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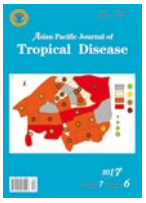

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Validated HPTLC technique for simultaneous evaluation of biomarkers gallic acid and quercetin in *Trichosanthes dioica* Roxb.: A systematic approach for quality control of herbals

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ABSTRACT

Objective: To investigate the simultaneous quantification of two biologically active elements, flavonoids gallic acid (GA) and quercetin (QE), in methanolic extract of *Trichosanthes dioica* that was customarily used to lower blood sugar and cholesterol.
Methods: Silica gel 60F254 having receded with aluminum plate was applied for the separation. We used toluene-ethyl acetate-formic acid (5:4:1, v/v/v) as a solvent system for the separation.
Results: At 270 nm in reflectance mode densitometric analysis was performed. Squeezed bands for GA and QE were achieved at R_f (0.31 ± 0.03) and (0.50 ± 0.02). The scheme was validated for sensitivity, specificity, limit of detection and quantification. Linear relationship was found between the concentration range of 100–1 000 ng spot^{-1} for GA and 150–900 ng spot^{-1} for GA and QE, respectively. The instrumental precision was noted to be 1.03–1.96 (% RSD) and 1.09–1.98 (% RSD) for GA and QE, separately. Methodology specificity was determined at three different study point with varying concentration for recovery studies. Mean % recovery was noted to be 99.4%–99.9% and 98.7%–99.3% and the content estimated as (31.00 ± 1.18)% and (39.00 ± 1.24)% for GA and QE, respectively.
Conclusions: The developed high performance thin layer chromatography scheme can be used for routine quality control analysis of *Trichosanthes dioica* and several other formulations containing these markers.

1. Introduction

Gallic acid (GA) and quercetin (QE) are present in different range of flora. GA and QE are well recognized for its multiple pharmacological effects ranging from anti-inflammatory to anti-hypertension activities[1]. Regular consumption of GA and QE has been shown protective effects in developing cancer and heart diseases[2-4]. Research interest increases in GA and QE because of its potential in improving public health. Concerning the estimation of GA and QE very tiny data are accessible for both qualitative and quantitative analysis. QE 3, 4-diglucoside, QE 4-monoglucoside and QE 3-monoglucoside are the most known glycoside and found in nature in the form of conjugated glycosides[5]. Many studies have reported QE as a major flavanol in apple, berries, carpers, onions and tea[6], and its protective role towards cardiovascular, cancer and inflammatory conditions[7]. The previous reports reveal that the GA and its derivatives possess wide spectrum of biological activities like antimicrobial[8], anticancer[9], antiviral, anti-inflammatory[10], analgesic[11] and anti-HIV activities[12]. These days, high performance thin layer chromatography (HPTLC) is used as conventional analytical technique due to some of its unique features such as high sample throughput, minimum sample requirement, multiple compounds can be analyzed at a time by using small volume of mobile phase that makes the process fast and economic as well[13-17]. Finger print analysis through HPTLC has appeared as an influential and current instrument that linking the botanical identity and chemical constituent's profile of the plant for the estimation of the markers (chemical and biochemical)[18]. The HPTLC-densitometric method is a renowned scheme for the partition and quantification of natural products. GA and QE have

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been estimated through HPTLC, LC-MS/MS and gas chromatography as an hyphenated techniques[19-24].

Trichosanthes dioica Roxb. (Cucurbitaceae) (*T. dioica*) is a vegetative herb usually cultivated in Malaysia, throughout Indian subcontinent and additionally in South Asia. It is usually familiar as "sespadula" in English or "parwal" in Hindi. Vitamin C and minerals like calcium, magnesium, sodium, potassium, copper and sulphur are found in rich source in *T. dioica*. Apart from fruits, leaves and tender shoots have also been used since antique era[24-27]. Medicinal properties like hypocholesterolemic, hypoglyceridimic, and hypophospholipidemic, have been identified in animals[28,29] and also in normal and diabetic human volunteers[30]. Direct feeding of *T. dioica* seeds have shown significant changes in serum lipid profile of normal and mild diabetic human and albino rabbits[31,32]. *T. dioica* effects in the powder form have also been studied on blood sugar and lipid profile of normal albino rabbits[33]. Seeds of the plants also showed antifungal and antibacterial activity and are extensively used in the management of acid dyspeptic ailments.

