

**BJMHR**

British Journal of Medical and Health Research

Journal home page: www.bjmhr.com

Evaluation of qualitative and quantitative phytochemical screening of *Thalictrum foliolosum*

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ABSTRACT

Thalictrum foliolosum DC is widely distributed plant in the Himalayan region extending India, Nepal, Bhutan, South East Tibet and Burma between an altitude ranges of 1000–3400 m. Within India, it was recorded in Jammu & Kashmir, Himachal Pradesh, Punjab, Uttar Pradesh, Delhi, Sikkim, Arunachal Pradesh, Meghalaya, Bihar, Orissa, Andhra Pradesh and Tamil Nadu. In traditional medicine, various parts of this plant, including its bark, stems, and leaves, have been employed to address a range of health concerns, such as malaria, cancer, mental disorders, and pain relief. This study aimed to explore the qualitative and quantitative phytochemical analysis of *T. foliolosum* leaves. Qualitative phytochemical analysis identified the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, and tannins while the quantitative phytochemical analysis showed that ethanolic extract is richest in phenolic and flavonoid content. This study on *T. foliolosum* leaves serves as a crucial diagnostic tool for species identification, and the development of quality parameters. The data obtained in this study may be regarded as a reference for future research endeavors.

Keywords: *Thalictrum foliolosum*, phenolic content, flavonoid content, qualitative analysis, quantitative analysis, etc.

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Received 22 September 2023, Accepted 24 October 2023

INTRODUCTION

The Himalayan region is a renowned treasure trove of unique medicinal plants, deeply rooted in the traditions of various global medicinal systems. With an impressive tally of approximately 20,000 medicinal plant species documented in this region, it's worth noting that around 500 of these plants find mention in ancient texts. Moreover, approximately 800 of these botanical species have been harnessed and incorporated into diverse indigenous systems of medicine. Extensive research into these medicinal flora resources has been conducted within the Indian Himalayan Region (IHR), shedding light on their remarkable therapeutic potential. India, in particular, is heavily reliant on the Himalayas for the production of medicinal products. To put it into perspective, India satisfies a substantial 80% of its Ayurvedic medicine demand, as well as 46% of its Unani drugs and 33% of allopathic drugs, predominantly drawing from the abundant botanical wealth of the Himalayas¹⁻⁴.

The Himalayan region's significance extends far beyond its geographical boundaries, as the knowledge and resources it offers have a global impact^{5,6}. These natural remedies are not only utilized in traditional healing systems but also serve as a foundation for modern pharmaceutical and herbal medicine industries worldwide. To safeguard the environment and preserve the rich traditions of local communities, sustainable management of these invaluable resources is of paramount importance⁷⁻¹⁰.

The genus *Thalictrum*, a member of the subfamily *Thalicthroideae* within the *Ranunculaceae* family, encompasses a diverse group of approximately 200 species. These species are distributed across several continents, including Asia, Europe, Africa, North America, and South America¹¹. One notable species within the *Thalictrum* genus is *Thalictrum foliolosum* DC. This plant has a wide distribution throughout the Himalayan region, encompassing parts of India, Nepal, Bhutan, South East Tibet, and Burma. It thrives at altitudes ranging from 1000 to 3400 meters.

In India, *Thalictrum foliolosum* has been recorded in various states and regions, including Jammu & Kashmir, Himachal Pradesh, Punjab, Uttar Pradesh, Delhi, Sikkim, Arunachal Pradesh, Meghalaya, Bihar, Orissa, Andhra Pradesh, and Tamil Nadu. In Himachal Pradesh, it can be found in several districts, such as Chamba, Kangra, Kinnaur, Kullu, Mandi, Lahaul-Spiti, Solan, Shimla, and Sirmour, growing at elevations of up to 3000 meters^{12,13}. This particular plant holds considerable ecological and economic importance, especially for tribal communities. It is considered one of the essential non-timber forest products in the region, contributing significantly to the income of different tribal communities. *Thalictrum foliolosum* is estimated to contribute between 19% to 32% of the total household income for

these communities¹⁴. Its widespread distribution and economic value make it a notable plant species in the Himalayan region.

