ESTIMATION OF BIOACTIVE COMPOUNDS IN *PAEONIA OFFICINALIS* BY USING TLC & HPLC

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Abstract

Phytochemicals are chemical compounds found in some plants that have medical significance and have a specific physiological effect on humans. In order to obtain pure compounds, it is common practice to use a variety of separation techniques, including Thin-Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC). The TLC analysis of *Paeonia officinalis* was performed for estimation of phenol & flavonoid. In case of flavonoid quercetin was used as standard. The Rf value for quercetin was found to be 0.60. The ethanolic extract possessed quercetin as flavonoid which is evident by Rf value. Further the aqueous extract can be supposed to have flavonoid other than quercetin. The results of TLC for phenol point towards fact that ethanolic extract contain gallic acid as phenol which is in accordance with the Rf value. Further, calibration curve of Quercetin was plotted by using HPLC. The sample was then passed in HPLC to analyze the flavonoids in sample. It was observed that for quercetin standard the Retention time was found to be 3.017. While in case of *Paeonia officinalis* extract retention time was noted to be 2.953, with the % assay of 0.27%. The study showed that the *Paeonia officinalis* are potential sources of phytochemicals that promote health and have strong biological activity that are therapeutically relevant.

Keywords: Phytochemical, *Paeonia officinalis*, Phenol, Flavonoid, TLC, HPLC

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DOI: 10.53555/ecb/2022.11.12.219

Introduction

Phytochemicals are chemical compounds found in some plants that have medical significance and have a specific physiological effect on humans. These phytochemicals have been utilized in herbal and homeopathic treatments to treat diseases since ancient times. These compounds, which are not nutrients, have a protective or disease-prevention function. The need for screening medicinal plants for bioactive chemicals as a foundation for additional biomedical research arises. Many of the medicinal plants' active ingredients have been identified and presented as useful drugs in contemporary medical systems thanks to advancements in phytochemical techniques. Alkaloids, flavonoids, tannins, and phenolic compounds are the most significant of these bioactive substances (Yadav and Agarwala, 2011; Arora *et al*., 2003)

The separation of plant extracts, which often consist of a mixture of different kinds of bioactive substances or phytochemicals with varying polarity, continues to be a significant obstacle to their identification and characterization. In order to obtain pure compounds, it is common practice to use a variety of separation techniques, including Thin-Layer Chromatography (TLC), column chromatography, flash chromatography, Sephardim chromatography, and High-Performance Liquid Chromatography (HPLC) (Ingle *et al*., 2017; Raaman, 2006).

In the study of natural products, TLC is the most widely used planar chromatographic technique. The analysis, isolation, and parameter-setting for column chromatography can all be done using this simple, affordable method. Typically, organic solvents (less polar) are utilized as the mobile phase and silica or alumina (more polar) are used as the stationary phase. Normal phase chromatography is the appropriate term for this circumstance. Reverse phase TLC is an alternative that uses a less polar (alkyl-bonded) silica or alumina as the stationary phase and a polar solvent (water, alcohol, etc.) as the mobile phase (Chakraborthy, 2010; Ganatra *et al*., 2013).

HPLC is a chromatographic technique that can separate a mixture of substances and is used in phytochemistry and analytical chemistry to identify, quantify, and purify the different components of the mixture. It is a flexible, reliable, and frequently used method for the isolation of natural products. As the primary option for fingerprinting research for the quality control of herbal plants, this analytical technique is currently rising in popularity among other analytical techniques. In order to completely describe a natural product's qualities, it is usually separated after a biological assay evaluates a relatively crude extract. For both analytical and preparative scale processing of such multi-component samples, HPLC's resolving power is the perfect tool (Khan *et al*., 2021; Russo *et al*., 2003).

Paeonia officinalis, also known as the European or Common peony, has long been grown in Europe. Since more than 2,000 years ago, the root has primarily been used medicinally to cure epilepsy and encourage menstruation. Convulsions and spasmodic nerve disorders like epilepsy have been successfully treated with root, which is also an antispasmodic, diuretic, sedative, and tonic. Additionally, it has been used to treat whooping cough, and the root can occasionally be converted into suppositories to treat hemorrhoids, varicose veins, and anal and intestinal spasms. It has been demonstrated through experimentation to have antihypertensive, abortifacient, and anti-ulcer properties. This plant's roots have significant medical value in both homeopathy and the unani system. The roots include protoanemonin, paeoniflorin, paeonin, paeonol, tannic acid, triterpenoids, volatile oil, asparagin, benzoic acid, flavonoids, and volatile oil. (Wenzel and Haskel, 1952; Ahmad & Tabassum, 2013). Thus, this study deals with estimation of bioactive compounds by using TLC & HPLC.

