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Determination of Quercetin a Biomarker in Hepatoprotective Polyherbal Formulation through High Performance Thin Layer Chromatography

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Abstract

Background: Quercetin was determined in bioactive fractions of *Ocimum gratissimum*, *Butea monosperma*, *Bauhinia variegate* and polyherbal hepatoprotective formulation by HPTLC method.

Methods: Polyherbal hepatoprotective formulation was developed by using five bioactive fractionated extracts of three plants namely *Butea monosperma, Bauhinia variegate* and *O. gratissimum*. All three plants contain quercetin. Chromatographic separation was performed on aluminium foil plates coated with 200 µm silica gel $60F_{254}$ Linear ascending development with toluene:ethyl acetate:formic acid, 5:4:0.1 (v/v/v) was performed at room temperature (25 ± 2°C) in a twin-trough glass chamber saturated with mobile phase vapor. Compact bands (R_f=0.38) were obtained for quercetin. Spectro densitometric scanning was performed in fluorescence mode at 380 nm. The method was validated for precision, recovery, specificity, detection and quantification limits.

Results: Linear regression analysis of the calibration plots showed a good linear relationship (R^2=0.9843 ± 0.0001) between peak area and concentration in the range 0.5-2.5 µg/band, respectively. The limits of detection and quantification were 0.089 and 0.26 µ/band. The recovery of the method was 97.33-99.11%.

Conclusion: The above method was a rapid and cost effective quality-control tool for routine analysis of quercetin in herbal extracts and in pharmaceutical dosage form.

Keywords: HPTLC; Quercetin; Liver protective polyherbal formulation

Introduction

Nature still obliges as the man's primary source for the cure of his ailments. Research in preventive medicine showed the importance of functional nutrition in reducing the risk factor of certain chronic diseases. Innate defense system of the human body may be insufficient for the damage caused by continued oxidative stress [1]. Quercetin and other flavonoids, have the structure to act as powerful antioxidants, and have often proven so *in vitro*. Quercetin, being a major constituent of the flavonoid intake, could be a key in fighting several chronic degenerative diseases [2]. Growing scientific evidence has shown adverse side effects, like liver damage and mutagenesis, of synthetic antioxidant [3]. Therefore, recently there has been an upsurge of interest in natural products as antioxidants, as they inhibit the free radical reactions and protect human body from various diseases, such as cancer and diabetes. Recent studies showed that a number of plant products including polyphenolic substances (e.g., gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol) and various plants or herbal extracts exert potent antioxidant actions, which are very well known for their healing powers [4].

Stem bark powder is used to apply on injury caused due to axe. Stem juice is applied on goitre of human being. Paste of stem bark is applied in case of body swellings. Bark is acrid, bitter, appetizer, aphrodisiac and laxative, anthelmintic, useful in fractures of the bones, diseases of theanus, dysentery, piles, hydrocele, cures ulcers and tumors. Bark is useful in biliousness, dysmenorrhea, liver disorder, gonorrhea and it also purifies the blood. The ash of young branch is prescribed in combination with other drugs in case of scorpion sting [5].

Bark

Kino-tannic acid, gallic acid, pyrocatechin. The plant also contains palasitrin, and major glycosides as butrin, alanind, allophanic acid, butolic acid, cyanidin, histidine, lupenone, lupeol, (-)-medicarpin, miroestrol, palasimide and shellolic acid [5].

Bauhinia variegata L. was widely used in traditional medicine to treat a wide range of complains. It contained many secondary metabolites which are suitable to be used as medicines. The phytochemical screening revealed that *Bauhinia variegata* contained terpenoids, flavonoids, and tannins, saponins, reducing sugars, steroids and cardiac glycosides. The pharmacological studies showed that *Bauhinia variegate* exertedanticancer, antioxidant, hypolipidemic, antimicrobial, anti-inflammatory, nephroprotective, hepatoprotective, antiulcer, immunomodulating, molluscicidal and wound healing effects [6]. The phytochemical screening of n-hexane chloroform, ethyl acetate and methanolic fractions of *B. variegata* flowers revealed the presence of terpenoids, flavonoids, tannins, saponins, reducing sugars, steroids and cardiac glycosides [7]. Its Constituents isolated from the leaves were included lupeol alkaloids, oil, fat glycoside, phenolics, lignin, saponins, terpinoids, β-sitosterol, tannins, kaempferol-3-glucoside , rutin, quercetin, quercitrin, apigenin, apigenin-7-O-glucoside, amides, carbohydrates, reducing sugars, protein, vitamin C, fibers, calcium and phosphorus [8,9]

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O. gratissimum is associated with chemo-preventive, anti-carcinogenic, free radical scavenging, radio protective and numerous others pharmacological use [10]. *O. gratissimum*is used to treat different diseases, e.g., upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever and conjunctivitis [11,12]. Earlier reports have shown the smooth muscle contracting lipid soluble principles, and antimutagenic activity in organic solvent extracts of *O. gratissimum* leaves [12,13]. This medicinal plant has also potential role as antibacterial [14,15], antifungal [16,17,18], antimicrobial [19,20] and anthelmintic [21]. The aqueous leaf extract and seed oil showed anti-proliferative and chemo-preventive activity on HeLa cells. Nangia-Makker et al. reported that, aqueous extract of *O. gratissimum* leaves inhibits tumor growth and angiogenesis by affecting tumor cell proliferation, migration, morphogenesis, stromal apoptosis and induction of inducible cyclooxygenase (COX-2) [22]. Ursolic acid was determined in dichloromethane and ethyl acetate fractions of methanolic extract of O. gratissimum in previously published report [23].

A limited number of study have been used for the determination of quercetin in *Butea monosperma* bark [24] and *Ocimum gratissimum* leaf and *Bauhinia vareigata* bark [25]. The quercetin concentrations were also determined by UV spectropho-tometry [26], liquid chromatography coupled with different types of detectors [27-31]. Even though these analytical procedures are suitable for the detection of quercetin in samples originating from plants, they have limitations with respect to their applications in the determination of quercetin in plant samples. The reported colorimetric method lacks sensitivity and is tedious and time-consuming. Even though high performance liquid chromatography (HPLC) is a method of choice, it is limited by extensive sample clean-up and requires expensive solvents and longer periods of column stabilization. In comparison to HPLC, HPTLC is a versatile analytical technique that requires less expensive instrumentation and expertise. The present study was carried out to develop a rapid, sensitive and accurate analytical method for estimation of quercetin in bioactive fractions of plant extracts and its pharmaceutical dosage form (hepatoprotective tablet formulation) for the routine analysis of a large number of plant extract samples and their formulations.

