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Research Article

Quantitative Determination of Quercetin in Michelia Champaca (L.) Flowers by HPTLC Technique

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ABSTRACT

A sensitive and reliable high performance thin layer chromatographic method has been developed for quantitation of quercetin in the dried flowers of *Michelia champaca*. The methanolic extract of flowers was chromatographed on silica gel 60 F254 plates with toluene: ethyl acetate: formic acid, 5: 4: 1 (v/v/v), as mobile phase. Detection and quantitation were performed by densitometric scanning at λ= 254 nm, by using deuterium lamp. The accuracy of the method was checked by conducting recovery studies using the standard addition method and the average recovery of quercetin was found to be 0.1439%w/w. The proposed HPTLC method provides a good resolution of quercetin from other constituents present in methanolic extract of dried flowers of *M.champaca*. The method is rapid, simple and precise.

Keywords: HPTLC, quercetin, *Michelia champaca.*

INTRODUCTION

Early humans recognized their dependence on nature to be healthy and fight against illnesses. Based on instinct, taste and experience, primitive men and women treated illnesses by using plants, animal parts and minerals. Physical evidence of use of herbal remedies dates back to 6000 years. Medicinal herbs are moving from fringe to main stream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals[1]. Many medicinal plants are used in modern medicine where they occupy a very significant place as raw material for important drugs and plants used in traditional system of medicine in pharmaceutical houses are collected from wild sources[2]. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals was used today for our various ailments [3]. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experiment. In view of this, local medicinal plants, which show suitable biological effect, could be standardized and similarly utilized. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant[4]. The chemical constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents[5]. The knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies[6]. High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials. HPTLC based method is being explored as an important tool in routine drug analysis. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. Additionally, numerous Samples can be run in a single analysis thereby dramatically reducing analytical time. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters[7,8].

MATERIALS AND METHODS

High Performance Thin Layer Chromatography (HPTLC) analysis

Standard preparation

About 20mg of quercetin standard was dissolved in 10ml of methanol. From the stock solution, dilute 1ml to 10ml with methanol. From this solution spot 0.5µl to 3.0µl containing concentration in the range of 100ng-600ng.

Sample preparation

About 100mg of methanolic extract was dissolved in 10ml of methanol. From this, 10-25µl of solution was used for the test.

Chromatographic conditions

Chromatography was performed on a 10*3 cm pre-coated HPTLC Silica gel 60 F_{254} plate (Merck). The plates were washed by methanol and activated at 60ºC for 5 minutes

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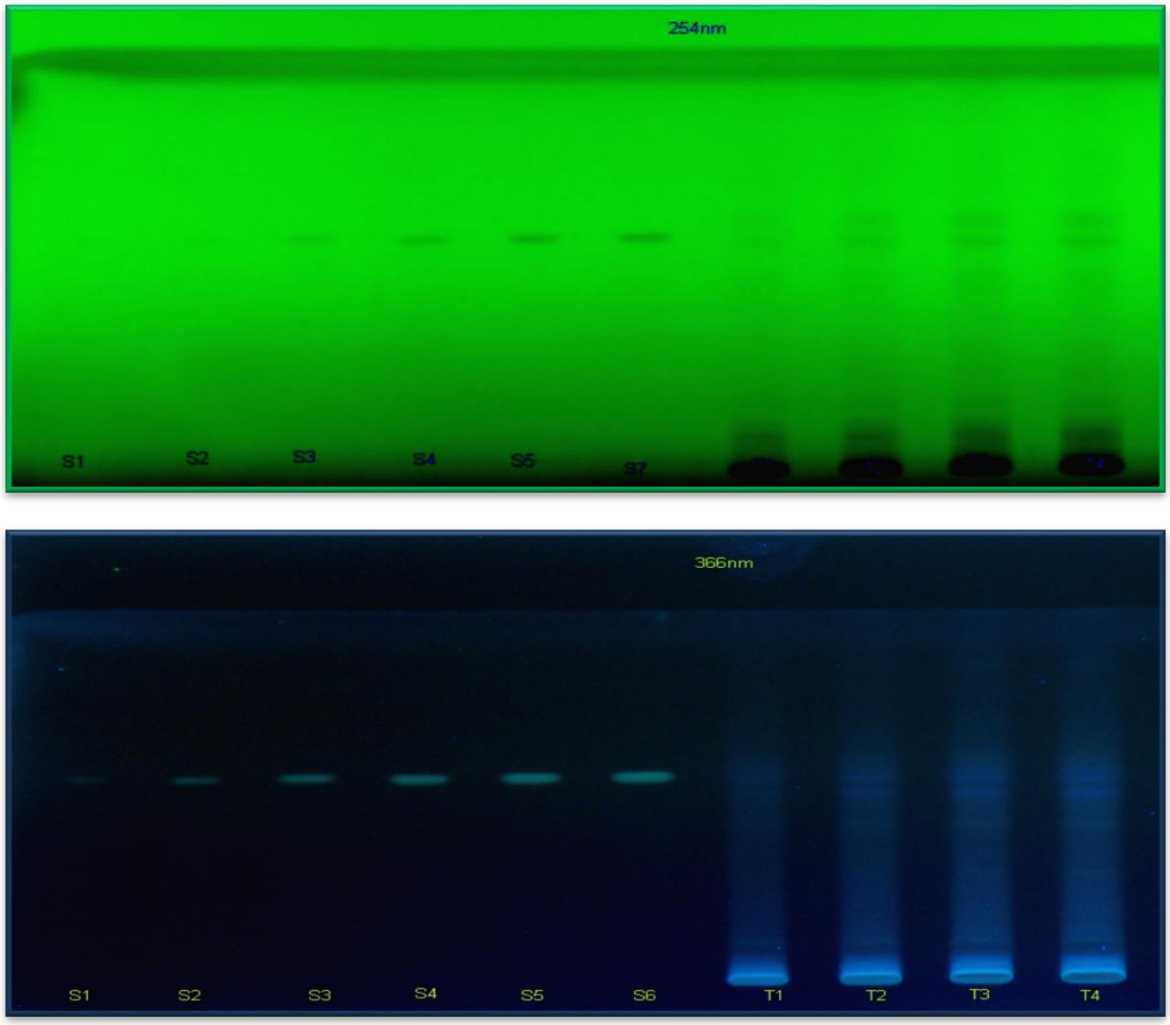


