RESEARCH ARTICLE

Simultaneous Determination of Pharmacological Active Phytocompound Gallic Acid and Quercetin in Aerial Parts of *Solanun indicum* and *Solanum xanthocarpum* by Validated High Performance Thin-layer Chromatography (HPTLC) Method

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ABSTRACT

Objective: Quantitative standardization of plant-based products is challenging albeit essential to maintain their quality. This study aims to develop and validate high-performance thin-layer chromatography (HPTLC) method for the simultaneous determination of gallic acid and quercetin from aerial parts of *Solanum indicum* and *Solanum xanthocarpum*.

Materials and Methods: The stock solution (1-mg/mL) of standard, gallic acid and quercetin in methanol: Water (1:1) was serially diluted and spotted (5 µL) on silica gel 60 F254 thin-layer chromatography plates. Toluene: Ethyl acetate: Formic acid: Methanol (3.5:4.8:1.0.:0.7, v/v/v/v) was selected as mobile phase for analysis at 280 nm.

Results: The developed method was robust and resolved gallic acid and quercetin at R_f 0.34 ± 0.03 and 0.55 ± 0.01, respectively. The limit of detection (26 and 47 ng band^{-1}) limit of quantification (78 and 141 ng.band^{-1}), recovery (99.6–99.8 and 98.5–99.7%), and precision (≤1.98 and 1.97) were satisfactory for gallic acid and quercetin respectively. Linearity range for gallic acid and quercetin were 100–1000 (r^2= 0.9993) and 150–900 ng band^{-1} (r^2= 0.9956) and the contents estimated as 0.63 ± 0.01% and 0.57 ± 0.01% w/w in SI and 0.51 ± 0.01% and 0.69 ± 0.01% w/w for gallic acid and quercetin respectively.

Conclusion: The developed HPTLC method was rapid, accurate, precise, reproducible, and specific for the concurrent estimation of gallic acid and quercetin. The method has been successfully applied in the analysis and routine quality control of herbal material and formulations containing *Solanum Species*.

Keywords: Gallic acid, Quercetin, High performance thin layer chromatography, *Solanum indicum*, *Solanum xanthocarpum*, Validation.

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INTRODUCTION

Phenolic compounds are plant substances that together have an aromatic ring with one or more hydroxyl groups. There are about 8,000 plant phenols, about half of which are flavonoids.[1] Phenols have a decent range of biochemical activities, including antioxidants, antimutagenicity, anti-carcinogenicity, and the ability to alter natural phenomena.[2] Phenols are the most important group of phytochemicals involved in some of the antioxidant activity of plants or plant products.[3] Flavonoids, both free and glycoside, are the largest group of current phenolic compounds found in many parts of plants. They have been found to have many biological activities including antibacterial, mitochondrial adhesion inhibition, anti-ulcer, anti-arthritis, anti-angiogenesis, anti-cancer, protein kinase inhibition, etc.[4] Flavonoids have two benzene rings separated by propane. Flavones and flavonols are the most important cosmopolitans of all phenols.[5] Flavonoids are especially useful because they function as antioxidants and protect against diseases, certain types of cancer, and age-related degeneration of cellular components. The properties of these polyphenols

