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COMPARATIVE ANALYSIS OF TRADITIONALLY USED MEDICINAL PLANTS FOR HEPATIC PROBLEMS FROM KUMAUN HIMALAYAS

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ABSTRACT

The Himalayan region is predominantly populated by individuals who live in communal settlements. These communities heavily depend on a wide range of plant resources for various purposes, including medicinal uses and other essential needs. In the present investigation, we conducted a comprehensive assessment of the comparative antioxidant efficacy within a specific set of medicinal plant species extracts. In the present study, firstly physoiochemical parameters were performed in both plants as shown in **Table 1**, the preliminary Phytochemical study for both extracts showed positive and negative results shown in **Table 2**. The total phenolics content in methanolic extract of *Cirisium wallichii* exhibited a total phenolics content of 59.25±0.0088 mg Gallic acid equivalent (GAE/g), while the methanolic extract of *Fraxinus micrantha* showed a total phenolics content of 136.97±0.0058 mg GAE/g. Additionally, the aqueous extract of *Cirisium wallichii* displayed a total phenolics content of 63.57±0.0088 mg GAE/g, whereas the aqueous extract of *Fraxinus micrantha* exhibited a total phenolics content of 122.81±0.0066 mg GAE/g. The flavonoid content was recorded in methanolic extract of *Cirisium wallichii*, as 128.28±0.0190 mg Quercetin equivalent while in the methanolic extract of *Fraxinus micrantha*, it was measured as 176.52±0.0017 mgQE/g. The aqueous extract of *Cirisium wallichii* had a flavonoid content of 96.72±0.0300 mgQE/g, whereas the aqueous extract of *Fraxinus micrantha* contained 149.49±0.0015 mgQE/g of flavonoids. The Tannin content was recorded in methanolic extract of *Cirisium wallichii* 24.57±0.0016 mg Tannic acid equivalent while in the methanolic extract of *Fraxinus micrantha*, it was measured as 35.49±0.0012 mgTAE/g. The aqueous extract of *Cirisium wallichii* had a Tannin content of 11.82±0.0017 mgTAE/g, whereas the aqueous extract of *Fraxinus micrantha* contained 29.67±0.0015 mgTAE/g of Tannins. The Antioxidant activity of both the extract showed the IC_{50} by spectrophotometric method by DPPH and ABTS free radical scavenging method. The aqueous extract of *Cirisium wallichii* and *Fraxinus micrantha* showed 109.65 µg/ml and 91.57µg/ml in methanolic 88.02µg/ml and 87.87µg/ml in DPPH. The radical scavenging effect of the aqueous extract of *Cirisium wallichii* and *Fraxinus micrantha* showed 120.19µg/ml and 120.00µg/ml, while in methanolic extract it was around 88.02µg/ml for *Cirisium wallichii* and 94.22µg/ml for *Fraxinus micrantha.* Both are found to be more potent than that of the synthetic antioxidant ascorbic acid. *Cirisium wallichii* and *Fraxinus micrantha* have demonstrated that an increased quantity of phenolic compounds correlates with a heightened efficacy in radical scavenging.

Keywords: Himalaya, Hepatoprotective, Uttarakhand, Antioxidant, Flavonoids, Phenolics

INTRODUCTION

Local healers predominantly utilized a vast array of plant species for traditional medicinal purposes, with a particular emphasis on procuring these botanical resources from remote and elevated regions. The utilization of plant components as medicinal agents is widely practiced by a significant portion of the global population. Notably, these botanical remedies are favored due to their perceived absence of adverse effects, in contrast to allopathic pharmaceuticals **[1]**. The Indian Himalaya is a haven for cultural and biological diversity, as well as a botanical paradise. Most people in the Himalaya live in villages and use plants for medicine, food, fodder, fire, timber, agricultural equipment, and a variety of other functions. Uttarakhand is India's newest Himalayan province and has been established as the 27th state of the Republic of India. The state is divided into two divisions, Kumaun and Garhwal, and has 13 districts. The Himalayas stretch for around 2500 kilometers from west to east in a huge arc. Kumaun is a district in Uttarakhand's hills that includes six districts: Nainital, U.S. Nagar, Bageshwar, Almora, Pithoragarh, and Champawat.