Being the constituent of this scientific study on the quantification of markers compound of the herbs, this paper explains development, validation and simultaneous estimation of GA and QE in *T. dioica* by HPTLC technique.

2. Materials and methods

2.1. Plant materials and chemicals

For research and experiment, we took fresh leaves of *T. dioica* from a district of the state of Bihar in India exactly from the basin of the Koshi river which was authenticated by Prof. (Dr.) Anjani Kumar Sinha, Principal, M L T Saharsa College Saharsa, Saharsa, Bihar and for the records a voucher specimen No. SHC 55/05 has been submitted in the herbarium, Department of Botany, M L T Saharsa College Saharsa, 852201, India. Marker drug pure GA (97%) and QE (99%) were procured as samples from Natural Remedies Pvt. Ltd, Bangalore, India. Toluene, ethyl acetate and formic acid all are analytical grade obtained from E. Merck, Mumbai, India. Silica gel 60F254 precoated with aluminum plate were purchased from E. Merck, Darmstadt, Germany.

2.2. HPTLC chromatographic conditions

The drugs were implicated by Linomat V (CAMAG, Muttenz, Switzerland) on precoated silica gel 60F254 (E. Merck, Darmstadt, Germany) with a thickness of 0.2 mm aluminium backed plate in the form of bands of thickness six (6 mm) with a CAMAG μL syringe. The solvent system (mobile phase) resided of toluene-ethyl acetate-formic acid (5:4:1, v/v/v). Linear ascending development was performed out in a 20 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland), earlier inundated & boosted with solvent system for 15 min at normal temperature (25 ± 2 °C) and comparative moistness of 60% ± 5%. The development comprises chromatogram track of 8 cm, 20 mL of solvent system, and duration time of 10 min. Five separate volumes (0.2, 0.4, 0.8, 1.0, 2.0 μL) of the standard solution of GA were applied on a 20 × 10 cm TLC plate for the preparation of the standard plot of GA. Similarly, five different volumes (0.3, 0.6, 1.2, 2.4, 4.8 μL) of a standard solution of QE were applied on a separate 20 cm × 10 cm silica gel aluminum backed plate for the making of the standard curve of QE. The slit measurement 3 mm × 0.45 mm and scanning speed were chosen as enhanced apparatus limitations. A persistent use or spraying rate of 160 nL s^{-1} and speed of scanning 20 mm s^{-1} were fixed for both GA and QE. Subsequent to the development, TLC plates were dried in a flow of air with the use of an air dryer. For densitometric scanning CAMAG TLC scanner III, (absorbance 270 nm) and run by winCATS software[13-17].

2.3. Preparation of marker drugs solutions

Ten milligrams of reference drugs GA & QE was weighed accurately transferred to 2 separate 10 mL volumetric flask and dissolve in methanol. For standard plot, GA reference solution (1–10 μL) was used to a HPTLC plate to furnish quantities in the range 100–1 000 ng $spot^{-1}$; however, QE reference solution (0.5–5 μL) was used to provide quantities in the range 150–900 ng $spot^{-1}$. These solutions were used for the preparation of standard curve of GA & QE, respectively.

2.4. Preparation of sample solutions

The leaves of *T. dioica* were air-dried and roughly grinded. A total of 500 g of the powdered material extracted thoroughly with a Soxhlet apparatus with methanol for 72 h. For obtaining a dark brown viscous mass (14.9%), the methanolic extract was concentrated at low pressure.

2.4.1. Method validation

The established scheme was validated according to the ICH guidelines (ICH, 2005) parallel to the other chromatographic HPTLC approaches reported by laboratory[25,34] which are in custom for the quality control of plant medicines.

2.4.2. Precision

The precision of the scheme was resulted by measuring the repeatability of the sample application and the dimension of peak areas for six imitates of the marker compound solution at the 3 amounts (150, 300 and 600 ng $spot^{-1}$ for GA & 200, 400 and 800 ng $spot^{-1}$for QE correspondingly). Inter-analyst precision was carried out by quoting the identical method by a different analyst. The intermediate precisions were determined regarding % RSD of the area.

