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# Nutraceutical potential of fast growing *Salix* species growing under different habitats of Kashmir Himalayas

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**Abstract**
*Salix* species possess a significant nutraceutical potential due to their rich content of bioactive compounds which exhibit anti-oxidant, anti-inflammatory, neuroprotective and other health-promoting effects. The study examined the phytochemical composition of *Salix alba* and *Salix fragilis* across diverse habitats in the Kashmir Valley and assessed the impact of environmental factors such as climatic conditions and soil properties on these phytochemicals. Phytochemical screening revealed significant variations in phytochemical profiles between the two species, with *Salix fragilis* exhibiting a higher overall concentration of various compounds. Analysis of climate data such as maximum temperature (4.2°C to 31.9°C), minimum temperature (-4.6°C to 19.7°C), precipitation (0mm to 294.6 mm) and relative humidity (61.2 to 93%) indicated distinct seasonal patterns and significant inter-annual variations, suggesting their role in influencing plant stress responses and secondary metabolite biosynthesis. Furthermore, habitat-specific differences were observed in soil properties, including available nitrogen (275 to 542.66 kg/ha), phosphorus (16.56 to 57.73 kg/ha), potassium (44.77 to 281.13 kg/ha), pH (5.55 to 7.45), and organic carbon content (1.57 to 2.52%). These heterogeneities in soil characteristics are posited to significantly impact plant growth, metabolism, and ultimately the biosynthesis and accumulation of specific phytochemicals. These findings highlight the influence of various environmental factors on plant secondary metabolism and suggest potential applications of these willow species as sources of bioactive compounds. The research provides a deeper understanding of the phytochemical diversity and ecological adaptations of *Salix* species in the Himalayan region. Furthermore, studies should be undertaken to identify and isolate the important compounds that possess anti-oxidant properties accountable for the observed indifferences between the different *Salix* species and across habitats. Also, research is necessary to study the influence of environmental stress (like heavy metal pollution) on the phytochemical composition and ecological functions of willows species.

**Keywords:** Bioactive, medicine, nutraceutical, phytochemistry, *Salix*, secondary metabolite

## Introduction

A diverse group of chemical compounds formed by plants, known as phytochemicals, are crucial for their survival, mediating interactions with their environment and playing a significant role in plant fitness (Bourgaud `*et al.*, 2001) [11]. Beyond their ecological importance, many of these secondary metabolites possess considerable nutraceutical potential, offering multiple benefits for human health, including anti-inflammatory, anti-bacterial, and anti-oxidant activities (Dias `*et al.*, 2021) [14]. *Salix* species possess a significant nutraceutical potential due to their rich content of bioactive compounds which exhibit anti-inflammatory, anti-oxidant, neuroprotective, and other health-promoting properties (Gligoric `*et al.*, 2019) [23]. Environmental stresses such as heavy metal exposure, salinity, and drought can significantly alter the phytochemical content of *Salix* (willow) species, leading to increased accumulation of certain compounds like flavonoids and phenolic acids, which act as a defence mechanism against the stress (Bajraktari `*et al.*, 2022; Khan `*et al.*, 2023) [7, 34]. Naturally, *Salix* trees have developed various mechanisms to fight against the toxic impact of environmental pollution by producing secondary metabolites. These secondary products play an imperative role in the adaptation and interacting with the ecosystem (Bourgaud `*et al.*, 2001) [11], such as through the activation of anti-oxidant systems to neutralize reactive oxygen. That is why plants are usually recognized for their anti-oxidant activities due to the accumulation of polyphenols in them (Bajraktari `et al., 2022) [7]. Studies have shown that different plant species possess rich sources of a diverse range of phytochemicals that have various biological potentials for anti-microbial and anti-oxidant effects (Gupta `et al., 2016; Javed `et al., 2021) [30, 33]. Phytochemicals with nutraceutical properties can be utilized to promote health, deter chronic diseases, slowing the process of aging, increase life expectancy or support the function or structure of the body (Nasri `et al., 2014) [44]. A nutraceutical is a food substance that provides medical or health benefits, including the prevention and treatment of disease (Prakash `et al., 2012) [49]. Simply 'nutraceutical,' composed of two words: nutrient and pharmaceutical, is a food supplement that play an important role in maintaining the healthy body and provides necessary supplements required for various metabolic processes to regulate body functions and thus preventing the body from diseases (Chanda `et al., 2019) [12]. Nutraceuticals produced by plants are generally categorized based of biosynthetic pathways into alkaloids, polyphenols, flavonoids, terpenoids and vitamins, playing a vital role in healthy foods (Alharbi `et al., 2022) [4]. These nutraceuticals are primarily associated with the amount of diverse phytochemicals, chiefly anti-oxidants and dietary fibre found in plant products. Moreover, plant nutraceuticals have a variety of bioactive constituents that exhibit their activity at the systemic stage by interacting with various targets in different tissues, even though the major effects are accomplished because of anti-oxidant, anti-proliferative, anti-inflammatory, anti-microbial, and hypocholesterolemic activities (Taroncher `et al., 2021) [58]. Among the phytochemicals, potential ones offering health benefits are alkaloids, flavonoids, polyphenols, isoflavonoids, phytoestrogens, anthocyanidins, terpenoids, saponins, carotenoids, limonoids, glucosinolates, phytosterols, and fibers (Prakash `et al., 2012; Gulzar `et al., 2024) [49, 29].