It's important to remember that medicinal plants contain numerous bioactive molecules, or phytochemicals, but only a small percentage of them are therapeutically useful. Furthermore, phytochemical yield is affected by plant parts (such as leaves, bark, flowers, fruits, roots, and seeds) and extraction methods **[2]**. A vast range of current natural medications are derived from phytochemicals found in medicinal plants. Traditional medical systems have a lot of advantages. Plant phytochemicals and derivative molecules are effective alternatives for boosting health, reducing illness, and reducing the risk of various diseases **[3, 4]**. Alkaloids, flavonoids, tannins, carbohydrates, glycosides, steroids, phenolics, and other families of chemical constituents are found in plants. Numerous investigations have been undertaken to confirm the efficacy of phytochemicals in the treatment of various pathological conditions, like diabetes, cancer, inflammation, neurodegenerative illnesses, malaria, bacterial infections, hepatic disorders, hypertension, etc. Some dyes, essential oils, and vegetable oils of plant origin is also extensively utilized in herbal preparations as effective medicines **[5, 6, 7]**.

Reactive oxygen species (ROS) are byproducts of regular oxygen metabolism that play an important role in signalling and homeostasis. However, under oxidative stress conditions, ROS levels can rise dramatically, causing damage to cellular proteins, lipids, and DNA, leading to fatal lesions such as ageing, cancer, and cardiovascular disease. Antioxidants can stop oxidation chain processes and suppress other oxidation reactions by eliminating free radical intermediates. Despite the fact that cells have complex systems of antioxidants such as glutathione, vitamin C, and vitamin A. Plant secondary metabolites with an aromatic ring containing at least one hydroxyl group are known as flavonoids and other phenolic substances. Over 8,000 phenolic compounds were detected in plants as naturally occurring chemicals. The two main enzymes for glucose metabolism are α-amylase and α-glucosidase. The breakdown of carbohydrate polymers into monomers is slowed when these enzymes are inhibited **[8, 9, 10]**.

This study aimed to evaluate the antioxidant capacities of two frequently utilized plant extracts from the Himalayan region. The assessment involved determining the levels of total phenolic compounds, flavonoids, and total tannins, as well as enzyme inhibitory activity. Additionally, the DPPH and FRAP assays were employed to measure the antioxidant potential of the selected plant extracts.

MATERIALS AND METHODS

- **Area descriptions**

The study area covers 10,192 Km^2. The survey was conducted in two districts, Almora and Pithoragarh, in the Kumaun Himalayas of Uttarakhand, during 2020–2022, in order to investigate medicinally important hepatoprotective herbs **(Figure 1)**. The Almora district lies between 29º 30' N to 30º 20' N latitudes and 79º 20' E to 80º 20' E longitudes, and Pithoragarh district is situated in the Soar valley in the Kumaun region of Uttarakhand. The district is located between latitude 29.4° N and longitudes 80° E and 81° E **[11, 12]**. A questionnaire was used to collect data from local residents. According to the literature, a standard approach was used for plant harvesting. During the flowering and fruiting seasons, field trips were made once a month. The samples were collected during each visit as per availability. The collected plants were dried to create herbaria.

Cirisium wallichii	***Fraxinus micrantha***

Figure 1: A map showing the specific locations within the study area

- **Collection of Plant Materials**

The fresh and healthy leaves of selected plants were collected between March and June from various sites in the Pithoragarh and Almora district of the Kumaun Himalaya, India. The plant specimens were identified in the Botany Department, D.S.B. Campus, Kumaun University, Nainital, and BSI Dehradun. Each specimen/plant material was labelled with the date of collection and locality, and their medicinal uses were recorded.