Material and Methods

Apparatus

HPTLC system (Linomat 5, Camag, Switzerland) automatic sample applicator, TLC scanner IV (Camag), flat bottom and twin- trough developing chamber (15 × 10 cm), pre-coated silica gel aluminum plate (E. Merck, Darmstadt, Germany), electronic analytical balance, Shimadzu (AUX-220), micro syringe (100 mL) (Hamilton).

Reagents and standards

Quercetin was purchased from Yucca enterprises, Wadala, Mumbai and methanol AR grade from S.d. fine-Chem Ltd., Mumbai.

Plant materials

Polyherbal hepatoprotective tablet was prepared by using fractions obtained from alcoholic extracts of *Butea monosperma*, *Bauhinia variegata* stem bark and *O. gratissimum* leaves (Figure 1). All these ingredients were collected from Maliba Pharmacy College campus and were authenticated by Prof. Minoo H. Parabia, Department of Bioscience, Veer Narmad South Gujarat University, Surat. Voucher specimen (No: MPC/13032010/01, 02 and 03) has been deposited in the Department of Bioscience.

Extraction and fractionation procedures:

The dried and powdered material of each plant (500 g) was extracted with methanol at room temperature for three weeks with shaking and stirring. Combined methanolic extracts were evaporated to dryness under reduced pressure below 40°C and then dissolved in distilled water and subjected to solvent-solvent fractionation.

***Butea monosperma* (Lam.) Taub:** Methanolic extract obtained was fractionated with petroleum ether, benzene, chloroform and acetone (AcO) in the order of increasing polarity to obtain respective fractions [32].

***Bauhinia variegate* L.:** Methanolic extract was fractionated with hexane, ethyl acetate (EtOAc) and n-butanol (n-ButOH) in the order of their increasing polarity to obtain respective fractions [18].

***Ocimum gratissimum* L.:** Alcoholic extract was fractionated with hexane, dichloromethane (DCM) and ethyl acetate (EtOAc) in the order of their increasing polarity to obtain respective fractions [33].

Each fraction was concentrated to dryness under reduced pressure and below (40-50°C) on a rotary evaporator to give Acetone fr. of *Butea monosperma* [yield 9.4% w/w], Ethyl acetate fr. [yield 2.2% w/w] and n-butanol fr. [yield 5.0% w/w] of *Bauhinia variegata* L. and dichloromethane fr. [yield 4.2% w/w] and ethyl acetate fr. [yield 4.8% w/w] of *Ocimum gratissimum* L. respectively.

Establishment of qualitative and quantitative phytoprofile of fractionated extracts

Qualitative phytochemical analysis: Each fraction was subjected to various qualitative chemical tests using reported methods to determine the presence or absence of metabolites viz., alkaloids, tannins, flavonoid, steroid, terpernoids and phenolic compounds, etc. [34].

Chemical test for flavonoids: Chemical tests were performed for flavonoids according to Macdonald et al. [35].

Quantitative phytochemical analysis

Determination of total phenols: Each sample was mixed with 1 mL Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na_2CO_3. The resultant mixture of was measured at 765 nm after 2 hr at room temperature. The mean of three readings was used and the total phenolic content was expressed in milligram of gallic acid equivalents/1 g extract. The coefficient of determination was found to be r^2=0.992 [36].

Determination of total flavonoids: Standard quercetin was used to make the calibration curve [0.04, 0.02, 0.0025 and 0.00125 mg/mL in 80% ethanol (v/v)]. The standard solutions and test samples (0.5 mL) of each fraction was mixed with 1.5 mL of 95% ethanol (v/v), 0.1 mL of 10% aluminum chloride (w/v), 0.1 mL of 1 mol/L sodium acetate and 2.8 mL water. The volume of 10% aluminum chloride was substituted by the same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture of each

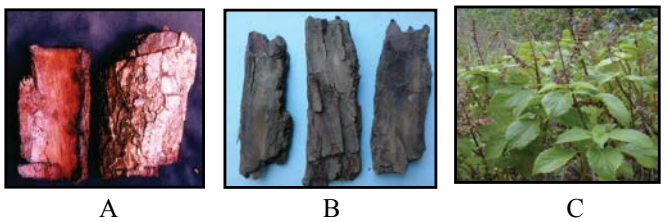

Figure 1: A: *Butea monosperma* stem bark, **B:** *Bauhinia variegata* stem bark, **C:** *O. gratissimum* leaves.

sample and standard solution were measured at 415 nm. The mean of three readings was used and the total flavonoid content was expressed in milligram of quercetin equivalents/1 g extract. The coefficient of determination was r^2=0.99020 [37].

Sample preparation

Preparation of standard solutions of quercetin: Stock solution of quercetin was prepared by dissolving 50 mg quercetin in 100 mL of methanol (500 µg/mL). Standard solutions of concentration 0.5, 1.0, 1.5, 2.0 and 2.5 in µg/mL were prepared by dilution of the stock solution with methanol.

Samples preparation from each plant extracts fractions: Accurately weighed 100 mg of each, acetone fraction of *Butea monosperma,* ethyl acetate and n-butanol fractions of *Bauhinia variegata* and dichloromethane and ethyl acetate fractions of *Ocimum gratissimum* was transferred to separate 10 mL volumetric flask and dissolved in 10 mL of methanol. These solutions were sonicated for 10 minutes and filtered through Whatman No. 1 filter paper to get solution containing 10 mg/mL each.

Sample preparation from polyherbal tablet: Polyherbal tablets equivalent to about 100 mg of mixture of fractionated extracts of *Butea monosperma, Bauhinia variegata* and *Ocimum gratissimum* was weighed and transferred to 10 mL volumetric flask containing 10 mL methanol to get solution containing 10 mg/mL. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for quercetin content [38].

Instrumentation and chromatographic conditions

HPTLC was performed on 15 cm × 10 cm aluminum backed plates coated with silica gel 60F254 (Merck, Mumbai, India). Standard solution of quercetin and sample solution were applied to the plates as bands 6.0 mm wide, 9.2 mm apart, and 15.0 mm from the bottomedge of the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µL Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28 ± 2°C), with toluene: ethyl acetate: formic acid, 5:4:0.2 (v/v/v), as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were dried with a hair dryer and then scanned at 380 nm with a Camag TLC Scanner with WINCAT software, using the deuterium lamp. The method was validated according to the ICH guidelines [11].

Calibration curve of quercetin

Different volumes of stock solution (500 µg/mL) were spotted on the TLC plate to obtain concentration 0.5, 1.0, 1.5, 2.0 and 2.5 µg /spot of quercetin, respectively. The data of peak areas plotted against the corresponding concentration.

Method Validation

The proposed method was validated as per ICH guidelines [39]. Samples were prepared as per the earlier adopted procedure given in the experiment.

