Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 892-897
Received: 08-07-2018
Accepted: 09-08-2018

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Phytochemical investigation of bioactive Emodin and quercetin in *Cassia fistula* and *Cassia tora* plant parts by HPTLC

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Abstract
In the present study, HPTLC method was developed and validated for the determination of a Emodin (anthraquinone) and Quercetine (flavonoid) in *Cassia fistula* and *Cassia tora* (family: Cesalpineceae) flower and stem extract. Appropriate mobile phases were found as n-hexane: ethyl acetate (7:3V/V) for Emodin (Compound 1) and toluene: ethyl acetate: formic acid (2.5:2:0.3V/V) for Quercetine (Compound 2). The densitometric determination was carried out for both compounds. These plates were scanned at 437nm & 254 nm absorbance / reflection mode respectively as Compound 1 & 2. Validation parameters such as linearity range, limit of detection and limit of quantification, regression analysis, sensitivity etc. Regression analysis shows the calibration data is in the range of 0.5 to3 µg for Compound 1, with correlation coefficient (r^2) value 0.977 whereas 2 to10 µg for Compound 2 with correlation coefficient (r^2) value0.991.The equation indicates that a unit increase in the concentration of Compound 1& 2 results in an increase in the detector response. Low magnitude of residual values indicates a good agreement between observed and calculated values. The accuracy of the proposed method is determined by performing replicate analysis, which indicates high accuracy as actual amount obtained close to theoretical amount. The robustness of proposed HPTLC method was checked with chamber saturation, as it has a pronounced influence on saturation profile.

Keywords: *Cassia fistula, Cassia tora* L, quercetin, emodin, HPTLC, flowers & stem extracts

Introduction
Cassia species belong to the family caesalpiniaceae. Cassia and Tamarind species are used for medicinal purposes. Some species of caesalpiniaceae yield dyes [1, 2]. All the species of Cassia have bright yellow flowers of characteristic shape. The typical flower consists of five similar sepals and petals. Cassia species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative and purgative uses [3-4]. Besides, they have been found to exhibit anti-inflammatory [5], hypoglycaemic [6] antiplasmodial [7]. Isolation and identification of quercetin and emodin from *Cassia tora* L as well as *Cassia Fistula* is *re*ported [8].

Pharmacological Importance of Quercetin and Emodin
Quercetin is one of the important bioflavonoids present in more than twenty plants material and which is known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities [9, 10]. Quercetin, a plant-derived aglycone form of flavonoid glycosides, has been used as a nutritional supplement and may be beneficial against a variety of diseases. Some of the beneficial effects include cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, anti-inflammatory activity, anti-diabetic, gastroprotective effects, antihypertensive, immunomodulatory, and anti-infective [11]. It was also found that quercetin and its conjugate metabolites could protect erythrocytes from the membranous damage that is caused by smoking [12]. Quercetin has a specific feature which inhibits fat accumulation in maturing human fat cells and simultaneously triggers apoptosis in existing fat cells [13, 14]. In addition, quercetin also blocks the uptake of glucose from the blood, blocks the fat cell production, and enhances fat cell necrosis [15, 16]. Quercetin has potential anticancer properties which include antiproliferative, growth factor suppression and antioxidant [17].
Emodin has been isolated from various botanical families such as Rhamnaceae, liliaceae, polygonaceae and Caesalpiniaceae [18]. Emodin, isolated from cassia family such as *Cassia fistula*, *Cassia auriculata* L possess potent antimicrobial activity against skin infecting pathogenic organisms [19, 20]. Emodin is a biologically active, naturally occurring anthraquinone derivative [21].

Isolation and identification of quercetin and emodin from *Cassia tora* L. in leaves was reported by TLC [22].

Literature survey revealed the wide applications of HPTLC [23]. Quantification of (-) epicatechin present in *cassia fistula* crude drug was testified by HPTLC method [24]. Determination of gallic acid and rutin in extracts *Cassia alata* and *Andrographis paniculata* [25]. HPTLC method validation of flavonoids in *Cassia auriculata* linn-a high Value medicinal plant [26].

