

## Research Article



## Effect of Seasonal Variations on the Phytochemical Composition and Antioxidant Activity of the Root Extract of *Thalictrum foliolosum*.

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### ABSTRACT

*Thalictrum foliolosum* DC, a perennial herb distributed throughout the world and is indigenously found across Himalayan region in India. Ethano-pharmacological relevance of this plant is well known and used as tonic, diuretic, antimalarial and antiproliferative etc. The present study aims to determine the effect of seasonal variations on the phytoconstituents and their antioxidant activities. The present study reported varied range of phenolics (25.22-136.09 mg/GAE/mg dw), flavonoids (16.14- 210.43 mg/QD/mg dw), alkaloids (29-37 mg/Caffeine/mg dw) throughout the year. The maximum contents of alkaloids were obtained in the month of March-April, phenolics and flavonoids were in November-December. The IC<sub>50</sub> value of *Thalictrum foliolosum* root extract ranges between 50-250 µg/ml throughout the year. The IC<sub>50</sub> value of *Thalictrum foliolosum* root extract during senescence stage of plant growth i.e. November to February reported to be 54 µg/ml by DPPH assay and 62.8 µg/ml by ABTS assay. The study concluded that senescence stage of plant growth was suitable for harvesting maximum natural antioxidant constituents.

**Keywords:** Antioxidant, Alkaloid, Flavonoid, Phenolics, Seasonality.

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### INTRODUCTION

Medicinal herbs and plants played important role in human health care since time immemorial. About 80% of the world's population relies on the use of traditional healthcare system based mostly on plant materials. Scientific studies on plants showed the presence of the promising phytochemicals that can be used for the treatment of many health problems. Phytochemicals are physiologically active secondary metabolites produced by the plants, well known for their therapeutic properties like antioxidant, anti-inflammatory, antibacterial etc. Various phytochemicals present in the medicinal plants are flavonoids, alkaloids, phenolics and tannins etc. Seasonality is one of the major factors that influence the formation and accumulation of the secondary metabolites in medicinal herbs and plants<sup>1</sup>. Production of secondary metabolites and antioxidant potentials of the plants are influenced by many factors soil type, genetic diversity, climate and extraction methods<sup>2</sup>. It is well known that the secondary metabolites are influenced by many factors, the variation in the ecological zone, varietal, geographical area, seasons and the growth years may result in the fluctuation phytochemical constituents and biomass. Medicinal plants could play

crucial role in human nutrition as they are rich source of mineral element and phytochemicals. The quantitative estimation of the phytochemicals and other mineral elements is important for determining the effectiveness of the medicinal plants in treating various diseases<sup>3</sup>. Antioxidant are mostly involved in the first line defense mechanism for maintaining overall wellbeing and protect body from the harmful effects of the free radicals. Antioxidants known to decrease risk of chronic diseases like cardiovascular, gastrointestinal diseases and cancer. The relation of total phenolic compounds and free radical scavenging activity describes the antioxidant potential of the medicinal plants. A number of researchers showed that the phenolic content can be used as the indicator of the antioxidant activity as phenolic acids scavenge free radicals and reduce their toxicity<sup>2</sup>.

*Thalictrum foliolosum* is a well-known medicinal plant traditionally used in treatment of dysentery, enteritis and various other diseases since time immemorial. Plant based natural products with medicinal properties has been used since a long time and are the main source of the drugs during human civilization<sup>4</sup>. Plants are rich and diverse source of potentially biodynamic compounds and these compounds can be explored for development of various drugs. Various phytochemicals produced by plants, such as alkaloids, flavonoids; phenols etc. are used for the treatment of a number of diseases. These phytochemicals are mostly secondary metabolites produced in response to environmental factors. Plant derived antioxidants are well known for their greater efficiency, lower toxicity and lower manufacturing cost as compared to chemically synthesized antioxidants, as plants has diverse bioactive compounds<sup>5</sup>.



The genus *Thalictrum* L., belongs to Ranunculaceae family having more than 200 species those are distributed throughout the World and is commonly known as “meadow rue”. Among these species 29 species were traditionally used in Mongolia and Tibet as folk medicine, about 67 species in southwest region of China<sup>6</sup> and seven species of *Thalictrum* genus are commonly found in India. This plant is traditionally used in India, China, and North America. *Thalictrum foliolosum* DC, a tall erect, rigid, perennial herbaceous flowering plant indigenous to the temperate Himalayan zone and Khasia hills of India<sup>7-9</sup>. In Himachal Pradesh *Thalictrum foliolosum* is distributed in Kullu, Mandi, Chamba, Kangra, Sirmour, Solan, Shimla, Kinnaur and Lahaul-Spiti districts up to an elevation of 3000 m<sup>10</sup>. *Thalictrum foliolosum* was traditionally used as tonic, diuretic, cathartic, antiperiodic, febrifuge, stomachic and purgative. The chemical constituent's viz. berberine, oxy berberine, columbamine, jatrorrhizine, thalifendine etc. has been reported from this plant<sup>11</sup>. *Thalictrum foliolosum* reported to have varying range of therapeutic potential viz. antioxidant, antimicrobial, antimalarial, anti-leishmanial, anti-proliferative, antipyretic, anti-epileptic activity etc. Bisbenxyloquinoline, an alkaloid isolated from *Thalictrum foliolosum* DC reported to inhibit DNA topoisomerase IB in a dose dependent manner and also exhibit anti-leishmanial activity<sup>12</sup>. Two new chloro-containing BIAs compounds having high antioxidant and anti-proliferative have been identified in this plant<sup>5</sup>. *Thalictrum foliolosum* roots were reported to exhibit notable *in-vitro* cytotoxic activity against human lung cancer cell lines<sup>6</sup>. Because of its wide range of medicinal properties this plant is recently added into Ayurvedic Pharmacopoeia<sup>9</sup>.