- **Plant extraction:**

The leaves of the identified plant were washed with distilled water and air-dried at room temperature (20±2°C). In an electric grinder, the dried material was crushed. 50 g of grounded powder was combined with 200 ml of solvents (w/v, 50 g/200 ml) in a 500 ml conical flask. To avoid evaporation, the mouths of the flasks were carefully closed with non-absorbent cotton and tightly wrapped in aluminium foil. Methanol and distilled water were used as extraction solvents. All mixes were shaken for 48 hours at 37 °C on a rotary incubator shaker at 190–220 rpm. The extract was filtered separately in a clean beaker after the mixtures were filtered through Whatman filter paper no. 1. The extracts were evaporated to dryness at 40 °C using a steam bath. The dried extracts were weighed and stored in sterile sample bottles at 4 °C in the refrigerator for further analysis.

- **Organoleptic Characteristics:** Organoleptic evaluation refers to the evaluation of the whole plant by colour, odour, taste, texture, touch, etc. **[13]**.
- **Physico-chemical parameter:** The various physicochemical parameters that were determined as per the guidelines include the following:

- **Moisture content**

In a previously weighed 100 ml beaker, 4 g of the sample was dried in an oven at 105°C for 5 hours. It was weighed after cooling in desiccators. The operation was repeated until the weight was constant. The sample's weight loss % was calculated **[14, 15]**.

- **Determination of Total ash value**

A precise 2 g sample was taken in a crucible dish. The material was equally distributed in a crucible and placed in a muffle furnace, which was heated up to 600^{o}C before being ignited. This process was repeated until the sample was free of carbon and turned white **[14, 15]**.

- **Determination of Acid insoluble ash value**

25 ml of 20% hydrochloric acid was added to the crucible dish containing the total ash and gently heated for 5 minutes while covered with a watch glass. The residue was washed

with hot water until the litmus became neutral. The insoluble material was recovered and re-placed in the same crucible for 6 hours until a constant weight was obtained. The residue was weighed after cooling in desiccators for 30 minutes **(Trease & Evans 1989; Kokate, 2014)**.

- **Determination of Water-soluble extractive value**

In a glass stopper flask, 4 g of the sample was taken, and 100 ml of distilled water was added. The flasks were shaken every 6 hours and then left to stand for 18 hours. The extract was filtered, and 25 ml of the filtrate was pipetted into a pre-weighed 100 ml beaker and dried on a water bath. It was placed in a hot air oven at 105°C for 5 hours before being cooled in a desiccator for 30 minutes and weighed. The operation was repeated until the weight was constant **[14, 15]**.

- **Determination of Alcohol soluble extractive Value**

The same technique was used as for the water-soluble extractive value. As a solvent, alcohol was used instead of water **[14, 15]**.

- **Determination of pH Value**

For the determination of pH, a 10% aqueous solution of the fresh sample was made, and the pH value was assessed using a pH meter **[14, 15]**.

- **Preliminary Phytochemical test:** Preliminary analysis of extracts was carried out to identify the presence of various phytoconstituents by employing standard protocols **[16, 17]**.
- **Phytochemical Analysis:**

The four extracts obtained from the powdered leaves were subjected to phytochemical tests to determine the presence of active secondary metabolites using standard procedures. The four extracts obtained from the powdered leaves of *Fraxinus micrantha* and *Cirisium wallichii* were subjected to a preliminary phytochemical screening using standard procedures for several classes of phytoconstituents using particular standard reagents. As each class has its own unique pharmacological significance, phytochemical screening assists in identifying chemical elements pertaining to a certain class.

- **Determination of total Phenolic Content:** The spectrophotometric technique was used to determine the total phenolic content. To summarize, 1 ml of material (1 mg/ml) was combined with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 minutes, 10 ml of a 7% Na_2CO_3 solution was added to the mixture, followed by 13 ml of deionized distilled water, and thoroughly mixed.

The mixture was maintained in the dark for 90 minutes at 23°C before the absorbance was measured at 750 nm. The TPC was calculated by extrapolating the calibration curve created by making gallic acid solution. The phenolic compound estimation was performed in triplicate. TPC was calculated using milligrams of gallic acid equivalents GAE per gram of dried material **[18, 19]**.

