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Isolation and Quantification of Quercetin from Roots of *Spermadicyton suaveolens* by High-Performance Thin-Layer Chromatography

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ABSTRACT

Roots of *Spermadicyton suaveolens* (SS) are used by local healers for the management of major as well as minor ailments. roots of *Spermadicyton suaveolens* are used for the management of diabetes life-threatening diseases. Isolation of phytoconstituents is the key factor in herbal treatment. Quercetins believed to stem from a multitude of plant extracts have proven to have ethnopharmacological significance. In the present study, we have to isolate, identify & quantify the quarcetinby high-performance thin-layer chromatography (HPTLC) in roots of *Spermadicyton suaveolens*. Following extraction, the extracts were quantified for the content of quercetin using HPTLC. The HPTLC analysis showed the presence of flavonoid Quercetin confirmed & quantified by comparison with a standard. The Rf value for the standard is 0.66 whereas for the root sample 0.65. During chromatographic investigations, extracts revealed peaks that corresponded to standard peaks.

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INTRODUCTION

Most of the present study is focused on the phytochemical analysis of plant species with ethnopharmacological value [1]. Phytochemical methods are crucial for evaluating bioactive constituents and identifying phytotoxins and phytoalexins [2]. The chemical structural assessment of various plant groupings, with a particular emphasis on secondary metabolites, is the emerging trend [3,4].

Phytochemical flavonoids are water-soluble compounds found in vascular plants bonded to sugar glycosides and attached as glycones occurring as a glycosidic combination. A two-dimensional chromatographic analysis of a direct alcoholic extract and onedimensional solubility profiles are used to determine flavonoids in plant tissue. Though flavonoids can be separated by different chromatographic and spectroscopical methods by using identified biomarkers [5-8].

Many flavonoids have the potential to scavenge free radicals, which is one of its most notable and therapeutically helpful qualities [9]. Besides free radical scavenging potential flavonoids are reported biological potential vasodilatory, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, antiallergic, antiviral and estrogenic effects [10]. They have been widely employed as chemotaxonomic markers[11].

Quercetin is a 3-ringed molecule with hydroxyl (–OH) groups attached, which is the same fundamental chemical structure as all other flavonoids. The high quercetin concentration of many therapeutic plants is responsible for their action. Several investigations have proven quercetin's substantial anti-inflammatory action as a result of direct suppression of inflammatory processes [12].

The *Spermadictyon suaveolens (SS)* commonly called *Forest Champa, Van-Champa, Gidesa, Jitsaya,* still waiting for diversified therapeutic application. This plant's stem powder is used by ayurvedic practitioners or vaidyas to treat viral ailments like herpes and diabetes [13].

Even though a variety of analytical methods for the isolation and characterization of plant constituents by high-performance thin-layer chromatography (HPTLC) and high liquid chromatography (HPLC) are available and reported in the literature, there is no systematic approach designed toward chromatographic quantification studies for selected plant extracts. As a result, we have strived to explore Phytoconstituents from *Spermadictyon suaveolens* in current research investigations.

MATERIALS AND METHODS

Plant Material

The root of SS was collected in the hilly vicinity of Panhala fort, Dist. Kolhapur, Maharashtra, in November. The roots were chopped into small pieces and dried before being used as raw material. To acquire powder, the roots were pulverized on a Rising Automatic DP Pulverizer. The ensuing powder was then surpassed through a 40 # sieve and stored in an airtight container.

Soxhlet extraction

Extraction was done using 50 g powdered root material *via* soxhlet apparatus by using ethanol solvent. After completion of extraction process, filtering and concentration of the residue to near about 50 mL on a water bath. The extract was then transferred to a previously weighed evaporating dish. The total weight of evaporating dish containing extract was measured and the extract was further placed on a water bath for evaporation till it becomes viscous. The difference in weight was calculated every 10 minutes until a constant weight was obtained.

Isolation, identification, and quantification of quercetin was carried out by HPTLC.