Over 400 plant species, including *Salix* species, have been identified to produce valuable phytocompounds which could be utilized in the pharmaceutical industry (Gligoric `et al., 2019; Agidew, 2022) [23, 1]. These phytochemicals contribute to their traditional uses and growing interest in modern pharmacology (Qadir `et al., 2020) [52]. Willows (*Salix* species) growing under diverse habitats of Kashmir valley from dry to moist habitats including riparian habitats and wetlands are promising, having multipurpose benefits (Malik `et al., 2020) [37]. A recent report revealed that *Salix* species have been demonstrated for anti-oxidant, anti-inflammatory, anti-arthritic, antipyretic, analgesic, and astringent properties clinically (Shivatare `et al., 2015; Chanda `et al., 2019) [55, 12]. Moreover, willow produces an original form of Salicin, a forerunner of an important modern drug, aspirin, having anti-oxidant, anti-pyretic and analgesic effects (Shivatare `et al., 2015) [55]. As per the latest study by Malik `et al. (2020) [37], there are 30 and 15 *Salix* species reported in India and Kashmir valley respectively. While in UT Jammu & Kashmir and Ladakh, out of the total 18 *Salix* species: 8 species are found abundantly in Kashmir valley, 4 species are confined to Ladakh Himalayas, and the remaining 6 species are common to both regions. Moreover, nearly 50 lakh willow trees are growing, which contributes 16 per cent of total broadleaf species in Kashmir valley. However, plant-derived nutraceuticals have received international recognition for their perceived safety and significant therapeutic and nutritional properties (Pandey `et al., 2011) [46]. Providentially, people have been growing *Salix* species in their surrounding environments to traditionally cure various ailments (Malik `et al., 2011) [38]. The process of synthesis and accumulation of these valuable bioactive compounds in plants are intricately linked to different environmental conditions, including climatic factors such as temperature, precipitation, and humidity, as well as crucial soil properties like nutrient availability, pH, and organic carbon content (Marschner, 2012; Prinsloo & Nogemane, 2018) [40, 50]. However, the specific impact of localized environmental conditions on the phytochemical composition of *Salix* species, especially in regions with unique ecological characteristics, remains an area requiring further investigation. This investigation aims to conduit this knowledge gap by assessing the nutraceutical potential and biological activity of *Salix alba* and *Salix fragilis* growing in Kashmir Himalayas. This research also provides a valuable insight into how to optimize the sustainable application of these plant resources for health and economic benefits.

## Materials and Methods

### Study area and sampling

The present study was conducted in Srinagar city of the Union Territory of Jammu and Kashmir. The city is located between 74º47´24´´ East longitude and 34º5´24´´ North latitude at an elevation of 1,585 meters above M.S.L. It is the largest city in the Kashmir Himalayas, lying along the Jhelum River banks and the shores of Anchar and Dal lakes, in between the Shankaracharya and Hari Parbat hills. After Kathmandu (capital of Nepal), Srinagar is the second largest metropolitan region in the Himalayas covering a total area of 1,979 sq. km (Census of India, 2011). There are a number of lakes and wetlands such as Dal, Nigeen, Khushal Sar, Gil Sar, Anchar, and Hokersar, and it also has a big dumping site, which is located at Achan. However, these aquatic ecosystems are polluted due to unscientific disposal of municipal wastes, biological wastes, hospital drainage and industrial effluents. The study area comprised 11 different polluted sites ($S_1$-$S_{12}$) and one unpolluted site ($S_0$): $S_1$= Anchar Lake, $S_2$= Sangam Lake, $S_3$= Khushal Sar, $S_4$= Tailbal, $S_5$= Baba Demb, $S_6$= Main Dal Lake, $S_7$= Cell no. 1 (Achan), $S_8$= Cell no. 2 (Achan), $S_9$= Cell no. 3 (Achan), $S_{10}$= Chinar colony, $S_{11}$= Zainakote, $S_{12}$= Hokersar wetland, and $S_0$=Harwan Sarband, a fresh water reservoir (served as a control) (Table 1).

The present experiment was performed at Nutrigenomics Laboratory, Division of Basic Sciences and Humanities, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, Kashmir. Both plant and soil samples were collected from selected sites of the Himalayan Kashmir (Table 1); the plant material, however, comprised of leaf, bark, and root of two *Salix* species, namely: *Salix alba* and *Salix fragilis*.

### Phytochemical analysis

Phytochemical investigation of the methanolic extract of plant species was investigated for the presence of various nutraceuticals.

### Preparation of methanolic extract

One gram of dried, ground plant material was weighed and crushed in 80% aqueous methanol until the residue became colorless. The extract was filtered and then, filtrate was dried using a water bath. The resultant dried extract was weighed and diluted with distilled water in a test tube to 10 ml mark for presence of nutraceuticals as:

**Alkaloids**

Wagner's test was employed to detect alkaloids. In this test, 2 ml of 1 M HCl and 2 ml of methanolic extract was added to a test tube and filtered. The Wagner's reagent was formulated by dissolving iodine in potassium iodide and the prepared filtrate was treated with it. The appearance of brown to reddish precipitate signified the presence of alkaloids in the plant extract (Banu & Cathrine, 2015) [8].

**Flavonoids**

NaOH test was employed for flavonoids. 2 ml of methanolic extract was taken in a test tube, then a few drops (100-200 µL) of 10% aqueous NaOH solution was added to it. The appearance of an intense yellow color that became colorless upon addition of a few drops of dilute HCl exhibited the presence of flavonoids.

**Sterols**

Salkowski test was employed to screen for sterols, in which 2 ml chloroform was added to 2 ml of methanolic extract. After this, with glass dropper 2 ml of concentrated $H_2SO_4$ was added and thoroughly shaken to mix the contents. The chloroform layer formed red while the acid layer appeared greenish-yellow fluorescence, indicating the presence of sterols (Banu & Cathrine, 2015) [8].

**Saponins**

Frothing test was used to detect saponins, in which 2 ml of methanolic extract was diluted with two volumes of water in a test tube and shaken for 5 minutes. The appearance of stable honeycomb froth was taken as indication for the presence of saponins (Banu & Cathrine, 2015) [8].

**Tannins**

Ferric chloride enol method was used to detect tannins. In this method, 2 ml of distilled water and 2 ml of methanolic extract were taken in a test tube. The immediate formation of a green precipitate after the addition of a few drops of 5% aqueous ferric chloride ($FeCl_3$) solution was taken as a positive sign for the presence of tannins (Banu & Cathrine, 2015) [8].