2.4.3. Robustness

The robustness of the suggested scheme was planned at 3

concentration, stages (100, 200 & 400 ng/spot for GA & QE respectively) in two dissimilar ways, *i.e.* by altering the composition of solvent phase and the UV wavelength. The % RSD of the area of peaks was intended to consider the robustness of the method. Solvent system (mobile phase) developed from toluene: ethyl acetate: formic acid (5:4:1, v/v/v) in diverse extents (5.6:3.4:1, v/v/v, 5.5:3.5:1, v/v/v, 5:3.5:1.5, v/v/v, and 6.5:2.5:1, v/v/v) were used for chromatography. The volume of solvent system and the time of saturated exploration were (20 ± 2) mL (18, 20 and 22 mL) and (20 ± 10) min (10, 20 and 30 min), sequentially. The TLC plates were initiated at (60 ± 5) °C for 2, 5 and 7 min earlier chromatography.

2.4.4. Limit of detection (LOD) and limit of quantification (LOQ)

To calculate the LOD and LOQ, blank methanol ($n = 6$) was applied and the standard deviation (σ) of the methodical rejoinder was ascertained.

LOD = 3.3(SD)/S

LOQ = 10(SD)/S

where SD is the official deviation of rejoinder (peak area) and S is the aggregate of the slope of the standard curve.

2.4.5. Recovery studies experiment

The accuracy of the current study was examined by using the scheme to drug samples to which known quantity of the standard drugs 50%, 100%, and 150% of the GA & QE had been added. Each concentration was examined in three times. The recovery of the standard drug at various concentration in the extract was estimated.

3. Results

3.1. Optimization of mobile phase

Under chamber saturated conditions chromatogram was developed for both GA and QE. Toluene: ethyl acetate: formic acid (5:4:1, v/v/v) was used as solvent phase (mobile phase) (Figure 1A and B) and was too used for the separation of *T. dioica* methanolic extracts (Figure 1D). The adjusted saturation period was found to be 10 min. Maximum absorbance's for the spots were observed at 270 nm under UV spectra. At 270 nm in reflectance mode densitometric analysis was performed (Figure 2). For GA & QE high resolution sharp, symmetrical, compact bands were obtained at R_f (0.31 ± 0.03) and (0.50 ± 0.02) separately (Figure 1C).

As per the author knowledge, to date, no studies have reported any HPTLC technique to concurrently estimate GA and QE in *T. dioica* herb or extracts. So, we made an attempt to produce a price operative HPTLC scheme to estimate natural constituent's in this herb. At R_f 0.31 and 0.50, GA and QE were well resolved (Figure 1A and B) from *T. dioica* methanolic extract. Visualization of the plates was done at dual altered wavelengths 254 and 270 nm, which shows the adaptable spectrum range. In accumulation, this also aids in differentiation of species by providing a better fingerprint data which helps in graphical documentation of separate compounds. This scheme was found to be reasonably effective because of its high baseline resolution. The uniqueness of the peaks of compounds 1–14 in the methanolic extracts was established by overlapping their UV absorption spectra at 270 nm with standards.