There are immense research on the quantitative estimation of phytochemicals present in this medicinal plants but effect of seasonality on its composition is still lacking which is well known to effect phytochemical composition. In spite of the wide range of medicinal properties of *Thalictrum foliolosum* and its populous use in traditional medicine system, there is not even a single study related to the effect of season based variation on the bioactive composition of the plant has been reported as per our knowledge. India is a huge country with diverse range of geographical areas, thus differing environmental conditions. Therefore, season based differences will affect the phytochemical composition of the plant and relevant studies will determine the effect of the seasonal differences on the phytochemical compositions. In this way, present study to evaluate effects of seasonality on phyto-constituents will help in the improved collection timing for the better pharmaceutical activities. So the main aim of this study was to evaluate the effect of season based variation on the bioactive composition and antioxidant properties over a course of a year.

## MATERIALS AND METHODS

### Chemicals

Methanol and chloroform (ACS grade) were obtained from Merck (Mumbai, India). DPPH and ABTS were obtained from CDH (New Delhi, India) and Sigma- Aldrich (Germany) respectively. All the chemicals and reagents used during experimentation were of analytical grade.

### Plant material

In order to determine the effects of season based variations on the phytochemical composition plant roots were collected from Kullu, Himachal Pradesh, India (31.8862° N, 77.1455° E) in different seasons of the year (March 2020 to Feb 2021) and brought to laboratory for further processing. Dried at room temperature in shadow and dried plant roots were used for extraction of pharmaceutically active ingredients. The plant sample was duly authenticated and a herbarium specimen (LKK2468) was submitted to the North regional center of Botanical Survey of India, FRI, Dehradun, Uttarakhand (India).

### Methanol/chloroform root extract preparation

Maceration technique used for the extraction of phytochemicals, involved soaking plant root sample (coarse or powdered) in a stoppered container with a solvent and kept at room temperature for 3-6 days while with frequent agitation. The process intended to soften and break the plant cell wall to release the soluble phytochemicals<sup>13</sup>. Methanol: chloroform (1:1) used as a solvent for the extraction of phytochemicals from the roots of *Thalictrum foliolosum*<sup>14</sup>. Dried plant roots crushed using mortar and pestle. Powered plant material then weighed and placed into a sterile reagent bottle. Methanol: chloroform (1:1) added to the vessel containing plant roots. Plant roots kept at room temperature for 6 days with frequent agitation. After 6 days solvent was filtered through Whatman no.1 filter paper and residue were dipped in the respective solvent again and then filtered. Solvents are then allowed to dry and dried root extract of *Thalictrum foliolosum* stored at 4 °C.

### Climate data

The climate data for all the harvests of plant roots were obtained from the Indian Meteorological Department through Climate Data Service Portal. The data obtained from March 2020 to Feb. 2021 and different meteorological variables were included in this study: average maximum and minimum temperature (°C), relative humidity (%), and average rainfall/precipitation (Table 1).

### Phytochemical composition of root extract of *Thalictrum foliolosum*

Phytochemical analysis of the methanol: chloroform (1:1) plant root extract for the qualitative identification of the phytochemicals viz., carbohydrates, soluble starch, tannins, alkaloids, steroids, flavonoids etc., were carried out using different qualitative methods<sup>15</sup>.



**Qualitative tests****1. Tests for carbohydrates****Fehling's test**

Fehling A solution containing copper sulfate in distilled water, Fehling B containing potassium tartrate and sodium hydroxide in distilled water were mixed in equal proportions and boiled with the methanol: chloroform (1:1) plant root extract in a water bath. The presence of reducing sugar was indicated by the formation of the brick red precipitates of cuprous oxide.

**2. Tests for alkaloids****Wagner's test**

Presence of the alkaloids were indicated by the formation of the brown precipitates with the Wagner's reagent containing 1.27 g iodine mixed with 2 g potassium iodide in 100 ml with distilled water.

**Dragondrof test**

Distilled water (5ml) was added to the 2ml of the plant root extract and then 2M HCl and 1ml of Dragondrof reagent was added. Orange/orange red precipitates indicate the presence of alkaloids.

**3. Tests for phenols/tannins****Ferric chloride test**

The presence of phenols/tannins in the plant root extract was indicated by the formation of the blue green color with 5% ferric chloride solution.

**4. Tests for steroids****Salkowski test**

1ml of the test sample was dissolved in 1ml of chloroform and equal volume of concentrated H<sub>2</sub>SO<sub>4</sub>. Formation of bluish red color to cherry color in chloroform layer shows the presence of steroids.

**5. Tests for terpenoids**

The plant extract (1ml), chloroform (2ml) and concentrated H<sub>2</sub>SO<sub>4</sub> (3ml) were mixed. The presence of terpenoids confirmed by the formation of the reddish brown precipitates at the interface of the plant root extract solution, chloroform and concentrated H<sub>2</sub>SO<sub>4</sub>

**6. Tests for soluble starch**

Plant extract (1ml) boiled with 1 ml of 5% KOH, cooled and acidified with H<sub>2</sub>SO<sub>4</sub>. Appearance of yellow color indicates the presence of the soluble starch.