- **Determination of total Flavonoid Content:** The flavonoids were identified using the aluminium chloride spectrophotometric technique. A 1.0 mg/ml extract was combined with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1.0 M potassium acetate, and 2.8 ml of distilled water. The mixture was vortexed for 30 seconds before being allowed to stand at room temperature for 30 minutes. At 415 nm, the absorbance was measured. The standard curve was plotted using quercetin as the standard. The flavonoid content was expressed as mg Quercetin equivalent/g dry weight **[18, 19]**.
- **Determination of total Tannin Content:** The total tannin content was calculated by making small changes to the technique. 1 ml of the plant extract was combined with 0.5 ml of Folin's phenol reagent, followed by 5 ml of 35% Na_2CO_3. The mixture was allowed to stand at room temperature for 5 minutes. A UV/visible spectrophotometer was used to read the blue colour produced at 640 nm. The tannic acid calibration curve was used to calculate tannin concentration, and the results were represented as tannic acid equivalent (mg/g). Phytochemical qualitative analysis Various methods were used to qualitatively identify the phytochemical components of methanol extracts obtained through various extraction techniques **[18, 19]**.
- **Determination of Antioxidant Activities:** The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of antioxidant activity as free radical scavenging activity (FRSA) of the plant extract. The stock solution was freshly prepared by mixing 0.2 mM of DPPH with 100 ml of methanol and stored at 20°C until required. A 3 ml aliquot of this freshly prepared DPPH solution was mixed with 100 µl of

methanol and various concentrations (20–160 µg/ml) of hydroalcoholic and aqueous extract samples. The reaction mixture was shaken well and incubated in the dark for 15 minutes at room temperature. Then the absorbance was taken at 517 nm. Ascorbic acid was used as a standard **[18, 20]**.

Percentage inhibition = [(Abscontrol–Abssample]/Abscontrol × 100

Where Abs control is the absorbance of DPPH radical with methanol, Abs sample is the absorbance of DPPH radical with sample extract/standard. All tests were performed in triplicate.

- **ABTS radical scavenging activity:** The cation scavenging activity of 2,20-azinobis (3-ethylbenzthiazoline-6-sulphonic acid), also known as ABTS, was tested. In brief, an ABTS solution (7 mM) was combined with a potassium persulfate (2.45 mM) solution and left in the dark overnight to produce a dark-colored solution containing ABTS radical cations. The ABTS radical cation was diluted with 50% methanol before use in the experiment for an initial absorbance of approximately 0.700.02 at 745 nm, with the temperature control set at 30°C. In a microcuvette, 300µl of test material was mixed with 3.0 ml of the ABTS working standard to determine free radical scavenging activity **[18, 20]**. The drop in absorbance was measured exactly one minute after mixing the solution, then every minute for the next six minutes. The % inhibition was estimated using the following formula:

Percentage inhibition = [(Abs control–Abs sample]/Abs control × 100

Where, Abs control is the absorbance of ABTS radical with methanol; Abs sample is the absorbance of ABTS radical with sample extract/standard. All test were performed in triplicate

- **Determination of Enzyme inhibitory activity:** The enzyme inhibitory activity of the extracts was analyzed for their α-amylase inhibitory activities **[21]**. 25 µl of sample extract was mixed with 50 µl of alpha amylase in phosphate buffer (pH 6.9 containing 6 mM sodium chloride). After 10 min at 37 °C, 50 µl of starch solution (0.05%) was added to start the reaction. After incubation at room temperature, the reaction was stopped using 25 µl of HCl (1M) and 100 µl of iodine- potassium iodide solution. Acarbose, is known to have α-amylase inhibitor, was used as a standard drug. The absorbance was measured at 630 nm. The α-amylase inhibitory activity was calculated by using the following equation **[22]**.