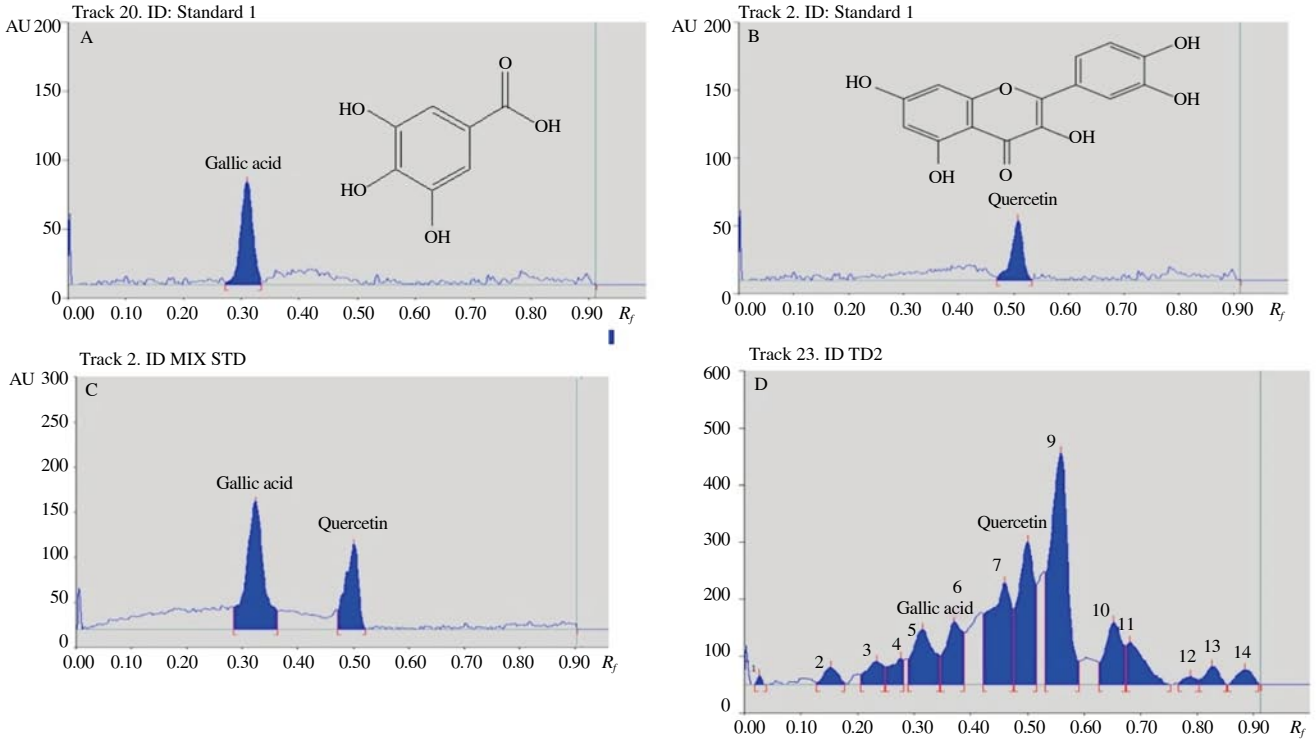

Figure 1. HPTLC chromatogram of (A) standard GA at R_f 0.31; (B) standard QE at R_f 0.50; (C) GA and QE simultaneously determined in by using toluene: ethyl acetate: formic acid (5:4:1, v/v/v) as solvent system scanned at 270 nm [GA (0.31) and QE (0.50)] and (D) methanolic extract of *T. dioica* scanned at 270 nm [peak 1–14; GA (0.31) and QE (0.50)].

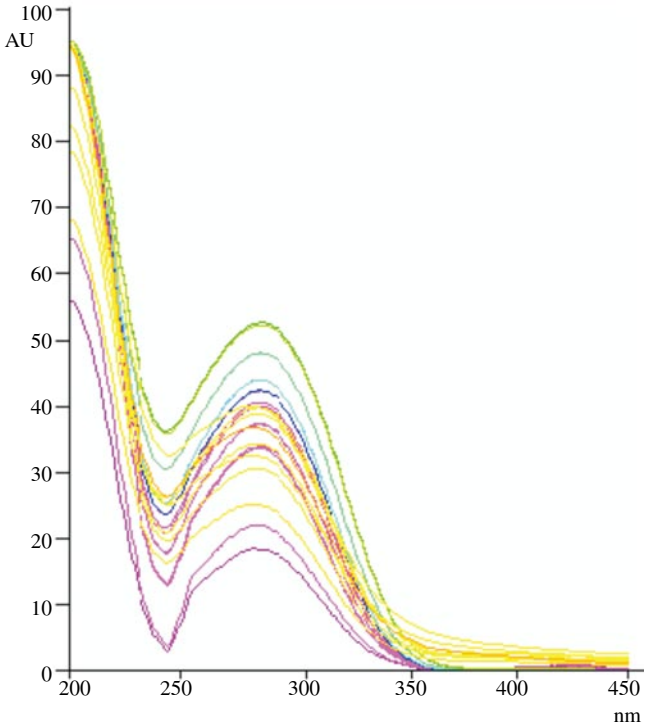

Figure 2. Overlay UV absorption spectra of standard GA, QE in sample methanolic extract of *T. dioica* and sample track at start, apex and end positions.