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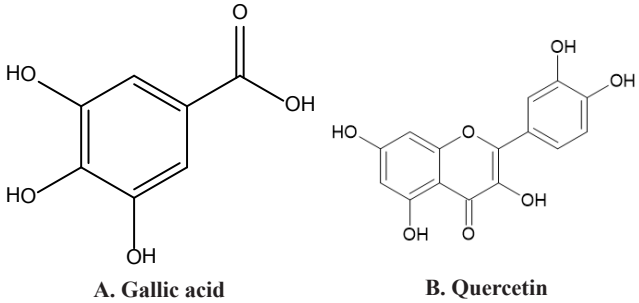
allow them to remove harmful free radicals such as superoxide and hydroxyl radicals.[6] Diffusion of plant flavonoids in food inhibits tumorigenesis in experimental animal models.[7] Biflavonoids have pharmacological effects such as inhibition of histamine release, platelet adhesion, and thus the effect of lens aldose reductase, suppress the inflammatory effect of hepatoxin, and have a cardiac stimulating effect.[8] The phytochemicals contained in fruits and vegetables are known to have antioxidant properties against oxidative damage caused by environmental chemicals.[9] However, their protective effect against chemically induced tissue damage depends on several factors such as dose, molecular polarity, and design of experiments.[10-12] This may be due to inadequate intestinal absorption in the biological system[13] or the need for different interstitial concentrations from tissue to tissue.[14] Among these compounds, acid (3,4,5 trihydroxybenzoic acid, GA) and quercetin (3,30,40,5,7 pentahydroxyflavone, QC) have both redox and structural properties and powerful antioxidants (Figure 1). This leads to high levels of antioxidant activity. Antioxidant activity 3 hydroxyl groups in GA,[15] then 4 hydroxyl groups in rings A and B and some double bonds, 4 keto groups and 3 hydroxyl groups C ring for basic QC.[8] Both GA and QC are found in Dominion Plantae and vegetable foods and beverages.[16] GA occurs not only in nuts, green tea and wine, but also in oak bark and galls as catechin derivatives and hydrolyzable tannins.[17] QC is rich in onions, apples, berries, cherries, broccoli, red grapes and tea. Both compounds are widely used in cosmetics and food additives to prevent oxidation processes that lead to product deterioration.[18,19] For example, acid (GA) acts as an antioxidant at high doses and has harmful effects on tissues,[20-22] but at low doses it acts as an antioxidant and has beneficial effects on human health. Brings cardiovascular disease, chemotherapeutic cancer, and mitochondrial damage to some tissues.[23-25] In addition, some studies using many phytochemicals such as quercetin (QC), hesperetin, naringenin, myricetin, and morin have shown that these flavonoids have both antioxidant and oxidative effects at different doses. It is shown to be. It has been proven.[26,27] The health benefits of GA and QC make it easier to use as an antioxidant in poly (vinyl alcohol) film formulations for food packaging.[28] Qualitative and quantitative analysis of phytochemical mixtures is one of the greatest challenges for natural chemists. The availability of advanced techniques such as ^{13}C-NMR, ^{1}H-NMR, mass spectrometry, capillary electrophoresis, and HPLC has improved the complex structure of phytochemicals and the accurate measurement of ml weights. However, there are still many loopholes. Apart from the exorbitant cost of these advanced techniques, the samples and data processing require advanced analytical skills and are not suitable for routine analysis. Given the widespread use of herbal products for the therapeutic and preventive effects of illness and health, it is essential to standardize them and ensure quality suitable for public consumption. High Performance Thin Layer Chromatography (HPTLC) is widely used for qualitative and quantitative evaluation and standardization of phytochemical products, and is inexpensive, fast and reproducible. It has established itself as an economical, simple and accurate technology.[29] HPTLC is rational for extending the chromatographic fingerprint to determine the most important active substances in medicinal plants. Separation and separation are much better, and the results are much more reliable and reproducible than DC. Combined with digital scanning profiling, it offers the main benefits of qualitative and quantitative *in-situ* measurements using scanning densitometry. In addition, colorful HPTLC images provide additional intuitive visible color and/or fluorescence parameters for parallel evaluation on the same plate. There was also better separation of individual secondary metabolites.[30]



A. Gallic acid **B. Quercetin**

Figure 1: A & B. Chemical structure of gallic acid and quercetin

Solanum indicum Linn. Commonly referred to as a Badi, The Sanskrit words Bhatkataiya (Hindi) and `Brihati` are the Ayurvedic medical system can be used as a single drug or as a one drug combination with other medicines. It' could be a much-forked perennial below a stinging shrub. The plant grows mainly within the nice and cozy warm parts 1500 meters above sea level. All plant parts viz. berries, leaves, roots, seeds and stem of this species are utilized in traditional system of medication and are useful in various diseases like bronchitis, asthma, dry cough, rhinitis, dysuria, leucoderma, sexual disorders, insomnia, cardiac weakness and pruritus.[31-34] The plant has been documented in Chinese folk medicine as anti-inflammatory and wound-healing agents and as an analgesic for toothache, rhinitis and carcinoma.[35] The species is among the ten medicinal plants whose roots are principally employed in preparation of Dashmularishta, a well-established Ayurvedic drug utilized in the treatment of fatigue and oral sores. Phytochemical profile of *S. indicum* reported the presence of alkaloids, flavonoids, phenols, and steroids. Clinical studies on anti-asthmatic and curing dental inflammation revealed the effective therapeutic potential of *S. indicum*. Exploration of assorted pharmacological effects from *S. indicum* emphasized the possible use of bioactive compounds to develop novel therapeutic drugs. It's a promising approach in search and development of the trendy & stylish medicine, supported traditional medicinal knowledge.[31]

