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Study Of Phyto-Chemicals And Antioxidant Activity Of Roots Extracts Of *Paeonia Emodi* Using UV And HPLC

Arvind Kumar Sharma[1]*, Vishal Gupta[2], Ashish Manigauha[3]

[1,2,3]Department of Pharmacy, Mansarovar Global University, M.P, India

***Corresponding author:-** Arvind Kumar Sharma
*Email id: aarvind2k@gmail.com

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Abstract

This study focuses on the comprehensive analysis of *Paeonia emodi* extract, including its phytochemical composition and pharmacological potential. Various solvents were used to extract bioactive compounds from *Paeonia emodi*, and their yields were determined. Phytochemical screening revealed the presence of glycosides, flavonoids, phenols, proteins, carbohydrates, and saponins in the extract. Quantitative estimation showed significant levels of total phenols and flavonoids, particularly in the ethanol extract. The extract exhibited notable antioxidant activity, as evidenced by its inhibition of DPPH and hydrogen peroxide radicals. Furthermore, quercetin, a potent antioxidant flavonoid, was quantitatively determined in the extract. These findings suggest that *Paeonia emodi* extract possesses promising antioxidant activity, which may have therapeutic implications in various health conditions.

Keywords: *Paeonia emodi*, phytochemical composition, antioxidant activity, total phenols, flavonoids, quercetin, DPPH assay, hydrogen peroxide assay, medicinal plants.

Introduction

Paeonia emodi, commonly known as Himalayan peony or Moutan, is a perennial herbaceous plant belonging to the Paeoniaceae family. Indigenous to the Himalayan region, *Paeonia emodi* has been traditionally used in Ayurvedic and traditional medicine systems for its therapeutic properties. The roots of *Paeonia emodi* are particularly valued for their medicinal benefits, including anti-inflammatory, analgesic, hepatoprotective, and antioxidant activities (Kumar et al., 2022).

Phytochemical analysis of plant extracts plays a crucial role in identifying and characterizing the bioactive compounds responsible for their pharmacological effects. Several studies have reported the presence of various phytochemicals in *Paeonia emodi*, including phenolic compounds, flavonoids, glycosides, tannins, and alkaloids. These phytochemicals have been linked to the plant's antioxidant properties and potential health benefits (Brusotti et al., 2014).

Antioxidants are compounds that can neutralize harmful free radicals in the body, thereby protecting cells from oxidative damage and reducing the risk of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Huy et al., 2008). Given the increasing interest in natural antioxidants as potential therapeutic agents, there is a growing need to explore the antioxidant potential of medicinal plants like *Paeonia emodi* (Ibrar et al., 2019).

UV (Ultraviolet) spectroscopy and HPLC (High-Performance Liquid Chromatography) are widely used analytical techniques for the qualitative and quantitative analysis of phytochemicals in plant extracts (Wu et al., 2013). UV spectroscopy allows for the identification of specific functional groups based on their characteristic absorption spectra, while HPLC enables the separation and quantification of individual compounds within complex mixtures (Butnariu et al., 2016).

In this context, the present study aims to investigate the phytochemical composition and antioxidant activity of roots extracts of *Paeonia emodi* using UV and HPLC techniques. By elucidating the chemical profile and antioxidant potential of Paeonia emodi extracts, this research contributes to the understanding of its therapeutic properties and potential applications in healthcare.

Material and Methods

Procurement of plant material

Roots of *Paeonia emodi* were collected from ruler area of Bhopal, the month of February, 2022. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant.

Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994).

Defatting of Plant Material

The shade dried roots of *Paeonia emodi* (65.3gm) were extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Successive extraction with different solvents by maceration method

Defatted plant material were extracted in four solvents of different polarity viz water, ethanol, ethyl acetate and chloroform (Mukherjee, 2007). Powdered plant materials were extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis.

Determination of extractive value (% yield)

The % yield of yield of each extract was calculated by using formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

Preliminary phytochemical screening is primarily an important aspect for establishing profile of given extract for its chemical compounds produced by plant. Phytochemical examinations were carried out extracts as per the following standard methods (Mukherjee, 2007).

Quantitative studies of phytoconstituents

Estimation of total phenol content

The total phenol content of the extracts was determined by the modified folin-ciocalteu method (Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extracts was dissolved in 10 ml methanol and

filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of each extract and 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 total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

In-vitro antioxidant activity ethanolic extract from *Paeonia emodi* using different methods

DPPH method

Total free radical scavenging capacity of the ethanolic extract from *Paeonia emodi* were estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10-100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Hydrogen peroxide method

In-vitro antioxidant activity of extract of *Paeonia emodi* using hydrogen peroxide was performed as Czochra and Widensk (2002) proposed. Added 2ml hydrogen peroxide (43 mol) and 1.0 ml hydroalcoholic sample [20-100 µl different extracts (4 mg / ml) ethanol] accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4). The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) has been calculated.

Quantitative study of marker compound (Quercetin) by HPLC

A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max}. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C_{18} (250 X 4.6 mm, 5µm) column, a Data Ace software.

