BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2018; 13: 137-141
ISSN: 1991-0088

HPTLC method for quantitative determination of hydroxycinnamic acid derivatives in *Solanum lycopersicum*

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Article Info

Received: 31 October 2017
Accepted: 11 April 2018
Available Online: 30 April 2018

DOI: 10.3329/bjp.v13i2.34461

Cite this article:
Ishwarya M, Narendhirakannan RT. HPTLC method for quantitative determination of hydroxycinnamic acid derivatives in *Solanum lycopersicum*. Bangladesh J Pharmacol. 2018; 13: 137-41.

Abstract

The study was aimed to validate and optimize high performance thin layer chromatography (HPTLC) method for the determination of phenolic compound hydroxycinnamic acid derivatives from the fruits of *Solanum lycopersicum* (tomato). The presence of caffeic acid and coumaric acid in the aqueous extracts *of S. lycopersicum* was observed in spectrophotometer at 200-400 nm. The phenolic functional group was recorded by FTIR analysis. In the HPTLC analysis, the pre-coated silica gel was used as the stationary phase. The solvent mixture containing toluene:ethyl acetate:formic acid:methanol (3:6:1.6:0.4) was identified as an optimum ratio were used as a mobile phase. The chromatograms of the extract was scanned by densitometer at 327 nm. The Rf values (0.67 and 0.69) and finger print data were recorded by WIN CATS software. The developed HPTLC methods for bioactive marker compounds present in the fruit were found to be simple, accurate, and economical.

Introduction

Natural product, such as plant extract, either as pure compound or as standardized extract, provides opportunities for new drug discovery. The premier steps to utilize the biologically active compound from the plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation (Sasidharan et al., 2011). These active compounds are considered to be the marker or chemicals that confirms the identity of the compound. It is a long process and challenging to identify specific marker compounds for all medicinally important plants. There are multiple active metabolites to be reported in each plant extract.

A chromatographic fingerprint for the extract is necessary to identify the pharmacologically active compound in it (Patil and Shettigar 2010). High performance thin layer chromatography (HPTLC) is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits. It is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks (Srivastava, 2011; Sethi, 2013).

There are many evidence from the nutritional studies indicating that regular consumption of raw tomatoes and tomato-based products are consistently associated with a reduction in the incidence of chronic degenerative diseases.

The main aim of this study was to identify the active phenolic compound, hydroxycinnamic acids from *Solanum lycopersicum* extract by HPTLC validation experiments. The four most widely distributed hydroxycinnamic acids in fruits are p-coumaric, caffeic, ferulic and sinapic acids (Macheix et al., 1990). Hydroxycinnamic acids usually occur in various conjugated

forms, the free forms being artefacts from chemical or enzymatic hydrolysis during tissue extraction. A variety of epidemiological studies have suggested that the intake of tomato-based foods is inversely related to the incidence of cardiovascular disease and cancer of different types (Shi and Maguer 2000; Erdman et al., 2009).

Materials and Methods

Reagents

Caffeic acid (99%) and coumaric acid (99%) standards were obtained from Sigma, India. All the mobile phases used were of analytical grade reagents.

Sample collection and Preparation

Fruits of *S. lycopersicum* was collected from a local market (Coimbatore, Tamil Nadu), after authentication from Botanical Survey of India, Coimbatore (Ref No: SI/SRC/5/23/2015/Tech./888), was used in this study. The fruits were dried, powdered and stored at room temperature. The extract was prepared by adding 0.5 g of the sample in 50 mL boiling tube containing 25 mL of distilled water. The sample was kept in 80°C for 30 min. later it was filtered and used for further analysis.

UV visible spectrophotometer analysis

The aqueous extracts were visualized for peaks under UV visible spectrophotometer ranging from 200 to 500 nm range. The bell curve peaks were recorded at the excitation range for caffeic acid and coumaric acid.

Preparation of stock solution and working standard solution

1 mg of the respective standard compounds, caffeic acid and coumaric acid were dissolved in 1 mL of methanol and used as the standard solution. The standard working solution was prepared at different concentration.

