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Quantitative Determination of Quercetin in *Wattakaka volubilis* (L.F) by HPTLC Technique

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Abstract : The study is mainly focused to establish the fingerprint profile of *W.volubilis* using high performance thin layer chromatography (HPTLC). A sensitive and reliable high performance thin layer chromatographic method has been developed for quantitation of quercetin in the dried flowers of *W.volubilis*. The methanolic extract 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 quantization were performed by densitometry 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 ofquercetin was found to be 0.0640%w/w. The proposed HPTLC method provides a good resolution of quercetin from other constituents present in methanolic extract of dried flowers of *Wattakaka volubilis*. The method is rapid, simple and precise.
Key words : *W.volubilis*, Quercetin, HPTLC analysis.

1. Introduction

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The substances having medical value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Products of primary metabolism such as aminoacids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance[1].Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the

Indian traditional health care system (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some need to be explored[2]. The photochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bio-activities[3]. Identification and quality evaluation of crude herbal extracts is a fundamental requirement. It is an accepted fact that the qualitative analysis of crude herbal extracts constitutes an important and reliable part of

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quality control protocol as any change in the quality of extract directly affects the constituents. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. 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[4,5,6].

Wattakaka volubilis is a stout, smooth, hoary or mealy, woody vine. Leaves are ovate or somewhat rounded, 7.5 to 15 cm long, 5 to 10 cm wide, rather leathery, rounded or pointed at the base, and pointed at the tip. Cymes are axillary or interpetiolar, and umbel-like. Flowers are green, about 1 cm across. Follicles are usually double, broadly lanceolate, 7.5 to 10 cm long, turgid, longitudinally ribbed, and velvety until mature. Seeds are elliptic, concave, smooth, shining, sharp-edged, and crowned with very fine, white, silky hairs .The leaves of *W. volubilis* are used traditionally in Andhra Pradesh, in a paste form in the treatment of fissures in the feet and in rheumatic pain [7] an ointment known as Hemajeevanti prepared from the leaves was found to be effective in the treatment of wounds, tineapedis, scabies, and in plantar psoriasis [8]. The leaves are applied to boils and abscesses to promote suppuration [9]. *W. volubilis* is distributed throughout the hotter parts of India, Taiwan, Cambodia, Nepal and Sri Lanka [10]. The chief phytoconstituents reported in the leaves and stems of *W. volubilis* are glycosides, flavonoids, triterpenoids and saponins[11]. Plant is also reported to possess mild CNS depressant, anthelmintic, antispasmodic, cytotoxic, antimutagenic and anticancer properties [12]. The roots of *W. volubilis* are reported to possess antipyretic activity [13].

2. Materials and Methods

TLC Details

T1-T6-1 to 4µl of Standard Quercetin, T1-T4-5 to 25µl of sample Solution

Identity Test

Sample Preparation : Macerate 5g of powdered drug in methanol for 24h.Filter the solution and concentrate to dryness. The dried residue dissolved in methanol and used for TLC analysis.

Standard Preparation : 20.5mg of quercetin standard was dissolved in 10ml of methanol. From the stock pipette out 1ml and dilute to 10ml.From the standard solution 1µl to 4µl was spotted containing concentration in the range of 200ng-800ng

Stationary phase : Silica Gel 60 F_{254}

Mobile phase : Toluene: Ethyl acetate: Formic acid (5:4:1)

Procedure : Applied 1.0µl to 4µl of standard solution and 5 to 25µl of test solutions on a precoated silica gel 60 F_{254} HPTLC plate (E.Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. Developed the plate in the solvent system to a distance of 8cm.Scanned the plate densitometrically at 254nm using TLC Scanner3.Observed the plate under UV light at 254nm & 366nm using CAMAG REPROSTAR3.