HPTLC fingerprints could be used in proper identification of medicinal plants, as a valuable analytical tool in the routine quality control and standardization of herbal drugs [27] and as a chemotaxonomical tool in the plant systematic [28], for determination of bioactive components of the herbal medicine [29].

It is applied specifically for standardization, quantification, identification of phytoconstituent, which may be marked as biomarkers in drug formulations. Anthraquinones which are potent anticancer agents well as active other substituted anthraquinones derivatives are definitely more potent for the tumor so the proposed work of standardization and validation of bioactive emodin an anticancer drug, and quercetin simultaneously using simple solvent system as a biomarker by HPTLC.

Materials and Method

Collection of the two medicinal plant species *Cassia tora and Cassia fistula* were collected from Western Pune Maharashtra, India, shade dried authentication was done by comparing with herbarium specimens preserved in Botanical Survey of India, Pune (Maharashtra). Authentication no of *Cassia tora* BSI/WC/Cert/2015/SG01, *Cassia fistula* is BSI/WC/Cert/2015/SG02.

Preparation of Extracts

Air shade dried, finely pulverized and exactly weighed plant material was utilized to prepare extracts with measured volumes of solvents like ethyl acetate, acetone, ethanol, methanol and distilled water. The freshly prepared extracts were analyzed to prevent any degradation. Solvents were removed under reduced pressure to get the crude mass of extracts. Weighed amounts of dried extracts were utilized for the study.

Chemicals utilized

Emodin (Compound 1) purchased from SRL Reagent CAS No. [11E33101]and Quercetin (Compound 2) purchased from Biochemica Reagent CAS No. [6151-25-3]

Method

A simple HPTLC method had been developed for the analysis and quantification of bioactive isolates, Compound 1 & Compound 2 present in different parts of *Cassia tora* and *Cassia fistula* plant in various extracts. This method provides the quantity present in each extract of each part of the plant which is a very reliable technique. Lower limit of quantification (LOQ) and limit of detection (LOD) indicates high accuracy of the developed method.

A suitable solvent system was acquired by attempting various mobile phases on pre-coated aluminium plate (Silica gel, Merck 60 F_{254}) for quantification of analytes. Appropriate mobile phases were found as n-hexane: ethyl acetate (7:3) for Compound 1 and toluene:ethyl acetate: formic acid (2.5:2:0.3) for Compound 2. The densitometric determination was carried out for both compounds. These plates were scanned at 437nm & 254 nm absorbance / reflection mode respectively as Compound 1 & 2.

The total content of the Compound 1 & 2 in extracts of plant parts was reported and estimated by comparing the peak areas with retardation factor. The proposed HPTLC method is found to be simple, faster and reliable for quantification of analytes.

Results and Discussion

A sensitive and reliable high performance thin layer chromatographic method has been developed for quantization of Compound 1& 2.The various extracts (semipolar to polar) of plant parts were chromatographed on aluminium precoated silica gel (60 F_{254}) plates, n-hexane: ethyl acetate (7:3) and toluene:ethyl acetate: formic acid (2.5:2:0.3) for Compound 1&2 with mobile phase. Visualization, detection and quantization was performed by densitometric scanning at most suitable wavelength (λ_{max}= 437nm& 254 nm) for Compound 1&2.

Validation parameters

Validation parameters such as linearity range, limit of detection and limit of quantification, regression analysis, sensitivity etc. are found out (Table1).

Regression analysis shows that the calibration data is in the range of 0.5 to3 µg for Compound 1 while 2 to10 µg for Compound 2.The equation indicates that a unit increase in the concentration of Compound 1& 2 results in an increase in the detector response. Low magnitude of residual values indicates a good agreement between observed and calculated values (Table 2). Residuals are distributed both above and below of the zero residual line indicating the random precision of the method.