FT IR analysis

The elutes were mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder, and FT-IR spectra were recorded in the range 4000–450 cm^{-1} in FT-IR spectroscopy (Perkin Elmer FT-IR Spectrometer, USA).

High performance thin layer chromatography (HPTLC)

Silica gel-coated TLC plates (F254 20 x 20 cm) were activated in an oven at 50°C for 30 min before usage. Samples were applied using a Linomat 5 (Camag, Switzerland) semi-automated HPTLC applicator with a 100 µL syringe (Hamilton, Switzerland) which was programmed using planar chromatography manager winCATS (Camag, Switzerland). Seven lanes were applied in each plate with a 6 mm band width, 10.3 mm between bands and 10 mm from the bottom of the plate. Volumes applied varied between 5.0 and 15.0 µL with the syringe rinsed with absolute ethanol between samples.

Solvent system

A mixture of toluene, ethyl acetate, formic acid and methanol was used as the mobile phase in HPTLC separations. The gradient elution was set up with the solvents mixed in ratio of 3:6:1.6:0.4 respectively and elution time was 15 min. Later the plates were allowed to dry completely. An AMD2 developer (Camag) was setup for the development of the TLC plates.

Rf value = Distance travelled by the substance/Distance travelled by the solvent

Photo documentation

The photo was taken with a TLC visualizer containing a G 16 powershot camera (Canon) using UV wavelengths of 366 and 243 nm. There was no further derivatisation required. Quantitative HPTLC analysis was performed using Video Scan software (Camag) with a minimum peak width of 7 pixel, peak heights of 400 pixel, and a minimum area of 1000 pixel, using a filter width of 7 nm. The retention factor values and finger print data were recorded by WIN CATS software.

Results

The results from the optimized method for determining the hydroxycinnamic acid present in the fruits of *S. lycopersicum* are given below. The peaks under UV visible spectrophotometer were obtained at 310 nm for standard caffeic acid and sample peak was noticed at 210 nm (Figure 1 and 2).

The band intensities in different regions of the FT IR spectra were analyzed and represented in Figure 3. The FTIR spectrum revealed a strong peak at ~ 3444.87 cm^{-1}, representing the characteristic of phenols.

HPTLC is a rapid and economic solvent method that enables several samples to be analysed simultaneously. An initial rapid screening of the compounds in molecular level present in the extracts was done using specific reagents. In HPTLC, the chromatographic conditions were based on isocratic separation with different mobile phase combinations. The mobile phase toluene:ethyl acetate:formic acid:methanol (3:6:1.6:0.4) was successful at separating caffeic acid and coumaric acid from the extract (Figure 4). In both FT IR and HPTLC, UV light was used for the detection of analyte compounds. On the HPTLC plate caffeic acid and coumaric acid produced blue bands at Rf values of 0.67 and 0.69, respectively, when observed under 366 nm UV light and green bands under 254 nm UV light. There was no specific derivatization required to visualize the bands on the TLC plates. There were similar results obtained on observation of both the plates. Thus,

