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PHYTOCHEMICAL INVESTIGATION AND CHROMATOGRAPHIC ANALYSIS OF ETHANOLIC EXTRACT OF *TINOSPORA CORDIFOLIA* WILD

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ABSTRACT
Chromatography techniques such as HPTLC and HPLC are commonly used to produce a fingerprint of Phytoconstituents. It allows identification and quantification of the main constituents within the plant. The aim of this study is to investigate the phytochemical constituents and comparing the HPTLC and HPLC quantification analysis of berberine in ethanolic extract of stem part of *Tinospora cordifolia* (ESTC). The HPTLC is effective for qualitative analysis, whereas HPLC is more accurate for quantitative analysis. A combination of these two methods may be useful in a quality control Testing as it would allow rapid qualitative analysis of herbal material while maintaining accurate quantification of extracts.

KEYWORDS: *Tinospora cordifolia* linn, HPTLC, HPLC, berberine, quantification and phytochemical constituents, T.cordifolia.

INTRODUCTION

Tinospora cordifolia is dried matured pieces of stem and it is widely distributed throughout tropical and sub-tropical India.[1] *it* is a large, glabrous deciduous climbing shrub found throughout tropical India, belonging to the family Menispermaceae. Stems are succulent with long filiform fleshly aerial roots from the branches. Bark is greyish brown (or) creamy white and warly. Leaves are membraneous, cordate with broad sinus. Flowers are small yellow or greenish yellow appearing when the plant is leafless.[2] The stems of *T.cordifolia* is used in traditional Indian medicine as a folkloric veterinary medicine and traditional ayurvedic medicine in India for its anti-inflammatory, immune modulatory, anti- pyretic activity, anti-oxidant activities and various other medicinal properties.[3] Ayurvedic system of medicine as a tonic, vitalizer and as a remedy for diabetic and metabolic disorders. Anti-oxidant activity of *Tinospora cordifolia* and inhibition of lipid peroxidation have been reported. The hypolipidemic action of *Tinospora cordifolia* greatly enhances its heart disposition.[4]

T.cordifolia (Menispermaceae) is an important medicinal plant of tropical and sub- tropical India. Its medicinal usage has been reported in the Indian herbal pharmacopoeia, the ayurvedic pharmacopoeia and in traditional systems of medicine such as Ayurveda, unani and siddha.[1,5] *T.cordifolia* called Guduchi in Sanskrit is a glabrous and succulent shrub. A variety of constituents have been isolated from *Tinospora cordifolia* belonging to different classes such as alkaloids, glycosides, diterpenoids, lactones, sesquiterpenoids and steroids. It contains about 11.2 percent protein and rich in calcium and phosphorus.[6]

The powdered stem of *T.cordifolia* (Guduchi) was extracted with different solvents of these, alcohol (like methanol and ethanol) and water, yield number of pharmacologically active alkaloids.[7,8] it also contain glycosides,diterpenoids, lactones and sesquiterpenoids.[9] Berberine and tinosporaside was major component obtained. The stem part contains various alkaloids like berberine, palmatine, jactrohizine and tembeterine. In this plant extracts cause reduction in fasting blood sugar in rabbit and rats. Aqueous extracts of the stem shows anti- inflammatory, analgesic and anti-pyretic actions in rats and immuno suppressive effect in rabbits and show antipsychotic activity in amphetamine challenged mice model.[10] Ethanolic extract of the stem exhibits protective effect in carbon tetrachloride induced hepatotoxicity show cardio protective activity in ischemia reperfusion induced myocardial infraction in rats.[11]

In the present study we have done the phytochemical screening of ethanolic extract of *Tinospora cordifolia*, HPLC & HPTLC fingerprint and quantitative study of presence of Berberine in *T.cordifolia*.

EXPERIMENTAL METHODS

Plant material

The plant was collected from Uthangudi village, Madurai during January 2019 and the plant was identified and authendified by Prof. Dr. Stephen Ph.D, Department of Botany, American College, Madurai, Tamil nadu, India.

Chemicals

Methanol, Ethanol, Petroleum ether, Ethyl acetate, Acetone, Formic acid and Water.