**Climate data**

The study utilized climate data acquired from the Indian Meteorological Department (IMD) located in Srinagar, Jammu and Kashmir, India. The data included monthly mean records of maximum temperature, minimum temperature, precipitation, and relative humidity. This dataset spanned the period from 2013 to 2024. The IMD is the primary agency responsible for meteorological observations and weather forecasting in India, ensuring the reliability and accuracy of the data.

**Soil analysis**

The soil was taken from beneath the canopy of sampled willow trees and adjacent fallow lands at depth of 0-30 cm with three replicates were sealed in air tight bags and transported to the laboratory. These soil samples were air dried, ground in a wooden pestle and mortar, passed through a 2 mm sieve, and then subjected to the following analysis: Alkaline Potassium Permanganate Method of Subbiah & Asija (1956) was utilized to determine the available nitrogen. Available phosphorus was analysed by the Method of Olsen & Sommers (1982). For potassium estimation, Flame Photometer Method (Jackson, 1973) [66] was used. Soil pH was assessed in a 1: 2.5 (soil: water suspension), with the help of pH meter (Jackson, 1973) [66] and the organic carbon content by the Method of Walkley and Black (1934).

**Statistical analysis**

The data acquired from the qualitative phytochemical screening of *Salix* plant extracts was analyzed descriptively based on the visual intensity of color changes observed during the respective tests. The quantitative data for soil properties (available nitrogen, phosphorus, potassium, pH, and organic carbon) were subjected to one-way Analysis of Variance to analyse significant variations between the sampling sites. Where significant differences were identified, Least Significant Difference (LSD) test was done to detect which site mean was significantly different at a probability level of $p \leq 0.05$.

**Results**

**Nutraceutical potential of *Salix* plant extracts**

The results related to the presence of phytochemicals in the leaf, bark and root of selected Salix plant extracts and detected by significant color changes are summarized in the Table 2 and Table 3. It was found that samples of both *Salix alba* and *Salix fragilis* collected from different sites possessed alkaloids, flavonoids, sterols, saponins and tannins as phyto-constituents.

The results pertaining to the presence of alkaloids in the leaf, bark, and root of *Salix alba* and *Salix fragilis* demonstrated a moderate presence of alkaloids, as represented in the Table 2 and Fig 1a.

A high presence of flavonoids was found in both screened plants, as depicted in table 2 and Fig 1b. However, among various plant parts, roots displayed a very high presence of flavonoids in both species collected from different sites.

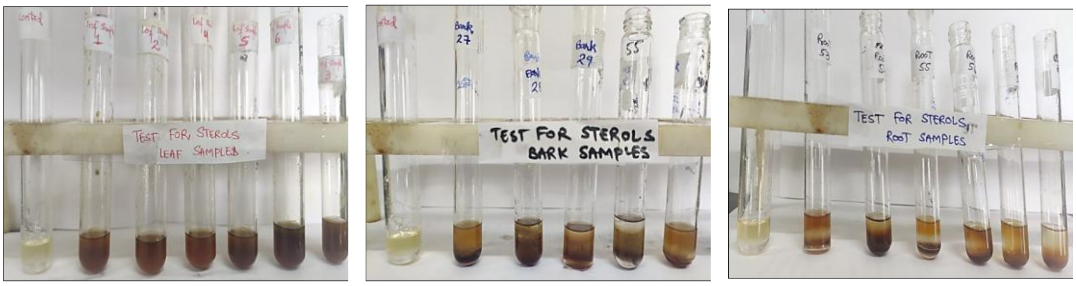
The data regarding sterols revealed that among the two selected species, *Salix fragilis* showed an appreciable presence of sterols in the bark, whereas *Salix alba* showed a nearly equal presence of sterols in both the leaves and bark (Table 2 & Fig 1c).

Like alkaloids, a moderate presence of saponins was observed in both *Salix alba* and *Salix fragilis* as shown in the Table 3 and Fig 1d.

A high presence of tannins was observed in the roots of both *Salix* species, as presented in the Table 3 and Fig 1e.

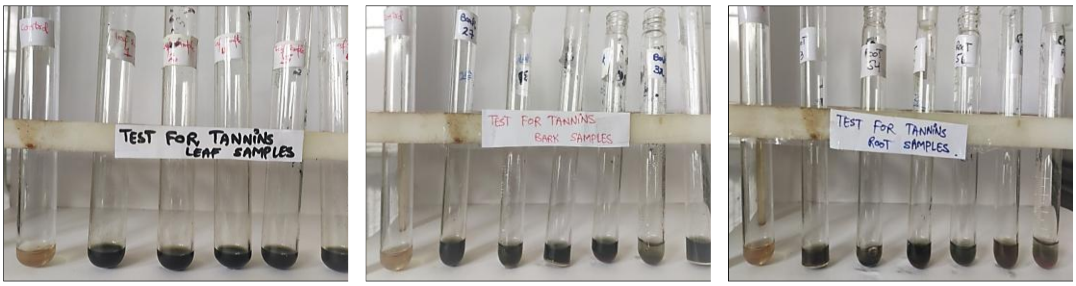

**a. Alkaloids**


**b. Flavonoids**




**c. Sterols**


**d. Saponins**




**e. Tannins**

**Fig 1:** Various phytochemicals among the leaf, bark and root of *Salix* species collected from different sites.

## Environmental factors

### Climate data

The monthly mean maximum temperature, minimum temperature, precipitation, and relative humidity in Kashmir Himalayas from 2013 to 2024 (Fig 2), revealing seasonal patterns and inter-annual variations for each climate variable as follows:

### Maximum and minimum temperatures

The monthly mean maximum temperature recorded in a study area from 2013 to 2024 is depicted in Fig 2a, varying from 4.2°C (Jan, 2017) to 31.9°C (July, 2024). Temperatures generally follow a seasonal pattern, with the lowest temperatures occurring in December and January, and the highest temperatures typically in May or June. There is some year-to-year variability in the monthly temperatures. For example, 2017 shows relatively lower temperatures in January compared to other years, while 2024 has a notably warmer January. Similar to the maximum temperatures, there's a distinct seasonal cycle in terms of monthly minimum temperatures, varying from -4.6°C (Jan, 2021) to 19.7°C (Aug, 2024) (Fig 2b). The coldest monthly minimum temperatures occur in December and January, while the warmest are typically in July and August. Minimum temperatures in December and January often drop below freezing (0°C), reaching lows of -4.6°C in 2021 and -3.6°C in 2018.

