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Research Article

QUANTIFICATION OF CATECHIN AND LYCOPENE IN *Calendula officinalis* EXTRACTS USING HPTLC METHOD

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ABSTRACT

Calendula officinalis is used as diuretic, diaphoretic, stimulant and possesses spermicidal activity because of its varied sources of biological activities like anti-inflammatory, antimutagenic, diuretic, and antispasmodic. High performance thin layer chromatography is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identifying the major chemical constituents of crude drug, and also for quality control of finished product. In the present study, (+) catechin and lycopene were detected against their standard in ethanolic extract of the floral part of *C. officinalis* by TLC using ethyl acetate: glacial acetic acid: formic acid: water [100:11:11:25, v/v/v/v/v] as solvent system. A migration distance of 75 mm with running time 30 min was required for the detection of the spots of standard lycopene and catechin with Rf =0.45 and 0.87, respectively. The highest content of catechin and lycopene were found to be 6.88 mg/g and 13.54 mg/g extract using the HPTLC method. Quantification of lycopene and catechin in *C. officinalis* floral part extract revealed its potential for further use in the prevention and treatment of various deadly diseases.

Key Words: *Calendula officinalis,* Catechin, HPTLC, Lycopene.

INTRODUCTION

Calendula officinalis Linn (Asteraceae) commonly known as English garden marigold is an aromatic annul herb[1]. It is native of Southern Europe and used in traditional system of medicine to treat various diseases[1]. The reported main constituents are flavonoids, flavonol, glycosides, and saponin[2]. This plant is used as diuretic, diaphoretic, stimulant and possesses spermicidal activity[3] because of its varied sources of biological activities like anti-inflammatory, antimutagenic, diuretic, and antispasmodic. High performance thin layer chromatography is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identifying the major chemical constituents of crude drug, and also for quality control of finished product[4]. 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.[5] .Thus in the present investigation an attempt was made to quantify the catechin and lycopene compound in alcoholic extract of *Calendula officinalis* by using HPTLC.

MATERIALS AND METHODS

Thin layer Chromatography (TLC) analysis was done as per the standard method described[6]. A solvent system was developed by using solvents of different polarity. The entire process of fractionation was conducted at Ethnoveterinary laboratory, Division of medicine, IVRI, Bareilly, U.P.

Sample preparation

The fresh flowers of *Calendula officinalis* were collected from the Bareilly district of Uttar Pradesh, in the month of January and were identified from the authentic sources. Dried flowers were crushed and 100 g each were macerated with 150 ml of absolute ethanol for 48 h with occasional shaking. The extracts were dried under reduced pressure at temperature 40^0C by using rotaevaporator and 10 mg of extract was further re-dissolved to 1 ml with ethanol, which were used for quantitative estimation.

Preparation of standard (+) catechin and lycopene solution

A 0.5 mg/ml solution of standard (+) catechin and lycopene (Sigma Aldrich) were made in absolute ethanol for preparation of the standard curve.

Procedure

The five standard levels (0.5, 1, 2, 3, 4 µg) of standard catechin were used for precalibration curve, for which 1, 2, 3, 4 and 5 µl of standard solution was applied on a TLC plate using a semi automatic Linomat 5 141140 applicator. Similarly the five standard levels (0.7, 1.4, 2.1, 2.8 and 3.5 µg) of standard lycopene solution were applied. The test extract (*Calendula officinalis*) of conc. 10 mg/ml was also applied band wise (band width 6 mm) with injection volume 8, 10, 12, 14 in duplicate. Total 18 applications were made on a 20 x 10 cm HPTLC plate (aluminium sheet precoated with silica gel 60 F254) with band 6 mm and tract distance 8 mm. A distance of 10 mm from lower edge of the plate was maintained at the time of application with delivery rate 4 µl/s using N_2 as spraying gas.

Chromatography

The plate was developed in a CAMAG twin trough horizontal developing chamber (20 x 10 cm) saturated with solvent (ethyl acetate: glacial acetic acid: formic acid: water 100:11:11:25 v/v) used as mobile phase. A migration distance of 75 mm with running time 30 min was required for the detection of the spots of standard lycopene and catechin with Rf =0.45 and 0.87, respectively (FIG 1)

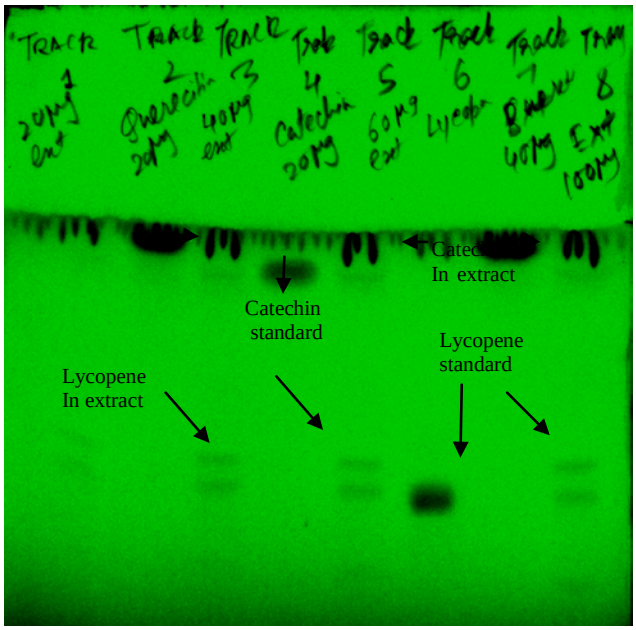

Fig 1: TLC profile showing detection of catechin and lycopene against their respective standard in *C. officinalis* ethanolic extract.