HPTLC Chromatographic conditions

Stationary Phase: Precoated silica gel plates Merck 60 F_{254}(10 x10,0.2mmthickness)

Mobile Phase: Toluene: ethyl acetate: ethanol: glacial acetic acid, 6:3:2:1(v/v/v/v)

Spotting device: Linomat V Automatic sample spotter, CAMAG (Switzerland).

Development Mode: CAMAG twin trough chamber, CAMAG Densitometer: TLC Scanner III, CATS software, CAMAG.

Preparation of standard solution

The stock solution of quercetin (20 g/ml) was made by properly weighing 2 mg of quercetin and dissolving it in 50 ml methanol in a 100 ml volumetric flask. It was then sonicated for 10 minutes, and the final volume of the solutions was built up to 100 ml with methanol to provide a quercetin solution with a suitable range.

Instrumentation and chromatographic conditions

The HPTLC was carried out on 20 cm10 cm aluminum packed plates covered with silica gel 60 F254 (Merck, Mumbai, India). Using a Camag (Muttenz, Switzerland) Linomat V sample applicator fitted with a 100 L Hamilton (USA) syringe, standard solution of quercetin and sample solution were applied to the plates as bands 8.0 mm wide, 30.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate. At room temperature (2820c), ascending development to a distance of 80 mm was carried out in a Camag glass twin-trough chamber previously saturated with mobile phase vapor for 20 minutes using Toluene: ethyl acetate: ethanol: glacial acetic acid, 6:3:2:1 (v/v/v/v) as mobile phase. After growth, the plates were air-dried and treated with 5% ethanolic $FeCl_3$solution and subsequently heated at 120^0 C for derivatization. These plates were scanned and visualized undervisiblelightat525nmandUVlightat254nmand366nmabsorbance/reflectionmodeusingreflectionmodebyCAMAG Scanner III and CATS.

HPTLC Quantification of the extracts

The quercetin content of different extracts was evaluated by comparing the area of the chromatogram with the concentration calibration curve. The Rf value of normal quercetin (0.65), as well as the Rf value of the extracts, were compared. The plate was quantified at 254 nm in the remission/absorption mode, with the following parameters: slit width 6.00x0.30mm, micro scanning speed 20mm/s, and data resolution 100 m step. The calibration settings were as follows: calibration mode- single level, statistics mode-cv, and evolution mode- peak height. The average quercetin concentration in separate extracts was represented as a percentage.

RESULT AND DISCUSSION

The HPTLC technique was refined to quantify the extract of the samples. Initially, different ratios of toluene, ethyl acetate, ethanol, and glacial acetic acid were explored. Rf = 0.65 was obtained using the mobile phase toluene: ethyl acetate: ethanol: glacial acetic acid, 6:3:2:1 (v/v/v/v). When the chamber was saturated with mobile phase for 20 minutes at room temperature, well-defined spots were produced. The TLC plates were observed under UV light at 254 nm. Figures 1 & 2shows an image of a TLC plate following chromatography of a quercetin standard and a methanolic extract of *Spermadictyon suaveolens (SS)*samples.