3.2. Calibration curve of GA and QE

Linear regression calculation and correlation factors were used to validate the linearity of compounds (GA and QE). A linear six-point calibration curve was obtained in the range of 100–1 000 ng spot^{-1}and 150–900 ng spot^{-1} for GA and QE, respectively. Developed method was found to be linear in nature as reflected form its regression equation and correlation coefficient of marker compound, $Y = 0.0048X + 0.012$ ($r^2 = 0.9991$) for GA and $Y = 0.033X - 0.017$ for QE ($r^2 = 0.9956$) (Table 1). The mean values (± SD) of the slope were 0.0048 ± 0.0003 and 0.033 ± 0.008 and intercepts were 0.012 ± 0.007 and 0.017 ± 0.002 for GA and QE, individually. No noteworthy alteration was perceived in the slopes of standard plots (ANOVA, $P > 0.05$).

3.3. Method validation

3.3.1. Precision

Precisions were determined and reported in terms of % RSD particularly intermediate. This incorporates data of inter-day, intra-day and inters analyst precisions. Accuracy of the developed methods indicated that the scheme was well adaptable for the repetitive study of GA and QE which was further supported by the low % RSD. The

Table 1

R_f, linear regression data for the calibration curve and sensitivity parameter for GA and QE.

Parameter	R_f	Linearity range (ng/spot)	Regression equation	Correlation coefficient (r^2)	Slope ± SD	Intercept ± SD	SE of slope	SE of intercept	LOD (ng/spot)	LOQ (ng/spot)
GA	0.31	100–1 000	Y=0.0048X + 0.012	0.9991	0.0048 ± 0.0003	0.012 ± 0.007	0.00017	0.0040	37.5	112.5
QE	0.50	150–900	Y=0.033X – 0.017	0.9956	0.0330 ± 0.0080	0.017 ± 0.002	0.00460	0.0011	69.0	207.0

Table 2

Precision of inter-day, intra-day and inters system analysis.

Elements	Conc. (ng spot^{-1})	Inter-day precision		Intra-day precision		Inter-system precision	
		Mean peak area ± SD	% RSD	Mean peak area ± SD	% RSD	Mean peak area ± SD	% RSD
Gallic acid	150	3 794.90 ± 34.78	1.90	3 908.50 ± 51.06	1.65	3 817.30 ± 63.18	1.80
	300	5 127.40 ± 27.49	1.54	5 190.10 ± 54.71	1.10	5 211.90 ± 49.34	1.77
	600	6 207.70 ± 39.77	1.07	6 311.70 ± 54.45	1.12	6 388.50 ± 67.98	1.03
Quercetin	200	1 708.70 ± 39.36	1.98	1 790.10 ± 49.96	1.73	3 817.00 ± 63.18	1.70
	400	2 582.90 ± 72.49	1.76	2 457.70 ± 44.43	1.89	5 211.00 ± 49.34	1.65
	800	2 867.50 ± 79.83	1.09	2 835.40 ± 54.45	1.17	6 388.00 ± 67.98	1.39

SD: Standard deviation; RSD: Relative standard deviation.

Table 3

Robustness of the method.

Mobile phase change (toluene-ethyl acetate-formic acid)			Mean peak area ± SD		% RSD	
Actual (v/v/v)	Used (v/v/v)	Level	GA	QE	GA	QE
5:4:1	5.5:3.5:1	–2	3 490.30 ± 59.62	1 880.70 ± 17.34	1.12	1.19
	5:4.5:0.5	0	3 375.80 ± 19.27	1 795.90 ± 39.55	0.53	0.61
	6:3:1	+2	3 205.50 ± 19.03	1 949.30 ± 40.36	1.09	1.22
Wavelength change						
Actual (270 nm)	Used (nm)	Level				
	268	–2	3 629.80 ± 39.36	1 859.70 ± 89.26	0.79	1.02
	270	0	3 567.30 ± 39.36	1 798.90 ± 73.69	0.98	0.69
	272	+2	3 709.50 ± 39.36	1 718.50 ± 94.98	1.73	1.87

RSD: Relative standard deviation.

results of the analysis are depicted in Table 2.