Solanum xanthocarpum Schrad and Wendl. (Solanaceae) is a thorny, diffuse, light green perennial herb commonly recognized as Indian night-shade or yellow berried nightshade (Kaṇṭakāri) plant.[36] It's well versed in India and Pakistan; often in wastage places, on roadsides and in open spaces likewise. Its fruit contains carpesterol, glucose, galactose, potassium chloride, variety of steroidal compounds and alkaloids mainly

within the kind of glycoalkaloids. The flavanoids quercitrin and apigenin glycosides were the key chemical constituents present within the fruits of *S. xanthocarpum*.[37,38] Many therapeutic activities of the fruits of this plant are reported. It's being employed for itching and fever, reduces adipose tissues likewise as seminal ejaculation.[39,40]

Based on the compelling evidence of biological activities of polyphenolic compounds gallic acid and quercetin, the study was focused on concurrent determination of content of GA and QC in methanolic extract aerial parts of SI and SX. The method has been validated as per the ICH guidelines and is suitable for application for the standardization of plant-based products.

MATERIALS AND METHODS

Plant Materials and Chemicals

Fresh aerial parts of plants SI and SX were collected from field area in roadside of Kishanganj (Bihar), India in February 2020. The sample (voucher number: IU Phar/Herbal/20/02/07/2020) was provided & certified by Dr. Muhammed Arif Associate Professor of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Integral University, Kursi Road, Lucknow, India. Standard gallic acid (purity: 98.7% w/w) and quercetin (purity: 98% w/w) were purchased from Natural Remedies Pvt. Ltd, Bangalore, India. All solvents used were chromatographic grade and the other chemicals used were analytical reagent grade (AR). Precoated silica gel 60 F_{254} HPTLC plate was obtained from E. Merck, Germany.

TLC Instrumentation and Conditions

Chromatography was performed on a 20 cm × 10 cm aluminum Lichrosphere HPTLC plate precoated with a 200 µm layer of silica gel 60F254 (E. Merck, Darmstadt, Germany) as previously described.[41-45] The samples were applied as strips 6 mm wide and 10 mm in distance using the Camag (Muttenz, Switzerland) sample applicator Linomat V with a 100 µL syringe. The constant application rate was 160 $nLs^{-1}uA$. Linear ascending development using Toluene: Ethyl acetate: Formic acid: Methanol (3.5:4.8:1.0.:0.7, v/v/v/v) as mobile phase was performed in a 20 cm × 10 cm double trough glass chamber (Camag). Saturation in the mobile phase was minimal at room temperature (25 ± 2°C) and relative humidity 60 ± 5%. The development distance was 8 cm (development time 10 minutes), and 20 mL of mobile phase was used. The plate was air dried at room temperature and heated (75°C. for 5 minutes) to identify a compact band. Densitometry analysis was performed at 280 nm in reflection mode using a CAMAG TLC Scanner III running on WinCATS software (version 1.2.0). The gap dimensions were 5 mm x 0.45 mm and the scan speed was 20 mm s^{-1}.

Preparation of Standard and Quality Control

A stock solution of gallic acid and quercetin (10 mg mL^{-1}) prepared in methanol and diluted appropriately to a standard in the concentration range of 0.1–1.0 mg mL^{-1}. For calibration, GA standard solution (1–10 µL) was applied to the HPTLC plate to provide quantities in the 100–1000 $ng.band^{-1}$. However, a quality control standard solution (0.5–5 µL) was used to provide an amount in the range of 150–900 $ng.band^{-1}$. The peak area and plot amount were processed by least squares linear regression. Each amount was applied 6 times. Low, medium, and high quality control samples with 50, 100, and 200 $ng.band^{-1}$ concentrations were taken with GA, and 100, 200 and 400 were examined with QC to validate the method.