Linearity and range

Linearity is expressed in terms of correlation coefficient of linear regression analysis. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 0.5, 1.0, 1.5, 2.0 and 2.5 µg /spot of quercetin respectively. The calibration curve of absorbance vs. concentration was plotted and correlation coefficient and regression line equations were determined.

Precision

Result of precision should be expressed as relative standard deviation (% R.S.D) or coefficient of variance (% C.V.).

Repeatability

Standard solutions were applied by Linomat 5 automatic sample applicator. Sample was spotted seven times for repeatability studies. The peak area obtained with each solution was measured and % C.V. was calculated.

Intraday precision

Mixed solution containing (1.0-2.0 µg/spot) of quercetin was analyzed three times on the same day and % C.V. was calculated.

Interday precision

Mixed solution containing (1.0-2.0 µg/spot) of quercetin was analyzed on three different days and % C.V. was calculated.

Accuracy

It was determined by calculating the recovery of quercetin by standard addition method.

Recovery studies

The accuracy of the method was established by performing recovery experiments at three different levels using the standard addition method. In 1 µl (1 µg/mL) of samples, known amounts of quercetin (0.5, 1.0 and 1.5 µg/spot) standard were added by spiking. The values of percent recovery and average value of percent recovery for quercetin were calculated.

Limits of detection and limit of quantization

The LOD and LOQ were estimated from the set of 5 calibration curves. The LOD and LOQ may be calculated as

LOD=3.3 × (SD/Slope)

LOQ=10 × (SD/Slope)

Where,

SD=Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope=Mean slope of the 5 calibration curves

Specificity

The specificity of the method was ascertained by analyzing the standard drug and extract. The spot for quercetin in the sample was confirmed by comparing the R_f values and spectra of the spot with that of the standard. The peak purity of the quercetin was assessed by comparing the spectra at three different levels, viz. peak start, and peak apex and peak end positions of the spot.

Results and Discussion

Phytochemical screening

Preliminary phytochemical screening of alcoholic extract and its fractions showed the presence of flavonoids, steroids, terpenoids,

tannins and phenolic compounds. The chemical tests analysis demonstrated that AcO fraction of *Butea monosperma*, EtOAc fraction and n-BtOH fraction of *Bauhinia variegata*, DCM and EtOAc fractions of *Ocimum gratissimum* were rich in phenolic compounds. The phenolic content in *Butea monosperma* (acetone fraction), *Bauhinia variegata* (Ethyl acetate and n-butanol fractions) and *Ocimum gratissimum* (Dichrolomethane and ethyl acetate fractions) were found to be 452 ± 1.6, 712.4 ± 2.4, 442.5 ± 1.1, 735 ± 2.1 and 1365 ± 1.4 mg gallic acid/1 gm fraction respectively. The flavonoid content in *Butea monosperma* (acetone fraction), *Bauhinia variegate* (Ethyl acetate and n-butanol fractions) and *Ocimum gratissimum* (Dichrolomethane and ethyl acetate fractions) were found to be 251 ± 1.8, 417 ± 2.2, 227 ± 3.2, 394.5 ± 2.4 and 717 ± 5.2 mg quercetin/1 gm fraction respectively. The phenol and flavonoid contents are responsible for hepatoprotective activity; hence these solvent fractions were selected for further study.

Optimization of mobile phase

Various ratios of solvents were tried as a mobile phase and optimum mobile phase was selected was toluene:ethyl acetate:formic acid, (5:4:0.1 v/v/v). This mobile phase allowed good resolution, dense, compact and well-separated spots at R_f value 0.38. Wavelength 380 nm was used for quantification of the drug. Since there is only one peak seen, is shown in Figure 3.

Quantification by HPTLC Method development

In HPTLC chromatogram, all tracks for standard quercetin at wave length 380 nm were shown in Figure 2. The R_f value of standard quercetin was found to be 0.38 and peak area was 9726 (Figure 3).

Method validation

Linearity and range: The linearity was determined for both drugs at five different concentration levels. The linearity of quercetin was in the range of 0.5-2.5 µg/spot and calibration curves are shown in Figure 3. Correlation co-efficient for calibration curve of quercetin was 0.9843.

The regression line equation for quercetin is as follows: y=9076x+6315 (Figure 4).

Precision

Repeatability: The data for repeatability are shown in Table 1. The % C.V for repeatability was found to be 0.5 (24341 ± 125) (Table 1)

Intra-day precision: The data for intra-day precision for quercetin are shown in Table 2. The % C.V of quercetin was found to be in range of 0.69%-0.97% (Table 2).

Inter-day precision: The data for inter-day precision for quercetin are shown in Table 3. The % C.V of quercetin was found to be in range of 0.77%-1.50% (Table 3).

Accuracy: Accuracy of the method was confirmed by recovery at three level of standard addition. Percentage recovery for quercetin was found to be in range of 97.33%-99.11%. The results are shown in (Table 4).

Limits of detection (LOD) and limit of quantitation (LOQ): Limit of detection and quantitation were determined by equation LOD=3.3 × (SD/s) and LOQ=10 × (SD/s) LOD and LOQ results are shown in (Table 5).

Estimation of Quercetin in Fractionated Extracts of *Butea monosperma*, *Bauhinia variegata* and *Ocimum gratissimum* and Polyherbal Formulation

The peak purity was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot. Good correlation (R^2=0.9843) was obtained between the standard and the samples in the range of 0.5-2.5 µg/spot.