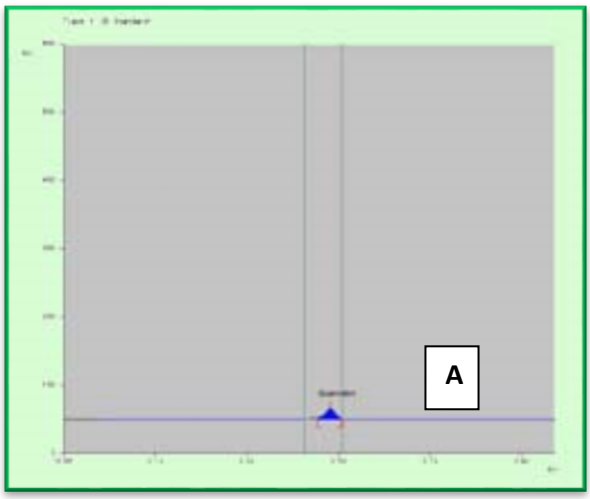
Figure 1: HPTLC finger printing of *Michelia champaca.*

Table 1: R_f values for methanolic extract of *M. champaca.*

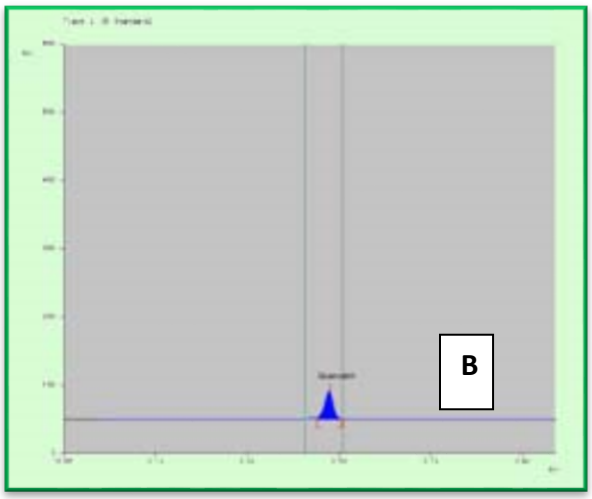
S. No	Start R_f	Start Height	Max. R_f	Max. Height	End R_f	End Height	Area %
Standard							
Track1	0.48	1.2	0.51	16.1	0.53	0.4	262.5
Track 2	0.48	2.1	0.50	41.9	0.53	0.1	582.1
Track 3	0.48	5.1	0.51	80.6	0.53	0.3	1144.9
Track 4	0.45	0.3	0.50	124.9	0.53	1.2	1915.3
Track 5	0.48	10.2	0.51	165.2	0.53	0.8	2533.8
Track 6	0.45	0.9	0.51	189.7	0.53	1.0	3023.5
Sample							
Track 7	0.47	0.5	0.49	47.9	0.51	0.1	617.9
Track 8	0.47	0.5	0.49	66.7	0.51	0.8	888.1
Track 9	0.47	0.2	0.49	85.1	0.51	0.3	1096.7
Track 10	0.47	0.6	0.49	97.0	0.53	0.0	1264.6