The accuracy of the proposed method is determined by carrying out replicate analysis, which indicates high accuracy as actual amount obtained close to theoretical amount.

In present work Camag Twin Trough glass chamber was saturated with solvent vapours for the time span of 20, 25 and 30 min. Saturation time for 25 min provide a good resolution of can be used for quantization. Different extracts were tested for the presence of Compound 1& 2 by comparing spectra at three different levels i.e. peak start, peak apex and peak end position of the spot.

The quantification of Compound 1 in various extracts of *Cassia Fistula* and *Cassia tora* plant parts such as stem and flower was performed & results are presented (Table 3&4, Fig.1&2).

Table 1: Validation parameters of Compound 1

Validation parameters	Compound 1
Linear range [µg/spot]	0.5-3
Regression equation (y=a+bx)	y = 4620.1x + 2150.7
Range (µg/ml)	0.5-3
Correlation coefficient (r)	0.9887
Correlation coefficient ($r2$)	0.9777
Moleculer absorptivity (lit mol^{-1} cm^{-1})	1.2485 x 10^{9}
Sandell's sensitivity (µg ml^{-1} cm^{-2})	2.1645 x 10^{-11}
Limit of quantification (LOQ) [ng / spot]	0.216
Limit of detection (LOD) [ng / spot]	0.716

Table 2: Regression analysis records

X value Conc. µg/spot	Y value Observed	Y value calculated	Residual values
0.5	3561.6	4460.1	898.5
1	7081.8	6770.2	-311.6
1.5	9879.2	9080.1	-799.0
2.0	11877.2	11390.2	-487.0
2.5	13585.2	13700.25	115.05
3.0	15430.2	16010.3	580.1

Table 3: Quantification of Compound 1 in plant parts of *Cassia fistula*

Extracts of Stem		Extracts of Flower		
B	C	A	B	C
0.0033	0.0033	0.0035	0.0011	0.0037

A, B, C: Ethyl acetate, Acetone & methanol extracts. Each value represents mean (n=3)

Table 4: Quantification of Compound 1 in plant parts in *Cassia Tora*

Extracts of stem		Extracts of Flower	
B	C	B	C
0.0031	0.0027	0.0035	0.0042

A, B, C: acetone, ethanol & methanol extracts. Each value represents mean (n=3)