### Precipitation

The monthly mean precipitation (mm) in the study area from 2013 to 2024 is demonstrated in Fig 2c. The area exhibits a distinct seasonal rainfall pattern, with the highest precipitation generally occurring during the summer monsoon months, typically July and August. There is significant year-to-year variability in precipitation for any given month. Similarly, other months show considerable fluctuations. For example, March precipitation ranges from a low of 5.1 mm in 2024 to a high of 294.6 mm in 2015. Similarly, November precipitation varies from 0 mm in multiple years to a high of 225.8 mm in 2019.

### Relative humidity
The relative humidity in the study area is generally quite high throughout the year, with most monthly averages staying above 60% (Fig 2d). Compared to temperature and precipitation, the monthly mean relative humidity shows less dramatic year-to-year variation. While there are some fluctuations, the overall patterns and ranges for each month remain relatively stable across the 12-year period. The consistently high relative humidity throughout the year suggests that the study location is likely to be influenced by significant source of moisture.

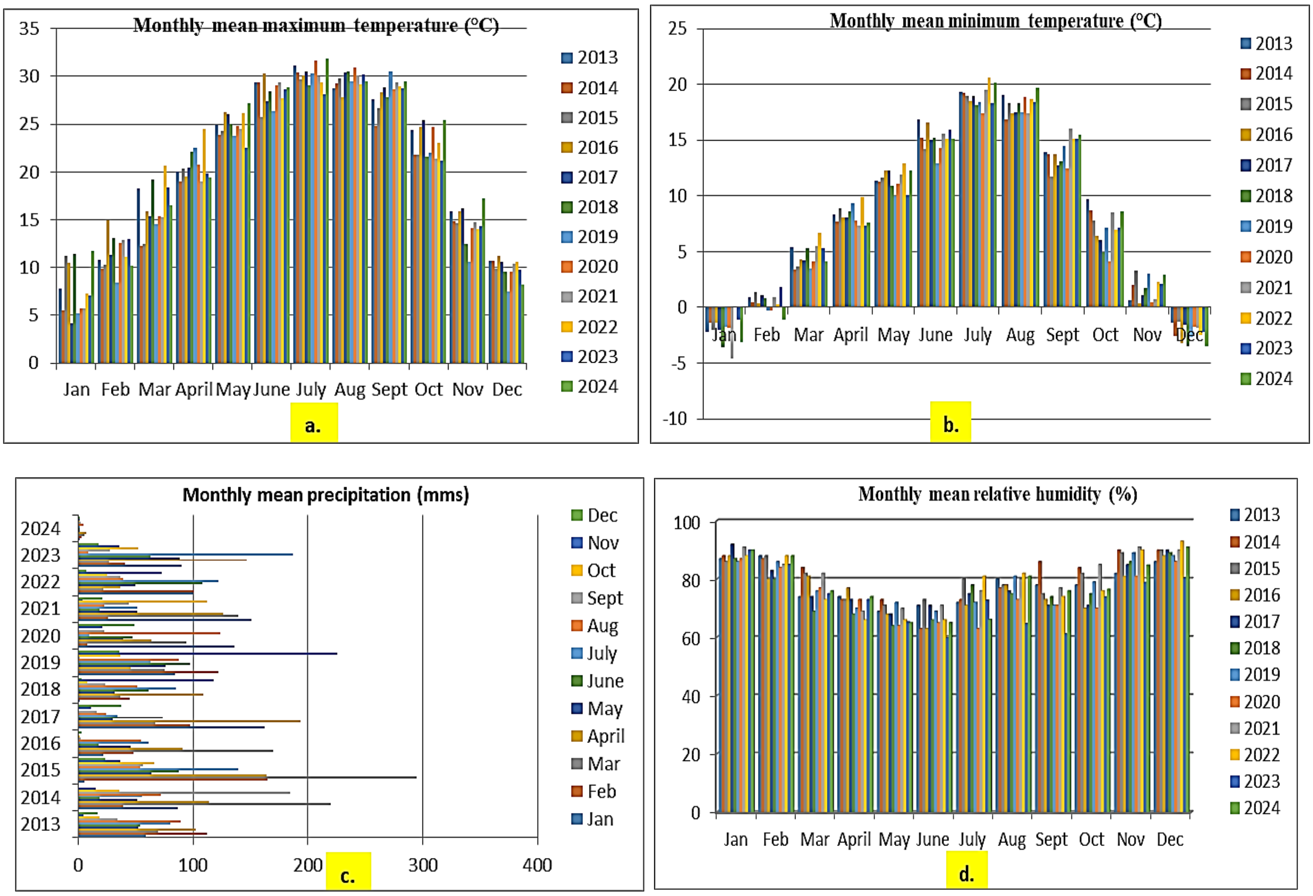



**Fig 2:** Representation of monthly mean maximum temperature, minimum temperature, precipitation, and relative humidity in Northwestern aHimalayas

### Soil properties
The soil properties varied considerably across the different sampling sites (Table 4), highlighting the significant variations in soil nutrients (available nitrogen (N), phosphorus (P), and potassium (K)), as well as pH and organic carbon (OC). Site $S_{11}$ appeared to have the highest levels of available phosphorus (57.73 kg/ha) and potassium (281.13 kg/ha) but one of the lowest levels of available nitrogen (275 kg/ha) and organic carbon (1.57%). Site $S_9$ had the highest available nitrogen (542.66 kg/ha) and high organic carbon (2.52%). Site $S_0$ had the highest pH (7.45-alkaline) but low levels of phosphorus (16.56 kg/ha) and organic carbon (1.81%). These variations in soil properties likely reflect differences in site characteristics, land utilization pattern, and other environmental conditions. The statistical significance of the differences in soil properties between sites, as indicated by the letters, suggests that these variations are likely to have a tangible impact on the phytochemical profiles of plants growing in these distinct locations.