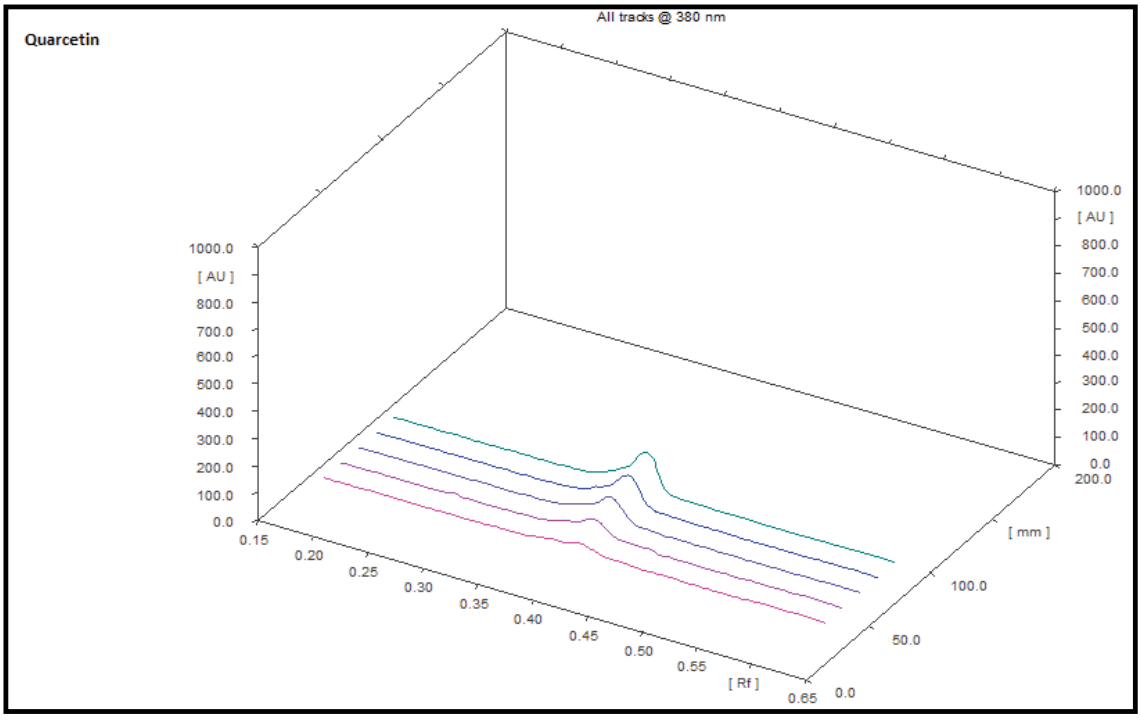

Figure 2: 3D-Chromatogram of quercetin (0.5-2.5 µg/spot).

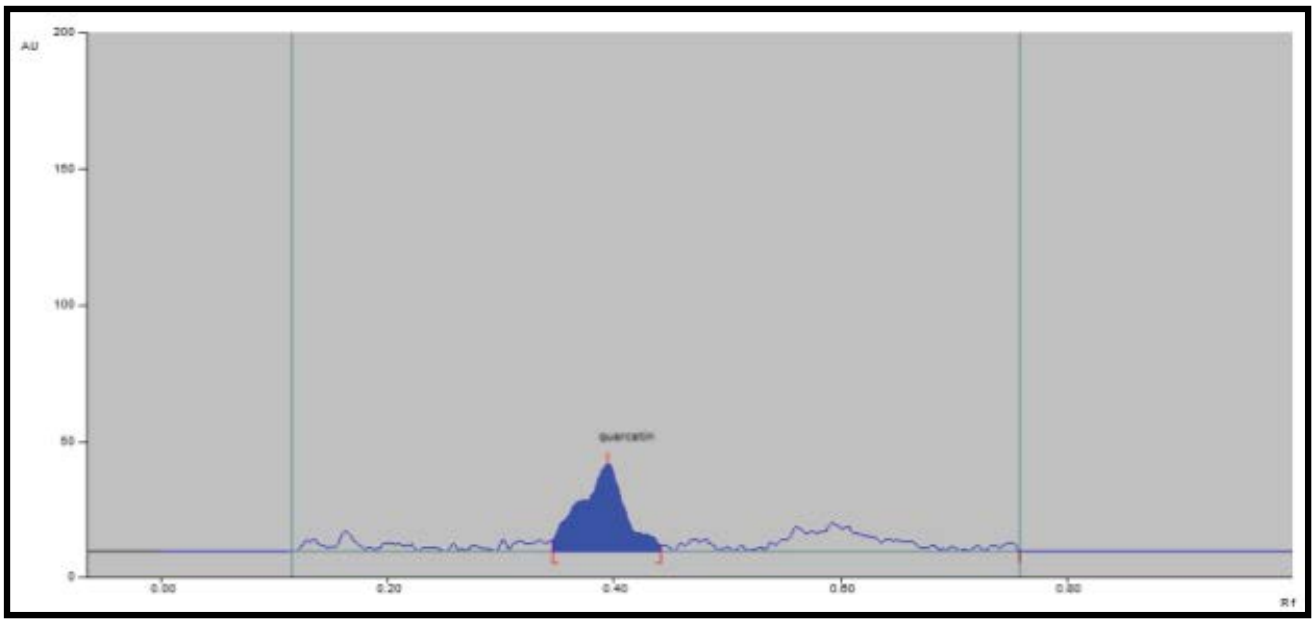

Figure 3: Densitogram of standard quercetin (0.5 µg/spot) at 380 nm.

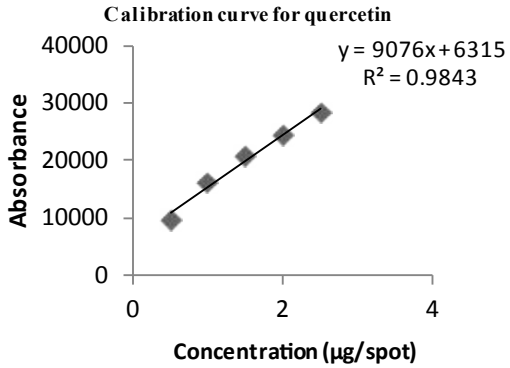

Figure 4: Calibration curve for quercetin (0.5, 1.0 and 1.5 µg/spot).

Concentration (µg/spot)	Peak area
2	24243
2	24558
2	24289
2	24289
2	24332
2	24467
2	24210
Average	24341.14 ± 125.97
% CV	0.51

Table 1: Repeatability data for estimation for quercetin.

Quercetin		
Concentration (µg/spot)	**Area Mean ± S.D. (n=3)**	**% C.V**
1.0	16256.91 ± 158.87	0.97
1.5	20777.82 ± 183.9448	0.88
2.0	24363.54 ± 170.40	0.69

Table 2: Intra-day precision data of estimation for quercetin.

Concentration (µg/spot)	Area Mean ± S.D. (n=3)	% CV
1.0	16274.1 ± 245.11	1.50
1.5	20777.82 ± 183.94	0.88
2.0	24364.79 ± 189.13	0.77

Table 3: Inter-day precision data of estimation for quercetin. Range of 0.77%-1.50%.

The identification of quercetin was done on the basis of R_f values. The concentrations of quercetin in acetone fraction of *B. monosperma*, ethyl acetate and n-butanol fractions of *B. variegata*, dichloromethane and ethyl acetate fractions of *O. gratissimum* were found to be 0.395, 0.174 4, 0.1382, 0.3229, 0.6734 (mg/10 mg) respectively. The results are shown in (Table 6). Acetone fraction of *Butea monosperma* showed eight peaks; the fourth peak R_f value (0.39) was coinciding with standard R_f value (Figure 5). The concentration of quercetin was found to be 0.395 (µg/10 mg).

