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Determination of Quercetin by HPTLC Method in Purple Dendrobium Flowers Extract

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ABSTRACT

Dendrobium species of plants are highly prized medicinal medicine. It includes a variety of bioactive like SG-168, dendroxine and anti –cancer (Phenanthraquinone). Phytochemical analysis of the flower extract showed the presence of alkaloids, carbohydrates, proteins, phytosterol, phenol, flavonoids etc. In the present study, an attempt was made to quantify the flavonoid quercetin in the Purple flower extract of Dendrobium. TLC was done to confirm the presence of quercetin and HPTLC method has been developed for quantification of quercetin in the methanol flower extract. TLC silica gel 60 F 254 plate was used as stationary phase and the solvent system toluene: chloroform: ethyl alcohol (4:4:1) as the mobile phase. Quantitative analysis was carried out in the absorbance range 200 to 400 nm. A good linear relationship 0.99926 was obtained between the concentration ranges of 100 – 300 ng spot^{-1}.

Keywords: Dendrobium, quercetin, TLC, HPTLC.

INTRODUCTION

The flavonoids, which occur both in the Free State and as glycosides, are the largest group of naturally occurring phenols. They are formed from three acetate units and phenylpropane units. They are widely distributed in nature but are more common in young tissues, where they occur in cell sap. Flavonoids have been referred to as nature's biological compound because of their inherent ability to modify the reaction taking place in the body due to allergies, virus and carcinogens [1]. They have been used extensively as chemotaxonomic markers and are abundant in the Polygonaceae, Rutaceae, Leguminosae, Umbellifarae, and Compositae.Whilemost are O-glycosides, a considerable number of C-glycosidesare also known. Many flavonoids-containing plants are diuretics or antispasmodics and some flavonoids have antitumor, antifungal and antibacterial properties as well as antihepatotoxic activity. The Flavanol quercetin (3, 3', 4', 5, 7pentahydroxyflavone) a phytoalexin, is one of the most potent biomedical agents known. Several types of diseases are inhibited by this biocompound such as cataract, coronary heart disease, and diabetes and cancer especially prostate cancer [2]

Methanol flower extract of Dendrobium was subjected to thin layer chromatography and high performance thin-layer chromatography, to find out the probable number of compounds present in them. Consequently,

the present study was focused on the quantitative estimation of the flavonoid quercetin by high performance thin-layer chromatography (HPTLC) in the herbal species Dendrobium.

Quantitative estimation of these compounds is important for current research and a variety of methods are required for this. TLC and HPTLC are the methods primarily used for separation, qualitative identification and semi-quantitative visual analysis of the samples [1]. 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].

The genus Dendrobium is one of the largest groups of family Orchidaceae [4]. Different species of these orchids are found in Northeast India, China and Japan and are recorded in the Chinese Pharmacopoeia. These species are not only valued for their ornamental beauty but also have important therapeutic properties [4, 5]. Various Dendrobium species have been reported to possess secondary metabolites such as phenols, alkaloids, coumarins, terpenes, flavonoids as the major therapeutic agents [6]. The previous studies on Dendrobium have shown them to be rich in certain biochemicals as carbohydrates, flavonoids, alkaloids, glycosides and other phytochemical contents which have great importance in medicinal field. The orchids were first put to medicinal use by the Chinese as herbal medicine [7]. A large number of Dendrobium have been empirically used for treatment of different diseases, thus, several studies have been undertaken to provide scientific proof to justify the medicinal use of various plants in the treatment of diseases [8].

Methanol flower extract of Dendroium was subjected to thin layer chromatography and high performance thin-layer chromatography, to find out the probable number of compounds present in them. Consequently, the present study was focused on the quantitative estimation of the flavonoid quercetin by high performance thin-layer chromatography (HPTLC) in the purple flower of Dendrobium species.

MATERIALS AND METHODS

Plant materials: The plant parts (flowers) of Dendrobium (Purple) were collected and the authenticity of the plant was confirmed by the Botany Dept. of M.D. College, Parel, Mumbai, India.

Preparation of the extract: The plants were cleaned, shade dried and powdered for the phytochemical study. The parts used were flower petals. The solvents used were methanol, ethanol and water. A dried and crushed flower of Dendrobium (Purple - 1 Kg) was extracted with methanol for 12 h and with water for 8 h. This water extract is transferred into a separatory funnel and treated with chloroform then organic phase is removed using rotary evaporator. Some amount of water extract refrigerated as such. After evaporation of solvent in vacuum, dark brown (Purple Dendrobium) coloured gummy mass (40 g) was obtained. The solubility of residue was checked in different solvent. The residue was analysed with TLC using different solvent system to get an idea about the number of components present. Based on the results obtained in the qualitative phytochemical analysis, the methanol extract of the flowers were taken for chromatographic analysis.