### Discussion
This study investigated the nutraceutical potential of *Salix alba* and *Salix fragilis* from the Kashmir Himalayas, with a specific focus on how localized environmental conditions, encompassing climate and soil properties, influence their phytochemical profiles and antioxidant activity (Bourgaud `*et al.*, 2001) [11]. The findings reveal a complex interplay between species, plant parts, and environmental factors in determining the accumulation of bioactive compounds.

### Variation in phytochemical composition
Alkaloids are biologically active heterocyclic basic chemical constituents containing nitrogen atom (Aniszewski, 2015) [6]. They comprise about 20% of all the secondary plant metabolites and constitute an important part in an organism's natural defence and human medicine. In plants, it promotes growth and development and provides protection against predators (Heinrich `*et al.*, 2021) [32]. The results pertaining to the presence of alkaloids in the leaf, bark and root of *Salix alba* and *Salix fragilis* demonstrated their moderate presence of the alkaloids. These results are in

parallel to the findings obtained by Qadir *`et al.* (2020) [52], Singh & Srivastava (2022) [57] and Gandhi *`et al.* (2023) [20] who have done preliminary phytochemical screening of methanolic extracts of *Salix* species and demonstrated the presence of alkaloids, terpenoids, flavonoids, tannins and proteins. Similar studies demonstrated that alkaloids and saponins are the appreciable secondary products in all crude *Salix* extracts (Gul *`et al.*, 2017 [28]; Marami *`et al.*, 2021 [39]; Agidew, 2022 [1]; Dou *`et al.*, 2022 [15]; Dubale *`et al.*, 2023) [17], which are in concordance with the present study results.

Flavonoid is a subclass of polyphenols group, made up of two aromatic rings with each having at least one hydroxyl group and is responsible for color, flavour and fragrance properties in plants (Beecher, 2003) [10]. They perform an essential function in plants by promoting cell growth and provide protection against environmental stresses including heavy metal exposure (Khan *`et al*., 2023) [34]. In humans, these act as anti-bacterial, anti-fungal, anti-inflammatory, anti-oxidant and anti-viral agents (Dias *`et al.*, 2021) [14]. The results exhibited a high presence of flavonoids in both *Salix* species. However, among various plant parts, roots showed very high presence of flavonoids in the both species collected from different sites. These studies are in accordance with Piatczak *`et al*. (2020) [47], Qadir *`et al.* (2020) [52], Aleman *`et al*. (2023) [3] and Gligoric *`et al.* (2023) [24] who have detected total flavonoids in leaf and bark of *Salix alba* and *Salix fragilis*. However, Gawlik-Dziki *`et al.* (2014) [21], Warminski *`et al.* (2021) [64] and Curtasu & Norskov (2024) [13] reported that *S. fragilis* shows high presence of flavonoids is in line with present study findings. However, among various plant parts, bark and roots showed high presence of flavonoids, this may be due to seasonal or environmental factors and developmental stages of plants (Tyskiewicz *`et al.*, 2019 [62]; Kohler *`et al*., 2020 [35]; Bajraktari *`et al.*, 2022 [7]; Dubale *`et al.*, 2023) [17].

Sterols are tri-terpenes which are based on the cyclo-pentane per hydrophenanthrene ring system (Goad & Akihisa, 2012) [25]. In plants, they play an essential part in many biochemical and physiological processes during development and stress resistance (Du *`et al*., 2022) [16]. They sustain the structure of cell membranes in living organisms and regulate life processes (Dufourc, 2008) [18]. The data regarding sterols revealed that among the two selected species, *Salix fragilis* showed an appreciable presence of sterols in bark whereas, *Salix alba* showed nearly equal presence of sterols in both the leaves and bark. The differences in sterol composition may be attributed to effortless hybridization of *Salix* species (Tienaho *`et al.*, 2021; Gandhi *`et al.*, 2023) [59, 20]. Similar investigations undertaken for the preliminary nutraceutical screening of methanolic extracts of *Salix* species revealed the presence of sterols (Qadir *`et al.*, 2020 [52]; Agidew, 2022 [1]; Dubale *`et al*., 2023) [17] confirms the present study results.

Saponins are generally glycosidic (with five sugar units) triterpenoids and soap like in nature (Vincken *`et al.*, 2007) [63]. They play diverse ecological functions in plants for instance protection and defense against herbivores and diseases (Mugford & Osbourn, 2012) [42]. The data related to sterols demonstrated a moderate presence of saponins in both *Salix alba* and *Salix fragilis*. This result is in congruence with those obtained by Qadir *`et al.* (2020) [52], Agidew (2022) [1] and Dubale *`et al.* (2023 [17]), who have detected the presence of saponins in *Salix* plants and revealed their varied content among species due to environmental factors and growth stage of the plant (Tienaho *`et al.*, 2021) [59]. Moreover, Marami *`et al.* (2021) and Dou *`et al.* (2022) [39, 15] reported saponins as the abundant secondary products in all crude extracts of *Salix* plants.