Ethyl acetate fraction of *Bauhinia variegata* showed eight peaks; the fourth peak R_f value (0.38) was coinciding with standard R_f value of quercetin (Figure 6). The concentration of quercetin was found to be 0.174 (µg/10 mg). n- butanol fraction of *Bauhinia variegata* showed six peaks, the third peak R_f value (0.38) was coinciding with standard R_f value (Figure 7). The concentration of quercetin was found to be 0.138 (µg/10 mg). Dichloromethane fraction of *Ocimum gratissimum* showed nine peaks, the third peak R_f value (0.38) was coinciding with standard R_f value (Figure 8). The concentration of quercetin was found to be 0.322 (µg/10 mg). Ethyl acetate fraction of *Ocimum gratissimum* showed seven peaks; the third peak R_f value (0.39) was coinciding with standard R_f value (Figure 9). The concentration of quercetin in ethyl acetate fraction of *Ocimum gratissimum* was found to be 0.673 (µg/10 mg) [39,40]. Polyherbal tablet of formulation showed eighteen peaks, the R_f value (0.38) of seventh peak was coinciding with standard R_f value. The HPTLC densitogram is shown in Figure 10. The concentration of quercetin was found to be 0.113 (µg/10 mg) [38].

Concentration of quercetin in sample (ng/spot)	Amount of quercetin standard added (µg/spot)	Total Concentration (µg/spot)	Mean concentration recovered (µg/spot)	% Recovery	% Recovery mean
1	0.5	1.5	0.49	0.98	
1	0.5	1.5	0.48	0.96	97.33
1	0.5	1.5	0.49	0.98	
1	1	2	0.98	0.98	
1	1	2	0.99	0.99	98.00
1	1	2	0.97	0.97	
1	1.5	2.5	1.49	0.99	
1	1.5	2.5	1.49	0.99	99.11
1	1.5	2.5	1.48	0.98	

Table 4: Recovery data for quercetin.

Quercetin	
Mean slope	9076
SD of intercept	6315
LOD (µg/spot)	0.08
LOQ (µg/spot)	0.26

Table 5: LOD and LOQ data for quercetin.

Tracks	Samples	R_f values	Concentration (mg/spot)
1	*Quercetin* (std.)	0.38	-
2	*Butea monosperma* (AcO)	0.39	0.395989423
3	*Bauhinia variegata* (EtOAc)	0.38	0.174416042
4	*Bauhinia variegata* (n-BtOH))	0.38	0.138276774
5	*Ocimum gratissimum* (DCM)	0.39	0.322939621
6	*Ocimum gratissimum* (EtOAc)	0.38	0.673424416
7	Polyherbal tablet	0.38	0.113155575

Table 6: Estimation of quercetin in fractionated extracts of *Butea monosperma, Bauhinia variegata, Ocimum gratissimum.*

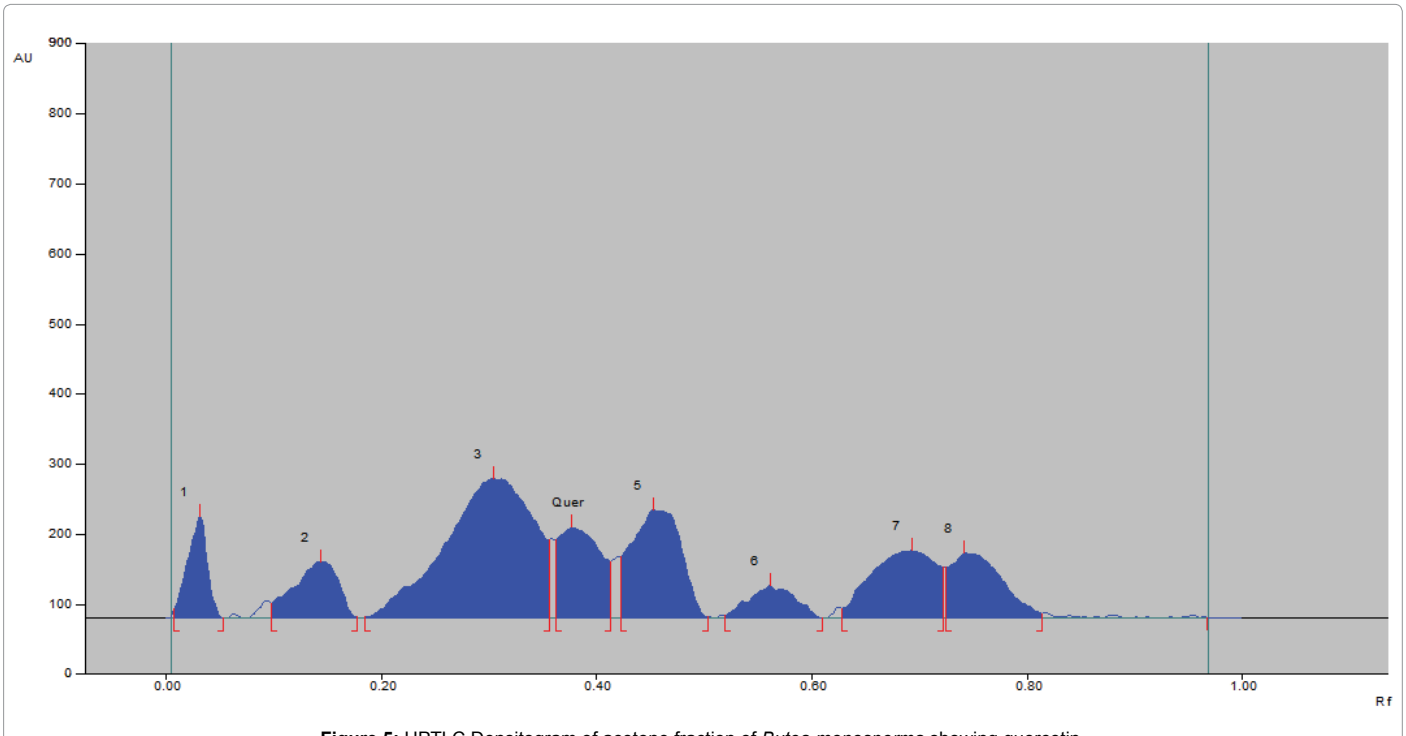

Figure 5: HPTLC Densitogram of acetone fraction of *Butea monosperma* showing quercetin.