**7. Bayer's test for Unsaturation**

Aqueous 1% KMnO<sub>4</sub> solution is purple in color and the disappearance of the purple color of the KMnO<sub>4</sub> and the appearance of a brown solid precipitates (MnO<sub>4</sub>) after drop wise addition of the plant extract solution indicates positive test for unsaturation.

**8. Test for sponins**

0.5 g of the powdered plant roots were dissolved in 10 ml distilled water and filtered. The 5ml of the filtrate shaken vigorously with 2.5 ml distilled water resulting in the formation of froth above the filtrate. Three drops of saturated oil added to the froth and again shaken vigorously. The presence of the sponins was indicated by the stable and persistent frothing and formation of emulsion.

**9. Test for phlobatannins**

The aqueous plant root extract mixed with 1% aqueous HCl and boiled. The deposition of red precipitates indicate presence of phlobatannins.

**10. Test for flavonoids**

3ml diluted ammonia mixed with 2ml of the plant root extract and mixed well. 1ml H<sub>2</sub>SO<sub>4</sub> added to above solution and the presence of flavonoids in the plant root extract indicated by the formation of the yellow color in the reaction mixture.

**11. Test for anthraquinones**

2ml aqueous plant root extract boiled with 4ml concentrated H<sub>2</sub>SO<sub>4</sub> and shaken well. Added 3ml of the chloroform to above mixture and shaken well. Chloroform layer separated and 1ml diluted ammonia added to it. Change in the color of the mixture to yellow indicate the anthraquinones presence in the plant root extract.

**Quantitative tests****1. Determination of Total Phenolic Content**

The modified Folin-Ciocalteu photometric method was used for the estimation of the total phenolic content present in the methanol: chloroform (1:1) root extract of *Thalictrum foliolosum* with some modifications<sup>16</sup>. The filtered plant root extract was oxidized with Folin-Ciocalteu's reagents and the reaction was neutralized with saturated sodium carbonate after 5 minutes. The neutralized plant extract solution was then immediately diluted with distilled water to the volume of 10 ml. After 90 minutes of incubation at room temperature the absorbance of the reaction mixture was measured at 750 nm against the blank. Gallic acid used as the phenolic standard.

**2. Determination of total flavonoid content**

The modified colorimetric method was used for the determination of the total flavonoid content in the methanol: chloroform (1:1) root extract of *Thalictrum foliolosum* with some modifications. The powdered plant root of extract was mixed with distilled water in a test-tube. Then 5% NaNO<sub>2</sub> was added to the above mixture, 10% AlCl<sub>3</sub> after 5 minutes and after another 5 minutes 1M NaOH followed by the addition of distilled water. After 15 minutes of incubation absorbance was measured at 510 nm against the blank. The standard curve was prepared



using different concentration of quercetin dehydrate as flavonoid standard<sup>17</sup>.

### 3. Determination of total alkaloid content

The titrimetric method given by Debnath et al. (2015)<sup>18</sup> was used for the determination of total alkaloid content in the methanol: chloroform (1:1) root extract of *Thalictrum foliolosum* with certain modifications. The powdered root extract of plant dissolved in n-butanol and equal volume of 0.1 N HCl solution added into it. The above mixture transferred to 100 ml separating funnel and shaken vigorously for 2-3 minutes. This results in the increased solubility of alkaloids into the reaction mixture. The alkaloids neutralized with 0.1 N HCl remain in the lower layer and upper layer contain n-butanol. The 0.1 N HCl portion separated from the n-butanol layer and the color of the solutions changes from transparent to slightly reddish on addition of 2-3 drops of the methyl red indicator. This portion is then titrated against 0.1 N NaOH, till color changes from red to pale yellow. The neutralization point was determined as color changes from red to yellow and experiment performed in triplicates.

Total amount

1ml of 0.1 N HCl = 0.0752 mg of alkaloid standard<sup>18</sup>.

### Antioxidant activity

The antioxidant activities of the methanol: chloroform (1:1) root extract of *Thalictrum foliolosum* were determined using DPPH radical scavenging, ABTS radical scavenging assay as previously prescribed with certain modifications<sup>19, 20</sup>. The antioxidant activity determined by a DPPH and ABTS radical scavenging assay were evaluated using a DPPH and ABTS reagent. Ascorbic acid, a water soluble vitamin used as antioxidant standard. The end point detection at 520nm and 510nm were employed for DPPH and ABTS radical scavenging activities respectively.

### Statistical Analysis

All the values of the experiment were presented as mean  $\pm$  SE (standard error) of triplicate measurement; they were examined statistically using analysis of variance (ANOVA). The p value <0.05 was considered statistically significant. The SPSS 17.0 used for performing ANOVA, and PAST software used for performing neighbor joining (NJ) cluster analysis and principal component analysis (PCA)<sup>21</sup>.

## RESULTS AND DISCUSSION

Pharmaceutical properties of medicinal plants are due to phytochemicals, which mostly secondary metabolites are produced in response to environmental factors viz. temperature, radiation, altitude, time and season of collection and rainfall. The plant growth stages were divided into five distinct stages viz., (1) Shoot bud initiation stage (2 months); (2) Vegetative growth stage (2 months); (3) Flowering and fruiting stage (2 months); (4) Preparation for senescence stage (2 months) and (5) Senescence stage which continues for the succeeding 4 months till the start of the upcoming growth cycle. The plant sample was

identified as *Thalictrum foliolosum* DC belonging to Ranunculaceae family and accessioned at BSD student herbarium, North regional center of Botanical Survey of India, FRI, Dehradun, Uttarakhand (India) with accession number-999.