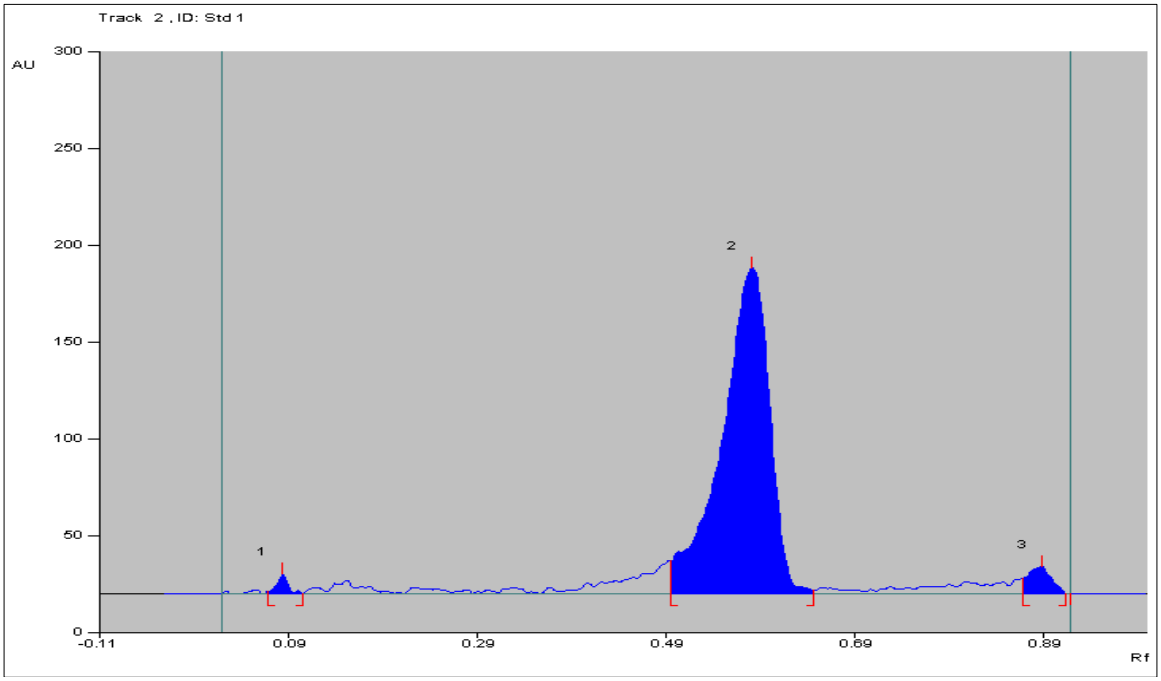

Fig 1: Compound 1

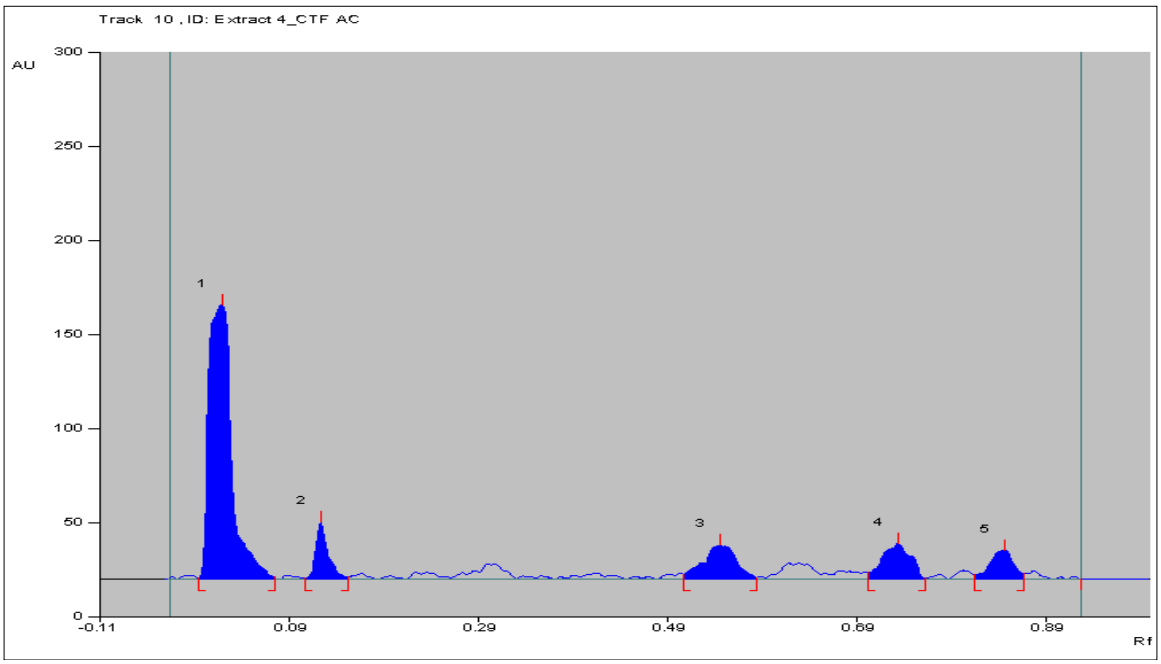

Fig 2: Quantification Compound 1 in test samples of plant extract.

Compound 2

For Compound 2 different composition of mobile phases are tested and desired phase is achieved as, toluene: ethyl acetate: formic acid (2.5:2:0.3) at λ_{max}254 nm.