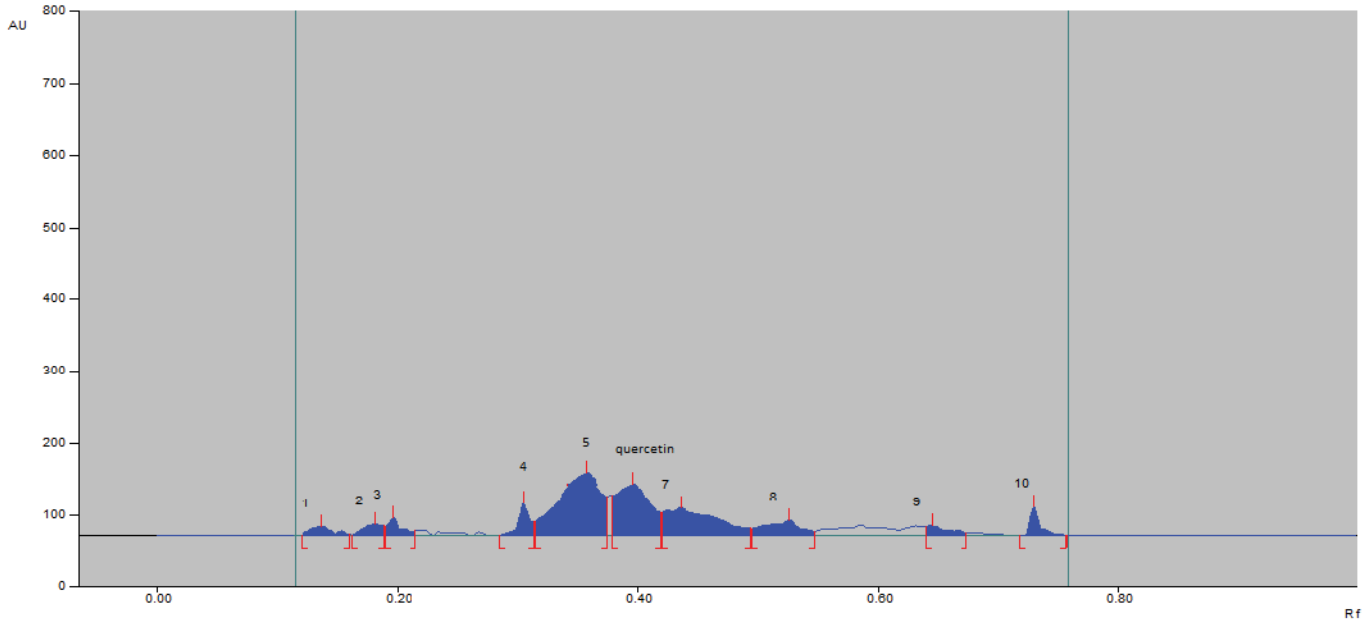

Figure 6: HPTLC Densitogram of ethyl acetate fraction of *Bauhinia variegata* showing quercetin n-butanol fraction of *Bauhinia variegata* showed six peaks, the third peak R_f value (0.38) was coinciding with standard R_f value (Figure 7). The concentration of quercetin was found to be 0.138 (µg/10 mg).

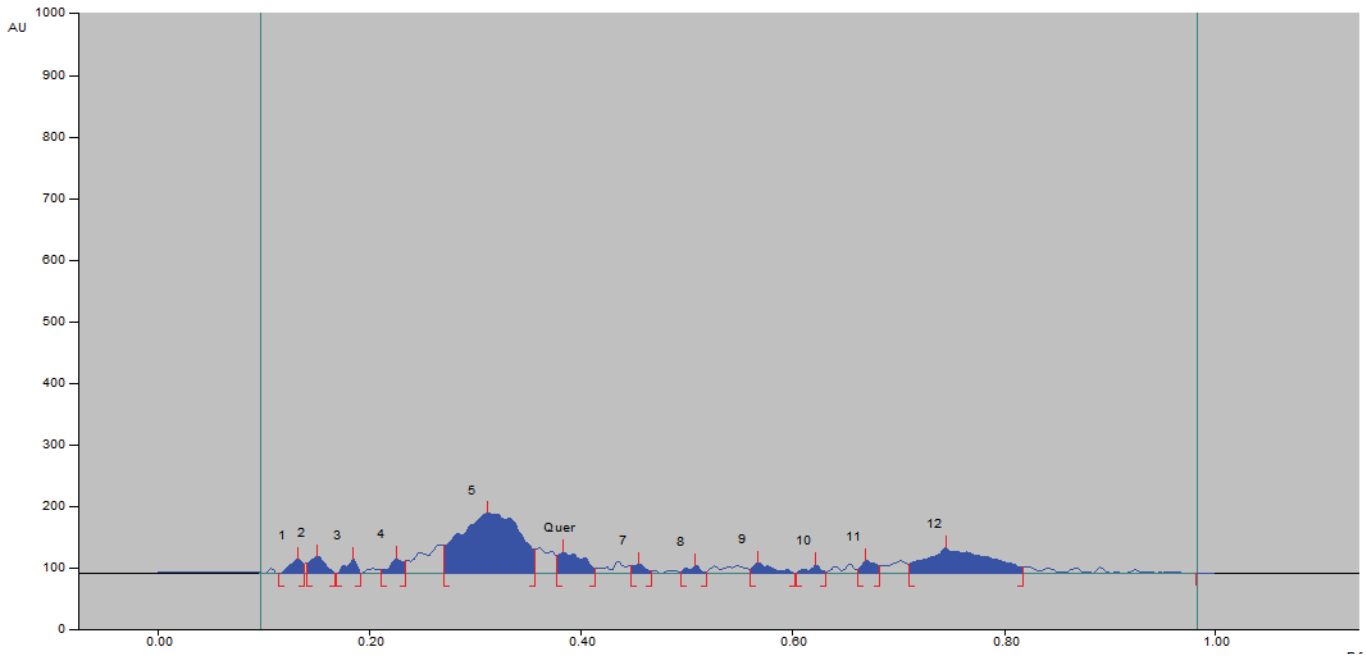

Figure 7: HPTLC Densitogram of n-butanol fraction of *Bauhinia variegata* showing quercetin.

Summary of validation parameters

The detailed summary of validation parameters is described in (Table 7)

Conclusion

A good correlation was obtained among the standard, samples of polyherbal formulation and fractionated extract of plant. An HPTLC method for quantitative estimation of quercetin present in fractionated extract of plants and polyherbal tablet has been developed and validated. The method can be used as a quercetin standard.