Wave length : 254 nm

3. Results

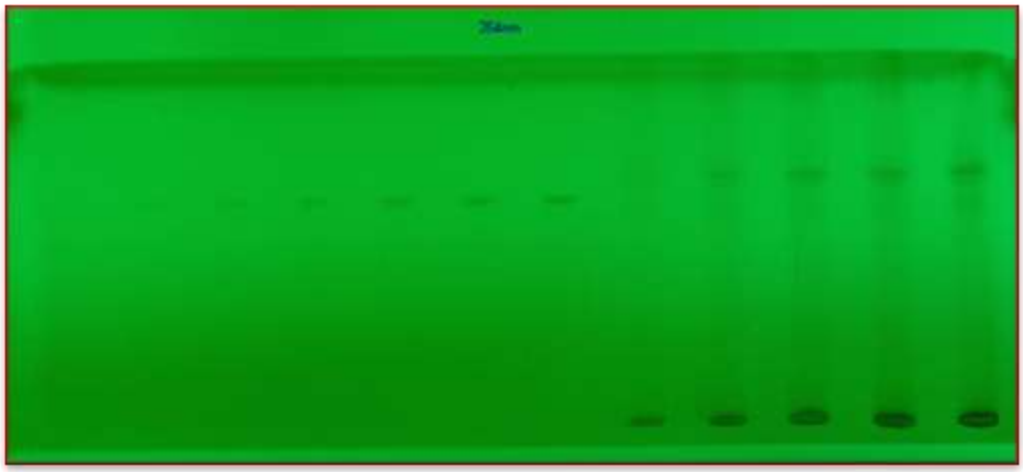
HPTLC Profile

Fingerprint analysis of sample was done through HPTLC method and the selected solvent system. Toluene: Ethyl acetate: formic acid (5:4:1) was suitable for quantitative analysis. HPTLC fingerprinting of the plants under study were presented photo documentation under 254 nm and 366 nm along with Rf values (**Figure1**). **Figure2** showed HPTLC chromatogram of methanolic extracts of *Wattakaka volubilis*. **Figure3** (1, 1.5, 2, 2.5,3, 3.5, 4, 4.5, and 5 µg of sample) the total number of spot was nine, respective maximum values RF values :(0.60, 0.59, 0.59, 0.59, 0.59, 0.59, 0.59, 0.60, 0.58). For quantitative analysis through HPTLC techniques, optimization of solvent system was found to be Toluene: Ethyl acetate: formic acid (5:4:1). Quantitative analysis was performed through HPTLC techniques using quercetin as standard marker compound

in the *Wattakaka volubilis*. In the HPTLC fingerprinting of methanolic extract gave a band corresponding to quercetin visible in test solution track. HPTLC photograph of standard quercetin visible in test solution track. HPTLC photograph of standard quercetin and methanolic extract of *Wattakaka volubilis* were presented **Figure**:**4**The percentage amount of quercetin in *Wattakaka volubilis* methanolic extract was found to be 0.2697% HPTLC ethanol confirms the presence of flavonoid such as quercetin in methanolic extract of *Wattakaka volubilis* were presented in the **figure5**.