This plant's root has long been used as a tonic, stomachic, febrifuge, diuretic, antiperiodic, and purgative. Atonic dyspepsia, edema, skin conditions, flatulence, jaundice, and visceral blockages are among the conditions they are used to treat¹⁵. When combined with *T. foliolosum*, the powdered roots of *Boerhavia diffusa* are used to treat eye conditions. It heals night blindness and corneal ulcers. Oriental sores, diarrhea, trachoma, diabetes mellitus type 2, hypercholesterolemia, and congestive heart failure have all been claimed to be treated by the plant¹⁶. To treat stomach pain and digestive issues, the powdered dried roots are combined with *Thymus linearis* in an equal amount and consumed on a regular basis. The ethnic tribes of the East Godavari District treat stomach aches with a root concoction. The Jaunsari tribes of Chakrata Tehsil in the Dehra Dun area of India utilize the root extract to cure snakebite, piles, and eye issues. The paste of the plant is used by the locals in Himachal Pradesh, India, for snake bites and skin ailments. On boils and zits, the leaf juice is applied. Diuretic, ophthalmic, purgative, salve, and stomachache are the root causes. In addition to treating fever, indigestion, and toothaches, it is a tonic cure for dyspepsia¹⁷. The rhizome is used by the Naga tribes in Manipur, India, to treat diarrhea, jaundice, piles, fever, and dyspepsia¹⁸. The plant is used by the Adi and Monpa tribes of Arunachal Pradesh to cure fever, pain, nematode worms, gastrointestinal system issues, and malaria^{19,20}. The current study focuses on investigating the qualitative and quantitative phytochemical analysis of *Thalictrum foliolosum* leaves.

MATERIALS AND METHOD

Plant Material

Thalictrum foliosum leaves were collected from the outskirts area of Mandi, Himachal Pradesh. The plant was identified, authenticated, and certified (HIMCOSTE/HPsBB/5067) by Dr. Pankaj Sharma, Himachal Pradesh State Biodiversity Board, Shimla, India.

Preparation of Extracts

The process began with the preparation of plant leaves, which were initially cleansed with water to eliminate any dirt and foreign particles. After this cleaning step, the leaves were separated and subsequently subjected to shade drying. Once adequately dried, the leaves were milled to achieve a coarse powder texture, and then passed through a No. 14 sieve to obtain a consistent particle size. The resulting dried and powdered leaves of *T. foliolosum*, measuring 20 grams, were then placed within the tube of a Soxhlet apparatus in the form of a thimble. In this apparatus, they were subjected to extraction using various solvents, namely ethyl acetate, methanol, and water. This extraction process occurred at a temperature range of 60–65°C and

lasted for approximately 3–4 hours. Subsequently, the extracts obtained from this process, which were respectively referred to as ethyl acetate (EAE), ethanol (EE), and aqueous (AE) extracts, were filtered while still hot to remove any particulate matter, and they were then dried by evaporation using a rotary vacuum evaporator. The final dried extract samples were stored at low temperatures in a refrigerator for further analysis. Moreover, the residue left behind from each of the extractions was dissolved in the same solvent used for the initial extraction, preserving these residues for further analysis and investigation.

Qualitative Analysis of the Phytochemicals

Various extracts of *T. foliolosum* leaves were subjected to phytochemical analysis. A series of identification tests were performed to detect presence of alkaloids, flavonoids, saponins, proteins and amino acids, fixed oils and fats, glycosides, tannins and steroids ²¹.

Quantitative Analysis of the Phytochemicals

Total Polyphenols and Flavonoid Contents

The total phenolic content (TPC) and flavonoid (TFC) content of each *T. foliolosum* leaf extracts were determined using the earlier reported method. TPC was expressed as mg of gallic acid equivalent (GAE) per 100 g of extract, while the TFC was expressed as mg of quercetin equivalents (QE) per 100 g ²¹.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

Various phytochemical analysis tests supported that the extracts contain alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins and glycosides, recorded in Table 3. The aqueous extract was found to be negative for the presence of alkaloids as compared to methanolic and ethyl acetate extract.

Table 1: Results of phytochemical screening of different extracts of *T. foliolosum* leaves extracts.