### Qualitative phytochemical composition of root extract of *Thalictrum foliolosum*

*Thalictrum foliolosum* is a familiar plant with an abundance of alkaloids and other phytochemicals. According to Ringmichon et al., (2013)<sup>22</sup> the rhizomes of *Thalictrum foliolosum* contain various phytochemicals viz., pectin, cellulose, mucilage, tannins, alkaloids, saponins, glucosides, starch, proteins and lipids<sup>22</sup>. In the present study, the phytochemical analysis of the methanol: chloroform root extract of *Thalictrum foliolosum* confirms the presence of carbohydrates, soluble starch, tannins, alkaloids, steroids, and flavonoids etc., at the different phases/stages of plant growth using different methods (Table 2).

### Quantitative phytochemical composition of root extract of *Thalictrum foliolosum*

Quantitative phytochemical composition of the methanol: chloroform root extract of *Thalictrum foliolosum* done to determine effect of seasonal variations. The parameters analyzed in the study involving the different samples collected throughout the year varied significantly ( $P < 0.05$ ) among different times of the collection. The experimental results revealed varying range of phenolics (25.22-136.09 mg/GAE/mg dw; Fig. 1) and flavonoids (16.14- 210.43 mg/QD/mg dw; Fig. 2), alkaloids (29-37 mg/Caffeine/mg dw; Fig. 3). The maximum contents of alkaloids were obtained in the month of March-April, phenolics and flavonoids were in November-December. The bioactive phytochemicals present in the plants are reported to be influenced by the seasons of the year. Bahukhandi et al. (2021)<sup>21</sup> reported that the secondary metabolites or phytochemical compositions of the medicinal plants vary eventually with the season and development stage. Bahukhandi et al. (2021)<sup>21</sup> and Wang et al. (2020)<sup>23</sup> outlined that phenolic and flavonoids content increases during winter season and also with the maturation of plant.

### Antioxidant activity of root extract of *Thalictrum foliolosum*

The antioxidant activity of the *Thalictrum foliolosum* by DPPH and ABTS assay presented in the form of % scavenging activities in Table 4 and figure 4 and 5 respectively. The antioxidant activity of the root extract reported to be maximum in senescence stage (Fig. 4 and 5) of plant growth i.e. November to February and with least activity during the early developing stage this study was supported by Afshar et al. (2021)<sup>2</sup>. The antioxidant activity of the root extract reported to have significant variations in the activity with difference in the season or time of collection. The IC<sub>50</sub> value is considered more appropriate method to represent effectiveness of antioxidant activity.



The IC<sub>50</sub> value of *Thalictrum foliolosum* root extract ranges between 50-250 µg/ml throughout the year. While IC<sub>50</sub> value ascorbic acid standard ranges between 20-80 µg/ml. The IC<sub>50</sub> value of *Thalictrum foliolosum* root extract during senescence stage of plant growth i.e. November to February reported to be 54 µg/ml by DPPH assay and 62.8 µg/ml by ABTS assay.

### Statistical Analysis

Neighbour-Joining cluster analysis performed using PAST software revealed the presence of two groups based on alkaloid, flavonoid and phenolic content and antioxidant activity (Fig.6). Based on phytochemicals and antioxidant activity the November- December represents the longest arm and indicated maximum potential. Principal component analysis (PCA) performed using PAST software reveals the presence of five components (PC-1=70.22%; PC-2 = 17.11%; PC-3 =9.31%; PC-4 = 3.31%; PC-5 = 0.03%)

(Fig. 7). The PC-1 indicates the interaction between months (September-October; November-December and January-February) and phenolics and flavonoids content, and antioxidant activity which shows inference of cluster analysis. PC-2 showed interaction between months (March-April; July-August) and alkaloid content. Loading plot showed correlation between phytochemical compounds and antioxidant activity of the methanol: chloroform root extract of *Thalictrum foliolosum* using multivariate assay. The phenols and flavonoids were reported to have direct relation with antioxidant activity<sup>24</sup>. While alkaloid contents<sup>25</sup> reported to inversely related to other secondary metabolites (Fig. 8). Outcomes revealed that phytochemicals constituents might show variations and emphasizing the months of November-December for harvesting natural antioxidant phytochemicals which has not been reported earlier in case of *Thalictrum foliolosum*

