

**HPTLC ESTIMATION OF BIOACTIVE COMPOUND PRESENT IN *SMILAX MACROPHYLLA*****Adarsh Kesarwani<sup>\*1</sup>, B.K. Dubey<sup>1</sup>, Deepak Kumar Basedia<sup>1</sup>, Sunil Kumar Shah<sup>2</sup>**<sup>1</sup>Technocrats Institute of Technology-Pharmacy, Bhopal (M.P.)<sup>2</sup>TIT-College of Pharmacy, Bhopal (M.P.)\*Corresponding Author's E mail: [adarshkesarwani1998@gmail.com](mailto:adarshkesarwani1998@gmail.com)

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**ABSTRACT**

*Smilax macrophylla* is a medicinal plant traditionally used for the treatment of various ailments due to its rich phytochemical composition. The present study aimed to evaluate the phytochemical profile and to estimate a bioactive flavonoid marker, quercetin, in the hydroalcoholic extract of *Smilax macrophylla* using High-Performance Thin-Layer Chromatography (HPTLC). The hydroalcoholic extract was prepared and subjected to preliminary phytochemical screening, which revealed the presence of glycosides, flavonoids, phenolic compounds, saponins, diterpenes, and tannins. Thin-layer chromatographic analysis was carried out using toluene:ethyl acetate:formic acid (5:4:1 v/v/v) as the mobile phase, and the presence of quercetin in the extract was confirmed by comparing R<sub>f</sub> values with the standard. HPTLC analysis was performed using silica gel 60 F<sub>254</sub> plates with densitometric scanning at 425 nm. Quantitative estimation showed that the total flavonoid and total phenolic contents of the extract were 0.82 mg/100 mg and 0.37 mg/100 mg of dried extract, respectively. The percentage content of quercetin in the hydroalcoholic extract was found to be 0.75%. The results demonstrate that HPTLC is a simple, precise, and reliable method for the identification and quantification of bioactive compounds in *Smilax macrophylla*, supporting its standardization and quality control.

**Keywords:** *Smilax macrophylla*, HPTLC, Quercetin; Flavonoids, Phytochemical screening, Herbal standardization.

**INTRODUCTION**

*Smilax macrophylla* is an important medicinal plant belonging to the family Smilacaceae and is extensively used in traditional systems of medicine for the treatment of inflammatory disorders, microbial infections, diabetes, and oxidative stress-related conditions<sup>1</sup>. The therapeutic efficacy of *Smilax macrophylla* is mainly attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, saponins, tannins, and glycosides<sup>2</sup>. Among these, flavonoids particularly quercetin and its derivatives are considered significant marker compounds due to their potent antioxidant, anti-inflammatory, and antimicrobial properties<sup>2,3</sup>.

Standardization and quality assessment of herbal drugs are essential to ensure their quality, safety, and therapeutic consistency. High-Performance Thin-Layer Chromatography (HPTLC) is a widely accepted analytical technique for the qualitative and quantitative estimation of bioactive compounds in plant extracts<sup>4</sup>. HPTLC offers several advantages, including high sensitivity, accuracy, minimal sample preparation, low solvent consumption, and the ability to analyze multiple samples simultaneously<sup>5</sup>.

Therefore, HPTLC estimation of bioactive compounds in *Smilax macrophylla* provides a reliable and reproducible method for the identification, quantification, and standardization of its active constituents. This approach supports scientific validation of traditional claims and ensures batch-to-batch consistency of herbal formulations<sup>4,5</sup>.

## **Material and Methods**

### **Material**

The materials used for the investigation included dried plant material of *Smilax macrophylla*, which was used for the preparation of the hydroalcoholic extract. Quercetin was employed as the reference standard for chromatographic analysis. Methanol was used as the solvent for the preparation of standard and sample solutions. Precoated silica gel 60 F<sub>254</sub> TLC plates were used as the stationary phase, while toluene, ethyl acetate, and formic acid were used for the preparation of the mobile phase. All chemicals and solvents used were of analytical grade, and distilled water was used wherever required during the study.

### **Methods**

#### **Collection of plant material**

The plants have been selected on the basis of its availability and folk use of the plant. Leaves of *Smilax macrophylla* were collected from local area of Bhopal in the month of March, 2025. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Smilax macrophylla* were preserved in plastic bags, closed tightly and powdered as per the requirements.