3.3.2. Robustness

The outcome of deliberate changes in the composition of solvent system (mobile phase) and detection wavelength was analyzed as % RSD and is presented in Table 3. Robustness of method was indicated by low percentage of RSD.

3.3.3. LOD and LOQ

We found LOD values 37.5 and 69.0 ng spot^{-1} for GA & QE respectively; however, LOQ values were found to be 112.5 and 207.0 ng spot^{-1} for GA and QE, respectively (Table 1), which shows the adequate assay sensitivity. The value of the lowest part of the calibration curve was used to represent LOD and LOQ. This proves good sensitivity of the proposed methods for the estimation of above marker drugs.

3.3.4. Recovery studies

At three quality control levels fortification of the samples were done which gives good repossessions for GA and QE. Afterward sample dispensation and spreading over percent recoveries for both markers were in the range of 99.4%–99.9% (GA) and 98.7%–99.4% (QE) as shown in Table 4.

Table 4
Recovery studies of GA and QE.

Concentration added to analyte (%)		Theoretical (ng)	Added (ng)	Detected (ng)	Recovery (%)	RSD (%)
GA	50		200	497.3	99.4	1.92
	100	300	400	698.6	99.80	1.51
	150		600	899.1	99.90	1.49
QE	50		100	197.5	98.7	1.74
	100	100	200	298.2	99.4	1.92
	150		300	397.4	99.3	1.27

3.4. Estimation of bioactive GA and QE in methanolic extract of TD

The percentages of GA and QE in methanolic extract of *T. dioica* estimated by developed method were (0.31 ± 1.18)% and (0.39 ± 1.24)%, respectively. A faster, precise, and cost effective HPTLC scheme has been established for the simultaneous quantitation of two biologically active markers in *T. dioica*.

4. Discussion

Quality of herbal medicines is judged by the presence of bioactive compounds. HPTLC fingerprint profile technique is considered as the golden standard to determine the bioactive components of the herbal medicine. HPTLC fingerprinting is accurate for herbal identification, authentication and standardization because of its liner and precise nature. This technique is also useful in quality control of herbal drugs and examination for the contaminant. It is also proved to be beneficial to the assessment of different pharmaceutical formulations in the market and plant orderly revisions[35,36]. As the demand for herbal products as medicines and cosmetics is growing, there is an urgent need for standardization of plant products. The HPTLC method is an important tool for standardization, phytochemical outlining and quantitation of compounds present in herbs[37]. Chromatographic fingerprinting is one of the rationale tools to fulfill the essential for economical and powerful quality assessment to folklore medicine throughout the earth. The enhanced chromatographic fingerprinting technique is also used to characterize the different chemical constituents widely found in various plant medicine and to preserve such "catalogue" for further multifacial justifiable analysis. HPTLC fingerprinting is proved to be a simple and reliable tool for the identification, authentication and quality control of herbal drugs.

Introducing HPTLC in pharmaceutical analysis is a major step in terms of quality assurance. In addition to high sensitivity and specificity, the developed HPTLC technique is simple and cost-effective for the simultaneous quantification of GA and QE compounds. This method is an effective tool for routine quality control of herbal materials as well as formulations containing any or both of these compounds as proved by statistical analysis.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Reis JF, Monteiro VV, De Souza Gomes R, Carmo MM, Costa GV, Ribera PC, et al. Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies. *J Transl Med* 2016; **14**(1): 315.

[2] Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. *Nutr Metab* 2015; **12**: 60.

[3] Godsey J, Grundmann O. Review of various herbal supplements as complementary treatments for oral cancer. *J Diet Suppl* 2016; **13**(5): 538-50.

[4] Bell L, Lamport DJ, Butler LT, Williams CM. A review of the cognitive effects observed in humans following acute supplementation with flavonoids, and their associated mechanisms of action. *Nutrients* 2015; **7**(12): 10290-306.