**Table 1:** Climate Data

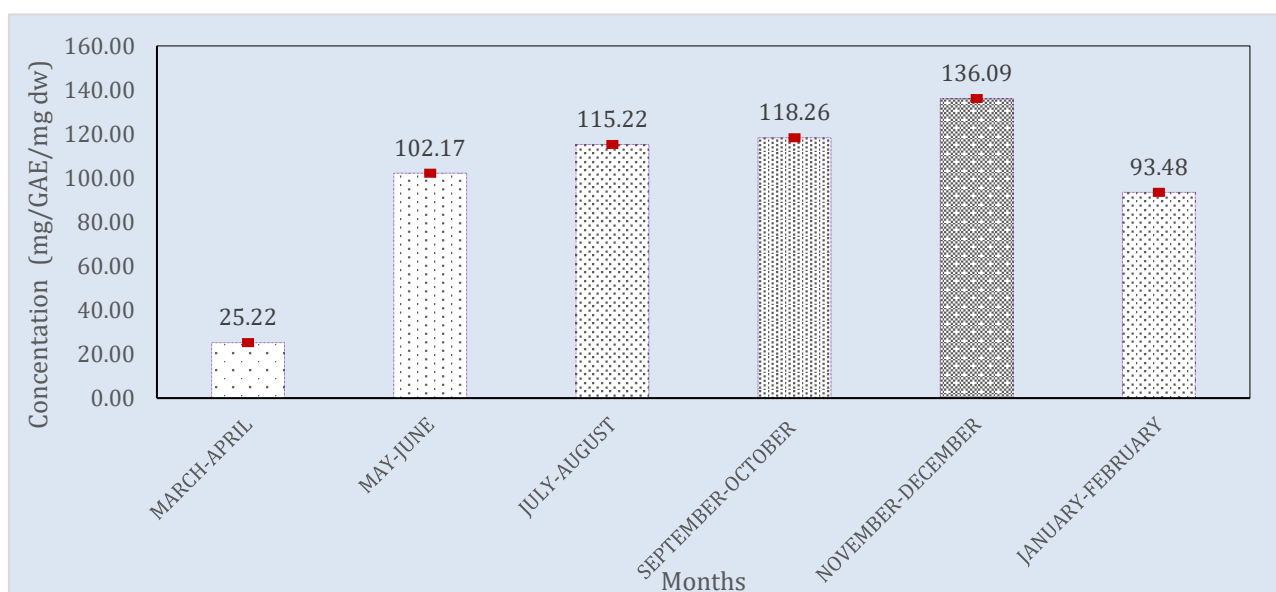
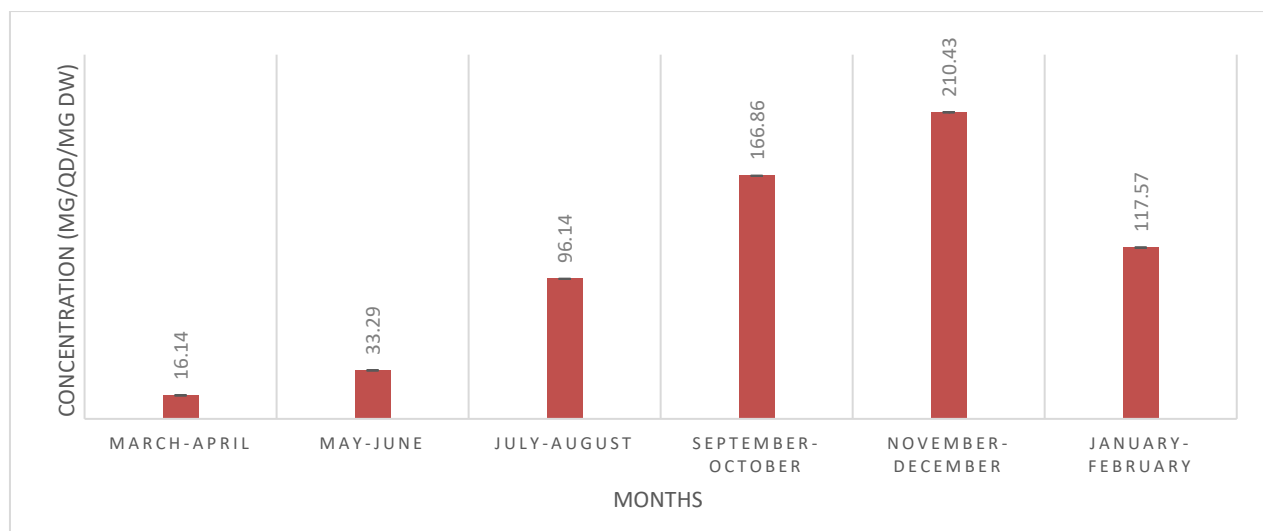
Climate Data				
Months	Precipitation (mm)	Maximum temperature (°c)	Minimum temperature (°c)	Relative humidity (%)
March-April	107.65	23.9	8.7	81
May-June	52.7	31.55	15.1	68
July- August	129.3	32.25	21.65	85.5
September –October	10.8	31.5	14.65	74.5
November- December	44.25	19.5	3.75	78.5
January- February	26.65	20.65	3.65	85.5

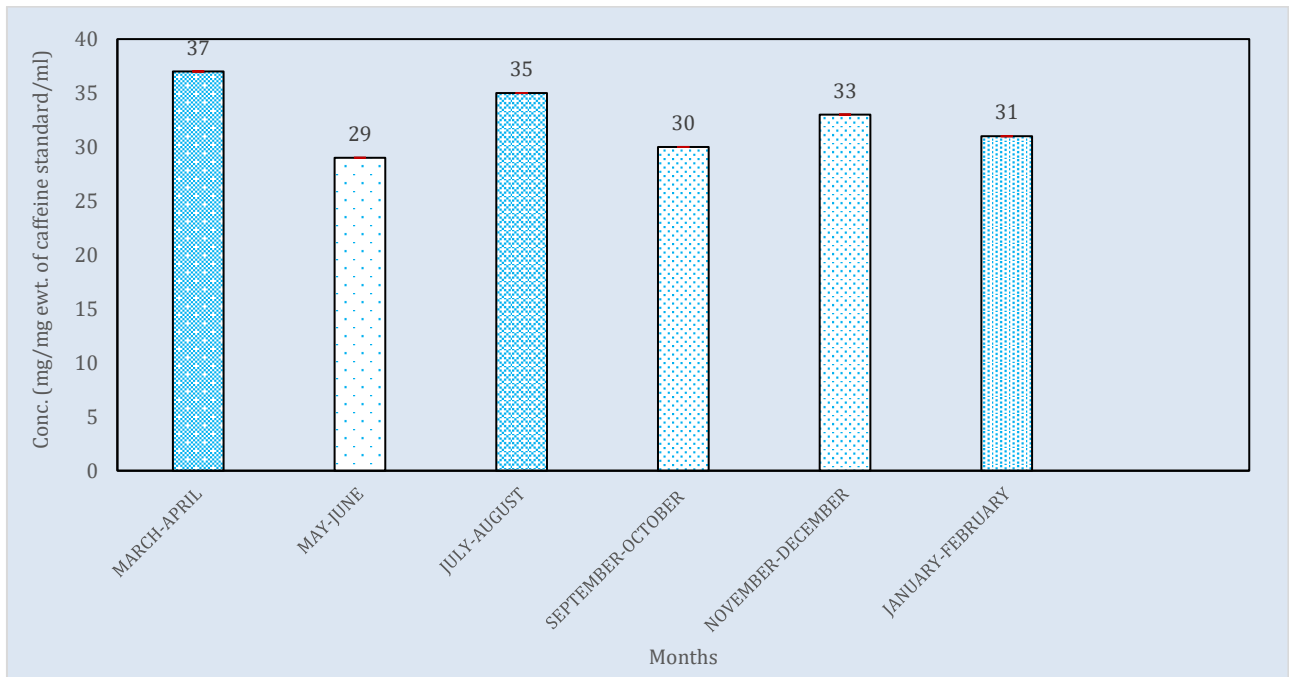
**Table 2:** Tests for qualitative phytochemical composition of root extract of *Thalictrum foliolosum*