#### **Extraction by Maceration**

50 gram shade dried leaves were coarsely powdered and subjected to extraction with petroleum ether by maceration process. The extraction was continued till the defatting of the material had taken place. Defatted powdered of *Smilax macrophylla* has been extracted with hydroalcoholic solvent (ethanol: water; 80:20v/v) solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C<sup>6</sup>. Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes: (1) promotes diffusion, (2) separates concentrated solution from the sample surface by adding new solvent to the menstruum for increasing the extraction yield.

### Determination of percentage yield

For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula, the percentage yield of extract was calculated:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

### Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods<sup>7</sup>.

### Separation and Identification of phytoconstituents by TLC

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors<sup>8</sup>. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) solvent system used. After pre-saturation with mobile phase for 20 min for development were used. The movement of the active compound was expressed by its retention factor (R<sub>f</sub>), values were calculated for different samples. The developed thin layer chromatographic plates were visualized in normal light, short UV light (254nm), and long UV light (365nm) using TLC cabinet (Electronic India). Once the chromatogram was developed the R<sub>f</sub> Value of spot was calculated using formula:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

### Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method<sup>9</sup>. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% AlCl<sub>3</sub> solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

### **Estimation of total phenolic content**

The total phenolic content of the extract was determined by the modified folin-ciocalteu method<sup>10</sup>. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

### **Estimation of Quercetin using HPTLC method**

A CAMAG HPTLC system (Switzerland) comprising CAMAG Linomat 5 applicator, CAMAG TLC scanner 3, CAMAG Wincats software, version 1.44, Hamilton syringe (100µl), CAMAG Reprostar 3, CAMAG TLC plate heater, CAMAG UV Cabinet were used for the study. The MFP-PARC, HPTLC facility used for present study<sup>11-13</sup>.

### **Preparation of the Standard**

1mg/ml of the standard, Quercetin was prepared with methanol. From this 100µl was diluted with 950µl of methanol and hence the concentration of the standard was 100 µg/ml.

### **Preparation of the extract Sample**

1mg/ml of the all extract was prepared with methanol separately. From this 100µl was diluted with 950µl of methanol and hence the concentration of the extract was 100 µg/ml.

### **HPTLC Analysis**

HPTLC analysis was performed using a CAMAG Automatic TLC Sampler equipped with win-CATS software. Chromatographic separation was carried out on precoated silica gel 60 F<sub>254</sub> TLC plates (20 × 10 cm) with a layer thickness of 0.2 mm. A total of eight tracks were applied on each plate with a band length of 6 mm. The mobile phase consisted of toluene, ethyl acetate, and formic acid in the ratio of 5:4:1 (v/v/v). The standard compound was obtained as a light-yellow powder, while the sample extract appeared as a brown powder; both were soluble in methanol. The standard solution was prepared at a concentration of 100 µg/ml and applied in volumes of 1, 2, 3, 4, and 5 µl, whereas the sample solution was prepared at a concentration of 1 mg/ml and applied in a volume of 2 µl per spot. Chromatographic development was carried out in a twin-trough chamber (20 × 10 cm) using ascending mode, with a migration distance of 75 mm. Densitometric scanning of the developed plates was performed at 425 nm for quantitative analysis.

### Preparation of the plates

The plates used for HPTLC was silica gel 60 F 254 (E.MERCK KGaA). 100 µg/ml of the Standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 1, 2, 3, 4, 5 and 6µl.

The concentration of the sample was 1.0 mg/ml, and the different amounts were 2µl. The mobile step used was Toluene: ethyl acetate: formic acid (5:4:1v/v/v). Built the chromatograph For 15 minutes, dried at room temperature and scanned at 425nm. The normal maximum peak area was measured. 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 herbal extracts

Estimation of Quercetin in extract of *Smilax macrophylla* the mean peak area of the sample was calculated and the content of Quercetin was quantified using the regression equation obtained from the standard curve.

### Results and Discussion

The percentage yield of the hydroalcoholic extract of *Smilax macrophylla* was found to be 8.25%, with a characteristic black color, indicating efficient extraction of polar and semi-polar phytoconstituents (Table 1). Preliminary phytochemical screening revealed the presence of glycosides, flavonoids, phenolic compounds, saponins, diterpenes, and tannins, while alkaloids and proteins were absent (Table 2). The presence of flavonoids and phenolic compounds supports the traditional medicinal importance of *Smilax macrophylla*.