prior to chromatography. Samples were applied on to the plate as 0.2 mm band using Linomat 5 sample applicator. The plate was developed in the solvent system to a distance of 8cm. The plate was scanned densitometrically at 254nm using TLC Scanner and observed under UV light at 254nm and 366nm using CAMAG REPROSTAR.

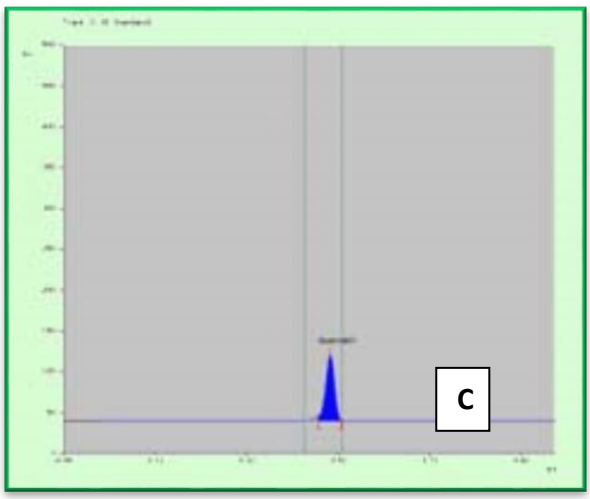
Stationary phase: Silica gel 60 F_{254}
Mobile phase : Toluene:Ethyl acetate:Formic acid (5:4:1)
Scanning wavelength: 254nm
Applied volume: 10,15,20 and 25µl
Development mode: Ascending mode
Evaluation: A band (Rf-0.50) corresponding to quercetin is visible in both reference and test solution tracks.



