International Journal of Pharma and Bio Sciences V1(1)2010

DETERMINATION OF QUERCETIN BY HPTLC IN *"CALENDULA OFFICINALIS"* EXTRACT

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ABSTRACTS

Calendula officinalis Linn (Asteraceae), is an aromatic annual herb, which is used in Traditional System of Medicine to treat various diseases like anti-inflammatory, antitumour, antispasmodic. The reported constituents are flavonoids, glycosides and saponins. From literature it was found that the quantification work was not carried out. It is considered to contain Quercetin, confirmed by TLC and by qualitative test. Thus it was quantified using HPTLC a sensitive method for development of marker compounds. The method was carried out on precoated TLC aluminum plates with silica gel 60 GF as stationary phase using solvent system as Chloroform: Methanol (9.5: 0.5) with Rf value of 0.43. Quantitative analysis was carried out in the absorbance at 366 nm. The linearity regression for the calibration showed r = 0.6548 and 0.999 with respect to height and area in a range of 0.5-5.0 g per spot. Thus HPTLC method provides a faster and cost effective quality control for the routine analysis of Quercetin in extracts containing *Calendula officinalis*.

KEYWORDS

Calendula officinalis, Quercetin, HPTLC analysis, Marker compound.

INTRODUCTION

Calendula officinalis Linn(Asteraceae) commonly known as English Garden Marigold or Pot marigold is an aromatic annual herb, which is used in traditional system of Medicine to treat various diseases. The reported main constituents are flavonoids, flavanol, glycosides and saponins[1]. This plant is native of southern Europe and it is used as diuretic, diaphoretic, stimulant and also posses spermicidal activity. This plant is used because of its varied source of biological activities like anti-inflammatory, Antimutagenic, antispasmodic. It is

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also used in gastro intestinal gynecological, eye disease, skin injuries and in some cases of burn[2]. So far no reports have been studied for its quantification of marker compounds present in the extracts. High Performance thin layer chromatography is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identity of crude drug and also for quality control of finished product[3]. However recent reviews shows that the thin layer chromatography and HPTLC techniques can be used to rectify many qualitative and quantitative analytical problems in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology[4,5].Thus in the present investigation an attempt was made to quantify the marker compound by using HPTLC. High Performance Thin Layer Chromatography is an important tool which can be used for both qualitative and quantitative analysis, which includes purity and identification of compounds.

MATERIALS AND METHODS

(i)Preparation of samples:

The fresh flowers of the plant *Calendula officinalis* were collected from the wild sources of the Dhulia district of Maharashtra, in the month of December and were identified from the authentic sources. Dried flowers were crushed and 100gms each were macerated with 150 ml of 50 % methanol for 48 h with occasional shaking. The extracts were separated and mark was again extracted twice with fresh 50 % methanol. The extract were pooled and concentrated in rotatory vacuum evaporated to get 100 ml of each sample separately[6]. One ml of each extract was further diluted to 5 ml with 50 % methanol, which were used for quantitative estimation. The extract was subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites.[7]

(ii)Standard Quercetin solution:

A 1 mg/ml solution of standard qurecetin (Sigma Aldrich) was made in 50 % methanol for preparation of the standard curve.

(iii)Procedure:

The five standard levels (1, 2, 3, 4 and 5 µg) of standard quercetin were used for calibration curve for which 1,2,3,4 and 5 µl of standard solution was applied in duplicate on a TLC plate using a semi automatic Linomat V sample applicator. The chromatograph was developed for 15 mints, dried at room temperature and the scanned at 366 nm; average peak areas of two standards were calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis) was prepared to get a regression equation by Win Cats software, which was used for the estimation of quercetin in Garden Marigold.

(iv)Chromatographic conditions:

Instrument: – Camag HPTLC system, consisting of Linomat V spotting device and scanner III with Win Cats 4 software
Stationary phase: – TLC aluminum sheets silica gel 60 F253 pre coated layer (20 cm X 10 cm), thickness 0.2 mm., no. of tracks : 18, band length : 6 mm.
Mobile phase: – Chloroform: Methanol (9.5: 0.5)
Development chamber: – Twin through chamber (20 X 10)
Distance run: – 75 mm
Scanning wavelength: – 366 nm
Slit dimension: – 6.00 x 0.45 mm, Micro
Measurement mode : – absorbance

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(v)Estimation of quercetin in test sample:

The mean peak height / area of duplicate sample were calculated and the content of quercetin was quantified using the regression equation obtained from the standard curve.