Materials & Methods

Collection of plant

The plant *Paeonia officinalis* was collected from local area of Bhopal.

Defatting & extraction

The plant material was powdered & subjected to defatting for 24 hours by petroleum ether further the extraction was carried out by maceration by using ethanol & water as solvent.

Thin layer chromatography

TLC plates were utilized as stationary phase. The mobile phase was created by mixing Toulene, ethyl acetate & formic acid in fixed proportion. The mobile phase was transferred into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase. The plant extract was applied on specific spot on TLC plate. Further the plate was placed in the TLC chamber. After the complete run, plate was removed from chamber, dried & Visualized in UV cabinet.

Quantitative study of marker compound (Quercetin) in *Paeonia officinalis* by HPLC

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min^{-1}. A small sample volume of 20 µL was used for sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm.

Preparation of standard solution

10mg of quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Analysis of extract

10 mg each extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min.

Results & Discussion

The TLC analysis of *Paeonia officinalis* was performed for estimation of phenol & flavonoid. In case of flavonoid quercetin was used as standard. The Rf value for quercetin was found to be 0.60. The ethanolic extract possessed quercetin as flavonoid which is evident by Rf value. Further the aqueous extract can be supposed to have flavonoid other than quercetin. The results of TLC for phenol point towards fact that ethanolic extract contain gallic acid as phenol which is in accordance with the Rf value. Further, calibration curve of Quercetin was plotted by using HPLC. The sample was then passed in HPLC to analyze the flavonoids in sample. It was observed that for quercetin standard the Retention time was found to be 3.017. While in case of *Paeonia officinalis* extract retention time was noted to be 2.953, with the % assay of 0.27%.

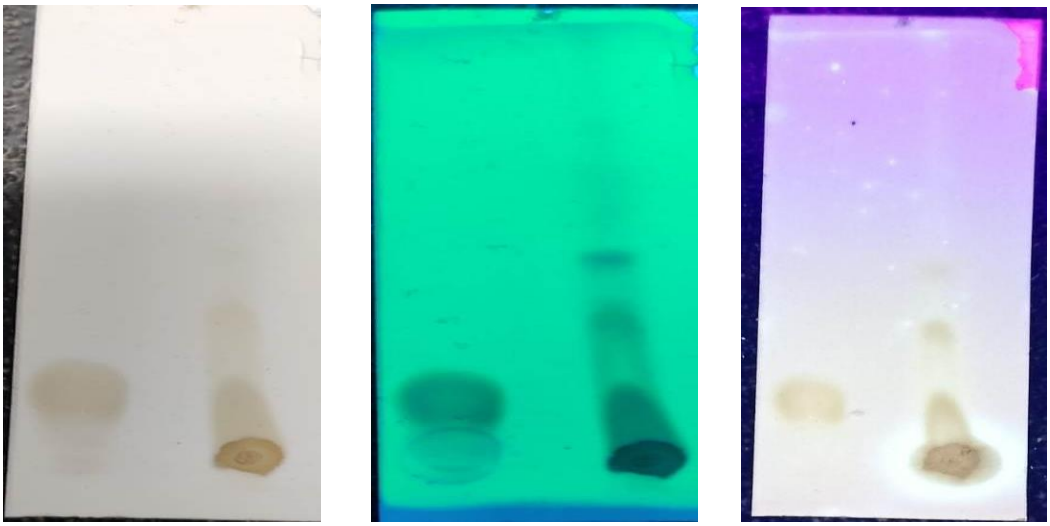
Table 1: TLC Analysis of *Paeonia officinalis* (Flavonoids)