Densitometric evaluation

After development, the plate was air dried and subsequently scanned by fluorescence at 254 nm using CAMAG TLC scanner and CATS evaluation software. A slit dimension of 6.00 x 0.30 mm micro was selected for scanning with speed 20 mm/s at data resolution 100 µm/step.

RESULTS AND DISCUSSION

In the present study, (+) catechin and lycopene were detected against their standard in ethanolic extract of the floral part of *C. officinalis* by TLC using ethyl acetate: glacial acetic acid: formic acid: water [100:11:11:25, v/v] as solvent system (FIG 1). The HPTLC spectrum of ethanolic extract of *C. officinalis* showed appearance of peaks of (+) catechin (peak 8 in FIG 2) and lycopene (peak 5 in FIG 2) with R_f 0.87 and 0.45, respectively (FIG 2). Calibration curves of the standard i. e. (+) catechin and lycopene were drawn by plotting concentration in µg vs peak height/area. A good linear relationship (between the concentration and peak area as well as peak height of the standards) was observed within the concentration ranges of 0.5-5.0 µg. The polynomial regression equation of the calibration curve of (+) catechin (FIG 3) was found to be Y= 298.828 + 2.5518*X + 0.002*X2 (Where Y = peak area X= concentration of catechin). The linear regression equation of the calibration curve of the standard lycopene was found to be Y= 180.831 + 445.482X with r^2 = 0.995 where Y is the peak area and X is the concentration of lycopene in µg. The calibration curve of lycopene was well fitted within the concentration range 0.5-3.5 µg (FIG 4). The absolute amount in µg of (+) catechin and lycopene present in the test extract were calculated from the calibration curves of catechin and lycopene with regression 0.999 and 0.995 respectively. The highest content of catechin and lycopene were found to be 6.88 mg/g and 13.54 mg/g extract using the present HPTLC method. Presence of lutein and lycopene in ethanolic extract of *Calendula officinalis* was reported by TLC[7] although literature are lacking regarding presence of catechin. The newly developed HPTLC method was found to be simple, specific and sensitive for monitoring catechin and lycopene content in the extract. Thus it can be employed for the analysis and standardization of raw materials at the time of formulating a preparation using calendula extract as well as for the quality control of finished product.

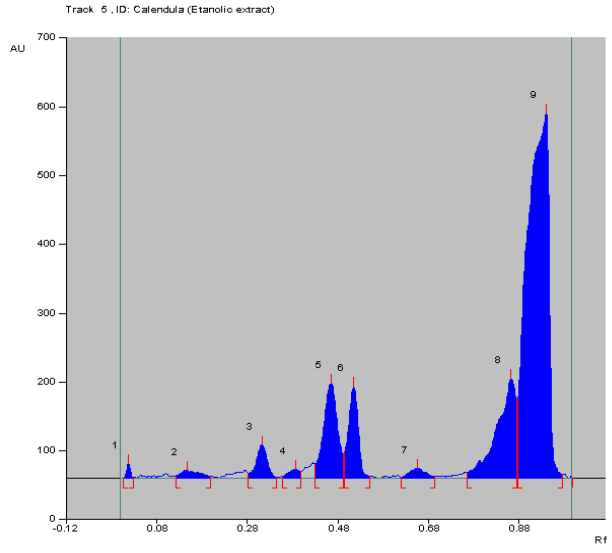

Fig 2: HPTLC profile showing R_f value of different ingredient in ethanolic extract of *C. officinalis* (catechin R_f value 0.45 and lycopene R_f value 0.87)

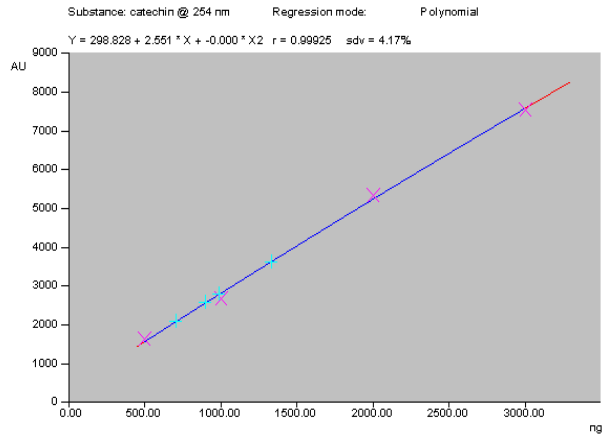

Fig 3: Calibration curve for catechin in ethanolic extract of *C. officinalis* against standard catechin.

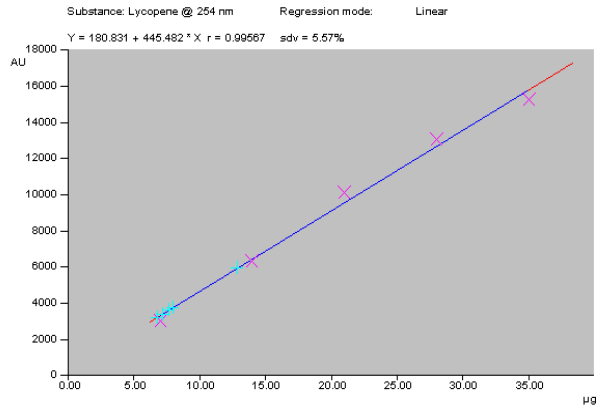

Fig 4: Calibration curve for lycopene in ethanolic extract of *C. officinalis* against standard lycopene.

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