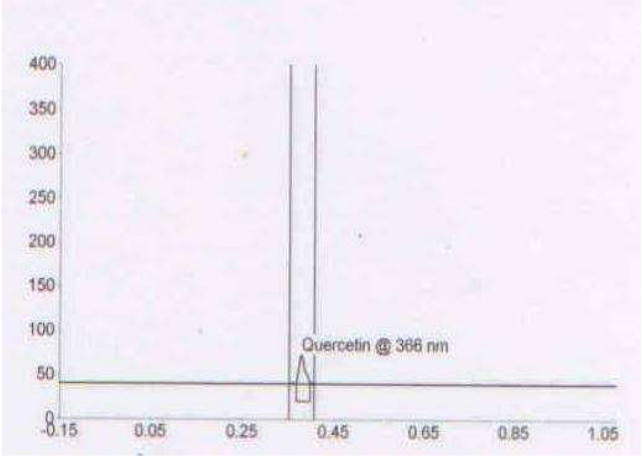
RESULT AND DISCUSSION

The standard quercetin has Rf value of 0.43. A good linear relationship (r^2 = 0.999 and 0.658 with respective to peak area and height, respectively) was observed between the concentration ranges of 1.0-5.0 µg. The regression equation was found to be Y= 35.859+0.049X with respective to height and Y=437.310+0.783X with respect to area, where Y is the peak height / area and X is concentration of quercetin. The highest content of quercetin was found to be 280.3 mg/100 gm using the present HPTLC method.

The UV spectrum of test sample is super imposable with that of standard quercetin indicating purity of peak. Simplicity, specificity and sensitivity of newly developed method makes it the apt choice for monitoring quercetin content for standardization of raw materials at the time of formulating a preparation as well as for the quality control of finished product

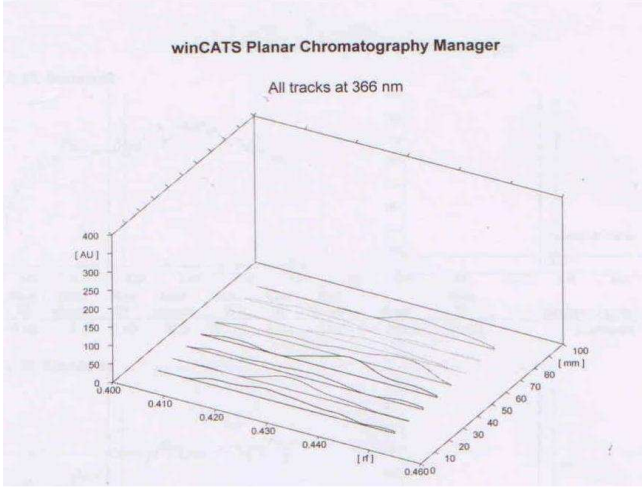
Graph 1:

Standard graph for Quercetin.



Graph 2

UV Spectrum of standard and sample superimposed at 366nm.



CONCLUSION

Lack of standardization techniques fails to identify the drug from its originality which thereby exploits the usage of drug from its Traditional System of Medicine. The plant *Calendula officinalis* is used from the ancient time for its great medicinal values as a remedy in day to day life but in this aspect adulterations are also done which leads to its extinct. It may be stated that the approach given for the standardization of

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quercetin using the HPTLC fingerprint method should be followed for standardization of all Unani and Ayurvedic compound. The scientific, quality assessment parameter accepted by the World Health Organization (WHO) evolved in the present investigation will be helpful in checking the identity and quality and to detect adulterants/ substitutes.

REFERENCES

1. Pietta P, Bruno AMP, Rava A, Journal of Chromatography, separation of flavonol-2-O-glycosides from *Calendula officinalis* and *Sambucus nigra* by High performance liquid and micellar electrokinetic capillary chromatography, 593: 165-170, (1992).
2. Krag K, Plants used as contraceptives by the North American Indians: an ethanobotanical study, Botanical Museum, Cambridge MA: Harvard University, (1976).
3. Sethi PD, Quantitative analysis of drugs in pharmaceutical formulations, New Delhi: CBS Publisher and Distributors, 589, (1997).
4. Weins C and Hauck HH, Advances and developments in thin layer chromatography, LC-GC International, 9: 710-7, (1996).
5. Kalasz H and Bathori M, Present status and future perspective of thin layer chromatography, LC-GC International, 10: 440-5, (1997).
6. Kokate CK, Practical pharmacognosy, Vallabh Prakashan, New Delhi, 110-111,(2005).
7. Harbone JB, Phytochemical methods – A Guide to Modern Techniques of Plant Analysis, Chapman and Hall London, 129, 189, 203, (1998).