S. No.	Mobile phase	*Rf* value
1.	**Quercetin (Standard)** Toluene: Ethyl acetate Formic acid (5:4:1)	0.60
2.	**(Ethanol extract)** Dis. travel by mobile phase= 6cm No. of spot at long UV = 4 No. of spot at short UV = 3 No. of spot at normal light= 1	Long- 0.55, 0.61, 0.83, 0.86 Short- 0.61, 0.73, 0.83 Normal- 0.61
2.	**(Aqueous extract)** Dis. travel by mobile phase= 6cm No. of spot at long UV = 1 No. of spot at short UV = 1 No. of spot at normal light= 0	Long-0.48 Short- 0.48 Normal- 0

Normal light **Short UV** **Long UV**

Table 2: TLC Analysis of *Paeonia officinalis* (Phenol)

S. No.	Mobile phase	*Rf* value
1.	**Gallic acid (Standard)** Toluene: Ethyl acetate Formic acid (7:5:1)	 0.45
2.	**(Ethanol extract)** Dis. travel by mobile phase= 6cm No. of spot at long UV = 2 No. of spot at short UV = 3 No. of spot at normal light= 2	 Long- 0.45, 0.52 Short – 0.45, 0.52, 0.58 Normal- 0.45, 0.52



Normal light **Short UV** **Long UV**

Table 3: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean AUC
1.	0	0
2.	5	665.580±6.32
3.	10	1345.458±8.15
4.	15	1984.324±5.85
5.	20	2675.985±7.32
6.	25	3345.478±9.85