Extraction of Plant Material for Analysis

Aerial parts of the plant SI and SX were air-dried and crushed. 500 g of the pulverized material was packed in a muslin cloth, separately subjected to a Soxhlet extractor, and continuously heat-extracted with methanol for 72 hours. Then, both SI and SX methanol extracts of SI and SX filterate through Whatman Paper No. 42 and the resulting filtrate were concentrated under reduced pressure and finally vacuum dried. The yields of methanol extract were 9.3% w/w and 10.7% w/w, respectively. The sample solution preparation protocol has been optimized for high quality fingerprints and efficient extraction of marker compounds. Since the marker compound is soluble in methanol, methanol was used for extraction. Fingerprinting of methanol extracts from SI and SX was performed by individually spotting 10 µL of a sample solution of appropriately diluted methanol extract on HPTLC plates. Each amount was applied 6 times. The peak area and plot amount were processed by least squares linear regression. The plates were developed and scanned as described above. The peak area was recorded and the amount of gallic acid and quercetin was calculated from the calibration curve.

Method Validation

Validation of the developed method has been conducted as per ICH guidelines for linearity range, precision, accuracy, limits of detection (LOD) and quantification (LOQ), and recovery.

Precision and Accuracy

Precision (inter and intraday) and accuracy of repeated analysis of quality control samples (n = 6) with gallic acid and 50, 100, and 200 $ng.band^{-1}$ at low, medium, and high concentrations of 100, 200 and 400 $ng.band^{-1}$ for quercetin evaluated respectively. Inter-day precision and accuracy were determined by repeating the intra-day assay on three different days. Precision is expressed as the coefficient of variation (CV, %) of concentration measured at each calibration level, and accuracy is expressed as % recovery [(drug detected / applied drug) x 100].

Selection and Optimization of Mobile Phase (Robustness)

Robustness was tested 3 times on a 400 $ng.band^{-1}$ with minor changes to mobile phase composition, mobile phase volume, mobile phase saturation time, and TLC plate activation. This will affect the results. I calculated and examined RSD (%) and SE of the peak area. Toluene: Ethyl acetate: Formic acid: Methanol (3.5:4.8:1.0.:0.7, v/v/v/v) to various ratios (3.8:4.5:1.0.:0.7, v/v/v/v, 3.0:5.0:1.0.:1.0, v/v/v/v, 4.5:4.5:0.5.:0.5, v/v/v/v and 5.0:4.0:0.5.:0.5, v/v/v/v) mobile phase were used for chromatography. The amount and duration of mobile phase saturation investigated was 20 ± 2 mL (18, 20, 22 mL) and 20 ± 10 minutes (10, 20, 30

minutes), respectively. Prior to chromatography, the plate was activated at 60 ± 5°C for 2, 5 and 7 minutes.

Sensitivity

To estimate the detection limit (LOD) and quantification limit (LOQ), blank methanol values were plotted 6 times to determine the standard deviation (σ) of the analytical response. LOD was expressed as the slope of the 3 σ/calibration graph and LOQ was expressed as 10 σ/slope of the calibration plot.

Recovery Studies

Recovery was investigated by applying this method to drug samples supplemented with known amounts of markers corresponding to 50, 100, and 150% of GA and QC. Each level was analyzed 3 times. It was used to confirm GA and QC recovery at various levels of the extract. Recovery rates of markers of various concentrations in the sample were measured.

RESULTS

Chromatography

The chromatogram uses toluene: ethyl acetate: formic acid: methanol (3.5:4.8:1.0:0.7, v/v/v/v) as the mobile phase or solvent system for gallic acid and quercetin under chamber saturation conditions (Figure 2 and 3). The same mobile phase was also used to separate methanolic extracts of SI and SX (Figure 4). We found that the optimized saturation time was 10 minutes. The UV spectrum measured for the spot showed maximum absorbance at about 280 nm, so densitometry analysis at 280 nm in reflection mode was performed as HPTLC. For gallic acid and quercetin, R_F 0.34 ± 0.02 and 0.55 ± 0.04 yielded sharp, symmetric, high-resolution compact bands (Figures 5 and 6).