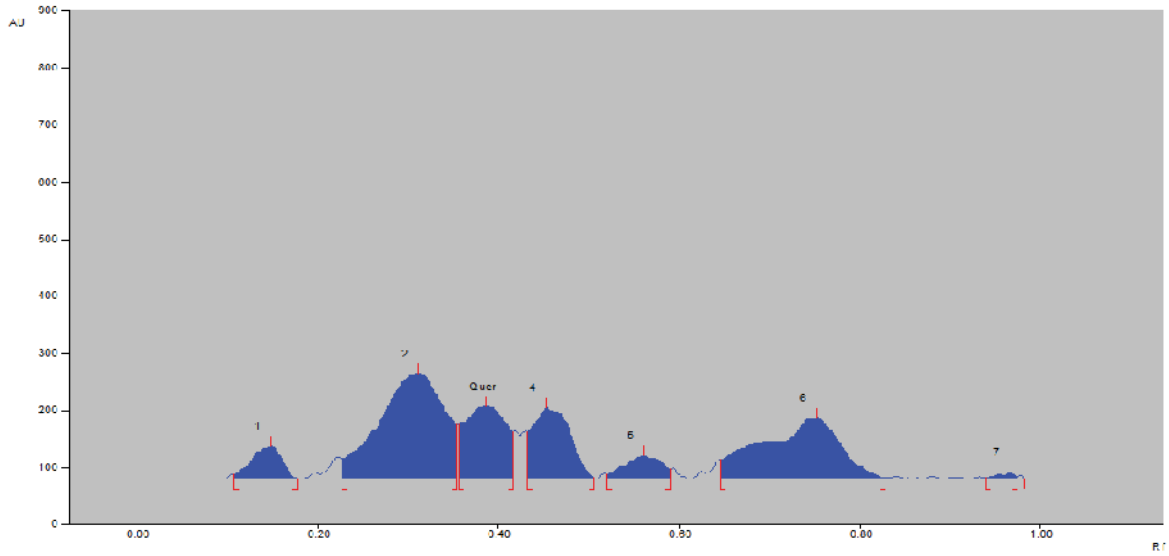

Figure 8: HPTLC Densitogram of dichloromethane fraction of *Ocimum gratissimum* showing quercetin.

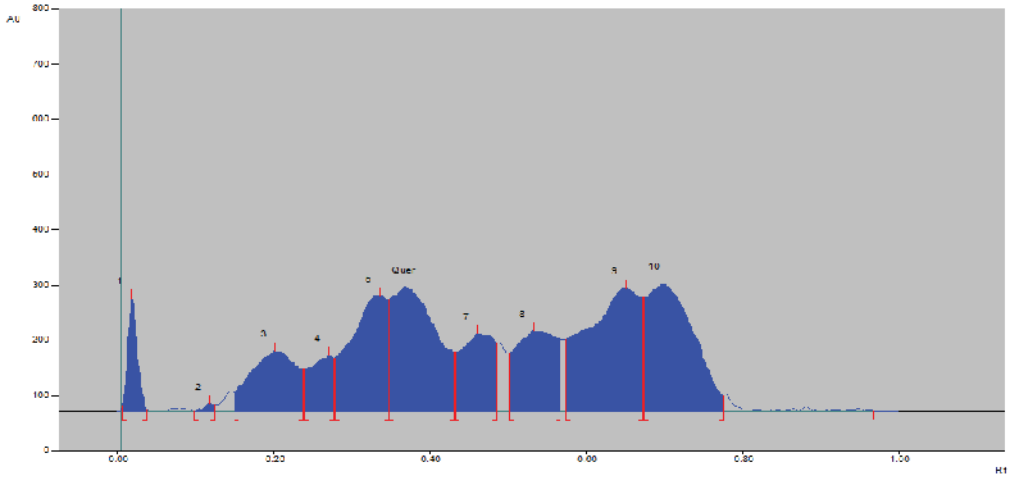

Figure 9: HPTLC Densitogram of ethyl acetate fraction of *Ocimum gratissimum* showing quercetin.

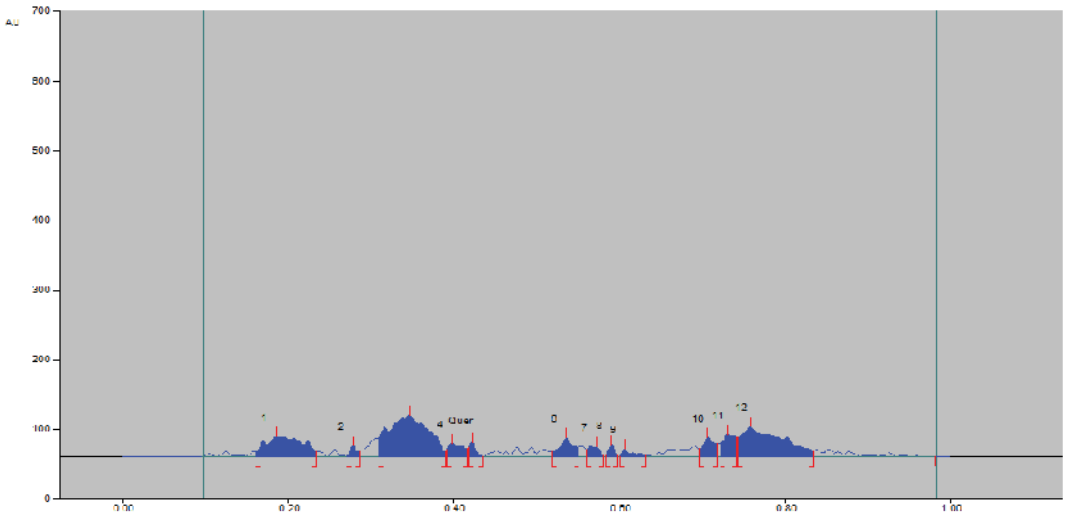

Figure 10: HPTLC Densitogram of polyherbal tablet of PTF-2 formulation showing quercetin.

Parameters	Result for quercetin
Linearity range	0.5-2.5 µg/spot
Correlation coefficient	0.9843
Precision (% CV)	
Repeatability (n=7)	0.5 (24341 ± 125)
Intraday precision (n=3)	0.69-0.97
Interday precision (n=3)	0.77-1.50
Accuracy (% recovery)	97.33-99.11
LOD (µg/spot)	0.08
LOQ (µg/spot)	0.26

Table 7: Summary of validation parameters.

Conflicts of interest

All authors have none to declare.