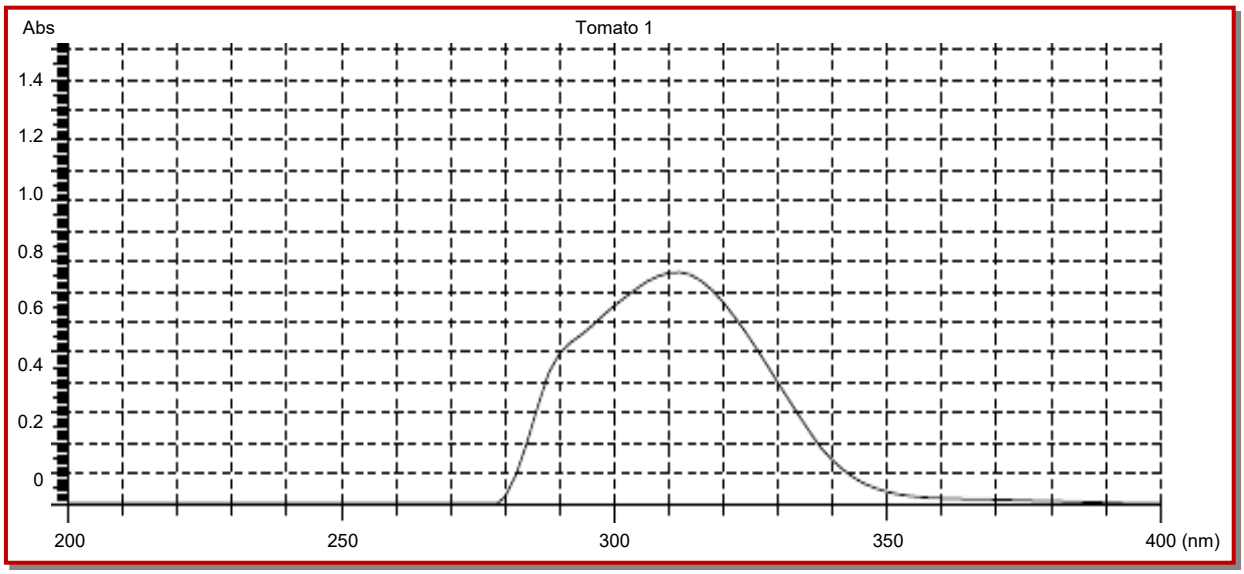

Figure 1: Peak obtained at 310 nm for the standard (Gallic acid)

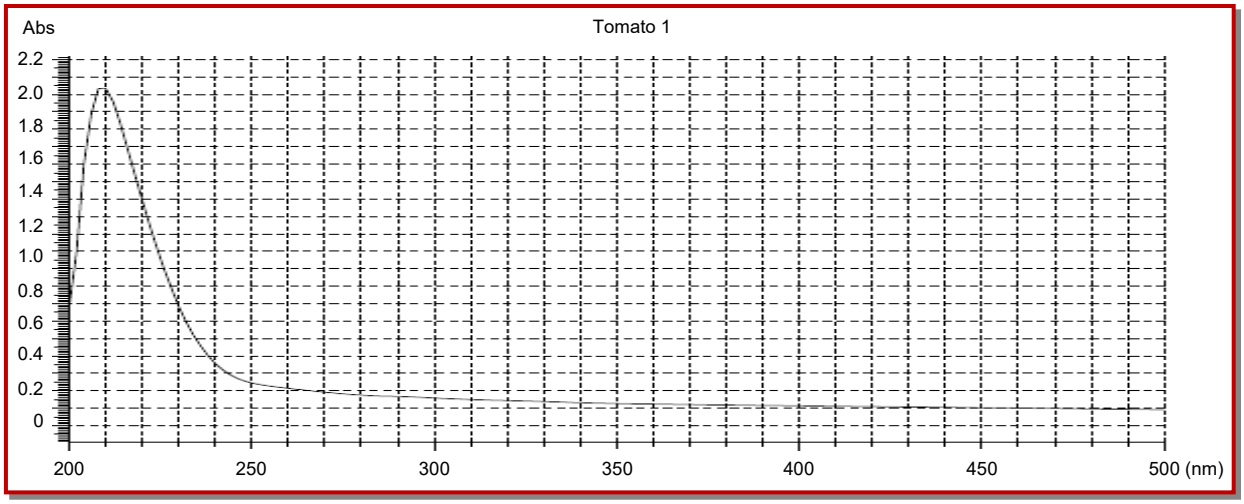

Figure 2: Peak observed at 210 nm for the sample (aqueous extract of *Solanum lycopersicum*)

it cannot be assumed that replicate plates are the same and further analysis is required if data from different plates is to be compared. The accuracy of the image analysis (i.e. variation between replicate images), was assessed by comparing images of the same plate. There was no significant variations observed, at times the software would recognise a single peak as a multiple peaks.

Calibration curve was constructed by plotting the peak area ratio versus different concentrations of caffeic acid and coumaric acid (5-10 µg). A good correlation coefficient (0.94 and 0.86 respectively) was obtained over the concentration range with respect to the volume.