Tannins are polymeric phenols (water-soluble polyphenols) derived from flavonoid units (Sieniawska & Baj, 2017) [56]. They are important deterrents to herbivores and play a significant role in medicine as well as human health (Tong *`et al.*, 2022) [60]. The results related to tannins showed its high presence in roots of both the selected Salix species. This finding is in accordance with studies (Gawlik-Dziki *`et al.*, 2014 [21]; Tyskiewicz *`et al.*, 2019 [62]; Qadir *`et al.*, 2020 [52]; Agidew, 2022 [1]; Aleman *`et al.*, 2023 [3]; Dubale *`et al.*, 2023 [17]; Gandhi *`et al.*, 2023) [20] which revealed that tannins comprise the major compounds of secondary plant metabolites in *Salix* species.

**Impact of climatic conditions on phytochemical composition**

The analysis of monthly mean maximum and minimum temperatures, precipitation, and relative humidity from 2013 to 2024 in the Kashmir Himalayas reveals distinct environmental patterns that likely exert significant influence on the phytochemical composition of *Salix* species. The observed seasonal temperature fluctuations, with cold winters and relatively warm summers, create a dynamic thermal environment that can impact plant growth, development, and the biosynthesis of secondary compounds (Prinsloo & Nogemane, 2018; Qaderi *`et al.*, 2023) [50, 51]. The wide temperature range, particularly the drop below freezing during winter months, may trigger specific stress responses, potentially causing accumulation of cryoprotective compounds or other such phytochemicals in plants (Zahra *`et al.*, 2021; Semmar, 2024) [65, 54]. Plants respond to fluctuations in temperature, water availability, and humidity by adjusting their metabolic processes, including the synthesis of stress-response compounds that have anti-oxidant effects (Khan *`et al.*, 2023; Bajraktari *`et al.*, 2022) [34, 7].

The precipitation data indicates a seasonal pattern with a tendency for higher rainfall during the summer monsoon. However, the substantial inter-annual variability highlights the potential for water stress or surplus conditions in different years. Water availability is a crucial factor affecting plant metabolism and the production of secondary compounds that possess anti-oxidant properties (Prinsloo & Nogemane, 2018) [50]. Drought stress, for instance, have shown enhanced accumulation of certain phenolic groups and essential oils in many plant species (Albergaria *`et al.*, 2020) [2]. Conversely, excessive moisture can influence nutrient uptake and potentially dilute the concentration of some phytochemicals and their biological activity (Lower & Orians, 2003) [36].

The consistently high relative humidity throughout the year suggests a moisture-rich environment. High humidity can affect transpiration rates, enzyme activity, and the overall physiological processes in plants, ultimately influencing the production and accumulation of phytochemicals (Amin *`et al.*, 2024) [5]. The relatively stable humidity levels compared to temperature and precipitation might indicate a more consistent influence on certain aspects of plant metabolism (Feller & Vaseva, 2014) [19].

The inter-annual variations observed in all climatic parameters underscore the complexity of the environmental factors influencing plant phytochemistry and antioxidant activity (Gololo, 2018) [26]. These fluctuations can lead to year-to-year differences in the amount and quality of bioactive compounds in aromatic and medicinal plants found in the North-western Kashmir Himalayas. Understanding these relationships is important for optimizing harvesting times, cultivation practices, and ensuring the consistent quality of plant-based products from this region.

Further research could explore the direct correlations between the observed climatic variations and the specific phytochemical profiles and biological activity of key plant species in the Kashmir Himalayas. Additionally, investigating the impact of extreme weather events, which are not explicitly captured by monthly means, on plant phytochemistry would deliver a thoughtful understanding of the environmental influences in this ecologically significant region.

**Impact of soil properties on phytochemicals**

The observed variations in soil properties are likely to play a vital part in influencing the composition of phytochemicals in plant species, found in these diverse habitats. Soil characteristics such as nutrient availability (nitrogen, phosphorus, potassium), pH, and organic carbon content are well-established factors affecting plant growth, metabolism, and the synthesis of secondary plant metabolites, which include various phytochemicals and their anti-oxidant effects (Marschner, 2012; Oliveira *`et al.*, 2023) [40, 43].

The significant differences in available Nitrogen (N) across the sites, with Site $S_9$ exhibiting the highest levels and Sites $S_{11}$ and $S_6$ the lowest, can have profound effects on plant primary and secondary metabolism. Nitrogen is a key element of amino acids, nucleic acids, and proteins, directly influencing plant growth, development and biomass production (Ohyama, 2010) [45]. However, the availability of nitrogen can also impact the synthesis of specific phytochemicals and their anti-oxidant activities. For instance, low nitrogen availability sometimes enhances the productivity of a few phenolic compounds in some plant species as a stress response (Gershenzon, 1984) [22].

Similarly, the variability in available Phosphorus (P), with Site $S_{11}$ showing the highest and Site $S_0$ the lowest levels, is critical for energy transfer, root development, and the biosynthesis of various metabolic intermediates. Phosphorus deficiency can limit overall plant growth but may also lead to the accretion of specific secondary plant metabolites, such as anthocyanins, in some plant species (Rengel & Marschner, 2005) [53].

Potassium (K), which was found in the highest amounts at Site $S_{11}$ and the lowest at Site $S_4$, plays a vital part in enzyme activation, osmotic regulation, and the transport of assimilates (Hasanuzzaman *`et al.*, 2018) [31]. Adequate potassium levels are mostly associated with enhanced overall plant health and can influence the biological activity and production of secondary plant products like alkaloids and terpenoids (Trankner *`et al.*, 2018) [61].

Soil pH, which ranged from acidic (Site $S_1$) to slightly alkaline (Site $S_0$), can significantly affect nutrient availability and uptake by plants (Barrow & Hartemink, 2023) [9]. Extreme pH values can limit the solubility of essential nutrients, thereby indirectly influencing phytochemical synthesis and biological activity (Prabhudev *`et al.*, 2023) [48]. For instance, acidic soils can enhance the availability of some micronutrients but decrease the availability of others, potentially altering metabolic pathways (Gondal *`et al.*, 2021) [27].