HPTLC Analysis

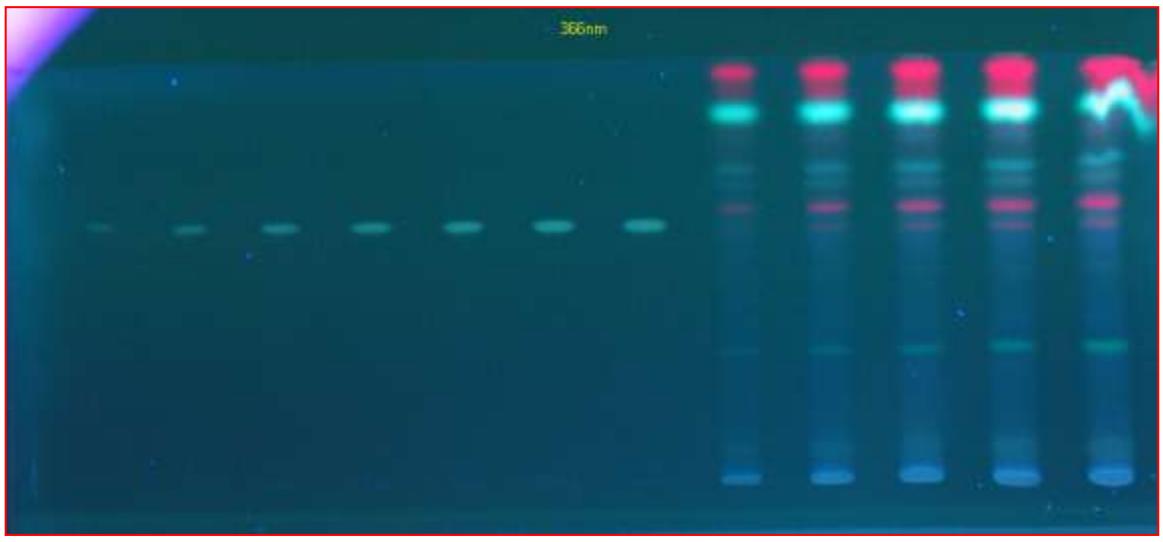
AT 254nm



S1 S2 S3 S4 S5 S6 S7 S8 T1 T2 T3 T4 T5

Fig : 1 Quantification of Quercetin in methanolic extract of *Wattakaka volubilis* PHOTO DOCUMENTATION UNDER UV

AT 366nm



S1 S2 S3 S4 S5 S6 S7 S8 T1 T2 T3 T4 T5

Fig 2 : Three dimensional representation of HPTLC chromatogram of *Wattakaka volubilis*.