Preparation of plant extract Treatment

Collected plant material was washed under running tap water to remove foreign earthy adherable matter. The plant materials are cut into pieces and dried under sun for two weeks time. The plant material were crushed into fine powder by using mechanical blender and kept in an air tight polythene bags for further use and stored at room temperature.

Extraction

Firstly, the stem part of plant was air dried and powdered coarsely. The powdered crude drug is defatted by using petroleum ether in soxhlet apparatus for 3 hours. The extraction was carried out by using ethanol as solvent and the extraction time is 8 hours. Rotary flash evaporator was used to recover the solvent and the extract was subjected to complete phytochemical screening.

THIN LAYER CHROMATOGRAPHY

Test plate: TLC pre-coated plates, silica gel $60F_{254}$
Format: 10×10 cm
Thickness: 250µm
Spotting volume: 10µl
Development chamber: normal TLC chamber **Mobile phase:** Ethyl acetate- Methanol- Formic acid.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Sample name: Ethanolic extract of *Tinospora cordifolia*

Standard name: Berberine/ Solubility: methanol

Mobile phase: Ethyl acetate: Methanol: Formic acid (6: 4: 0.5)

Detection range: 366nm

Sample preparation

1mg of the extract was dissolved in 1ml of Ethanol and filtered through 0.2 µ membrane filter.

Standard preparation

1mg of Berberine was dissolved in 1ml of Ethanol (stock.) 100 µL of the stock was diluted to 1ml with Ethanol (100 µg/ml, working standard), filtered through 0.2 µ membrane filter.

Linearity

1to 5 µL (0.1-0.5 µg)of the working standard was applied as 6mm band on a silica precoated aluminium plate using Linomat 5 applicator.

Sample: 2-6 µL of 1mg/ml sample was applied.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Sample name: Ethanolic extract of *Tinospora cordifolia*
Standard name: Berberine/ Solubility: methanol
Mobile phase: Methanol :(0.1%) Formic acid.

Detection range: 266nm

Sample preparation

1mg of the extract was dissolved in 100 ml of Ethanol and filtered through 0.2 µ membrane filter.(0.01mg/ml).

Standard preparation

1mg of Berberine was dissolved in 10 ml of Ethanol (0.1mg/ml).

RESULTS AND DISCUSSION

The percentage yield of crude ethanolic extract of *Tinospora cordifolia* - 4.96%w/w.

Table 1: phytochemical investigation of Ethanolic extract of *T.cordifolia.*

Phytochemical analysis	Ethanolic extract of T.cordifolia
Molisch's test	+ve
Flavonoid test	+ve
Terpenoid test	+ve
Xentoprotic test	-ve
Gelatin test	+ve
Alkaloid test	+ve
Ninhydrin test	+ve
Steroid	-ve
Phenol test	+ve
Saponins	+ve
Diterpenes	+ve

HPTLC Fingerprint analysis

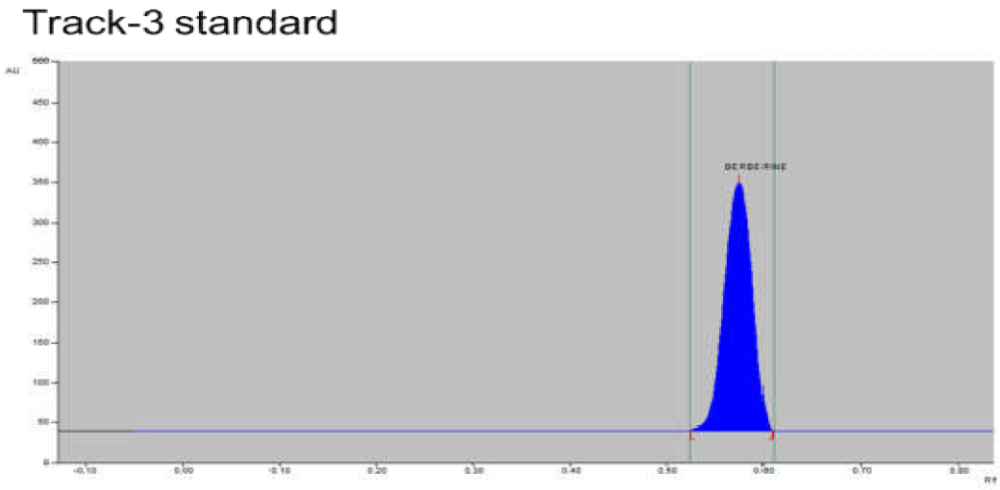

Figure 3: HPTLC chromatogram of standard berberine.