TLC analysis of the hydroalcoholic extract using toluene:ethyl acetate:formic acid (5:4:1 v/v/v) as the mobile phase showed a well-defined spot for standard quercetin with an R<sub>f</sub> value of 0.52 under normal light, short UV, and long UV conditions (Table 3). The extract exhibited multiple spots, among which one corresponded with the R<sub>f</sub> value of quercetin (R<sub>f</sub> 0.52), confirming the presence of quercetin in the extract. Additional spots observed in the extract indicate the presence of other flavonoid constituents.

The total flavonoid content and total phenolic content of the hydroalcoholic extract were found to be 0.82 mg/100 mg and 0.37 mg/100 mg of dried extract, respectively, indicating a higher concentration of flavonoids compared to phenolic compounds (Table 4). The HPTLC chromatogram of standard quercetin showed a sharp and well-resolved peak (Figure 1), while the chromatogram of the hydroalcoholic extract demonstrated a corresponding peak at the same R<sub>f</sub> value, confirming the identity of quercetin in the extract (Figure 2).

Quantitative HPTLC analysis revealed that the percentage content of quercetin in the hydroalcoholic extract of *Smilax macrophylla* was 0.75% (Table 5). The results demonstrate good resolution, reproducibility, and reliability of the developed HPTLC method for the estimation of quercetin.

**Table 1: Percentage yield of *Smilax macrophylla* extract**

| S. No. | Extract        | Colour | Percentage yield |
|--------|----------------|--------|------------------|
| 1.     | Hydroalcoholic | Black  | 8.25%            |

**Table 2: Phytochemical screening of extract of *Smilax macrophylla***

| S. No. | Constituents         | Hydroalcoholic extract |
|--------|----------------------|------------------------|
| 1.     | <b>Alkaloids</b>     |                        |
|        | Mayer's Test         | -ve                    |
|        | Wagner's Test        | -ve                    |
|        | Dragendorff's Test   | -ve                    |
| 2.     | <b>Glycosides</b>    |                        |
|        | Legal's Test         | +ve                    |
| 3.     | <b>Flavonoids</b>    |                        |
|        | Lead acetate test    | +ve                    |
|        | Alkaline test        | +ve                    |
| 4.     | <b>Phenol</b>        |                        |
|        | Ferric chloride test | +ve                    |
| 5.     | <b>Proteins</b>      |                        |
|        | Xanthoproteic test   | -ve                    |
| 6.     | <b>Carbohydrates</b> |                        |
|        | Molisch's Test       | -ve                    |
|        | Benedict's Test      | -ve                    |
|        | Fehling's Test       | +ve                    |
| 7.     | <b>Saponins</b>      |                        |
|        | Froth Test           | +ve                    |
| 8.     | <b>Diterpenes</b>    |                        |
|        | Copper acetate test  | +ve                    |
| 9.     | <b>Tannins</b>       |                        |
|        | Gelatin Test         | +ve                    |

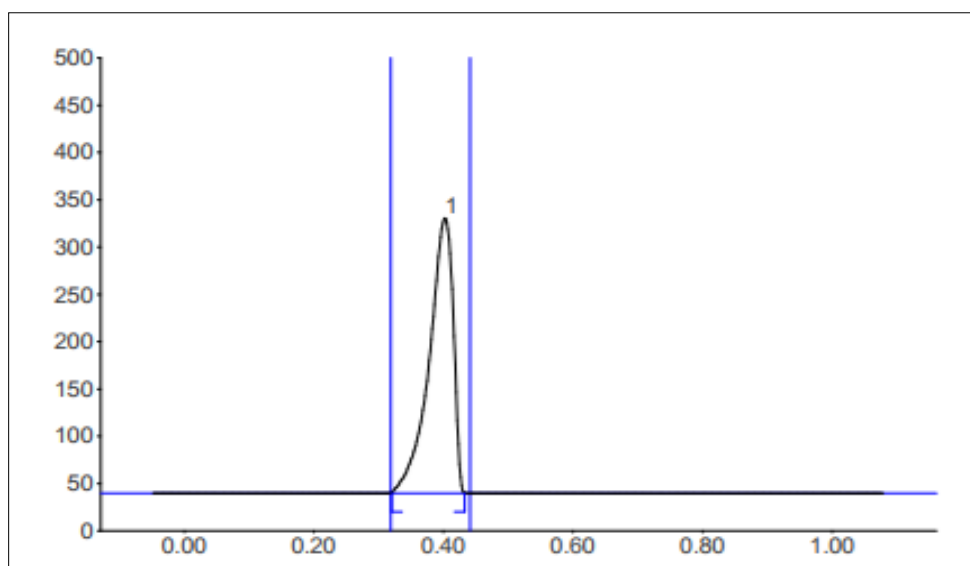
[+ve= positive; -ve= negative]