Preparation of the Standard: 1mg mL^{-1} of the standard quercetin was prepared with methanol. From this 50µL was diluted with 950µL of methanol and hence the concentration of the standard was 50 µg m L^{-1}.

Preparation of the Sample: 5.0 mg of the sample was diluted with 1 mL of methanol. Thus, the concentration of the sample was 5.0 mg m L^{-1}.

TLC study: Thin layer chromatography was conducted to study the number of compounds present in the extract. The adsorbent used for thin layer chromatography was silica gel 60 F 254. The pre-coated TLC plate (Merck, Germany) was heated in an oven for activation. 1ml of the standard flavonoid quercetin and

the methanol stem extract were applied dried and then kept in the developing tank. The chamber was saturated with the solvents for 20 min at room temperature. Several solvent systems were tried to identify a suitable developing solvent system for the separation of compounds. After the development of the plate, it was air-dried then the numbers of spots were noted and Rf values were calculated.

HPTLC Analysis Instrument: CAMAG Linomat5" Linomat 5-080222'S/ N080222(1.00.13) TLC Sampler with win CATS software. Stationary phase: TLC plates silica gel 60 F 254 pre coated layer (10.0 cm X 10.0 cm), thickness 0.2 mm., No. of tracks: 3 and 4, band length: 8.0 mm. Mobile phase: Toluene: Chloroform: Ethyl alcohol (4:4:1) for Pink flower . Standard: Brown powder Sample : Brown powder Solubility: Methanol Standard concentration: 50µg m L^{-1} Standard Injection volumes (µL): 1, 2, 4,6,8,10,12,14,16,18 Sample concentration: flower – 5.0 mg m L^{-1} Sample application volumes (µL): 5, 10, 15, 20,(Violet), 5, 7 and 10 (Pink) Development chamber: Twin trough chamber (20 X 10) Development mode: Ascending mode Distance run: 75 mm Scanning wavelength: 386 nm Lamp: D2 Slit dimensions 4.00 x 0.30 mm, Micro Measurement mode: absorbance.

Preparation of the plates: The plates used for HPTLC was silica gel 60 F 254 (E.MERCK KGaA).50 µg m L^{-1} of the standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 µL. The sample concentration was 5.0 mg m L^{-1} and the different volumes were 5, 10, 15, 20, (Violet) and 5, 7 and 10 (Pink) µL. The mobile phase used were toluene: Chloroform: ethyl alcohol (4:4:1) for Pink flower and n hexane: acetone (3:2) for violet flower..The chromatograph was developed for 15 min, dried at room temperature and scanned between 190 to 400 nm. Average peak area of the standard was 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.

Estimation of quercetin in methanol flower extract of Dendrobium: The mean peak height / area of the sample were calculated and the content of quercetin was quantified using the regression equation obtained from the standard curve.

Limits of Detection and Limit of Quantification: The limit of detection (LOD) was the lowest amount of the analyte in the sample which can be detected. The limit of quantification was the lowest amount of the analyte in the sample which can be quantitatively determined. The signal-to-noise ratios were 3:1 and 10:1 respectively.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of methanol extract of the flower of Dendrobium (Purple) revealed the presence of alkaloids, amino acids, phenols, sterols, terpenoids, carbohydrates, flavonoids, and tannins. The TLC procedure was optimized with a view to separate the compounds and to identify one of the phytochemical flavonoid in the extract. Initially toluene: ethyl acetate: chloroform: ethylalcohol: n hexane: acetone in varying ratios was tried along with several combinations of other solvents. The developing system consists of toluene: Chloroform: ethylalcohol (4:4:1 v/v/v/v) and n hexane: acetone (3:2 v/v) gave a sharp and well-defined band with Rf = 0.71 for quercetin (Table-1-3).

Table 1. Preliminary phytochemical screening of different extracts of Dendrobium flowers (Purple)

Phytoconstituents	M	EA
Flavonoids	+	+
Phenols &Tannins	+	+
Saponins	+	+
Alkaloids	+	+

M, Methanol Extract, EA,Ethanol extract, +, Present, -, Absent

Table 2. HPTLC results of methanol Flowers extract of Dendrobium (Purple) and the standard quercetin

Track 1	Peak	Rf	Height	Area	Assigned substance
1	1	0.71	42.0	829.8	Quercetin
2	1	0.70	64.7	1253.8	Quercetin
3	1	0.73	60.5	1172.3	Quercetin

Table 3. Data pertaining to HPTLC fingerprint of different flowers (Purple) extracts of Dendrobium at 300 nm.