There is no HPTLC method for simultaneously quantifying GA and QC in SI and SX herbs or extracts. Therefore, to quantify the bioactivity marker component of this herb, we attempted to develop and verify an inexpensive, simple and sober HPTLC technology with a hyphen. Gallic acid and quercetin were well resolved at RF 0.34 and 0.56 from SI and SX methanolic extract samples in the solvent system, respectively, as in the standard case (Figures 2 and 3). The plate was visualized at two different wavelengths, 254 nm and 280 nm, as the compound was found to absorb in a variable range of spectra. In addition, this helped generate better fingerprint data, making it possible to better distinguish species by

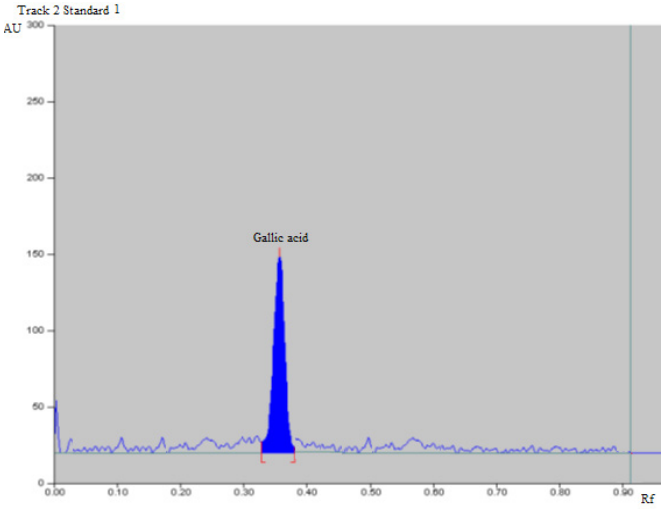


Figure 2: HPTLC chromatogram of standard gallic acid at R_F 0.34

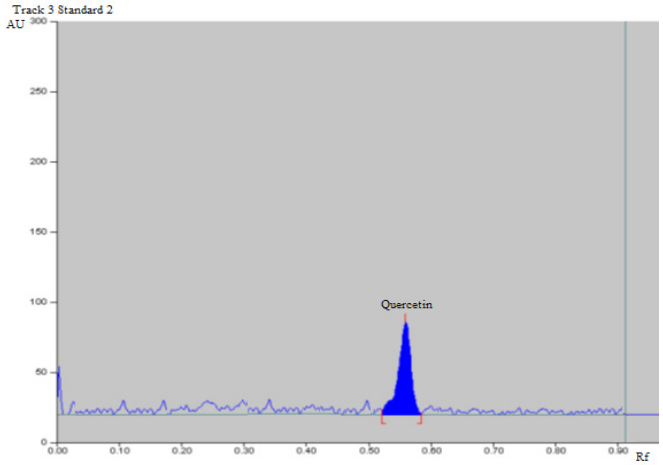


Figure 3: HPTLC chromatogram of standard Quercetin at R_F 0.55

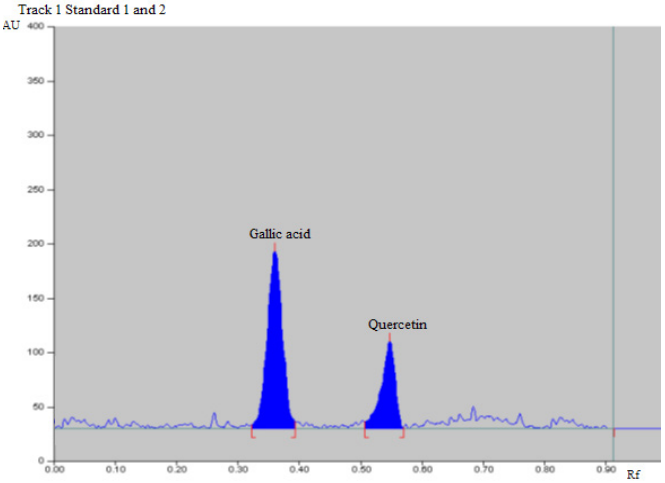


Figure 4: HPTLC chromatogram of methanolic extract of SI and SX scanned at 280 nm [peak 1-11 and 1 - 8; GA (0.34) and QE (0.55)]