**Table 3: TLC of hydroalcoholic extract of *Smilax macrophylla* (Flavanoids)**

| S. No.                                                    | Extract                              | Rf Value         |
|-----------------------------------------------------------|--------------------------------------|------------------|
| Mobile phase (Toluene: Ethyl acetate: Formic acid; 5:4:1) |                                      |                  |
| 1.                                                        | Quercetin                            |                  |
|                                                           | Normal light =1 spot                 | 0.52             |
|                                                           | Short UV=1 spot                      | 0.52             |
|                                                           | Long UV=1 spot                       | 0.52             |
| 2.                                                        | Extract of <i>Smilax macrophylla</i> |                  |
|                                                           | Normal light =2 spot                 | 0.52, 0.70       |
|                                                           | Short UV=2 spot                      | 0.52, 0.70       |
|                                                           | Long UV=3 spot                       | 0.52, 0.70, 0.72 |

**Table 4: Estimation of total flavonoids and phenol content of extract of *Smilax macrophylla***

| S. No. | Extract        | Total flavonoids content<br>(mg/ 100 mg of dried extract) | Total phenol content<br>(mg/ 100 mg of dried extract) |
|--------|----------------|-----------------------------------------------------------|-------------------------------------------------------|
| 1.     | Hydroalcoholic | 0.82                                                      | 0.37                                                  |

**Figure 1: Chromatogram of standard Quercetin**

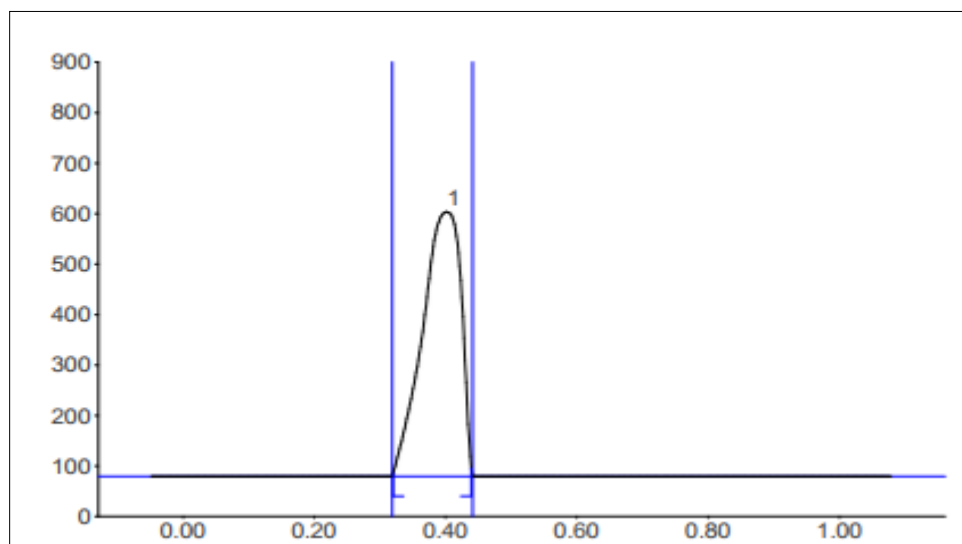


**Figure 2:** HPTLC chromatogram of hydroalcoholic extract of *Smilax macrophylla*

**Table 5:** Results of percentage content of Quercetin in extract

| S. No. | Hydroalcoholic extract    | % Found |
|--------|---------------------------|---------|
| 1      | <i>Smilax macrophylla</i> | 0.75%   |

## CONCLUSION

The present investigation successfully demonstrated the phytochemical evaluation and HPTLC estimation of a bioactive flavonoid marker in the hydroalcoholic extract of *Smilax macrophylla*. Preliminary phytochemical screening confirmed the presence of important secondary metabolites such as flavonoids, phenolic compounds, glycosides, saponins, diterpenes, and tannins, supporting the medicinal potential of the plant. TLC and HPTLC analyses confirmed the presence of quercetin in the extract by matching  $R_f$  values and chromatographic profiles with the standard. Quantitative HPTLC results revealed that the hydroalcoholic extract contained appreciable amounts of total flavonoids and phenols, with quercetin present at a concentration of 0.75%. The developed HPTLC method was found to be simple, precise, and reliable, making it suitable for routine quality control and standardization of *Smilax macrophylla* and its herbal formulations. These findings provide scientific validation for the traditional use of the plant and support its further pharmaceutical and nutraceutical applications.

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