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 each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm.

Preparation of standard stock 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.

Preparation of working standard solution

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 and Discussion

The extractive values of *Paeonia emodi* were determined using different solvents, with varying percentages of yield obtained, as shown in Table 1. Among the solvents used, the aqueous extract exhibited the highest yield at 6.65%, followed by ethanol (3.54%), ethyl acetate (2.41%), and chloroform (0.58%). These values indicate the efficiency of different solvents in extracting phytochemicals from the plant material.

Subsequently, phytochemical screening of the chloroform extract of *Paeonia emodi* was conducted, and the results are summarized in Table 2. The screening revealed the presence of various phytoconstituents such as glycosides, flavonoids, diterpenes, phenols, proteins, carbohydrates, and saponins, while alkaloids and tannins were found to be absent. The presence of these phytochemicals suggests the potential pharmacological activities of the plant extract.

Table 3 presents the estimation of total phenol and flavonoids content in different extracts of *Paeonia emodi*. The ethanol extract exhibited the highest total phenol content of 0.568 mg/100 mg of dried extract, followed by the aqueous extract (0.421 mg/100 mg). Similarly, the ethanol extract also showed the highest total flavonoids content of 0.967 mg/100 mg, indicating its richness in these bioactive compounds.

Furthermore, the antioxidant activity of *Paeonia emodi* extract was evaluated using the DPPH and hydrogen peroxide methods, and the results are depicted in Tables 4 and 5, respectively. The extract demonstrated significant antioxidant activity, as evidenced by its percentage inhibition of DPPH and hydrogen peroxide radicals. The IC50 values indicate the concentration of the extract required to inhibit 50% of the radicals, with lower values suggesting higher antioxidant potency.

In Figure 1, the chromatogram of the standard quercetin is shown, serving as a reference for comparison with the chromatogram of the ethanolic extract of *Paeonia emodi* presented in Figure 2. The quantitative estimation of quercetin in the extract revealed a percentage assay of 0.071%, indicating the presence of this flavonoid compound in the plant extract.

the findings suggest that *Paeonia emodi* possesses significant antioxidant potential due to its high phenol and flavonoids content, as well as its ability to inhibit free radicals. These results support the traditional medicinal uses of *Paeonia emodi* and warrant further exploration of its therapeutic applications.

Table 1: Extractive values ofextracts of *Paeonia emodi*

S. No.	Extracts	Colour	% Yield* (W/W)
1	Chloroform	Dark green	0.58%
2	Ethylacetate	Green	2.41%
3	Ethanol	Dark green	3.54%
4	Aqueous	Light brown	6.65%

Table 2: Result of phytochemical screening of *Paeonia emodi*chloroform extract

S. No.	Constituents	Chloroform extract	Ethylacetate extract	Ethanol extract	Aqueous extract
1	**Alkaloids** Hager's Test:	 –Ve	 –Ve	 –Ve	 –Ve
2	**Glycosides** Legal's Test:	 –Ve	 –Ve	 +Ve	 –Ve
3	**Flavonoids** Lead acetate Test: Alkaline test:	 –Ve –Ve	 +Ve +Ve	 +Ve –Ve	 +Ve –Ve
4	**Diterpenes** Copper acetate Test:	 –Ve	 +Ve	 +Ve	 +Ve
5	**Phenol** Ferric Chloride Test:	 –Ve	 –Ve	 +Ve	 +Ve
6	**Proteins** Xanthoproteic Test:	 –Ve	 +Ve	 +Ve	 +Ve
7	**Carbohydrate** Fehling's Test:	 –Ve	 –Ve	 +Ve	 +Ve
8.	**Saponins** Froth Test:	 –Ve	 +Ve	 +Ve	 +Ve
9.	**Tannins** Gelatin test:	 –Ve	 –Ve	 –Ve	 –Ve

(+Ve =Positive, –Ve= Negative)

Table 3: Estimation of total phenol and flavonoids content of *Paeonia emodi*

S. No.	Extracts	Total phenol content	Total flavonoids content
		(mg/ 100 mg of dried extract)	
1	Chloroform	–	–
2	Ethylacetate	–	0.142
3	Ethanol	0.568	0.967
4	Aqueous	0.421	0.341

Table 4: % Inhibition of ascorbic acid and ethanolic extract of *Paeonia emodi* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	*Paeonia emodi* extract

1	10	43.16	11.89
2	20	50.12	14.99
3	40	54.06	29.20
4	60	72.62	32.56
5	80	77.26	39.28
6	100	84.92	52.20
IC_{50}		**22.83**	**98.67**

Table 5: % Inhibition of ascorbic acid and ethanolic extract of *Paeonia emodi* using hydrogen peroxidemethod

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	*Paeonia emodi* extract
1	20	45.54	8.11
2	40	64.04	9.59
3	60	66.54	11.07
4	80	75.72	12.17
5	100	80.84	14.20
IC 50		**19.82**	**594.52**

