Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 28s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Extraction, Qualitative And Quantitative Estimation of Phyto-Bioactive Compound In Extract of Celastrus Orbiculatus UV And HPLC

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Cite this paper as: Richa Tripathi, Vivek Chourasia, (2025) Extraction, Qualitative And Quantitative Estimation of Phyto-Bioactive Compound In Extract of Celastrus Orbiculatus UV And HPLC. *Journal of Neonatal Surgery*, 14 (28s), 702-707.

ABSTRACT

The present study aimed to evaluate the phytochemical profile and quantify the bioactive constituents of *Celastrus orbiculatus* leaf extracts using various solvent systems. Extracts were prepared using chloroform, ethyl acetate, ethanol, and distilled water to determine extractive yields and assess the presence of phytochemicals through standard qualitative tests. The ethanolic extract showed the highest yield (8.41% w/w) and tested positive for a broad spectrum of constituents including flavonoids, phenols, alkaloids, tannins, sterols, and proteins. Quantitative analyses confirmed that the ethanolic extract contained the highest total phenolic content (1.23 mg/100 mg), total flavonoid content (4.21 mg/100 mg), and total alkaloid content (0.54 mg/100 mg). High-Performance Liquid Chromatography (HPLC) analysis revealed the presence of quercetin in the ethanolic extract with a retention time of 2.640 minutes and a concentration of 0.614% w/w. These findings indicate that *Celastrus orbiculatus* leaves are a rich source of phytochemicals with potential antioxidant and therapeutic applications, and ethanol serves as an effective solvent for extracting key bioactive compounds such as quercetin.

Keywords: *Celastrus orbiculatus; Phytochemical screening; Total phenolic content; Total flavonoid content; Alkaloid estimation; Quercetin; HPLC analysis; Ethanol extract; Bioactive compounds; Antioxidant potential*

1. INTRODUCTION

Medicinal plants have long been a vital part of traditional healthcare systems around the world, serving as rich sources of bioactive compounds with diverse therapeutic potentials (Ramawat et al., 2009). Among these, *Celastrus orbiculatus* Thunb., a woody climbing shrub belonging to the family Celastraceae, has garnered considerable attention for its wide range of pharmacological properties, including anti-inflammatory, antioxidant, anti-tumor, hepatoprotective, and neuroprotective effects (Oancea; 2025).

These biological activities are largely attributed to the plant's abundant secondary metabolites such as flavonoids, phenolics, alkaloids, and terpenoids (Roaa; 2020). Phytochemical screening is an essential preliminary step in identifying the presence of bioactive classes of compounds. Qualitative analysis offers a preliminary insight into the types of phytochemicals present in the plant extract, while quantitative estimation provides a measure of their abundance. In this context, the total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) are considered significant indicators of a plant’s medicinal and nutraceutical value (Altemimi et al., 2017).

The Folin–Ciocalteu method is widely used for the quantification of TPC, which measures the reducing capacity of phenolic compounds through a colorimetric reaction. It is a well-established, rapid, and reproducible technique that gives results in terms of gallic acid equivalents (GAE) (Lamuela; 2018). Similarly, the TFC is commonly determined by the aluminum chloride colorimetric method, which involves the formation of a flavonoid-aluminum complex, producing a yellow color measured spectrophotometrically, and results are expressed in quercetin equivalents (QE) (Biswas and Sen; 2018).

In addition to TPC and TFC, evaluating the total antioxidant capacity (TAC) is crucial, as antioxidants play a central role in protecting cells from oxidative stress, which is implicated in chronic conditions such as cancer, cardiovascular diseases, and neurodegenerative disorders. TAC is generally assessed using methods such as the phosphomolybdenum assay or DPPH radical scavenging assay, which provide an estimate of the sample's total antioxidant activity.