Organic carbon content, an indicator of soil fertility and microbial activity, also varied across the sites, with the highest levels observed in Sites $S_1$, $S_5$, $S_8$, $S_9$, and $S_{10}$, and the lowest in Site $S_{11}$ and $S_0$. Organic carbon influences soil structure, water retention, and nutrient cycling, all of which can indirectly affect the production of phytochemicals by influencing overall plant health and stress responses (Martins-Noguerol *`et al.*, 2023) [41].

Soil pH also varied significantly across sites, ranging from highly acidic to alkaline. These differences in nutrient availability and soil acidity are known to influence plant metabolic pathways, potentially leading to differential synthesis and accumulation of phytochemicals like flavonoids and phenolics, which directly contribute to antioxidant activity. For example, nutrient stress or specific soil compositions can induce the production of secondary metabolites as a defence mechanism (Marschner, 2012; Prinsloo & Nogemane, 2018) [40, 50].

Future investigation should aim to directly correlate the phytochemical composition of specific plant species found at these sites with the measured soil properties to elucidate the specific relationships and identify key soil factors driving the production of important bioactive compounds that possess biological activity in the Kashmir Himalayas. Understanding these links is crucial for maximizing the sustainable utilization and conservation of the region's rich medicinal and aromatic plant resources.

**Conclusion**

Alkaloids, flavonoids, sterols, saponins and tannins were the main phyto-constituents found in *Salix alba* and *Salix fragilis* collected from different sites. Between the two selected species, *Salix fragilis* demonstrated high overall composition of nutraceuticals and have potential to be used in pharmaceutical industries as source of bioactive compounds. Crucially, this study underscores the profound impact of environmental attributes on the phytochemical composition of *Salix* species. The distinct seasonal and inter-annual variations in climatic parameters (temperature, precipitation, and relative humidity) in the Kashmir Himalayas likely contribute to the observed fluctuations in secondary metabolite biosynthesis, possibly triggering stress responses that enhance the production of certain compounds that possess anti-oxidant activity. Furthermore, the significant heterogeneity in soil properties across the sampled sites, including nutrient levels, pH, and organic carbon content, directly correlated with differences in plant health and, consequently, their phytochemical profiles. Sites with varying levels of essential nutrients and diverse pH conditions appeared to modulate the sequestration of specific bioactive compounds. The study highlights the potential impact of environmental attributes on the nutraceutical potential of plants and provides a valuable contribution to the understanding of *Salix* phytochemistry and its ecological significance in Kashmir valley. Different plant parts exhibit variations in phytochemical composition, signifying their specific functions and responses to environmental stresses.

In essence, our findings not only confirm the rich phytochemical diversity of *Salix alba* and *Salix fragilis* in this region but also provide critical insights into how localized environmental stressors and resource availability shape their chemical makeup and the consequent biological properties. This understanding is vital for developing effective strategies for the sustainable harvesting and cultivation of these medicinal plants. By considering both climatic variability and soil characteristics, we can optimize the production of nutraceutically rich *Salix* resources, ultimately contributing to their potential utilization in therapeutic applications and the economic benefit of the Kashmir Himalayas. Furthermore, studies should be undertaken to isolate and detect the specific compounds responsible for the perceived indifferences between the different *Salix* species and across habitats. Also research is required to study the impacts of various environmental stress (e.g., heavy metal pollution) on the phytochemical composition and ecological functions of willows species.

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**Table 1:** Description of various sampling sites under study area

| S. No. | Sampling location | Site name | Latitude | Longitude | Altitude (m) | Habitat type |
|---|---|---|---|---|---|---|
| 1. | $S_0$ | Harwan | 34°09´33.8´´ | 74°54´37.6´´ | 1692 | Fresh water reservoir |
| 2. | $S_1$ | Anchar | 34°08´32.6´´ | 74°47´41.7´´ | 1596 | Anchar & Khushalsar Lake |
| 3. | $S_2$ | Sangam | 34°07´36.6´´ | 74°46´53.2´´ | 1589 | |
| 4. | $S_3$ | Khushal Sar | 34°06´48.7´´ | 74°48´08.9´´ | 1604 | |
| 5. | $S_4$ | Tailbal | 34°09´34.5´´ | 74°51´55.1´´ | 1595 | Dal Lake |
| 6. | $S_5$ | Baba Dam | 34°05´13.8´´ | 74°49´05.7´´ | 1581 | |
| 7. | $S_6$ | Main Dal | 34°06´13.4´´ | 74°50´12.9´´ | 1579 | |
| 8. | $S_7$ | Cell no. 1 | 34°07´28.9´´ | 74°47´19.6´´ | 1540 | Dumping site |
| 9. | $S_8$ | Cell no. 2 | 34°07´35.1´´ | 74°47´19.3´´ | 1578 | |
| 10. | $S_9$ | Cell no. 3 | 34°07´32.2´´ | 74°46´57.7´´ | 1584 | |
| 11. | $S_{10}$ | Chinar Colony | 34°06´28´´ | 74°43´30.6´´ | 1586 | Wetland (Ramsar site) |
| 12. | $S_{11}$ | Zainakote | 34°06´28.1´´ | 74°43´44.6´´ | 1594 | |
| 13. | $S_{12}$ | Main Hokersar | 34°06´33.0´´ | 74°43´09.9´´ | 1595 | |