3D DISPLAY @ 254nm

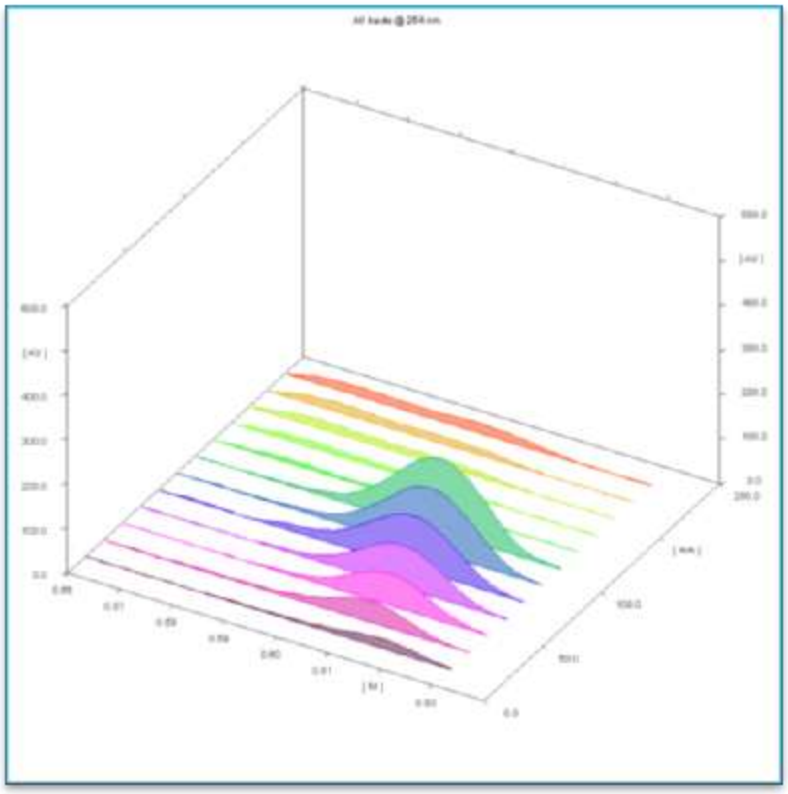


Figure 3: HPTLC Chromatogram of methanolic extract of *Wattakka volubilis*

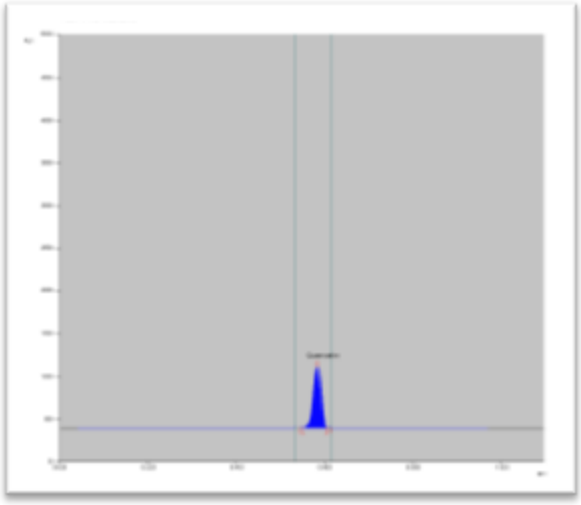
Peak Display (1µl of Standard)

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.59	1.8	0.62	25.1	100.00	0.63	1.3	264.5	100.00	Quercetin

Peak Display (1.5µl of Standard)

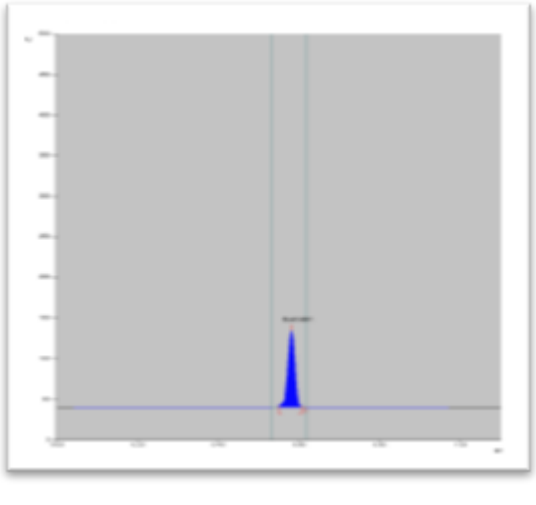
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.59	1.8	0.61	46.0	100.00	0.63	0.0	638.7	100.00	Quercetin

Peak Display (2µl of Standard)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.58	0.4	0.61	71.2	100.00	0.63	0.9	982.8	100.00	Quercetin

Peak Display (2.5µl of Standard)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.58	0.2	0.61	94.0	100.00	0.63	0.7	1353.8	100.00	Quercetin

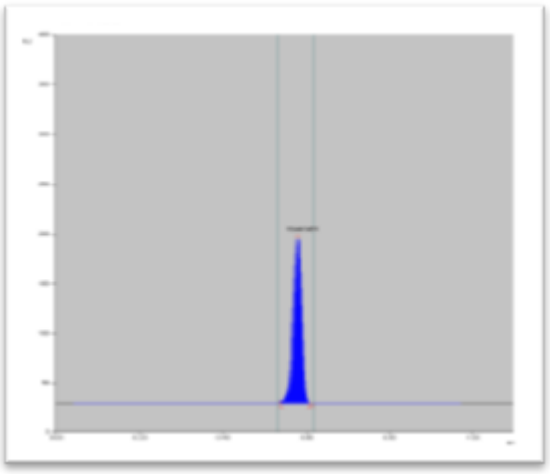
Peak Display (3µl of Standard)

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.58	1.0	0.61	118.5	100.00	0.63	1.0	1868.5	100.00	Quercetin

Peak Display (3.5µl of Standard)

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.57	1.9	0.61	141.7	100.00	0.63	0.9	2187.8	100.00	Quercetin

Peak Display (4µl of Standard)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.57	1.6	0.61	163.6	100.00	0.63	1.3	2560.4	100.00	Quercetin

Peak Display (25µl of Sample)