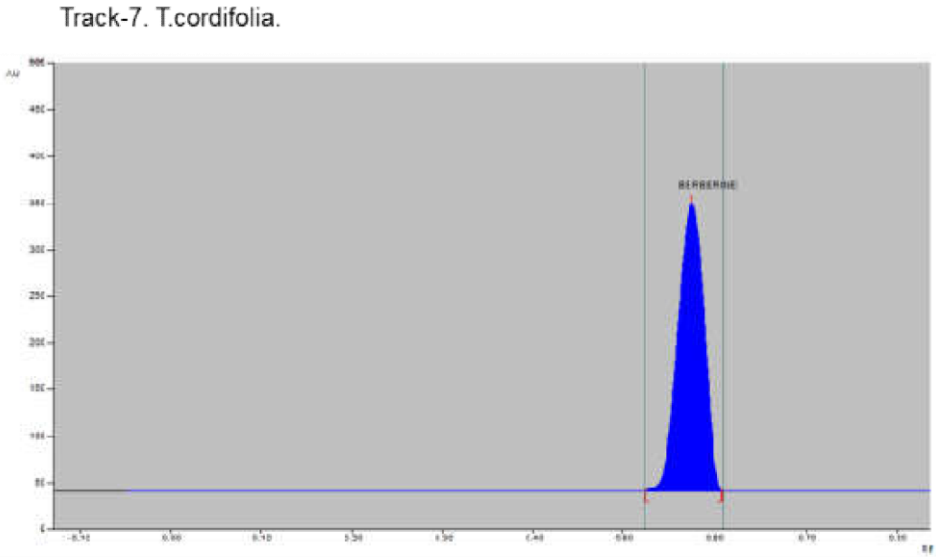

Figure 4: HPTLC chromatogram of sample (Ethanolic of extract of *T.cordifolia*).

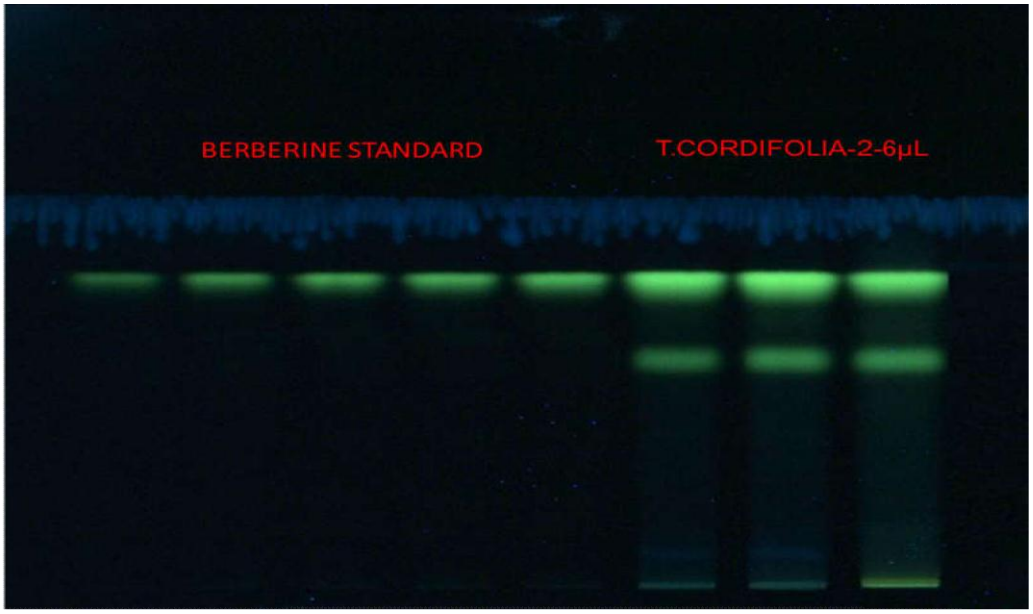

Fig 5: Image of HPTLC plate under the UV light.