Phytoconstituents	Leaf Extracts		
	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	Present	Present	Present
Glycosides	Present	Absent	Present
Flavonoids	Present	Absent	Present
Phenolic compounds	Present	Present	Present
Tannins	Present	Absent	Present
Saponins	Present	Absent	Present
Steroids	Present	Absent	Present

Qualitative phytochemical screening

Total Phenolic Content (TPC)

The determination of the total phenolic content (TPC) was chosen as the method to assess the phenolic levels in the plant extracts. These phenolic compounds are known for their redox

properties, which enable them to function as potential antioxidants. For this assessment, the phenolic content in each extract was measured using the Folin-Ciocalteu reagent, and the results were expressed as gallic acid equivalents (GAE) per gram of dry extract weight (Table 1). The findings from this analysis indicate that the methanol extract (ME) exhibited a significantly higher TPC in comparison to the ethyl acetate extract (EAE) and the aqueous extract (AE). Specifically, the TPC for the ME was approximately 64.94 ± 0.22 mg GAE per gram of dry extract weight, while the EAE had a TPC of 35.23 ± 0.13 mg GAE per gram of dry extract weight, and the AE had a TPC of 11.12 ± 0.15 mg GAE per gram of dry extract weight. To calculate the TPC, a linear equation was used, which was derived from the calibration curve of gallic acid. The equation is expressed as $y = 0.006x + 0.459$, with an R^2 value of 0.981. This calibration curve allowed for the conversion of the data obtained through the Folin-Ciocalteu reagent into quantitative values, using gallic acid as a reference standard.

Total Flavonoid Content (TFC)

Flavonoids, as secondary metabolites, are known for their antioxidant properties, and the effectiveness of these properties is influenced by the number and position of free OH (hydroxyl) groups. To quantitatively determine the flavonoid content in selected plant extracts, we employed a colorimetric method using aluminum chloride. The results were expressed in terms of quercetin equivalents (QE) per gram of dry extract weight. The findings of this analysis revealed that the ethanolic extract (EE) exhibited a notably higher Total Flavonoid Content (TFC) when compared to the ethyl acetate extract (EAE) and the aqueous extract (AE). Specifically, the TFC for EE was approximately 54.34 ± 0.11 mg GAE (Gallic Acid Equivalent) per gram of dry extract weight, while EAE had a TFC of 24.64 ± 0.14 mg QE (Quercetin Equivalent) per gram of dry extract weight, and AE had a TFC of 13.45 ± 0.13 mg GAE per gram of dry extract weight. The determination of TFC was accomplished using a linear equation derived from the calibration curve of quercetin, expressed as $y = 0.006x + 0.351$, with an R^2 value of 0.986. This calibration curve allowed for the conversion of the colorimetric data into quantitative values for the flavonoid content, using quercetin as a reference standard.

Table 2: Total phenolic and flavonoid content

Extracts	Phenolic Content (mg/g GAE)	Flavonoid (mg/g QE)
EE	64.94 ± 0.22^a	54.34 ± 0.11^a
EAE	35.23 ± 0.13^b	24.64 ± 0.14^b
AE	11.12 ± 0.15^c	13.45 ± 0.13^c

All values represent means $\pm$ SEM of three replicates. EE: Ethanolic extract; EAE: Ethyl acetate extract; AE: Aqueous extract. Statistical significance was determined at $p < 0.05$ and is indicated with different letters.

CONCLUSION

The current study, which involves a comprehensive blend of qualitative and quantitative analyses, sheds valuable light on the constitution of the ethanolic extracts derived from *T. foliolosum*. This investigation reveals a diverse range of secondary metabolites present in these extracts, encompassing alkaloids, phenolic compounds, glycosides, flavonoids, and tannins. Notably, the assessments of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) accentuate the significant abundance of these secondary metabolites within the ethanolic extracts. This suggests that the extracts are particularly rich in phenolic compounds and flavonoids, positioning them as a valuable source of these bioactive constituents. These ethanolic extracts stand out as a prolific reservoir of secondary metabolites, notably including alkaloids, phenolic compounds, glycosides, flavonoids, and tannins. The elevated TPC and TFC values underscore their potential for diverse applications, underscoring the imperative for further exploration to unlock their therapeutic and commercial promise.

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