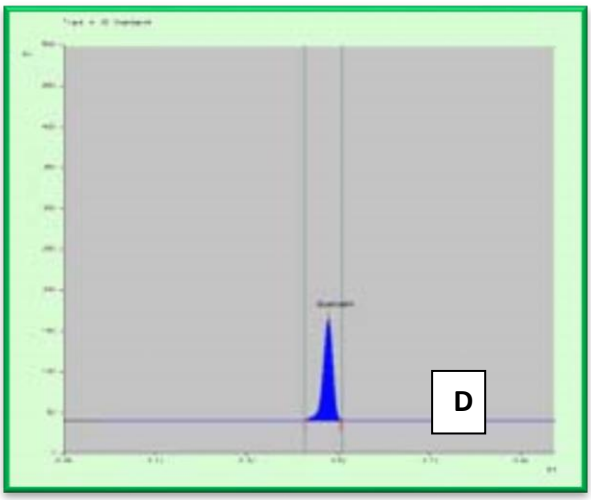
Peak display at 0.5µl



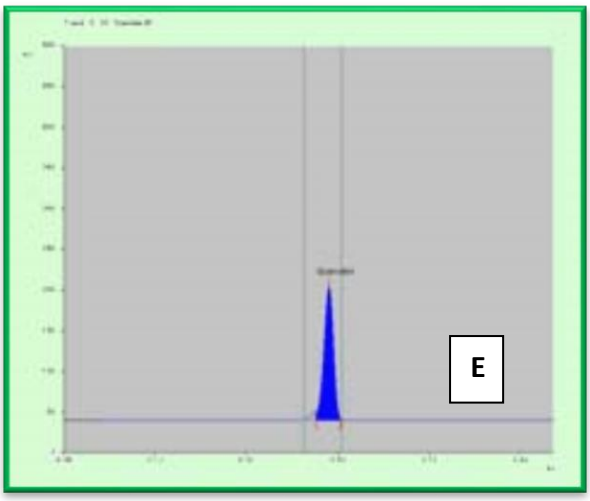
Peak display at 1.0µl



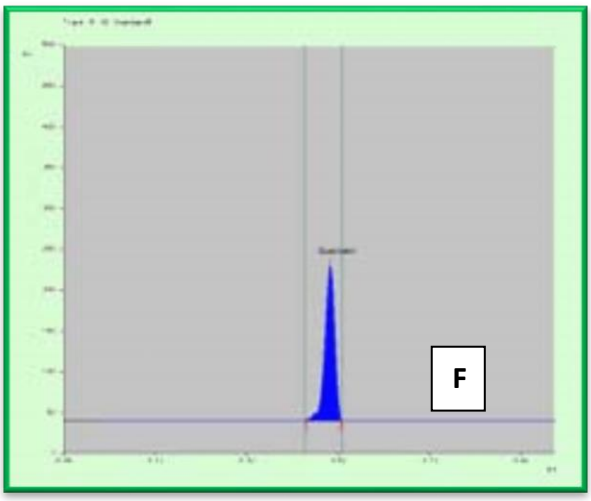
Peak display at 1.5 µl



Peak display at 2.0 µl



Peak display at 2.5 µl



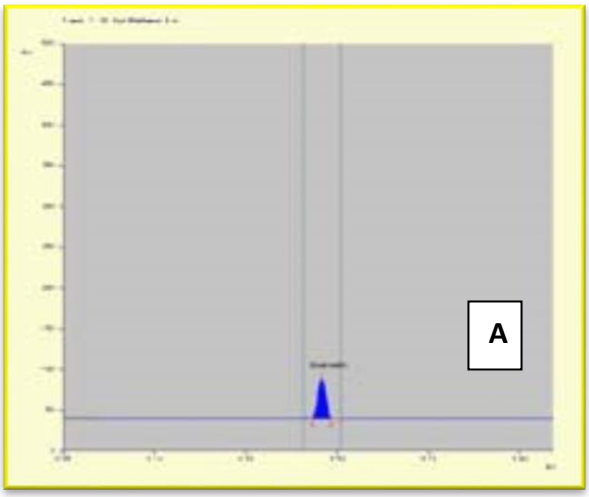
Peak display at 3.0 µl

Figure 2 (A-F): HPTLC chromatogram of Quercetin standard.

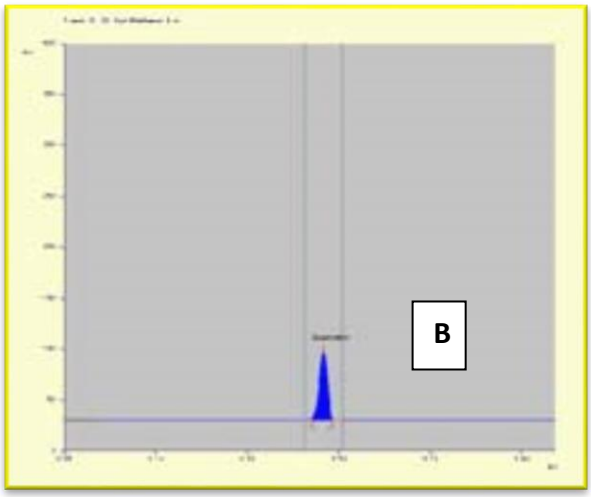
The plates were developed in the chosen mobile phase and were photo documented at 254 nm and 366 nm. The chromatogram of developed plate was recorded at 254 nm.

RESULTS AND DISCUSSION

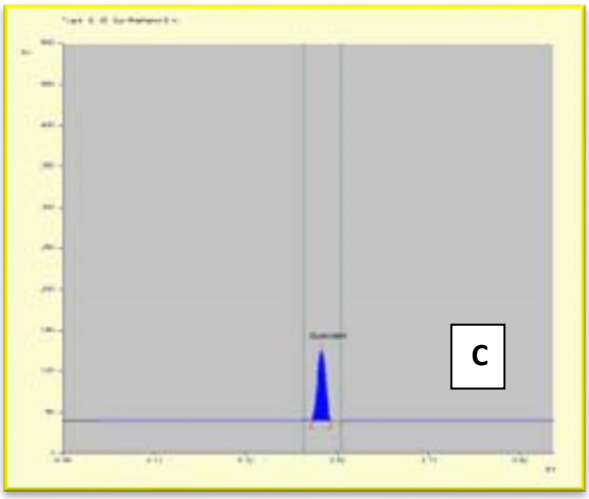
High Performance Thin Layer Chromatography (HPTLC) analysis