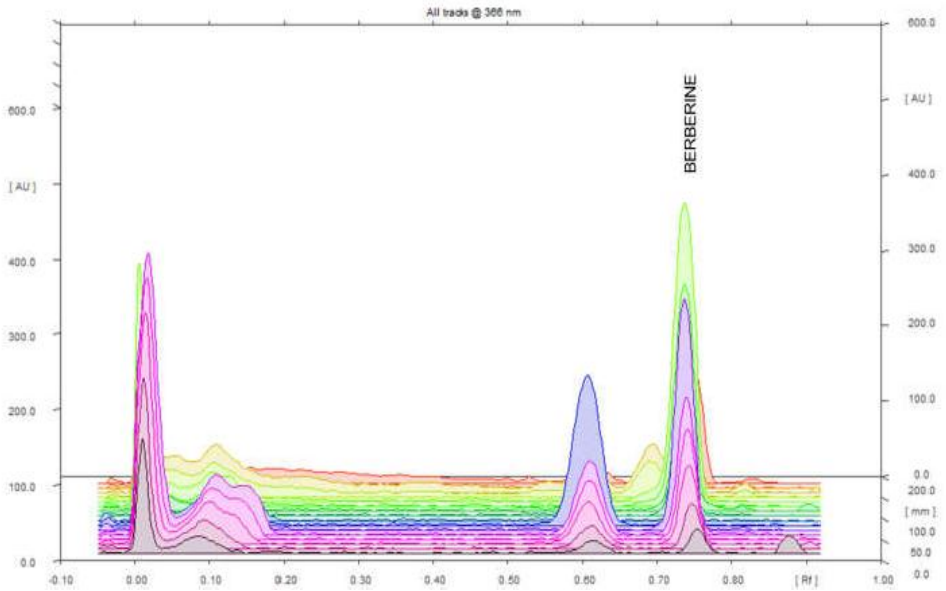

Fig 6: winCATS planar chromatography manager.

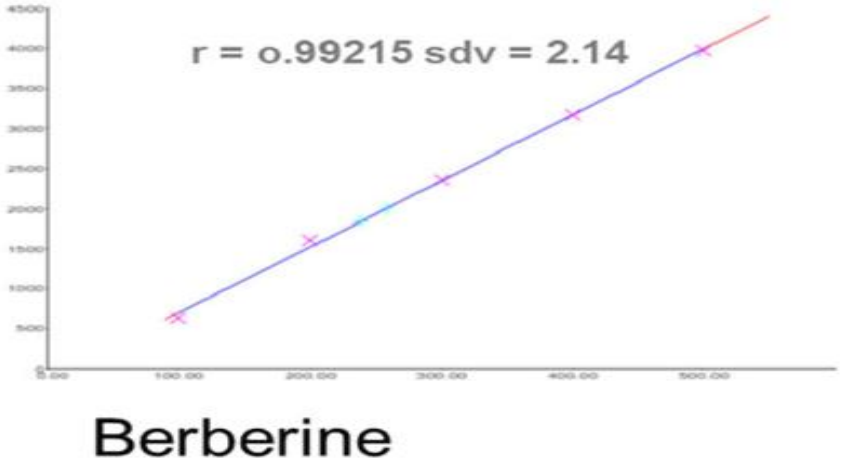

Fig 7: linearity curve of standard berberine.