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.59	16.3	0.60	20.4	100.00	0.62	0.1	332.4	100.00	Quercetin

Linearity

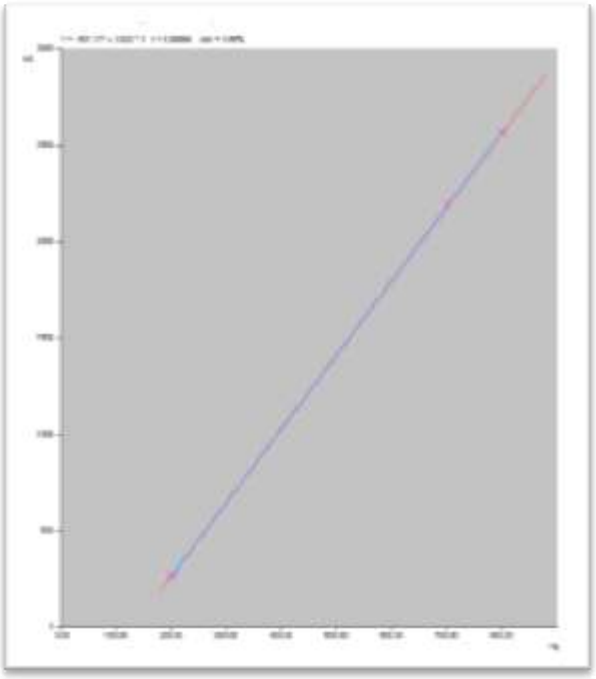


Figure4: Standard curve of Quercetin.

Spectral Comparison For Purity

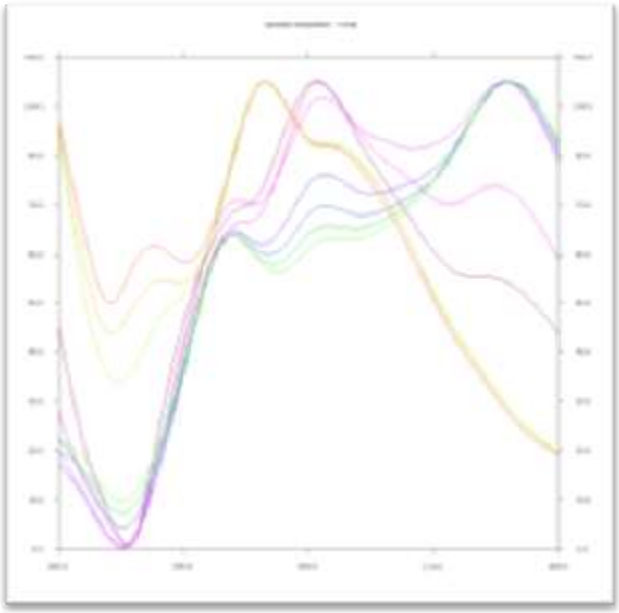
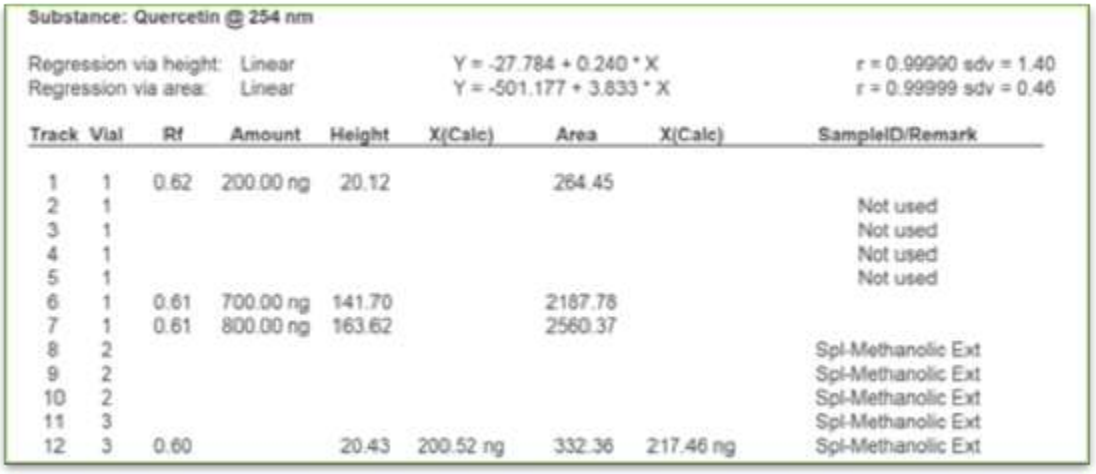