Tests for qualitative phytochemical composition of the root extract of <i>Thalictrum foliolosum</i> .						
Tests	March-April	May-June	July-August	September – October	November-December	January-February
Tests for carbohydrates	+	+	+	+	+	+
Tests for alkaloids	++	++	+	+	+	+
Tests for phenols/tannins	+	++	+	+	+++	++
Tests for steroids	–	–	–	–	–	–
Tests for terpenoids	++	+	+	+	+++	++
Tests for soluble starch	+	+	+	+	+	+
Bayer’s test for Unsaturation	+	+	+	+	++	+
Test for sponins	+	+	+	+	+	+
Test for phlobatannins	–	–	–	++	+	–
Test for flavonoids	+	+	++	++	++	++
Test for anthraquinones	–	–	–	+	+	+

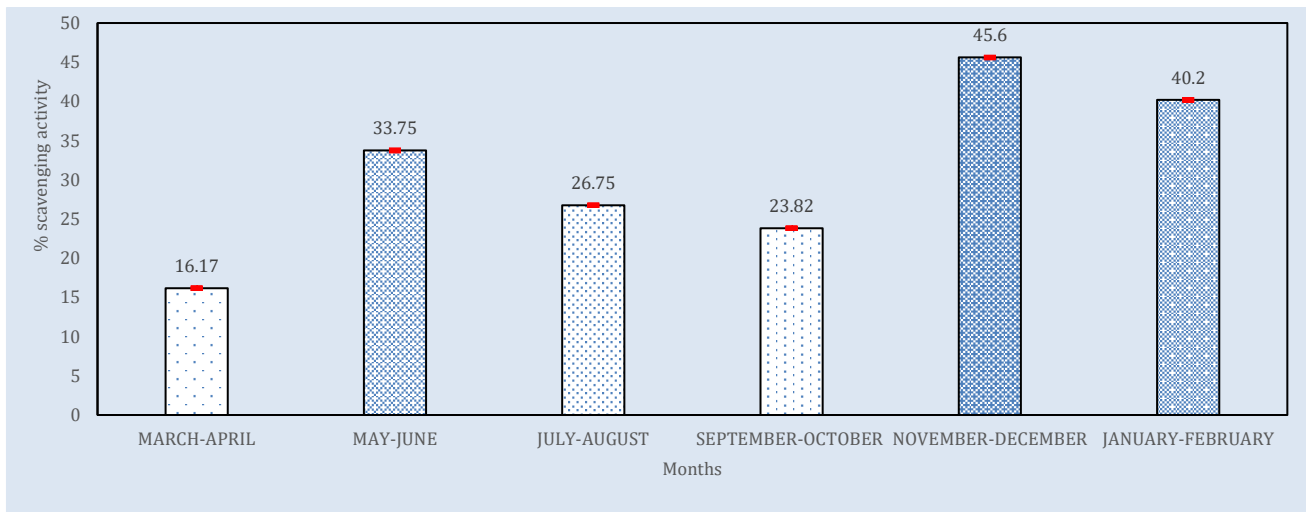
**Table 3:** Quantitative phytochemical composition of root extract of *Thalictrum foliolosum*.

Quantitative phytochemical composition of root extract of <i>Thalictrum foliolosum</i> .			
Months	Total phenolic content (mg/GAE/mg dw) $\pm 0.003$	Total flavonoid content (mg/QD/mg dw) $\pm 0.003$	Total alkaloid content (mg/mg Caffeine dw) $\pm 0.002$
March-April Shoot bud bursting phase	25.22	16.14	37
May-June Vegetative growth phase	102.17	33.29	29
July- August Flowering phase	115.22	96.14	35
September –October Preparation of Senescence phase	118.26	116.86	30
November- December Senescence phase	136.09	210.43	30
January- February Senescence phase	93.48	117.57	31