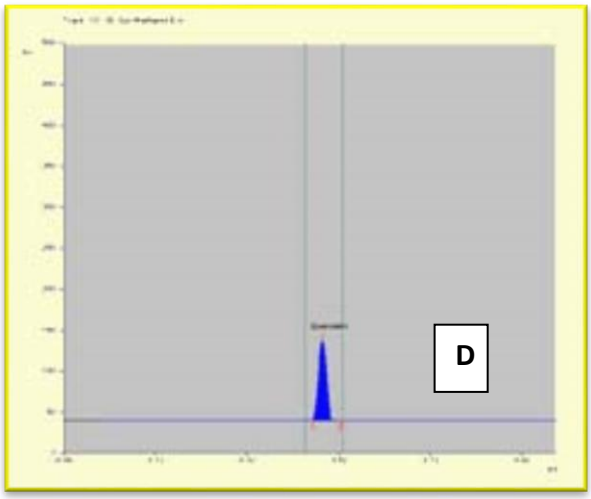
Peak display at 10 µl



Peak display at 15 µl



Peak display at 20 µl



Peak display at 25 µl

Figure 3(A-D): HPTLC chromatogram of flower extract of *M. champaca*.

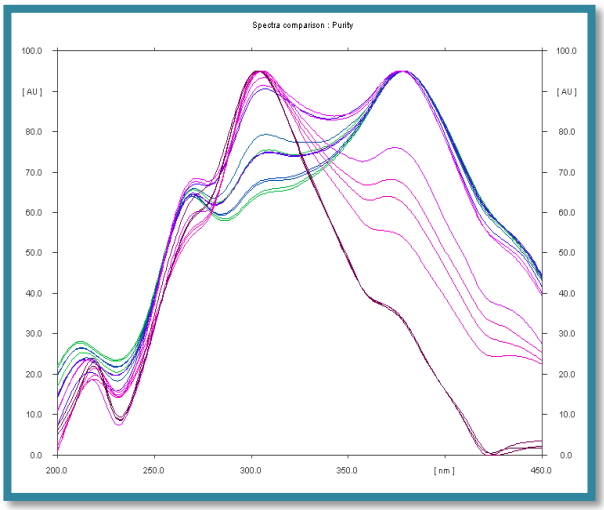


Figure 4: Spectral comparison of purity of sample tracks with Standards at selected wavelength.

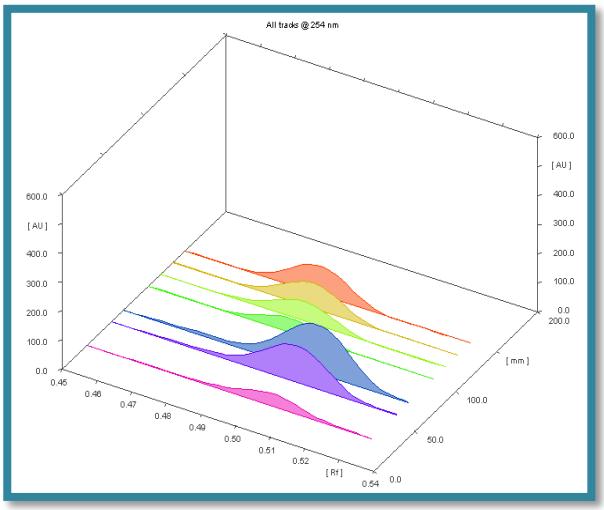


Figure 5: HPTLC-3D display of *Michelia champaca*.

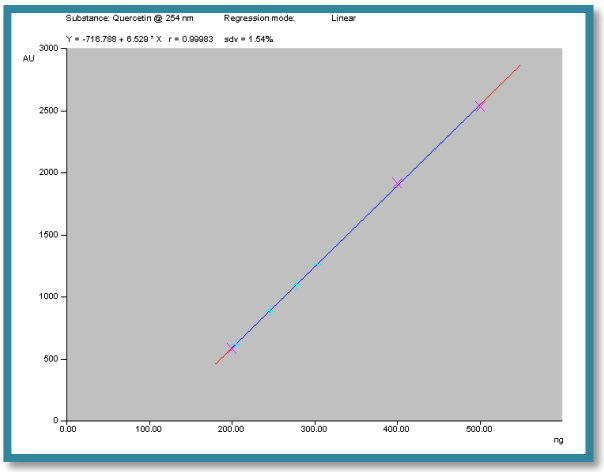
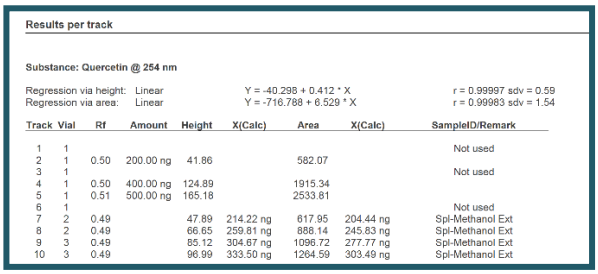


Figure 6: Standard curve of Quercetin.