Precent Inhibition (%) = [Abs. control – Abs. sample/Abs. control] x100

- **RESULTS AND DISCUSSION**

Organoleptic Characteristics:

***Crisium wallichii*:** Thistle plants are odourless and are distinguished by white or pale pink flower heads with spiky, dilated points on the inner involucre bracts. Spines are restricted to the edges of the leaf at the ends of the lobes rather than the surface; taller plant, typically reaching a height of 2.5 meters; leaves unevenly pinnately lobed, sessile, cottony on the bottom surface, lobe tips with strong sharp spines, heads are around 2-4 cm diameter, solitary or clustered; outer involucre bracts are lanceolate, culminating in firm spreading spines; and inner bracts have dilated tip.

Fraxinum micrantha: *Odour:* Characteristics, Taste: Slightly sweet. The small-flowered Himalayan ash tree is a huge deciduous tree with grey bark. The young shoots have rusty hair, and clusters of small white blooms emerge in enormous branching clusters up to 25 cm across. Flowers do not have petals. Pinnate leaves with 5-9 elliptic-lance shaped, long-pointed, saw-toothed, stalked leaflets that are 6–15 cm long. Fruits are winged, 4.5 cm long, and 8 mm wide.

Physico-chemical parameter:

Different physical parameters were studied, such as total ash, acid-insoluble ash, water-soluble extractive value, ethanol-soluble extractive value, loss on drying at 105 ^{o}C and pH, etc., as listed in **Table 1**.

- **Phytochemical constituents:**

The phytochemical investigation of the hydroalcoholic and aqueous extracts revealed that alkaloids, saponins, carbohydrates, glycosides, tannins, proteins, and flavonoids were present in the extracts in higher and lower amounts **(Table 2)**.

Table 1: Physicochemical parameters of *Cirisium wallichii and Fraxinus micrantha*

S. No.	Name of Parameters	FM *(Fraxinus micrantha)*	CW *(Cirisium wallichii)*
1.	pH (10% aqueous solution (v/w)	4.41±0.312	8.35±0.210
2.	Loss on drying at 105^{o}C (% w/w)	8.96±0.17	8.92±0.20
3.	Total Ash (% w/w)	5.80±0.14	6.30±0.16
4.	Acid-insoluble ash (% w/w)	1.95±0.07	2.22±0.06
5.	Water-soluble extractive (% w/w)	47.07±1.67	37.49±1.96
6.	Alcohol-soluble extractive (% w/w)	65.71±1.68	56.08±1.09

Table 2: Phytochemical screening of the methanolic and aqueous extracts of *Cirisium wallichii* and *Fraxinus micrantha*

Phytochemical constituents	Test Applied	CWM	CWA	FMM	FMA
Alkaloids	Mayer's test	+	+	+	-
	Wagner's test	+	+	+	-
Saponins	Frothing test	+	+	+	+
Carbohydrates	Molisch's test	-	+	-	+
	Fehling's test	-	+	-	+
Glycosides	Keller Killiani test	+	-	+	-
Tannins and phenolic contents	Ferric chloride test	+	-	+	-
	Lead acetate solution test	+	-	+	-
Flavonoids	Alkaline reagent test	+	+	+	+
	Shinoda test	+	+	+	+
Test for acidic compounds	Litmus paper test	-	-	+	+
Test for Proteins	Biuret test	+	+	+	+
	Ninhydrin test	+	+	+	+

(+) present; (-) absent. *Cirisium wallichii* methanolic (CWM), *Cirisium wallichii* aqueous (CWA), *Fraxinus micrantha* methanolic (FMM) and *Fraxinus micrantha* aqueous (FMA)

- **Phenolic and Flavonoids content:**

Flavonoids are polyphenolic chemicals found in plants that serve a variety of biological activities. Many flavonoids have been shown to be effective against viral infections, particularly at the molecular level by inhibiting virus growth. Because of the presence of a hydroxyl group, phenols, another primary aromatic compound present in plants, have free radical scavenging characteristics. Natural polyphenolic compounds prevent mutagenesis and carcinogenesis in humans. Avian assault is deterred by a high polyphenol content.