Validation parameters

Validation parameters such as linearity range, limit of detection and limit of quantification along with sensitivity are noted (Table 5, Fig. 6). Regression analysis using regression equation over a range is reported (Table 6).

The data of peak area are plotted against the corresponding concentration of standard Compound 2. Quantification of Compound 2 in various extracts of plant parts such as stem and flower is performed and results are presented (Table 7&8, Fig.4 & 5).

Table 5: Validation parameters for compound 2.

Validation parameters	Observations
Linear range [µg/spot]	2-10
Regression equation(y=a+bx)	y = 2617x + 14561
Range(µg/ml)	2-10
Correlation coefficient (r)	0.9955
Correlation coefficient (*r*2)	0.9912
Moleculer absorptiity (lit mol^{-1} cm^{-1})	7.9095x10 ^{8}
Sandell's sensitivity (µg ml^{-1} cm^{-2})	3.821 x 10 $^{-9}$
Limit of quantification (LOQ) [ng / spot]	0.382
Limit of detection (LOD) [ng / spot]	0.012

Table 6: Regression analysis records.

X value Conc. µg/spot	Y value observed	Y value calculated	Residual values
2	19092.2	19595	503
4	25301.2	25029	-272.2
6	28716.2	30263	1546.8
8	35640.2	35497	-143.2
10	41906.2	40731	-1775.2
12	45501.2	45965	464

Table 7: Quantification of Compound 2 in plant parts of *Cassia fistula.*

Extracts of Stem		Extracts of Flower		
B	C	A	B	C
0.00044	0.00040	0.00044	0.00048	0.00032

A, B, C: Ethyl acetate, Acetone & methanol extracts. Each value represents mean (n=3)

Table 8: Quantification of Compound 2 in plant parts in *Cassia Tora*

Extracts of stem		Extracts of Flower	
B	C	B	C
0.00046	0.00043	0.00049	0.00055

B & C: Acetone & Methanol extracts. Each value represents mean (n=3)