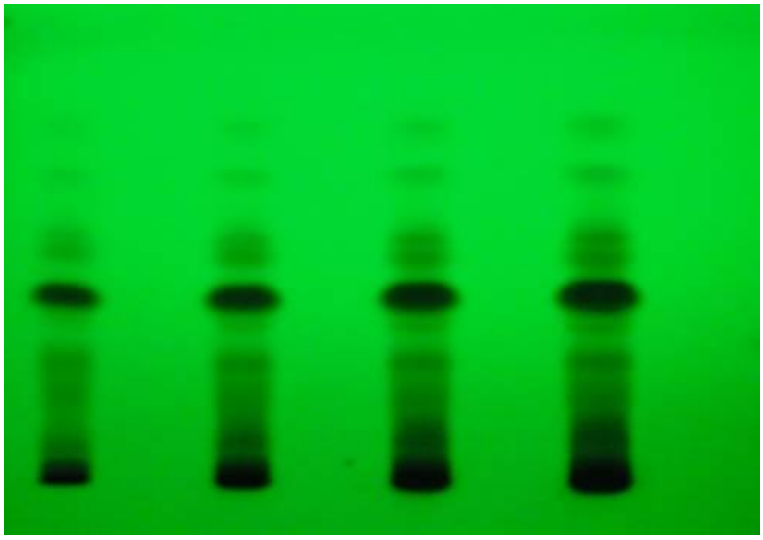

Figure No 1: High-performance thin-layer chromatography chromatograms of *Spermadictyon suaveolens* root and standard for Quercetin under UV at 365 nm

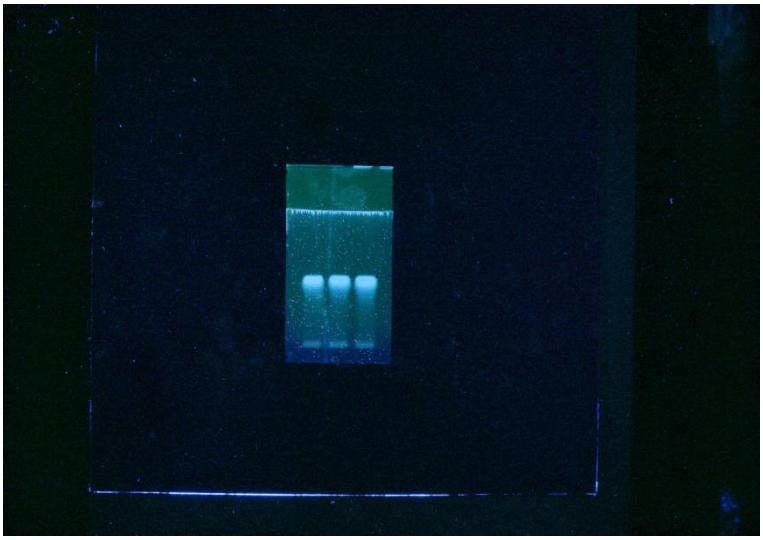

Figure No 2: High-performance thin-layer chromatography chromatograms of *Spermadictyon suaveolens* root and standard for Quercetin under UV at 254 nm

The identification of the quercetin bands in the sample chromatogram was validated by comparing it to the chromatogram obtained from the reference standard solution. The Rf values obtained for the plant extracts closely matched the Rf values obtained for normal quercetin, making it a relevant fingerprint parameter. The chromatogram of standard quercetin is shown in Figure A, and the chromatograms of quercetin detected in *Spermadictyon suaveolens*root samples are presented in Table 1 displays the Rf values obtained for each sample. The peak from the sample solution corresponding to quercetin (0.66, 0.66) exhibited nearly the same retention factor as normal quercetin (0.65).

Table No. 1: Quercetin HPTLC Chromatographic analysis

Sample	Rf	Max. Height	Area
Spermadictyon suaveolens root	0.65	72.4	1829.2
Standard	0.66	702.5	35971.5

The presence of large amounts of components in the extracts makes it challenging to isolate and characterize plant compounds. Although 200–300 flavonols aglycone is known, only three generally recognized flavonols, kaempferol, quercetin, and myricetin, are found in substantial amounts in plant extracts [14].

The result indicated that the method used in this work resulted in good peak shape and enabled good resolution of quercetin from *Spermadictyon suaveolens* samples. The isolation and identification of quercetin by HPTLC, HPLC, and TLC studies have also been reviewed by many authors.

CONCLUSION

Based on the findings of this investigation, it was concluded that quercetin was effectively extracted from *Spermadictyon suaveolens*. A speedy, simple, accurate, and specific HPTLC technique for quantifying quercetin in *Spermadictyon suaveolens* root has been devised. This technique offers great ability to be widely used in direct routine analysis and quality assurance of related extracts and medicines.

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