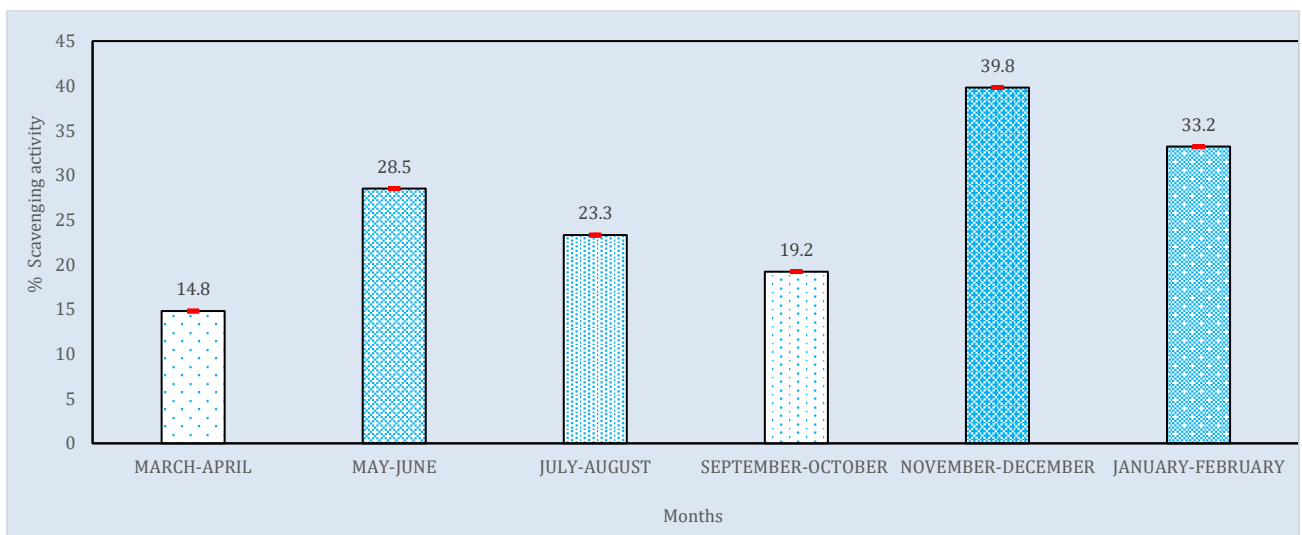
**Figure 1:** Total phenolic content in the root extract of *Thalictrum foliolosum*.**Figure 2:** Total flavonoid content in the root extract of *Thalictrum foliolosum*.



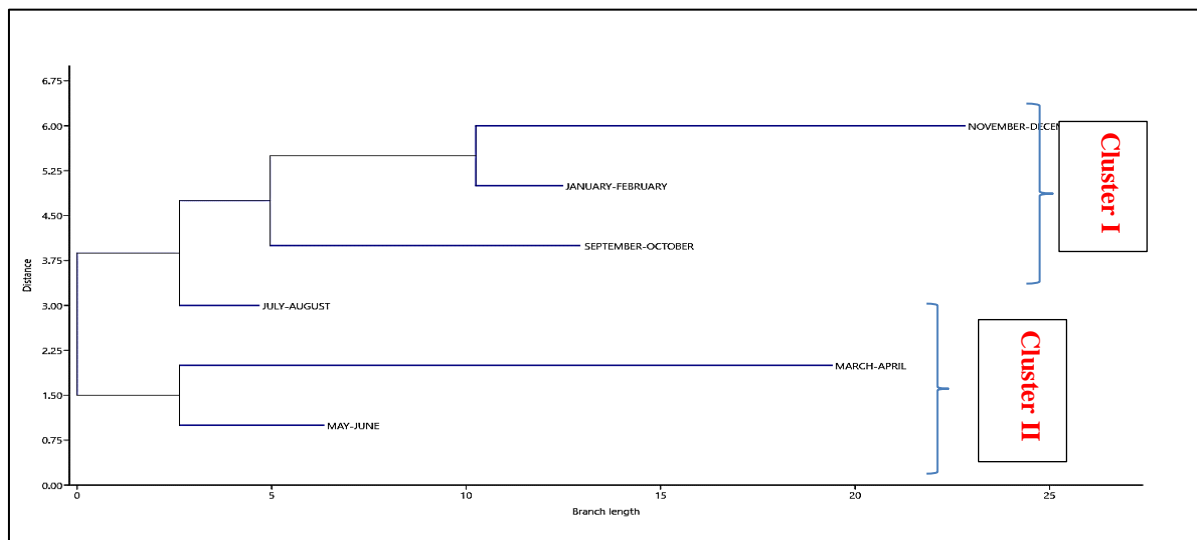
**Figure 3:** Total alkaloid content in the root extract of *Thalictrum foliosum*.