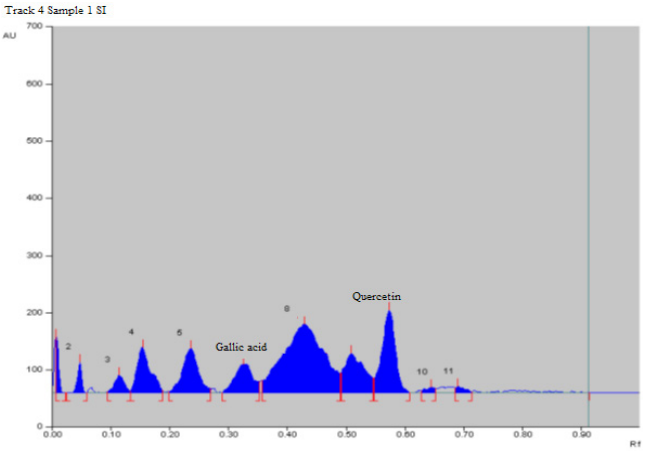


Figure 5: Chromatogram of GA and QE simultaneously determined in by using toluene–ethyl acetate–formic acid (5:4:1, v/v/v) as solvent system scanned at 280 nm [GA (0.34) and QE (0.55)]

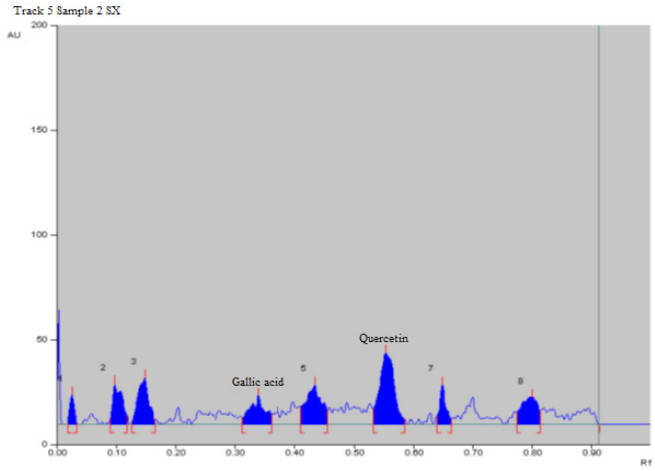


Figure 6: HPTLC chromatogram of methanolic extract of Solanum xanthocarpum (SX) scanned at 280 nm [peak 1 - 8; GA (0.34) and QE (0.55)]

Table 1: TLC fingerprints of methanolic extract of SI and SX at 280 nm

Plants	*Solvent system*	*R_f value*
S. indicum,	(Toulene: ethyl acetate:formic acid; Methanol, 3.5:4.8:1.0.:0.7, v/v/v/v)	0.02, 0.06, 0.13, 0.17, 0.24, 0.34, 0.47, 0.51, 0.55, 0.64, 0.69
S. xanthocarpum		0.03, 0.08, 0.13, 0.34, 0.43, 0.55, 0.65, 0.79.

Table 2: R_F, linear regression data for the calibration curve and sensitivity parameter for GA and QE

Parameter	*Gallic acid*	*Quercetin*
R_F	0.34	0.55
Linearity range (ng.band^{-1})	100–1000	150–900
Regression equation	Y=0.0058X+0.014	Y=0.043-0.018
Correlation coefficient (r^2)	0.9993	0.9956
Slope ± sd	0.0058 ± 0.0001	0.043 ± 0.005
Intercept ± sd	0.018 ± 0.006	0.017 ± 0.008
Standard error of slope	0.00021	0.0046
Standard error of intercept	0.0030	0.0021
LOD	26	47
LOQ	78	141

improving the visual identification of individual compounds. The method developed here proved to be highly selective with good baseline resolution for each compound. Band identity of compounds 1-11 for SI and 1 - 08 for SX in the sample extract was confirmed by superimposing their UV absorption spectra on the standard spectrum at 280 nm (Table 1).

