phase volume, saturation time.It was noted that the chromatograms did not significantly change.(Table no.3).

Table 3: Result of robustness

Factor	Level	Area	Rf
Saturation time			
10 min	-5	12170	0.33
15 min	0	12173	0.44
20 min	5	12178	0.50
	S. D± R. S. D		± 0.
Total mobile phase	level	Area	Rf
8.5ml	-1	12170	0.33
9.5 ml	0	12173	0.44
10.5 ml	1	12178	0.50
	S. D± R. S. D		1 ± 0

Conclusion

The quantification and identification ofactive component in Momordica dioica's herbal extract.For the identification and measurement of berberine, a new HPTLC approach has been created.A rapid, selective, sensitive, reliable and robust HPTLCa technique has been created and verified for the determinationof Berberinein herbal formulations.The average berberine recovery rate was (0.041). According to ICH criteria, the procedure was successfully verified.

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