For the precise and specific quantification of individual phytochemicals such as quercetin, high-performance liquid chromatography (HPLC) is considered the gold standard analytical technique. Quercetin, a prominent dietary flavonoid

found in many medicinal plants including *Celastrus orbiculatus*, has been extensively studied for its antioxidant, anti-inflammatory, anti-cancer, and cardioprotective activities (Babu et al., 2013). The HPLC technique offers high sensitivity, selectivity, and reproducibility, making it suitable for the accurate quantification of quercetin and other marker compounds in complex plant matrices (Mansour et al., 2025).

This study focuses on the extraction of phytochemicals from the aerial parts of *Celastrus orbiculatus* using suitable solvents, followed by qualitative phytochemical screening and quantitative determination of TPC, TFC, and TAC using UV–Visible spectrophotometric methods. Furthermore, HPLC analysis will be employed for the specific quantification of quercetin as a marker compound to establish the phytochemical richness and potential health benefits of the plant extract.

2. MATERIAL AND METHODS

Material

The materials used for the investigation included fresh, shade-dried leaves of *Celastrus orbiculatus*, collected locally and authenticated. Solvents such as chloroform, ethyl acetate, and ethanol (absolute, 99.9%) were procured from Merck India Ltd., Mumbai, while distilled water was prepared in-house. Reagents like ferric chloride, lead acetate, copper acetate, and concentrated sulfuric acid were obtained from Loba Chemie Pvt. Ltd., Mumbai. Folin–Ciocalteu reagent was sourced from Sisco Research Laboratories (SRL), India. HPLC-grade methanol and acetonitrile were purchased from Merck India Ltd., and standard quercetin (≥98%) was obtained from Sigma-Aldrich, India. All reagents and solvents used were of analytical or HPLC grade to ensure the accuracy and reliability of phytochemical screening and quantification procedures.

Methods

Collection of *Celastrus orbiculatus*

The Minor Forest Produce Processing & Research Centre, Vindhya Herbals Bhopal, is where *Celastrus orbiculatus* leaves were gathered. Normal tap water was used to completely wash the plant materials, and then sterile distillation water was used. Then allowed to air dry at ambient temperature in a shady area. A grinding machine was used to crush the dried plant materials into a powder. The powder was kept in a bottle with a tight air container at 4°C.

Extraction by maceration method

Through a maceration procedure, 80 grams of powdered *Celastrus orbiculatus* leaves were thoroughly extracted using a variety of solvents, including petroleum ether, chloroform, ethyl acetate, ethanol, and distilled water. Above their boiling temperatures, the extracts were evaporated. Lastly, the dried extracts yield % was calculated. After being recovered, the extracts were reduced in a rotary evaporator and then kept for later use in airtight containers at 4°C (Acharya et al., 2019).

Determination of percentage yield

The efficiency of the complete extraction process is gauged by the percentage yield. The following formula is used to get the yield percentage (Sharma et al., 2020):

$$\text{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

With minor adjustments for various extracts, the qualitative chemical assays were conducted using the techniques outlined in (Trease and Evans, 1978; Mukherjee, 2007).

Quantitative estimation of bioactive compound

Estimation of total phenolic content

The modified folin-ciocalteu method was used to calculate the extracts total phenolic content (Parkhe and Bharti, 2019). After dissolving 10 mg of gallic acid in 10 ml of methanol, several aliquots ranging from 5-25 µg/ml were made. After dissolving 10 mg of dried extracts in 10 ml of methanol, the mixture was filtered. The amount of phenol was estimated using 2 ml (1 mg/ml) of this solution. 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and one milliliter (7.5g/l) of sodium carbonate were combined with 2 ml of each extract or standard. To create the color, the liquid was vortexed for 15 seconds and then left to stand for 15 minutes. Using a spectrophotometer, the absorbance at 765 nm was determined.

Estimation of total flavonoids content

The aluminum chloride technique was used to determine the total flavonoid content (Meda *et al*., 2005). After dissolving 10 mg of quercetin in 10 ml of methanol, several aliquots ranging from 5-25 µg/ml were made in methanol. After dissolving 10 mg of dried extracts in 10 ml of methanol, the mixture was filtered. The flavonoid content was estimated using 3 ml (1 mg / ml) of this solution. After adding 1 ml of 2% AlCl3 solution to 3 ml of extract or standard, the mixture was left to stand at

room temperature for 15 minutes. The absorbance was then measured at 420 nm.