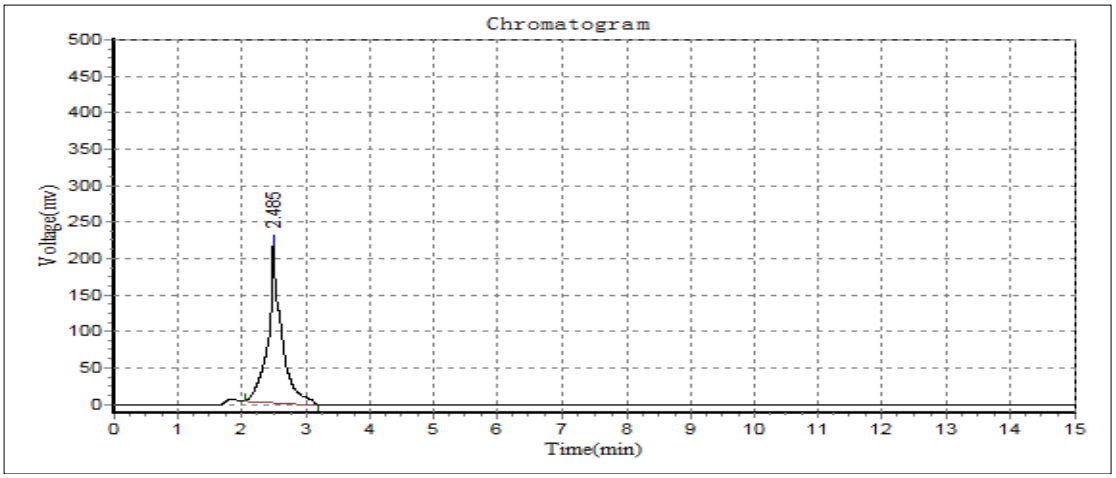

Figure 1: Chromatogram of standard Quercetin

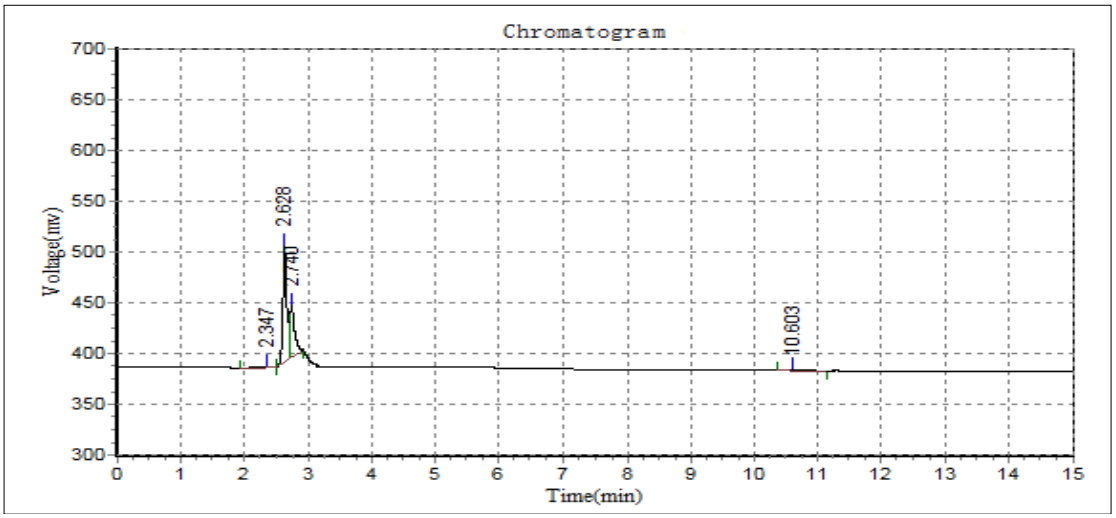

Figure 2: Chromatogram of ethanolicextract of*Paeonia emodi*

Table 6: Quantitative estimation of Quercetin in extract

S. No.	Standard/ Ethanolicextract	RT	Area	% Assay
1.	Quercetin	2.485		
2.	*Paeonia emodi*	2.628	37.471	0.071

Conclusion

The comprehensive analysis of *Paeonia emodi* extract presented in this study provides valuable insights into its phytochemical composition and pharmacological potential. The extractive values revealed the efficacy of different solvents in extracting bioactive compounds from the plant material, with the aqueous and ethanol extracts showing the highest yields. Phytochemical screening confirmed the presence of various phytoconstituents, including glycosides, flavonoids, phenols, proteins, carbohydrates, and saponins, indicating the diverse chemical profile of the extract. Quantitative estimation demonstrated significant levels of total phenols and flavonoids, particularly in the ethanol extract, highlighting its antioxidant potential. The antioxidant activity assays further supported these findings, showing notable inhibition of DPPH and hydrogen peroxide radicals by the extract. Additionally, the presence of quercetin, a potent antioxidant flavonoid, was quantitatively determined in the extract, further corroborating its antioxidant properties.

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