[5] Nam JS, Sharma AR, Nguyen LT, Chakraborty C, Sharma G, Lee SS. Application of bioactive quercetin in oncotherapy: from nutrition to nanomedicine. *Molecules* 2016; **21**(1): E108.

[6] Utrera M, Estévez M. Impact of trolox, quercetin, genistein and gallic acid on the oxidative damage to myofibrillar proteins: the carbonylation

pathway. *Food Chem* 2013; **141**(4): 4000-9.

[7] Vidak M, Rozman D, Komel R. Effects of flavonoids from food and dietary supplements on glial and glioblastoma multiforme cells. *Molecules* 2015; **20**(10): 19406-32.

[8] Giovinazzo G, Grieco F. Functional properties of grape and wine polyphenols. *Plant Foods Hum Nutr* 2015; **70**(4): 454-62.

[9] Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. *Nutr J* 2016; **15**(1): 99.

[10] Sourani Z, Pourgheysari B, Beshkar P, Shirzad H, Shirzad M. Gallic acid inhibits proliferation and induces apoptosis in lymphoblastic leukemia cell line (C121). *Iran J Med Sci* 2016; **41**(6): 525-30.

[11] Dorniani D, Saifullah B, Barahuie F, Arulselvan P, Hussein MZ, Fakurazi S, et al. Graphene oxide-gallic acid nanodelivery system for cancer therapy. *Nanoscale Res Lett* 2016; **11**(1): 491.

[12] Hussain MS, Azam F, Nazeer Ahamed KFH, Ravichandiran V, Alkskas I. Anti-endotoxin effects of terpenoids fraction from *Hygrophila auriculata* in lipopolysaccharide-induced septic shock in rats. *Pharm Biol* 2016; **54**(4): 628-36.

[13] Hussain MS, Sheeba F, Ali M. Simultaneous HPTLC–UV530nm analysis and validation of bioactive lupeol and stigmasterol in *Hygrophila auriculata* (K. Schum) Heine. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S612-7.

[14] Hussain MS, Sheeba F, Ali M. Phtyochemical investigation and hyphenated chromatographic analysis of bioactive lupeol and stigmasterol in *Trichosanthes dioica* extract. *J Nat Pharm* 2012; **3**(1): 43-53.

[15] Hussain MS, Sheeba F, Ali M. Hyphenated chromatographic analysis of bioactive gallic acid and quercetin in *Hygrophila auriculata* (K. Schum) Heine growing wildly in marshy places in India by validated HPTLC method. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S477-83.

[16] Hussain MS, Sheeba F, Ali M. Validation of the method for the simultaneous estimation of bioactive marker gallic acid and quercetin in *Abutilon indicum* by HPTLC. *Asian Pac J Trop Dis* 2012; **2**(Suppl 1): S76-S83.

[17] Hussain MS, Nazeer Ahmed KFH, Ravichandiran V, Ansari MZH. Free radical scavenging capacity of natural terpenes from *Hygrophila auriculata* (Schum) Heine. *Asian J Tradit Med* 2009; **4**(5): 179-87.

[18] Hussain MS, Nazeer Ahmed KFH, Ansari MZH. Studies on diuretic activity of *Hygrophila auriculata* (Schum) Heine in rats. *Int J Health Res* 2009; **2**(1): 59-64.

[19] Ahmad S, Kamal YT, Singh M, Parveen R, Athar MT, Ahmed A. Development and validation of high performance thin layer chromatographic method for the determination of gallic acid in crude drugs and herbal formulations. *Asian J Chem* 2011; **23**: 469-70.

[20] Ahmad S, Singh M, Kamal YT, Parveen R, Ahmed FJ. Development and validation of high performance thin layer chromatographic method for estimation of glycyrrhizic acid in herbal formulation. *Asian J Chem* 2011; **23**: 2098-100.

[21] Sethiya NK, Mishra S. Simultaneous HPTLC analysis of ursolic acid, betulinic acid, stigmasterol and lupeol for the identification of four medicinal plants commonly available in the Indian market as Shankhpushpi. *J Chromatogr Sci* 2015; **53**(5): 816-23.