Dendrobium flowers	Extract	Solvent System	No. of Peaks	Rf values	% Area
Pink	Methanol extract		04	0.47,0.53,0.61,0.73	4.72,13.14,35.43,46.70

This showed the presence of the bioactive compound flavonoid. The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution [9]. HPTLC analysis of the methanol flower extract of Dendrobium (Purple) was carried out along with the standard flavonoid quercetin and toluene: chloroform: ethyl alcohol: (4:4:1 v/v) and n hexane: acetone (3:2 v/v) as the mobile phase. The number of bands obtained was seven to eight (Pink). The identity of the bands of quercetin in the methanol extract was confirmed by comparing the UV-Vis absorption spectra (Fig. 3-5) with those of standards using a CAMAG TLC scanner 3 (Fig.1 A,B,C and 2). The standard quercetin has Rf value of 0.70 (Table-2). A good linear relationship (r2 = 0.99826 and 0.99926 with respective to height and peak area, respectively) was observed between the concentration ranges of 100-600 ng $spot^{-1}$ (Fig.3, 4). The use of standard ensures the concentration and ratio of the test compound in the flower. This result coincides with the study of Sachin U. Rakesh et al., (2009) who finds a good correlation (r = 0.9998) between the standard and the sample of quercetin in the dried flowers of Nymphaea stellata. The limit of detection and limit of quantification was found to be 100 ng and 300 ng respectively. The regression equation was found to be Y= 29.69+0.8307*X with respective to height and Y=290.5+19.31*X with respect to area, where Y is the peak height / area and X is concentration of quercetin. With the help of above statistical data, the content of quercetin was determined in the methanol flower extract of Dendrobium which was found to be 198.2 mg $100g^{-1}$ (Table-3).The peak purity of quercetin was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot. HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant [10]. HPTLC technique could be considered as an accurate and precise method for the determination of flavonoid in Clerodendrum vicosum vent root samples [11].

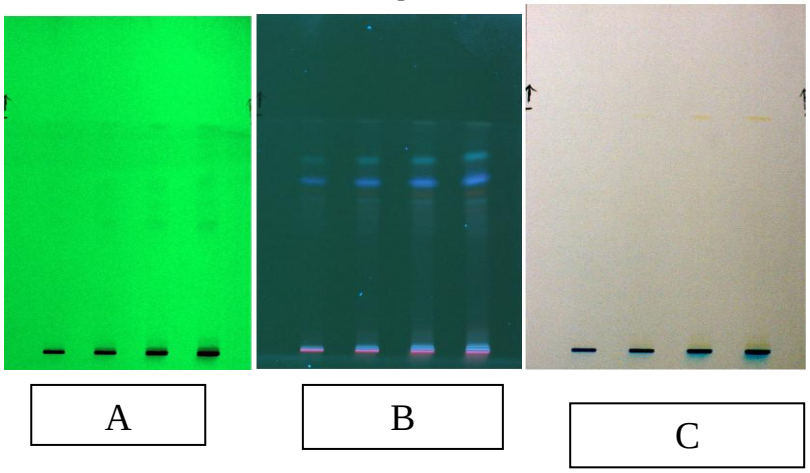

Fig. (1 A,B,C) HPTLC profile of flowers extract of Dendrobium (Purple)

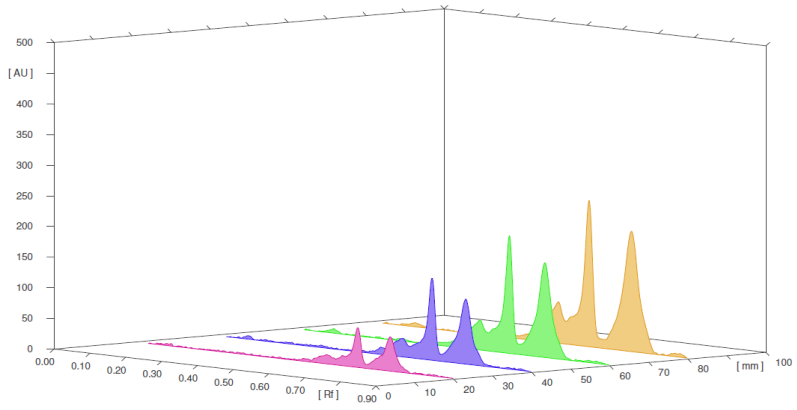

Fig 2. Three dimensional representation of HPTLC chromatogram of Dendrobium flowers (Purple) methanol extract measured at 300 nm.