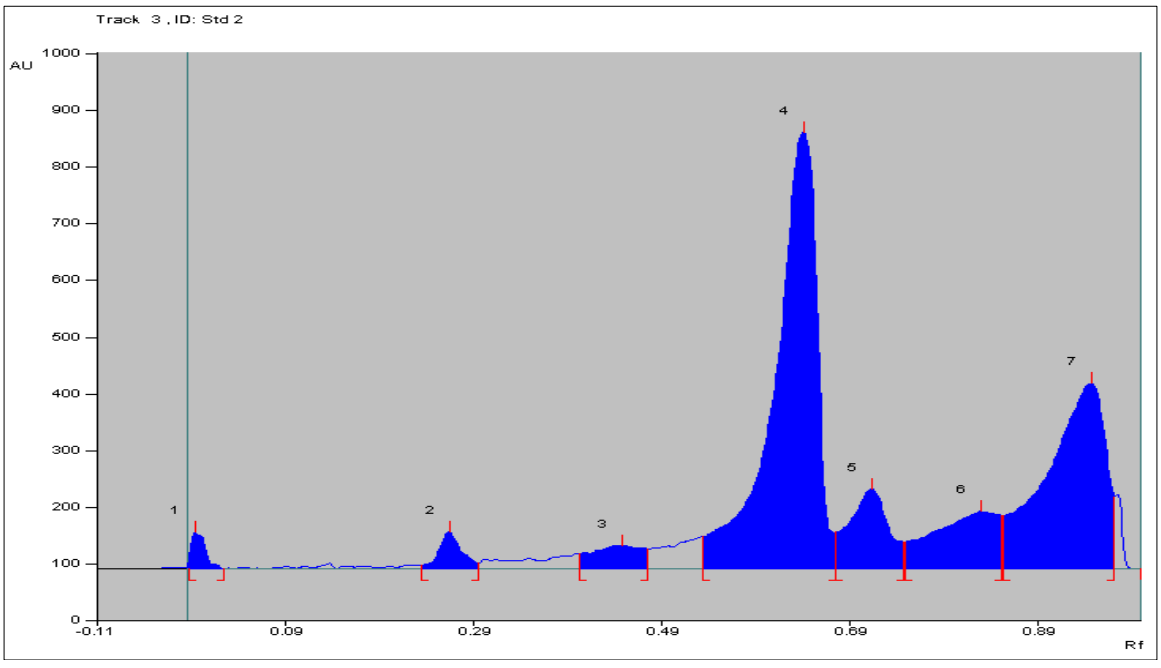

Fig 3: Compound 2

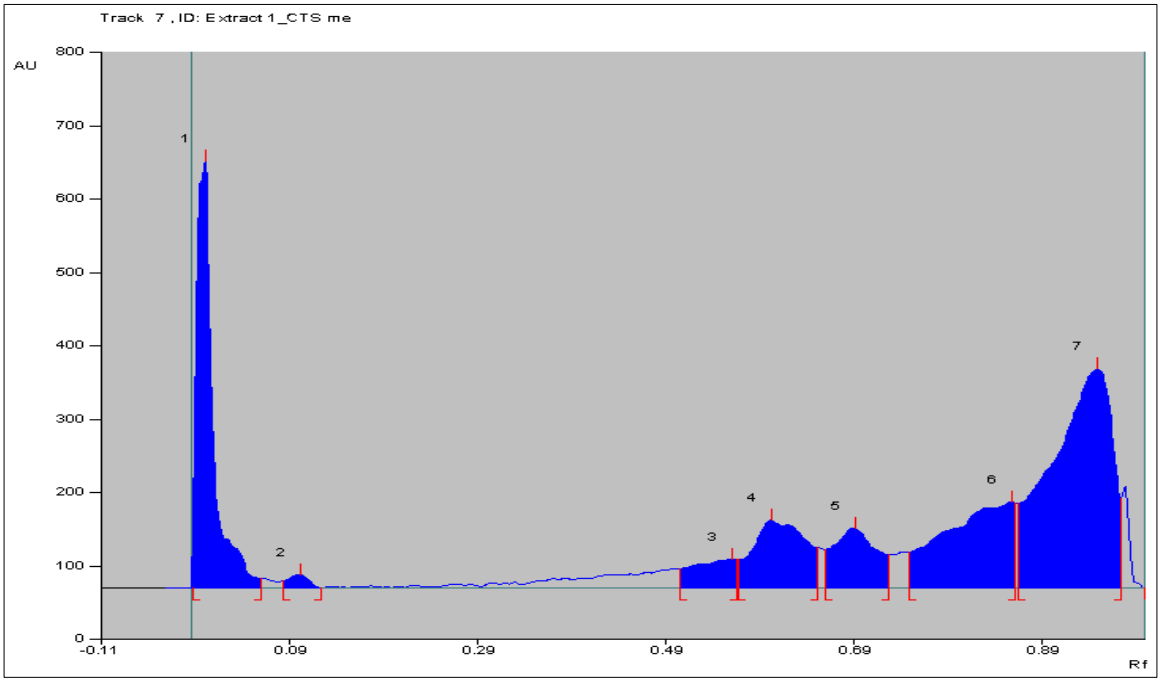

Fig 4: Quantification Compound 2 in test samples of plant extract

Conclusion

The proposed work of standardization and validation of bioactive emodin an anticancer drug, and quercetin simultaneously using simple solvent system as a biomarker by HPTLC. HPTLC fingerprinting of this plant species will also provide basic information useful for the isolation, purification and characterization of marker chemical compounds. This work will be positively helpful to the mankind. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved. Further work will be carried out on characterization and quantitative estimation of bioactive chemical constituent.

Acknowledgment

The authors are thankful to the principal, S.P. College, Pune-411030, India for providing the necessary facility and are grateful to the University Grant Commission for financial support under Minor Research Project scheme (F.47- 1119/14 /General/ 45/ WRO - XII Plan)

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