Figure 5: Spectral comparison of purity of sample tracks with Standards at selected wavelength.



Substance: Quercetin @ 254 nm

Regression via height: Linear Y = -27.784 + 0.240 * X r = 0.99990 sdv = 1.40
Regression via area: Linear Y = -501.177 + 3.833 * X r = 0.99999 sdv = 0.46

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1	0.62	200.00 ng	20.12		264.45		
2	1							Not used
3	1							Not used
4	1							Not used
5	1							Not used
6	1	0.61	700.00 ng	141.70		2187.78		
7	1	0.61	800.00 ng	163.62		2560.37		
8	2							Spl-Methanolic Ext
9	2							Spl-Methanolic Ext
10	2							Spl-Methanolic Ext
11	3							Spl-Methanolic Ext
12	3	0.60		20.43	200.52 ng	332.36	217.46 ng	Spl-Methanolic Ext

Discussion

HPTLC analysis

HPTLC finger analysis and quantitative analysis of marker compound using modern analytical techniques. In the last few decades (HPTLC) has become known as an important tool for the qualitative, semi qualitative and quantitative phytochemical analysis of herbal drugs and formulation. The major advantage of HPTLC in several samples can be analyzed simultaneously using small quantity of marker compound and mobile phase with very less time [14].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 0.0640%w/w. Well defined spots of quercetin were found in the extract matching the retention factor (Rf) of standards that were visualized under UV light. From the regression analysis, the concentration of quercetin determined in the methanolic leaf extract of *Wattakaka volubilis* was found to be 0.0640%w/w. This result coincides with the previous study[15]. 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[16]. 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 [17-19]. 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[20]. It is known to exert shielding effect on the damaged β-cells in STZ induced diabetic rats[21]. The inhibition of aldose reductase enzyme by quercetin prevents glucose conversion to s -orbitol 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.

In the presence investigation the flavonoid compounds were analyzed by HPTLC method. A densitometry HPTLC analysis was; performed for the progress of characteristic fingerprint sketch which may be used as marker for quality evaluation and standardization of the herbal drug.

Conclusion

HPTLC fingerprint analysis not only gives the ideafor the authentication of the plant extracts and itsconstituents but also provides the parameters forquality of herbal formulations. The chromatographic fingerprint, therefore is suitable for monitoring theidentity and purity profile of a plant extract. Inaddition to qualitative detection, HPTLC techniquealso provides semiquantitative information about the major active phytoconstituents present in a plantextract, thus enabling an assessment of plant extractquality.HPTLC fingerprint analysis can be used as adiagnostic tool for the correct identification of theplant. Though further

work to characterize the otherchemical constituents and perform quantitativeestimation with marker compounds is also necessarythese data can also be considered along with the other values for fixing standards to this plant.

The HPTLC fingerprinting results showed that quercetin is present in the methanol extract of *W.volubilis* was estimated to be 0.0640%w/w. In future, these Inconclusion, the results obtained from qualitativeevaluation of HPTLC fingerprint images will behelpful in the identification and quality control of thedrug and ensure therapeutic efficacy. It can beconcluded that HPTLC fingerprint analysis of leaf extract of*W.volubili s*can be used as a diagnostic toolfor the correct identification of the plant and it isuseful as a phytochemical marker and also a goodestimator of genetic variability in plant population.

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