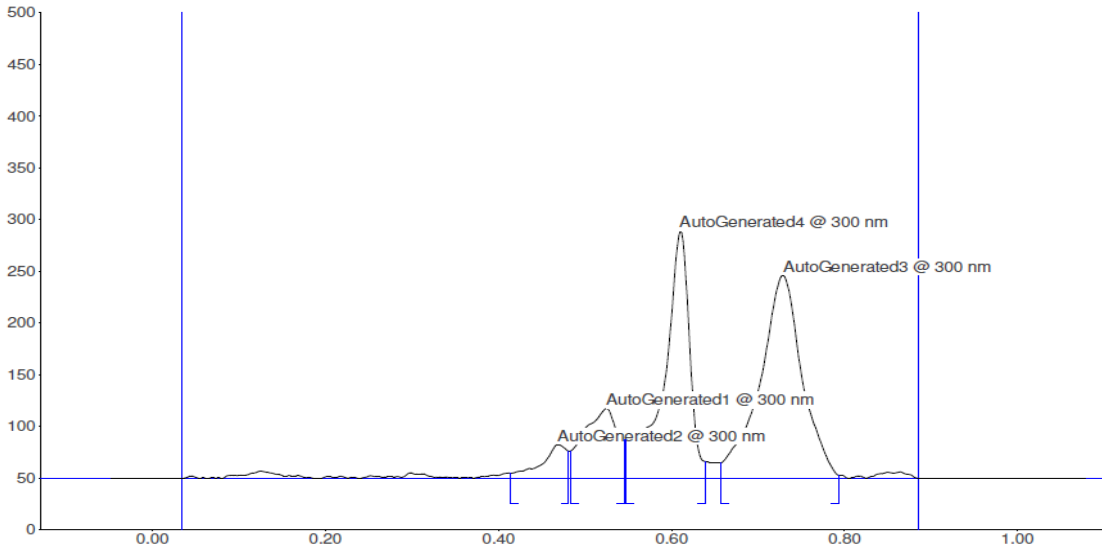

Fig 3. Chromatogram of methanol extract of Dendrobium flowers (Purple) at 300 nm.

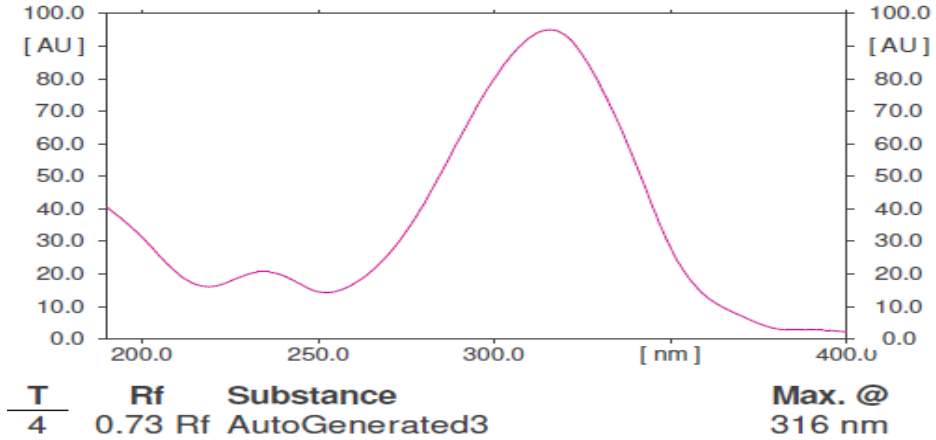

Fig 4. UV absorption spectrum of Dendrobium Pink flowers at 316 nm

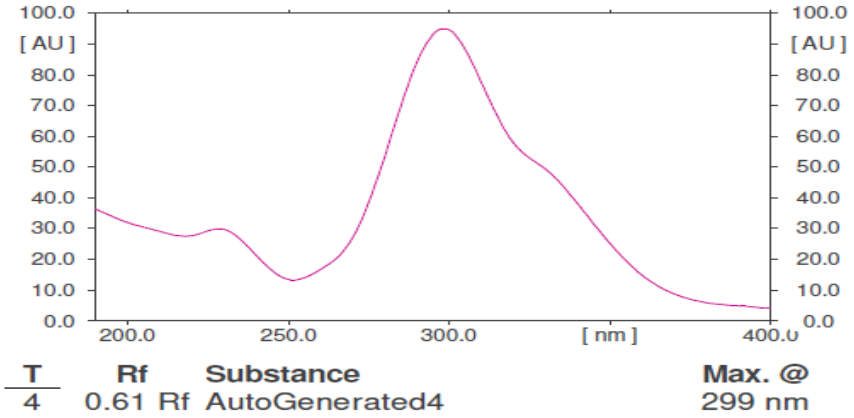

Fig 5. UV absorption spectrum of Dendrobium (Purple) flowers at 299 nm

APPLICATIONS

The chromatographic studies useful that the methanol flower extract of Dendrobium contain an appreciable amount of flavonoid quercetin, which confirms its medicinal value.

CONCLUSIONS

The phytochemical studies showed the presence of most of the biologically active compounds in the plant. It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The chromatographic studies conducted with the methanol flower extract of Dendrobium revealed an appreciable amount of flavonoid quercetin, which confirms its medicinal value.

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