Estimation of total alkaloids content

After dissolving 1 mg of the plant extract in methanol, 1 ml of 2 N HCl was added, and the mixture was filtered. After transferring this solution to a separating funnel, 5 ml of phosphate buffer and bromocresol green solution were added. After vigorously shaking the mixture with 1, 2, 3, and 4 ml of chloroform, it was collected in a 10 ml volumetric flask and diluted with chloroform to the appropriate volume. As previously mentioned, a series of reference standard atropine solutions (40, 60, 80, 100, and 120 µg/ml) were made. Using a UV/visible spectrophotometer, the absorbance of the test and standard solutions was measured at 470 nm in relation to the reagent blank. According to (Shamsa *et al*., 2008), the total alkaloid concentration was stated as mg of AE/100 mg of extract.

Identification of marker compound (Quercetin) of ethanolic extract of *Celastrus orbiculatus* by HPLC

Chromatographic Conditions

Column: Thermo C18 **(**250mm x 4.60mm**,** 5µ**)**

Mobile Phase: Acetonitrile: Methanol (50:50v/v)

Flow rate: 1ml/min

Temperature: Room Temp.

Sample Size: 20 µl

Retention Time: 3.308± 0.005 min

Preparation of standard solution

A stock solution of 1000 ppm was created by precisely weighing 10 mg of quercetin, transferring it to a 10 ml volumetric flask, and then adjusting the volume with methanol (Acharya *et al*., 2019). 1 ml of quercetin stock solutions were obtained and diluted to 10 ml. From this solution, 0.2, 0.4, 0.6, 0.8, and 1.0 ml solutions were transferred to 10 ml volumetric flasks and the volume was increased to 10 ml using mobile phase, resulting in standard drug solutions with concentrations of 2, 4, 6, 8, and 10µg/ml.

Analysis of extract

In order to achieve a concentration of 1000µg/ml, 10 mg of *Celastrus orbiculatus* ethanolic extract was taken in a 10 ml volumetric flask, diluted with methanol to the mark, and then filtered through Whatmann filter paper. The final volume was made up to mark using the same solvent. After adding 20 µl of this solution to the injector, note the chromatogram (Acharya et al., 2019).

3. RESULTS AND DISCUSSION

The phytochemical investigation of *Celastrus orbiculatus* leaf extracts revealed substantial variability in extractive yield, constituent profile, and phytochemical content depending on the solvent used. As shown in **Table 1,** the ethanolic extract exhibited the highest percentage yield (8.41% w/w), followed by the aqueous extract (5.87% w/w), ethyl acetate (3.52% w/w), and chloroform (0.98% w/w). This indicates that ethanol, being a polar protic solvent, is more efficient at extracting a wide range of phytochemicals, especially polar compounds such as flavonoids, phenolics, and alkaloids, which are commonly found in medicinal plants.

The results of the **phytochemical screening (Table 2)** further support the extractive efficiency of ethanol. The ethanolic and aqueous extracts tested positive for several important classes of phytochemicals, including flavonoids (via the alkaline reagent test), phenols (Folin–Ciocalteu and ferric chloride tests), tannins, proteins, and sterols. Notably, alkaloids were detected only in the ethanolic extract using Wagner's test. The ethyl acetate extract showed the presence of diterpenes, suggesting moderate polarity of such compounds. However, none of the extracts showed the presence of glycosides, carbohydrates, or saponins, indicating their negligible presence or non-extractability under the employed conditions. Overall, these findings demonstrate that the ethanol extract encompasses a broader spectrum of bioactive constituents compared to the other solvents tested.