References

1. Halliwell B (1994) Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? The lancet 344: 721-724.
2. Bouktaib M, Lebrun S, Atmani A, Rolando C (2002) Hemisynthesis of all the O-monomethylated analogues of quercetin including the major metabolites, through selective protection of phenolic functions. Tetrahedron 58: 10001-10009.
3. Singh R, Singh M, Chandra L, Bhat D, Arora M, et al. (2012) In vitro Antioxidant and free radical scavenging activity of Macrotyloma uniflorum (Gahat dal) from Kumauni region. Int J Fundam Appl Sci 1: 9-11.
4. Kiran B, Lalitha V, Raveesha K (2013) Psoralea corylifolia L. a potent medicinal plant with broad spectrum of medicinal properties. Int J Fundam Appl Sci 2: 20-22.
5. Burli D, Khade A (2007) A comprehensive review on Butea monosperma (Lam.) Kuntze. Pharmacogn Rev 1: 333-337.
6. Al-Snafi A (2013) the pharmacological importance of Bauhinia variegata–a review. Int J Pharm Sci Res 4: 160-164.
7. Uddin G, Sattar S, Rauf A (2012) Preliminary Phytochemical, In vitro Pharmacological Study of Bauhinia alba and Bauhinia variegata flowers. Middle-East Journal of Medicinal Plants Research 4: 75-79.
8. Dhale D (2011) Phytochemical screening and antimicrobial activity of Bauhinia variegata Linn. J ecobiotechnol 3: 4-7.
9. Spilková J, Hubik J (1992) Biologische wirkungen von flavonoiden. II. Pharmazie in unserer Zeit 21: 174-182.
10. Gupta SK, Prakash J, Srivastava S (2002) Validation of traditional claim of Tulsi, Ocimum sanctum Linn. as a medicinal plant. Indian J Exp Biol 40: 765-773.
11. Ilori M, Sheteolu AO, Omonigbehin EA, Adeneye AA (1996) Antidiarrhoeal activities of Ocimum gratissimum (Lamiaceae). J Diarrhoeal Dis Res 14: 283-285.
12. Onajobi FD (1986) Smooth muscle contracting lipid-soluble principles in chromatographic fractions of Ocimum gratissimum. J Ethnopharmacol 18: 3-11.
13. Obaseiki-Ebor EE, Odukoya K, Telikepalli H, Mitscher LA, Shankel DM (1993) Antimutagenic activity of extracts of leaves of four common edible vegetable plants in Nigeria (west Africa). Mutat Res 302: 109-117.
14. Nakamura CV, Ueda-Nakamura T, Bando E, Melo AF, Cortez DA, et al. (1999) Antibacterial activity of Ocimum gratissimum L. essential oil. Mem Inst Oswaldo Cruz 94: 675-678.
15. Orafidiya LO, Oyedele AO, Shittu AO, Elujoba AA (2001) The formulation of an effective topical antibacterial product containing Ocimum gratissimum leaf essential oil. Int J Pharm 224: 177-183.
16. Lemos Jde A, Passos XS, Fernandes Ode F, Paula JR, Ferri PH, et al. (2005) Antifungal activity from Ocimum gratissimum L. towards Cryptococcus neoformans. Mem Inst Oswaldo Cruz 100: 55-58.
17. Nwosu MO, Okafor JI (1995) Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolus and some other pathogenic fungi. Mycoses 38: 191-195.
18. Silva M, Oliveira J, Fernandes O, Passos X, Costa C, et al. (2005) Antifungal activity of Ocimum gratissimum towards dermatophytes. Mycoses 48: 172-175.
19. Nakamura CV, Ishida K, Faccin LC, Filho BP, Cortez DA, et al. (2004) In vitro activity of essential oil from Ocimum gratissimum L. against four Candida species. Res Microbiol 155: 579-586.
20. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, et al. (2004) Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol 35: 275-280.
21. Pessoa LM, Morais SM, Bevilaqua CM, Luciano JH (2002) Anthelmintic activity of essential oil of Ocimum gratissimum Linn. and eugenol against Haemonchus contortus. Vet Parasitol 109: 59-63.
22. Nangia-Makker P, Tait L, Shekhar MP, Palomino E, Hogan V, et al. (2007) Inhibition of breast tumor growth and angiogenesis by a medicinal herb: Ocimum gratissimum. Int J Cancer 121: 884-894.
23. Gupta A, Navin RS, Sonia P, Dinesh RS, Jitendra SY (2013) Determination of ursolic acid in fractionated leaf extracts of *Ocimum gratissimum Linn* and in developed herbal hepatoprotective tablet by HPTLC. Pharmacognosy Journal 5: 156-162.
24. Geeta R, Prakash R, Navgeet S, Neeru V, Sumit J (2011) Butea monosperma (Lam.) Kuntze: A Review. IJRP 2: 98-108.
25. Jash S, Roy R, Gorai D (2014) Bioactive constituents from Bauhinia variegata Linn. Int J Pharm Biomed Res 5: 51-54.
26. Mahapatra SK, Chakraborty SP, Das S, Roy S (2009) Methanol extract of Ocimum gratissimum protects murine peritoneal macrophages from nicotine toxicity by decreasing free radical generation, lipid and protein damage and enhances antioxidant protection. Oxid Med Cell Longev 2: 222-230.
27. Aaby K, Hvattum E, Skrede G (2004) Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with coulometric array detection: relationship to antioxidant activity. J Agric Food Chem 52: 4595-4603.
28. Alonso-Salces RM, Ndjoko K, Queiroz EF, Ioset JR, Hostettmann K, et al. (2004) On-line characterisation of apple polyphenols by liquid chromatography coupled with mass spectrometry and ultraviolet absorbance detection. J Chromatogr A 1046: 89-100.
29. Lommen A, Godejohann M, Venema DP, Hollman PC, Spraul M (2000) Application of directly coupled HPLC-NMR-MS to the identification and confirmation of quercetin glycosides and phloretin glycosides in apple peel. Anal Chem 72: 1793-1797.
30. Nielsen SE, Freese R, Cornett C, Dragsted LO (2000) Identification and quantification of flavonoids in human urine samples by column-switching liquid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry. Anal Chem 72: 1503-1509.
31. Rodríguez-Delgado MA, Malovaná S, Pérez JP, Borges T, García Montelongo FJ (2001) Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. J Chromatogr A 912: 249-257.
32. Sharma A, Chakraborti K, Handa S (1991) Antihepatotoxic activity of some Indian herbal formulations as compared to silymarin. Fitoterapia 62: 229-235.
33. Chattopadhyay RR (2003) Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: part II. J Ethnopharmacol 89: 217-219.
34. Khandelwal K (2001) Pharmacognosy: Techniques and Experiments. Nirali Prakashan 8: 3-40.
35. Macdonald IO, Oludare AS, Olabiyi A (2010) Phytotoxic and Anti-microbial activities of Flavonoids in Ocimum gratissimum. Life Science Journal 2: 37-40.
36. Yuvaraj P, Louis T, Madhavachadran V, Gopinath N, Rekha S., 2011. Total Phenolic Content And Screening of Antioxidant Activity Of Selected Ayurvedic Medicinal Plants. 91: 25-31.
37. Kosalec I, Bakmaz M, Pepeljnjak S, Vladimir-Knezević S (2004) Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acta Pharm 54: 65-72.
38. Alam P, Ali M, Singh R, Shakeel F (2011) A new HPTLC densitometric method for analysis of swertiamarin in Enicostemma littorale and commercial formulations. Nat Prod Res 25: 17-25.
39. ICHHT G (2005) Validation of analytical procedures: text and methodology Q2 (R1). IFPMA: Geneva.
40. Prakash J, Gupta SK (2000) Chemopreventive activity of Ocimum sanctum seed oil. J Ethnopharmacol 72: 29-34.