HPLC Fingerprint analysis

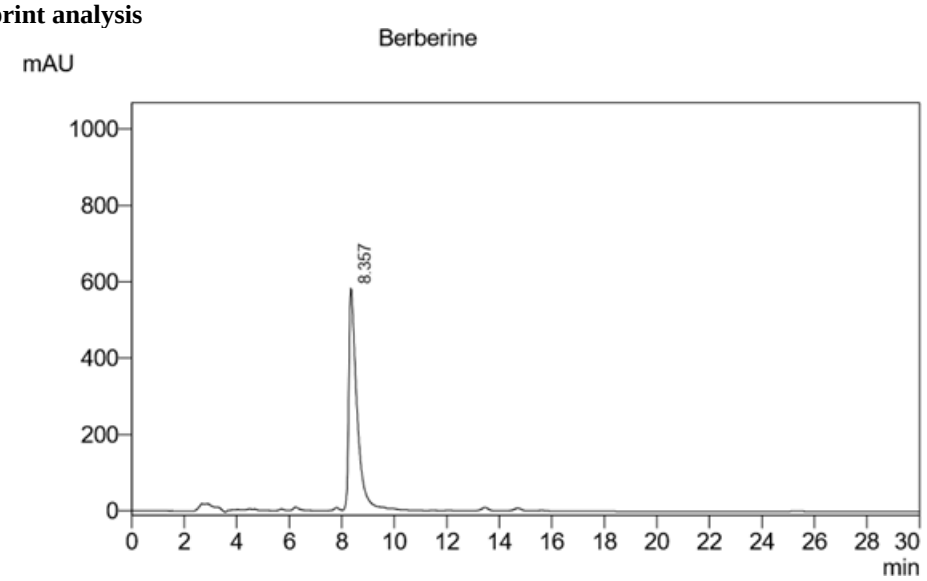

Figure 1: HPLC chromatogram of standard berberine.

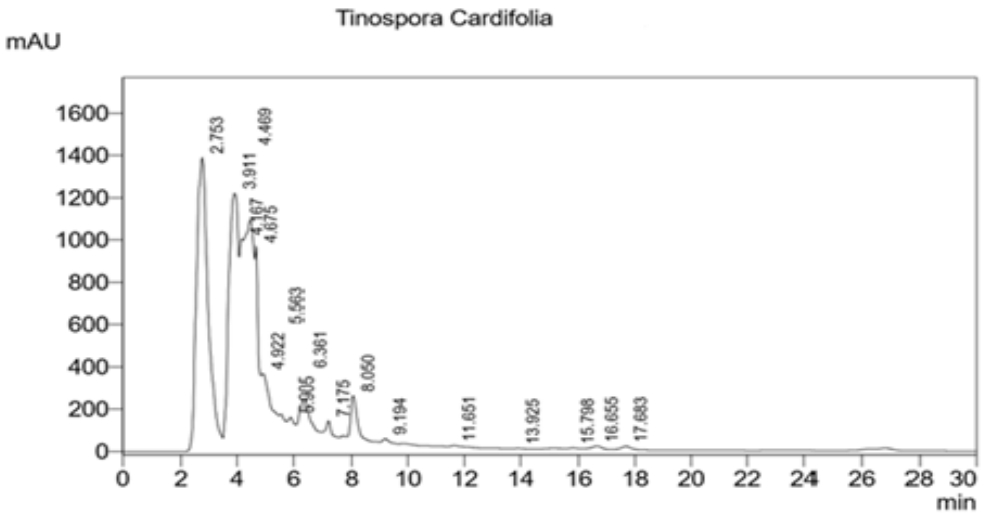

Figure 2: HPLC chromatogram of sample (Ethanolic of extract of *T.cordifolia*).

The amount of berberine in ESTC by HPTLC method is 0.37%w/w

The amount of berberine in ESTC by HPLC method is 0.34%w/w

CONCLUSION

The phytochemical test on ethanolic extract of Tinospora cordifolia stem extract Showed the presence of various phytoconstituents like Carbohydrates, Flavonoids, Terpenoids, Alkaloids, Saponins, Proteins, Diterpenes, Gelatin and Phenol. The proposed HPLC and HPTLC method was found to be rapid, simple and accurate for quantitative estimation of berberine in Tinospora cordifolia. The results of linearity range and correlation coefficient show that within the concentration range, a good correlation between peak area and corresponding concentration of berberine. HPLC and HPTLC fingerprint profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. This parameter can also be a very important tool if some adulteration is suspected in plant material. The present HPLC and HPTLC fingerprint profile can be used as identification tool to identity and to determine the quality and purity of the plant material in future studies. This method allows reliable identification and quantification of berberine in Tinospora cordifolia.

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