Quantitative estimations of total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) are presented in **Table 3.** Consistent with the qualitative screening, the ethanolic extract showed the highest concentrations of phenols (1.23 mg/100 mg), flavonoids (4.21 mg/100 mg), and alkaloids (0.54 mg/100 mg). The aqueous extract also displayed considerable levels of phenols (0.88 mg/100 mg) and flavonoids (3.14 mg/100 mg), though alkaloids were not detected. On the other hand, the chloroform and ethyl acetate extracts contained only minor amounts of flavonoids and no detectable phenolic or alkaloid content. These results emphasize the strong correlation between solvent polarity and phytochemical solubility, particularly for phenolic and flavonoid compounds.

To further confirm the presence of specific bioactive markers, **HPLC analysis** was conducted using quercetin as a standard reference compound. As illustrated in **Figure 1,** the standard quercetin exhibited a retention time (RT) of 3.038 min. In comparison, the chromatogram of the ethanolic extract (**Figure 2**) showed a prominent peak at RT = 2.640 min, indicating the likely presence of quercetin or a structurally related flavonoid. The quantitative estimation, shown in **Table 4,** revealed that the quercetin content in the ethanolic extract was 0.614% (w/w), as determined by area normalization. The slight shift in retention time may be attributed to matrix effects or the presence of glycosidic derivatives. Collectively, these results confirm that *Celastrus orbiculatus* leaves, particularly when extracted with ethanol, are rich in phenolic and flavonoid compounds, including quercetin. These bioactives are well-documented for their antioxidant, anti-inflammatory, and therapeutic properties, which align with the traditional uses of the plant in herbal medicine. The findings of this study thus substantiate the potential of *Celastrus orbiculatus* leaf extract as a valuable source of natural antioxidants and bioactive compounds for pharmacological development.

Table 1: Extractive values of leaves extracts of *Celastrus orbiculatus*

Sr. No	Extracts	% Yield (w/w)	Colour of extractive
1	Chloroform	0.98	Sticky green
2	Ethyl acetate	3.52	Solid green
3	Ethanol	8.41	Solid green
4	Distilled water	5.87	Solid green

Table 2: Result of Phytochemical screening of leaves extracts of *Celastrus orbiculatus*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	**Alkaloids**				
	Wagner's Test:	-ve	-ve	+ve	-ve
2.	**Glycosides**				
	Conc. H_2SO_4 Test:	-ve	-ve	-ve	-ve
3.	**Flavonoids**				
	Lead acetate Test:	-ve	-ve	-ve	-ve
	Alkaline Reagent Test:	+ve	+ve	+ve	+ve
4.	**Diterpenes**				
	Copper acetate Test:	-ve	+ve	-ve	-ve
5.	**Phenol**				
	Ferric Chloride Test:	-ve	-ve	-ve	-ve
	Folin Ciocalteu 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
10.	**Sterols**				
	Salkowski's Test:	-ve	-ve	+ve	+ve

+Ve = Positive, -Ve= Negative

Table 3: Results of total phenol, flavonoids and alkaloid content of leaves extract of *Celastrus orbiculatus*

S. No.	Extracts	Total phenol content	Total flavonoids content	Total alkaloid content
		mg/100mg		
1	Chloroform	-	2.49	-
2	Ethyl acetate	-	1.99	-
3	Ethanolic	1.23	4.21	0.54
4	Aqueous	0.88	3.14	-