Calibration

Linearity of compounds (gallic acid and quercetin) was validated by the linear regression equation and correlation coefficient. The six-point calibration curves for gallic acid and quercetin were found to be linear in the range of 100–1000 ng.band^{-1} and 150–900 ng.band^{-1}. Regression equation and correlation coefficient for the reference compound were: Y= Y=0.0058X+0.014 (r^2=0.9993) for gallic acid and Y=0.04w3-0.018 for quercetin (r^2=0.9956), which revealed a good linearity response for developed method and are presented in Table 2. The mean values (± sd) of the slope were 0.0048 ± 0.0003 and 0.043 ± 0.005, and intercept were 0.018 ± 0.006 and 0.017 ± 0.008, respectively for gallic acid and quercetin. No significant difference was observed in the slopes of standard plots (ANOVA, $p > 0.05$).

Method Validation

Precision and Accuracy

Table 3 presents intra-day and inter-day precision (as coefficient of variation, (%CV) and accuracy of the assay for GA and QE at three QC levels (150, 300 and 600 ng.band^{-1}). Intra-day precisions (n = 6) for GA and QE were ≤1.84% and ≤1.92%, however the inter-day precisions were ≤1.98% and ≤1.96%, respectively, which demonstrated the good precision of proposed method. Intra-day accuracy gallic acid and quercetin were 98.8–100.2% and 98.8–100.1%, however inter-day accuracy for gallic acid and quercetin were 99.7–99.9% and 98.8–99.6%, respectively. These values are acceptable, so the method was accurate, reliable, and reproducible.

Robustness

SD and %RSD were calculated for GA and QC. The low SD and% RSD values (<2) after making small deliberate

Table 3: Precision and accuracy of the method

Gallic caid				*Quercetin*			
Nominal concentration[a]	*Obtained*[a,b]	*Precision*[c]	*Accuracy*[d]	*Nominal concentration*[a]	*Obtained*[a,b]	*Precision*[c]	*Accuracy*[d]
Intraday batch							
150	148.7	1.84	98.8	200	197.7	1.83	98.8
300	299.4	1.69	99.7	400	398.1	1.92	99.7
600	600.8	1.65	100.2	800	801.6	1.68	100.1
Interday batch							
150	149.5	1.98	99.7	200	197.5	1.95	98.8
300	299.5	1.86	99.6	400	396.8	1.97	99.2
600	598.7	1.79	99.9	800	798.4	1.96	99.6

[a]Concentration in ng.band^{-1}
[b]Mean from six determinations (n=6)
[c]Precision as coefficient of variation (CV, %) = [(standard deviation)/(concentration found)] × 100
[d]Accuracy (%) = [concentration found)/(nominal concentration)] × 100

Table 4: Robustness of the method

	Gallic acid		*Quercetin*	
Optimization condition	*SD*	*%RSD*	*SD*	*%RSD*
Mobile phase (Toulene: ethyl acetate:formic acid; 5.5:3.5:1, v/v/v, 5:4.5:0.5, v/v/v, 5.5:4:0.5, v/v/v and 6:3:1, v/v/v)	1.79	1.82	1.91	1.65
Mobile-phase volume (18, 20, and 22 mL)	1.25	1.77	1.62	0.89
Duration of saturation (10, 20, and 30 min)	1.98	1.63	1.09	1.01
Activation of TLC plates (2, 5, and 7 min)	1.89	1.78	1.53	1.22

Table 5: Recovery studies of GA and QE

Concentration added to analyte (%)	*Theoretical (ng)*	*Added (ng)*	*Detected (ng)*	*Recovery (%)*	*RSD (%)*
Gallic acid					
50		200	498.3	99.6	1.94
100	300	400	697.6	98.8	1.57
150		600	899.8	99.8	1.44
Quercetin					
50		100	197.5	98.5	1.78
100	100	200	298.8	98.4	1.94
150		300	397.7	99.7	1.39

Table 6: GA and QE contents estimated in methanolic extract of SI and SX by developed method

	Gallic acid *		*Quercetin* *	
	Content (ng.spot^{-1})	*%RSD*	*Content (ng.spot^{-1})*	*%RSD*
S. indicum (SI)	63.0	1.08	57.0	1.10
S. xanthocapum	51.0	1.16	69.29	1.22

*Volume applied in each replicate was ten microlitres

changes to the method indicated that the method was robust. (Table 4).