Discussion

Hydroxycinnamic acid is the major phenolic compound to be reported. Simple phenolics have absorption maxima between 220 and 280 nm (Yang et al., 2012). Ferulic acid exhibits a maximum absorbance at 215 nm with additional absorbance at 287 and 312 nm. Coumaric acid displays a maximum absorbance at 286 nm with additional absorbance at 209 and 220 nm (Holser, 2012). Tomato plant is rich in polyphenols compounds (flavonoids and hydroxycinnamic acids), such as chlorogenic, caffeic, and ferulic acid, and rutin flavonoid (Chen, 2014; Rivero, 2003) and these compounds have phenolics rings and hydroxyl groups that act trapping the free radical and inhibiting the generation of reactive oxygen species. Several flavonoids have been identified and reported from tomato plant (Samsonowicz et al., 2015).

It has been accepted that a spectral band obtained at 3400 cm^{-1} showed a broad and strong indication of presence of –OH group, exclusively phenols (Nirma-

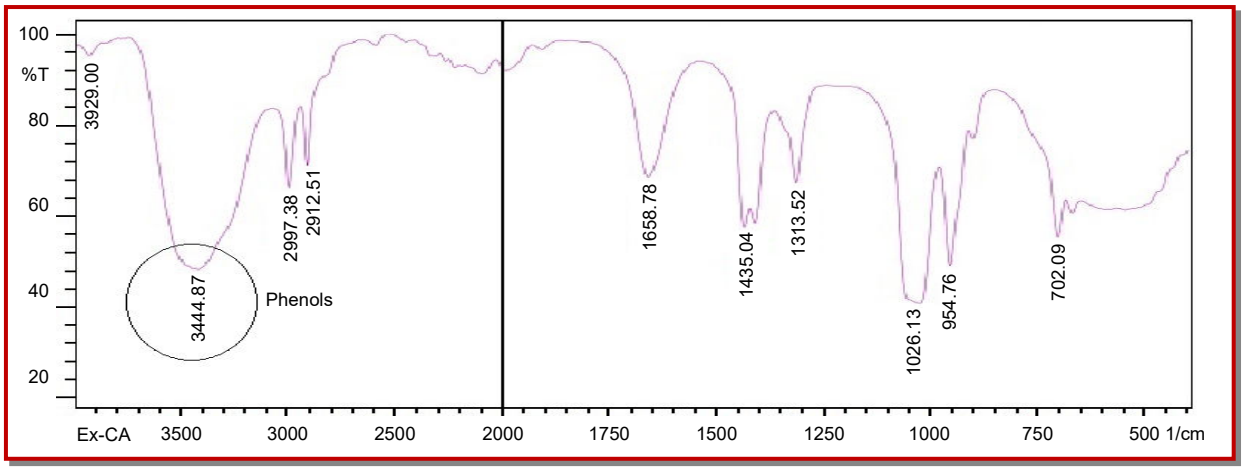

Figure 3: FTIR analysis of optimized *Solanum lycopersicum* extract

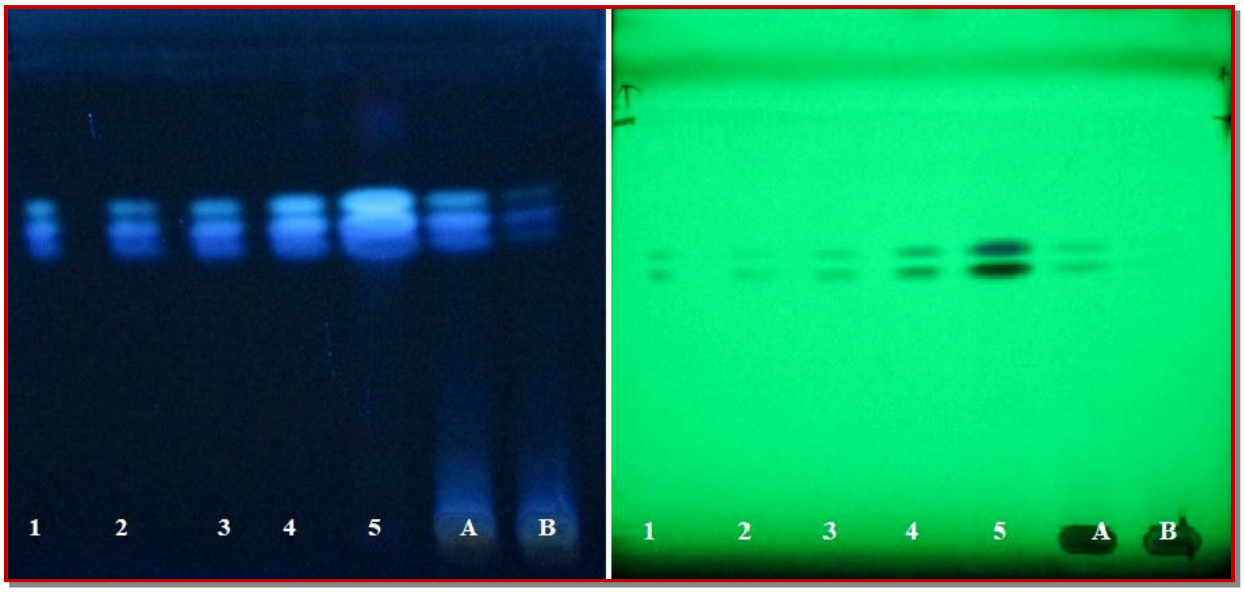

Figure 4: A developed HPTLC plate seen under 366 and 254 nm UV light. Lanes 1 and 5 contain the mixture of standards caffeic acid and coumaric acid at various concentrations ranging from 5 to 10 µg. Lanes A and B contains the hot water extract and sonicated + extract of *Solanum lycopersicum* fruits

ladevi, 2010). These band range are assigned to the stretching vibration of hydroxyl groups that interact by H bonding (Guerrero 2014). A detailed band assignment of transmission and ATR-FTIR spectra of isolated tomato fruit cuticles ranges from 1500-1600 cm^{-1} (Ramírez et al., 1992; España et al., 2014). The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity. Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds (Bravo, 1998).

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte (Shivatare et al., 2013). Semi-automated HPTLC applicators have allowed greater control of substance application onto HPTLC plates (Loeschera et al., 2014). In order to assess the extent of variation due to instrumental and handling errors, 2 identical plates were set up and run under the same conditions.