[22] Hashim S, Beh HK, Hamil MS, Ismail Z, Majid AM. High-performance thin-layer chromatography method development, validation, and simultaneous quantification of four compounds identified in standardized extracts of *Orthosiphon stamineus*. *Pharmacogn Res* 2016; **8**(4): 238-43.

[23] Saurabh S, Lalit M, Chauhan MG. Pharmacognostic study of male leaves of *Trichosanthes dioica* with special emphasis on microscopic technique. *J Pharmacogn Phytother* 2010; **2**(5): 71-5.

[24] Kharbanda C, Alam MS, Hamid H, Javed K, Bano S, Ali Y, et al. Ameliorative effects of *Trichosanthes dioica* extract in suppressing inflammatory mediators and attenuating oxidative stress. *Planta Med* 2015; **81**(5): 348-56.

[25] Kabir SR, Rahman MM, Tasnim S, Karim MR, Khatun N, Hasan I, et al. Purification and characterization of a novel chitinase from *Trichosanthes dioica* seed with antifungal activity. *Int J Biol Macromol* 2016; **84**: 62-8.

[26] Ratnesh KS, Sanjukta C, Devendra KR, Shikha M, Rai PK, Watal G, et al. Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants. *J Med Plants Res* 2009; **3**(11): 944-8.

[27] Bhattacharya S, Haldar PK. Chemopreventive property of *Trichosanthes dioica* root against 3-methylcholanthrene-induced carcinogenesis in albino mice. *J Environ Pathol Toxicol Oncol* 2012; **31**(2): 109-19.

[28] Rai PK, Mehta S, Gupta RK, Watal G. A novel antimicrobial agents *Trichosanthes dioica*. *Int J Pharm Biol Sci* 2010; **1**(3): 1-9.

[29] Rai PK, Mehta S, Gupta RK, Watal G. Glycemic properties of *Trichosanthes dioica* leaves. *Pharm Biol* 2008; **46**(12): 894-9.

[30] Bhattacharya S, Haldar PK. The triterpenoid fraction from *Trichosanthes dioica* root suppresses experimentally induced inflammatory ascites in rats. *Pharm Biol* 2013; **51**(11): 1477-9.

[31] Kirana H, Srinivasan BP. *Trichosanthes cucumerina* Linn. improves glucose tolerance and tissue glycogen in noninsulin dependent diabetes mellitus in rats. *Indian J Pharmacol* 2008; **40**(3): 103-6.

[32] Rai PK, Dolly J, Rakesh KS, Rajesh KG, Geeta W. Antihyperglycemic profile of *Trichosanthes dioica* seeds in experimental models. *Pharm Biol* 2008; **46**(5): 360-5.

[33] Khan S, Ali A, Ahmad S, Abdin MZ. Affordable and rapid HPTLC method for the simultaneous analysis of artemisinin and its metabolite artemisinic acid in *Artemisia annua* L. *Biomed Chromatogr* 2015; **29**(10): 1594-603.

[34] Kamal A, Kamal YT, Ahmad S, Ahmad FJ, Saleem K. Simultaneous HPTLC determination of strychnine and brucine in *Strychnos nux-vomica* seed. *J Pharm Bioallied Sci* 2012; **4**(2): 134-9.

[35] Chester K, Tamboli ET, Singh M, Ahmad S. Simultaneous quantification of stevioside and rebaudioside A in different stevia samples collected from the Indian subcontinent. *J Pharm Bioallied Sci* 2012; **4**(4): 276-81.

[36] Manjula K, Rajendran K, Eevera T, Kumaran S. Quantitative estimation of lupeol and stigmasterol in *Costus igneus* by high-performance thin-layer chromatography. *J Liq Chromatogr Relat Technol* 2013; **36**(12): 197-212.

[37] Hussain A, Rahman MA, Hussain MS, Mirza MA, Iqbal Z, Harwansh R, et al. HPTLC method for analysis of sertraline in pure bulk drug and lipidic nano delivery system: a stress degradation studies. *J Liq Chromatogr Relat Technol* 2013; **36**(6): 700-16.