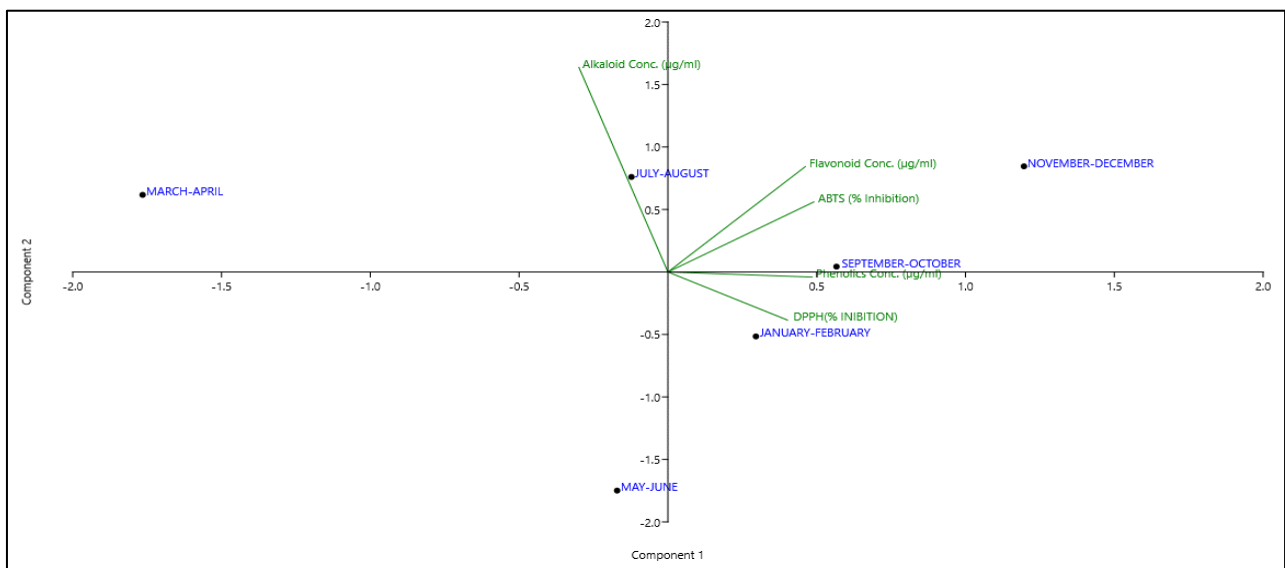
**Figure 4:** Antioxidant activity of the root extract of *Thalictrum foliosum* using DPPH method.



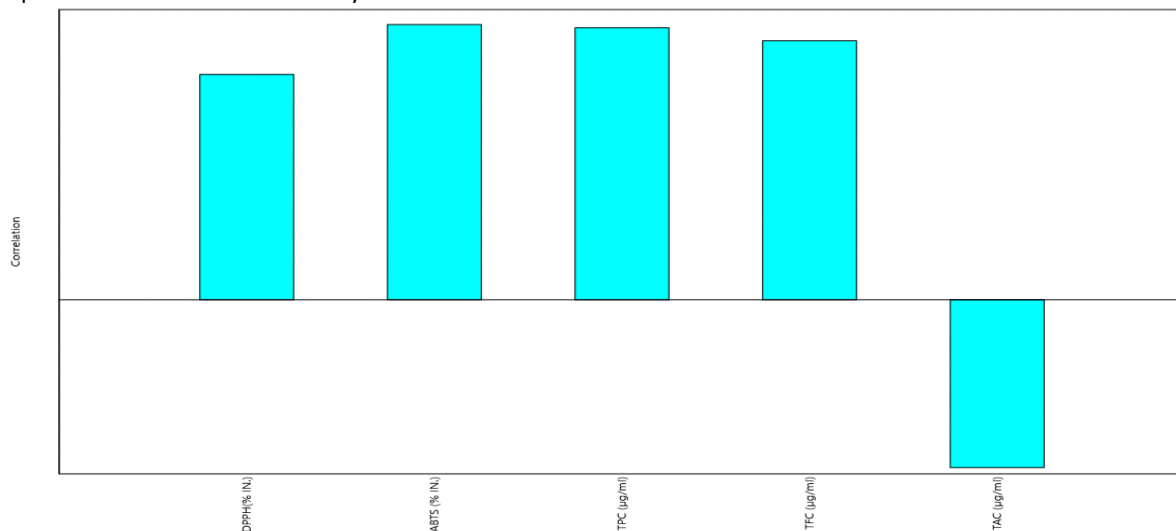
**Figure 5:** Antioxidant activity of the root extract of *Thalictrum foliosum* using ABTS method.



**Figure 6:** Neighbour-joining cluster analysis of the root extract of *Thalictrum foliolosum* based on the phytochemical compounds and antioxidant activity in different months.



**Figure 7:** Principal components analysis of the root extract of *Thalictrum foliolosum* based on the phytochemical compounds and antioxidant activity in different months.



**Figure 8:** Loading plot showing correlation between phytochemical compounds and antioxidant activity of the root extract of *Thalictrum foliolosum* using multivariate assay.



**Table 4:** Antioxidant activity of the root extract of *Thalictrum foliolosum*.

Months	Antioxidant Activity	
	DPPH Assay (% Scavenging Activity/50 µg/ml) (±0.012)	ABTS Assay (% Scavenging Activity 50 µg/ml) ( ±0.002)
March-April Shoot bud bursting phase	16.17	14.8
May-June Vegetative growth phase	33.75	28.5
July- August Flowering phase	26.75	23.3
September –October Preparation of Senescence phase	23.82	19.2
November- December Senescence phase	<b>45.6</b>	<b>39.8</b>
January- February Senescence phase	40.2	33.2

## CONCLUSION

In conclusion the present study led to the determination of the effect of the seasonal variation on the phytochemical composition and antioxidant activity of *Thalictrum foliolosum*, as seasonal variation remarkably affect their biosynthesis and these changes are vary throughout seasons. The quantitative evaluation of the phytochemical revealed that alkaloid content was highest during March-April while phenolic and flavonoid content was highest during November-December. The antioxidant activity was also reported to be highest during senescence stage i.e. November-December and lowest during early development stage. Therefore, this study recommends that while collecting medicinal plant seasonal variations should be considered, which will help producer to select best period of time for harvesting and for obtaining maximum amount of desired compound for their pharmaceutical applications.

## Additional Requirements

No additional requirements.

## Author Contributions

Lalita Kaushal was responsible for conceptualization, validation, formal analysis, investigation, writing original draft and visualization. Kumari Alka was responsible for helping in the reviewing and editing. All the authors contributed towards the elaboration of the manuscript and have given their approval of the final version.

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## Supplementary Material

The supplementary material is added at the end of this article.

## Data Availability Statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

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