Results per track

Substance: Quercetin @ 254 nm

Regression via height: Linear Y = -40.298 + 0.412 * X r = 0.99997 sdv = 0.59
Regression via area: Linear Y = -716.788 + 6.529 * X r = 0.99983 sdv = 1.54

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1							Not used
2	1	0.50	200.00 ng	41.86		582.07		
3	1							Not used
4	1	0.50	400.00 ng	124.89		1915.34		
5	1	0.51	500.00 ng	165.18		2533.81		
6	1							Not used
7	2	0.49		47.89	214.22 ng	617.95	204.44 ng	Spl-Methanol Ext
8	2	0.49		66.65	259.81 ng	888.14	245.83 ng	Spl-Methanol Ext
9	3	0.49		85.12	304.67 ng	1096.72	277.77 ng	Spl-Methanol Ext
10	3	0.49		96.99	333.50 ng	1264.59	303.49 ng	Spl-Methanol Ext

Under chromatographic conditions, a densitometry HPTLC analysis was performed for the development of characteristic finger print profile for methanolic flower extract of *M.champaca* (Figure 1) which may be used as markers for quality evaluations and standardization of the drug. The densitometric quantification obtained for standard and sample exhibited same R_f value as shown in Table 1. The Chromatograms of standard quercetin are shown in Figure 2 (A-F) and that of quercetin in *Michelia champaca* are shown in Figure 3 (A-D). Spectral comparison of purity for quercetin reference standard with quercetin in samples is shown in Figure 4. The 3D spectra of all tracks scanned at 366 nm are shown in Figure 5. From the regression equation, y = -716.788x6.529, a good linear relationship (r =0.99983 with respective to height and peak area, respectively) was observed between the concentration ranges 100-600ng (Figure 6). The use of standard ensures the concentration and ratio of the test compound in the flowers. The concentration of the test sample was estimated to be about 0.1439%w/w. Well defined spots of quercetin were found in the extract matching the retention factor (R_f) of standards that were visualized under UV light. From the regression analysis, the concentration of quercetin determined in the methanol flower extract of *M. champaca* was found to be 0.1439%w/w. This result coincides with the previous study[9]. The study has reported the quantitative analysis of quercetin in leaves of *M. champaca* by high performance thin layer chromatographic method and finds a good correlation
(r = 0.9993) between the standard and the sample of quercetin in the dried leaves and stem bark of *Michelia champaca*. Based on the earlier observation the presence of quercetin and its derivatives[10], the well known antioxidants in *C. auriculata*, the HPTLC analysis was performed to understand the influence of aforementioned phytoconstituents on the efficiency of the plant extracts. Flavonoids are a large group of natural polyphenolic substances widely distributed in the plant kingdom that can act as antioxidants in biological systems. Quercetin (3, 3', 4', 5, 7- pentahydroxy flavone), one of the most abundant flavonoids, is present in large amounts in vegetables, fruits, tea, and olive oil. It contains a number of phenolic hydroxyl groups and is a potent oxygen free radical scavenger and a metal chelator[11]. It has been demonstrated that quercetin exhibits its therapeutic potential against many diseases, including ischemic heart diseases, atherosclerosis, liver fibrosis, renal injury, and chronic biliary obstruction[12-14]. Quercetin, a bioflavonoid is a well known antioxidant which brings about the formation of considerably less reactive species from highly reactive free radicals by its reactivity[15]. It is known to exert shielding effect on the damaged β-cells in STZ induced diabetic rats[16]. The inhibition of aldose reductase enzyme by quercetin prevents glucose conversion to sorbitol and might be one of the ways of restoring the normal glycemic condition in diabetic rats. Occurrence of quercetin in the extracts from different parts of the plant, supplements the bioactivity of the plant in regulating the diverse factors responsible for ageing and cellular damages.

CONCLUSION

The HPTLC fingerprinting results showed that quercetin is present in the methanol extract of *M. champaca* was estimated to be 0.1439%w/w. In future, these fingerprinting images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy.

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