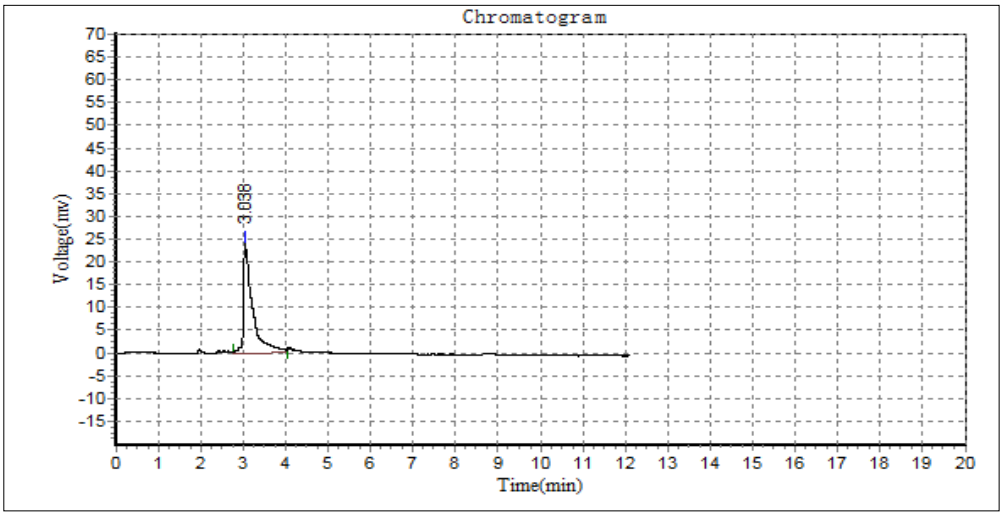

Figure 1: Chromatogram of standard Quercetin

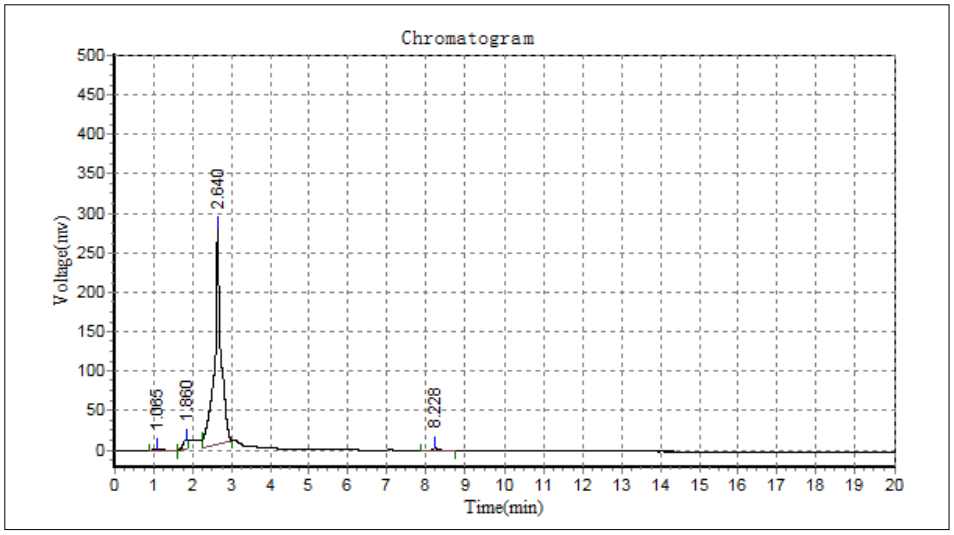

Figure 2: Chromatogram of ethanolic extract of *Celastrus orbiculatus*

Table 4: Quantitative estimation of Quercetin in *Celastrus orbiculatus* extract

S. No.	Standard/ Extracts	RT	Area	% Assay
1.	Quercetin	3.038		
2.	Ethanolic extract	2.640	1245.658	0.614

4. CONCLUSION

The study successfully demonstrated that *Celastrus orbiculatus* leaves possess significant phytochemical constituents with potential medicinal value. Among the various solvents used, ethanol proved to be the most effective for extracting a wide range of bioactive compounds, showing the highest extractive yield and the richest profile of secondary metabolites, including phenols, flavonoids, alkaloids, tannins, and sterols. Quantitative analysis confirmed that the ethanolic extract contained the highest levels of total phenolic and flavonoid content. Furthermore, HPLC analysis validated the presence of quercetin in the ethanolic extract, with a measurable concentration of 0.614% w/w, highlighting its antioxidant potential. These findings support the traditional use of *Celastrus orbiculatus* in herbal medicine and provide a scientific basis for further pharmacological studies and the development of natural therapeutic agents derived from this plant.

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