Sensitivity

The LOD values for GA and QE were 26 and 47 ng.band^{-1}, respectively. However, the LOQ values were 78 and 141 ng. band^{-1}, respectively (Table 2), indicating sufficient assay sensitivity. LOD and LOQ were determined from the slope at the bottom of the calibration plot. This showed that the proposed method had good sensitivity for the quantifying the above compounds.

Recovery Studies

Good recoveries were achieved by concentrating the sample at three quality control levels, GA and QC. The results showed in Table 5, the recovery rates for both the sample treatment and post-application are in the range of 99.6–99.8% (gallic acid) and 98.5–99.7% (quercetin) (Table 5).

HPTLC-UV$_{280\ nm}$ analysis of bioactive GA and QC in methanolic extract of SI and SX

The contents of gallic acid and quercetin in the methanol extracts of *S. indicum* and *S. xanthocarpum* were estimated according to the proposed method and the results obtained are summarized in Table 6. The proportions of gallic acid 0.63 and 0.51 and quercetin were 0.57 and 0.69 in the methanolic extract SI and SX, respectively. For the first time, a simple, accurate and fast HPTLC method has been developed for the simultaneous quantification of two bioactive compounds, SI and SX.

DISCUSSION

Plant identification and impurity detection need phytochemical analysis and finger printing.[46] HPTLC successfully separates diverse elements and delivers accurate, reliable, and cost-effective qualitative and quantitative data for a number of applications, including quality control and standardization of food and commercial herbal formulations. GA and QC fingerprints were previously reported using HPTLC.[47] The validation of an HPTLC-based, robust, sensitive, and accurate approach for simultaneously detecting GA and QC at 280 nm using a well-defined Rf value is presented here for the first time. The presence of GA and QC is indicated by HPTLC fingerprints of methanolic extracts of SI and SX, which can be measured using the established approach. We present a novel HPTLC-based simultaneous quantification approach for two polyphenols, such as GA and QC that was successfully employed to standardize SI and SX in this work. The GA and QC levels of the ground section of SI and SX are reported for the first time. These had previously been reported to be found all over the plant.

CONCLUSIONS

The most appropriate method for analyzing secondary metabolites found in plant products is densitometric HPTLC.

Using a fixed mobile phase composition [(Toluene: Ethyl acetate: Formic acid: Methanol (3.5:4.8:1.0.:0.7, v/v/v/v)] and densitometric analysis at 280 nm, a sensitive, accurate, and robust HPTLC technique for measurement of GA and QC in methanolic extract of SI and SX was developed. The current study is the first to describe a constant mobile phase composition for effective GA separation, and QC was effectively used to estimate these biomarkers in methanolic extracts of SI and SX. This method might be used to estimate these biomarkers in different herbal extracts, cutting down on time and making it a more cost-effective tool for study.

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AUTHOR CONTRIBUTIONS

Mr. Murtaza Iqubal designed the whole work and wrote the manuscript, Satish Kumar Sharma supervised the work, Md. Sarfaraj Hussain and Mohd. Mujahid provided the quercetin and gallic acid, helped in designing and revising the whole submitted manuscript.

LIST OF ABBREVIATIONS

AR: Analytical reagents; CV: Coefficient of variations; HPTLC: High performance thin layer chromatography; HPLC: High-pressure liquid chromatography; GA: Gallic acid; QC: Quality control; QC: Quercetin; ^{13}C NMR: Carbon Nuclear Magnetic resonance; ICH: International Council for Harmonization; ^{1}H NMR: Proton Nuclear magnetic resonance; TLC: Thin layer chromatography; LOD: Limit of quantification; LOQ: Limit of detection; UV: Ultra violet; SD: Standard deviation.

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