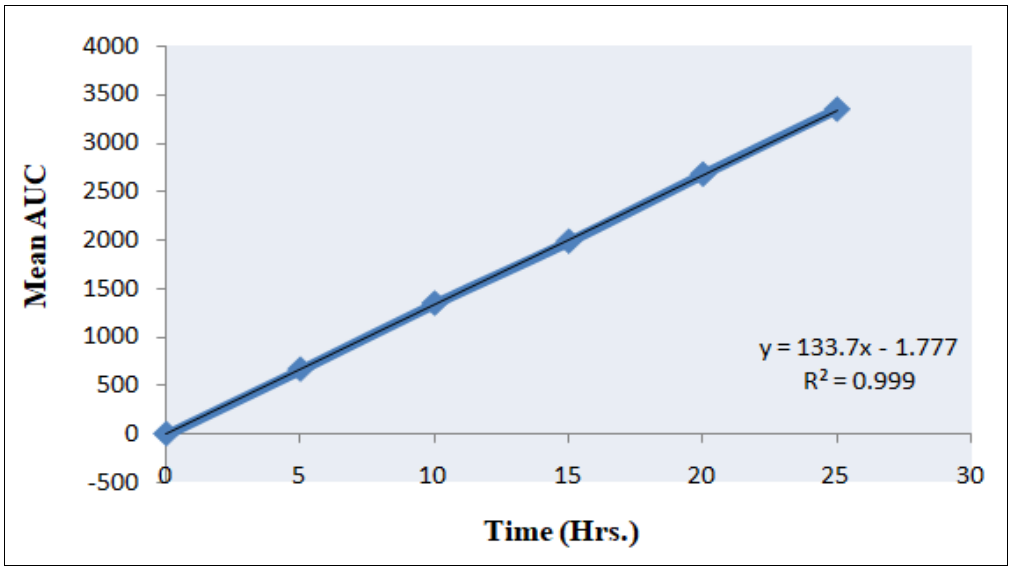


Figure 1: Calibration curve of Quercetin

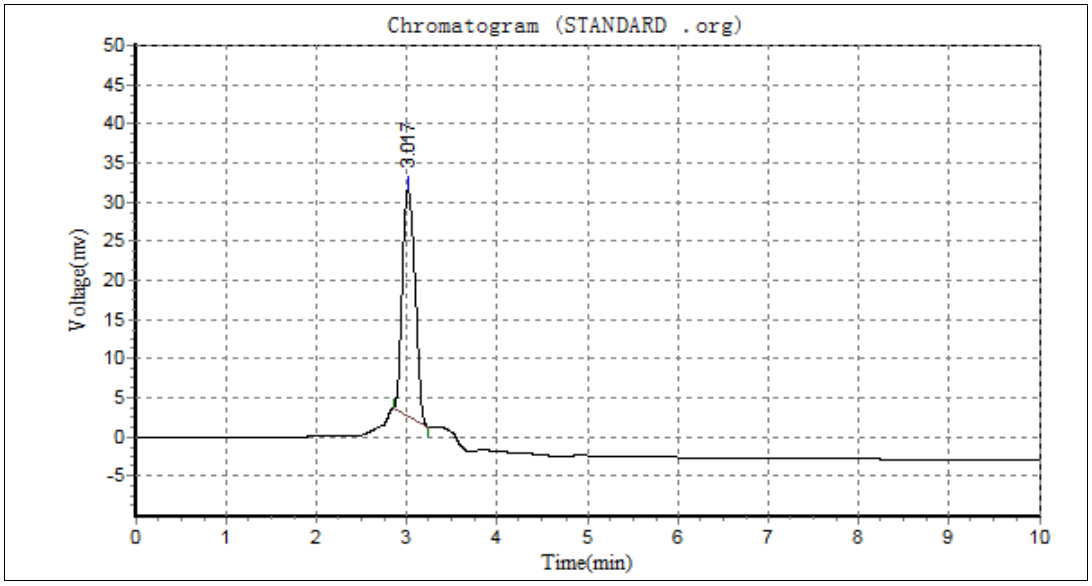


Figure 2: Chromatogram of standard Quercetin

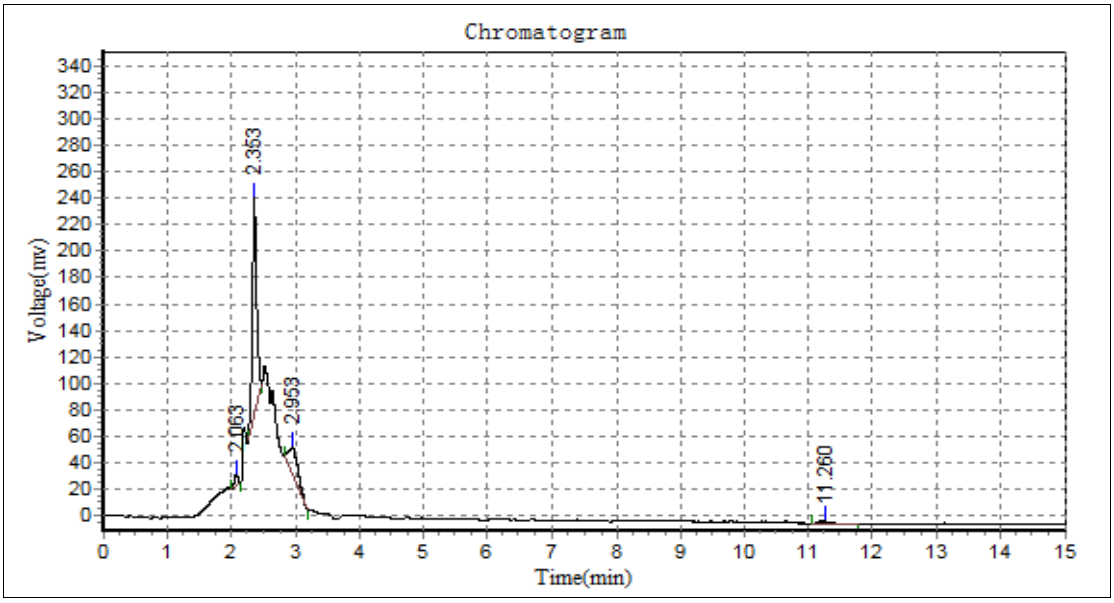


Figure 3: Chromatogram of ethanolic extract of *Paeonia officinalis*

S. No.	Standard/ Extract	RT	% Assay
1.	Quercetin	3.017	-
2.	*Paeonia officinalis*	2.953	0.27

Conclusion

A phytochemical analysis of *Paeonia officinalis* extracts already revealed the presence of substances from several different chemical classes, including proteins, alkaloids, glycosides, flavonoids, tannins, steroids, carbohydrates, and polyphenols. TLC and HPLC fingerprints were used to establish the presence of several phytochemicals. In both the extract, the TLC fingerprinting revealed a number of spots. Different phytochemicals were present, according to an HPLC examination. A fingerprint using HPLC and TLC would be useful for determining the quality of *Paeonia officinalis* raw materials, as well as for identifying and separation of crucial phytochemicals for medicinal purposes.

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