HPTLC is still progressively discovering its way in pharmaceutical industrial analysis. With the various encroachments in the stationary phases and the introduction of densitometers as detection equipment, the technique achieves for given applications a precision and trueness comparable to high-performance liquid chromatography (HPLC) (Shivatare et al., 2013).

HPLC methods, on the other hand, are generally considered more robust, and are capable of higher degrees of precision on replication and quantitation. With advances in HPTLC technology, other aspects of finger-

printing such as the use of gradient mobile phases and detection also need to be considered to determine the effectiveness of HPTLC as a quality control technique and potential applications in a quality control setting (Loeschera et al., 2014). Both HPTLC and HPLC face similar limitations when developing a method for fingerprint profiling, however, HPTLC has the advantage of having both coloured bands and retention factors to identify individual substances. It also allows samples to be run simultaneously making it a quick simple process of comparing chemical profiles.

Conclusion

Recent advances in instrumental technology have allowed the improvement of TLC to HPTLC analysis. So, it can provide useful qualitative and quantitative data in short work time and also the determination of key standard in samples. The results of this study shows the quantitative analysis of caffeic acid and coumaric acid by HPTLC method.

Acknowledgements

This work was funded by Department of Science and Technology- Science and Engineering Research Board (Project sanction NO. SB/YS/LS-262/2013), Government of India. We would like to express sincere gratitude to all the persons who helped us in carrying out the work and Department of Bioscience and Technology, Karunya Institute of Technology and Sciences, Coimbatore for providing necessary facilities.

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