**Table 2:** Phytochemical screening of the leaf, bark and root extracts of selected *Salix* species collected from different sites

| Parameter | Alkaloids | | | | | | Flavonoids | | | | | | Sterols | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | *Salix alba* | | | *Salix fragilis* | | | *Salix alba* | | | *Salix fragilis* | | | *Salix alba* | | | *Salix fragilis* | | |
| Sites | Leaf | Bark | Root | Leaf | Bark | Root | Leaf | Bark | Root | Leaf | Bark | Root | Leaf | Bark | Root | Leaf | Bark | Root |
| Control | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $S_0$ | + | + | + | + | + | + | + | +++ | ++ | ++ | +++ | +++ | + | ++ | + | ++ | ++ | ++ |
| $S_1$ | + | + | + | + | + | + | + | + | +++ | + | ++ | + | ++ | ++ | + | ++ | ++ | ++ |
| $S_2$ | + | + | + | + | + | + | + | + | +++ | + | ++ | ++ | ++ | ++ | + | + | ++ | + |
| $S_3$ | + | + | + | + | + | + | + | + | +++ | + | ++ | +++ | + | + | + | + | ++ | + |
| $S_4$ | + | + | + | + | + | + | + | + | ++ | + | ++ | +++ | + | + | + | + | ++ | + |
| $S_5$ | + | + | + | + | + | + | + | + | +++ | + | ++ | +++ | ++ | ++ | + | ++ | ++ | + |
| $S_6$ | + | + | + | + | + | + | + | + | ++ | + | ++ | +++ | ++ | ++ | + | + | ++ | + |
| $S_7$ | + | + | + | + | + | + | + | ++ | +++ | + | ++ | +++ | ++ | + | + | + | ++ | + |
| $S_8$ | + | + | + | + | + | + | + | ++ | +++ | + | ++ | +++ | + | + | + | + | ++ | + |
| $S_9$ | + | + | + | + | + | + | + | ++ | +++ | + | ++ | +++ | + | ++ | ++ | + | ++ | + |
| $S_{10}$ | + | + | + | + | + | + | + | + | ++ | + | ++ | +++ | ++ | ++ | + | + | ++ | ++ |
| $S_{11}$ | + | + | + | + | + | + | + | ++ | + | ++ | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| $S_{12}$ | + | + | + | + | + | + | + | + | ++ | + | ++ | +++ | ++ | ++ | ++ | + | + | ++ |

* +=Moderate, ++= high and +++= very high significant visibility of color change

**Table 3:** Phytochemical screening of the leaf, bark and root extracts of selected *Salix* species collected from different sites

| Parameter | Saponins | | | | | | Tannins | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | ***Salix alba*** | | | ***Salix fragilis*** | | | ***Salix alba*** | | | ***Salix fragilis*** | | |
| **Sites** | **Leaf** | **Bark** | **Root** | **Leaf** | **Bark** | **Root** | **Leaf** | **Bark** | **Root** | **Leaf** | **Bark** | **Root** |
| Control | - | - | - | - | - | - | - | - | - | - | - | - |
| $S_0$ | + | + | + | + | + | + | ++ | + | ++ | ++ | + | ++ |
| $S_1$ | + | + | + | + | + | + | ++ | ++ | +++ | ++ | + | +++ |
| $S_2$ | + | + | + | + | + | + | ++ | ++ | ++ | ++ | + | ++ |
| $S_3$ | + | + | + | + | + | + | ++ | + | ++ | ++ | + | ++ |
| $S_4$ | + | + | + | + | + | + | + | + | ++ | ++ | + | ++ |
| $S_5$ | + | + | + | + | + | + | + | + | + | + | + | ++ |
| $S_6$ | + | + | + | + | + | + | ++ | + | ++ | ++ | + | ++ |
| $S_7$ | + | + | + | + | + | + | ++ | ++ | +++ | ++ | ++ | ++ |
| $S_8$ | + | + | + | + | + | + | ++ | ++ | ++ | ++ | ++ | ++ |
| $S_9$ | + | + | + | + | + | + | ++ | ++ | ++ | ++ | ++ | ++ |
| $S_{10}$ | + | + | + | + | + | + | ++ | ++ | ++ | ++ | + | ++ |
| $S_{11}$ | + | + | + | + | + | + | ++ | ++ | ++ | ++ | + | ++ |
| $S_{12}$ | + | + | + | + | + | + | ++ | ++ | ++ | ++ | ++ | ++ |

* + =Moderate, ++= high and +++= very high significant visibility of color change

**Table 4:** The different soil properties across various selected sites in the Kashmir Himalayas

| Sites | Available N (kg/ha) | Available P (kg/ha) | Available K (kg/ha) | pH | OC (%) |
|---|---|---|---|---|---|
| $S_0$ | 370.00[e] | 16.56[k] | 46.65[hi] | 7.45[a] | 1.81[c] |
| $S_1$ | 484.66[b] | 49.80[c] | 68.71[g] | 5.57[f] | 2.48[a] |
| $S_2$ | 402.66[d] | 48.06[d] | 55.86[h] | 6.85[b] | 2.37[ab] |
| $S_3$ | 431.33[c] | 53.00[b] | 155.44[e] | 5.59[f] | 2.38[ab] |
| $S_4$ | 403.66[d] | 42.13[f] | 44.77[i] | 6.02[de] | 2.37[ab] |
| $S_5$ | 477.33[b] | 53.40[b] | 276.00[ab] | 5.55[f] | 2.48[a] |
| $S_6$ | 296.66[f] | 23.48[i] | 135.12[f] | 6.40[cd] | 2.36[ab] |
| $S_7$ | 496.00[b] | 23.60[i] | 128.20[f] | 5.58[f] | 2.37[ab] |
| $S_8$ | 495.00[b] | 38.46[g] | 253.66[c] | 5.58[f] | 2.52[a] |
| $S_9$ | 542.66[a] | 21.06[j] | 139.28[f] | 5.66[ef] | 2.52[a] |
| $S_{10}$ | 401.66[d] | 44.80[e] | 265.00[b] | 5.88[ef] | 2.51[a] |
| $S_{11}$ | 275.00[f] | 57.73[a] | 281.13[a] | 6.47[bc] | 1.57[c] |
| $S_{12}$ | 400.33[d] | 24.33[h] | 181.04[d] | 5.99[e] | 2.17[b] |
| **Mean** | 421.30 | 38.18 | 156.22 | 6.04 | 2.30 |
| **CD ($p \leq 0.05$)** | 25.02 | 0.61 | 11.08 | 0.38 | 0.28 |

a*CD ($p \leq 0.05$)-least significant difference at $p<0.05$

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