In our study, total phenolics content was found (59.25±0.00882, in methanolic extract of *Cirisium wallichii* 136.97±0.00057 in methanolic extract of *Fraxinus micrantha, followed by* 63.57±0.00882 in aqueous extract of *Cirisium wallichii* and 122.81±0.00664 aqueous extract of *Fraxinus micrantha in* mg Gallic acid equivalent (GAE/g) in both selected plants *as shown in* **(Table 3).** A study done by **[23]** on *F. excelsior* total phenolic content was reported 109.8 µgGAE/mg in methanolic extract of fruit, in another study on *Cirsium japonicum* roots 22.36 and 17.73 mg Tan/g **[24].** Among phenolics, *Fraxinus* species contains Tyrosol, (Z, Z)-6,9-cis-3,4-epoxy nonadecadiene, β-sitosterin, (+)-Ascorbic acid 2,6-dihexadecanoate, Fraxetin, Caffeic acid, Ferulic acid, Apigenin and Esculetin **[26, 27, 28, 29],** (8,3,4,5-tetrahydroxy-7-O-a-glucosyl isoflavone)-6-8-(4-hydroxy--O-a-glucosyl isoflavone) biflavone, 5,5,7-trihydroxy-3,7- dimethoxy-4-4-O-biflavone, ethyl-(2,3-dihydroxy-5,6-dimethoxy-4-acetyl) benzoate, azelaic acid, 3-hydroxyazelaic acid and 6-2-dihydroxy-5-methoxy isoflavonone-4-O-a-D-glucopyranoside **[30, 31]**.

Most of the phenolics in *Fraxinus micrantha* as well as *Cirisium wallichii* exhibit antioxidant activity **(Figure 2).** In the present study, the content of flavonoids was also recorded in both of the selected plants' methanolic and aqueous extracts (176.52±0.001732, 128.28±0.019079, 149.49±0.001528 and 96.72±0.030097 mg *Quercetin equivalent* (mgQE/g) of both selected plants shown in **Table 3, Figure 3**. Similarly, some other studies by Yin et al, in 2008 on *Cirsium japonicum* showed flavonoid content ranging from 13.04±0.36 to 0.34±0.04 mgQE/g in methanolic and water extracts. Hence, results showed that the roots and leaves of both are also good sources of phenolic as well as flavonoid compounds. Therefore, they have been found to have high medicinal value due to their rich source of phenolics and flavonoid compounds. The present study was conducted to check the efficiency of these selected plants for polyphenolic and phenolics content.

- **Tannin Content:**

Tannins are naturally occurring compounds found in plants, seeds, bark, wood, fruits, etc. and possess various biological properties and medical uses. They've been linked to plant defence mechanisms against mammalian herbivores, birds, and insects. Tannins are phenolic compounds and plant secondary metabolites that have beneficial effects on protein metabolism in ruminants, decreasing rumen protein degradation and enhancing amino acid absorption in the small intestine **[32]**. In our study, total tannin content was found (24.57±0.001155, 35.40±0.001202, 11.82±0.001732 and 29.67±0.00145 mgTAE/g in selected plants, *Cirisium wallichii* and *Fraxinus micrantha*, both methanolic and aqueous extracts, as shown in the above **Table 3, Figure 4**. Similar studies on *Cirisium sp*. were done and found tannins % range from 1-7.6% **[33]**. Tannins serve multiple ecological functions, so it is likely that structural diversity has evolved to benefit plants in these capacities. significant effects of tannin structure on biochemical activity.

- **Antioxidant Activity: By DPPH**

The most commonly used technique for determining the free radical scavenging activity (FRSA) of plant extracts in vitro is the DPPH method. In this approach, DPPH are free radicals that are neutralized by antioxidant compounds such as vitamins, phenolic compounds, tannins, and many other active molecules. The antioxidant potential of the extracts based on DPPH radical scavenging was assessed using the IC_{50} measure in this investigation **(Table 4)**.

The DPPH technique revealed that the plant portions of *Cirisium wallichii* and *Fraxinus micrantha* exhibited remarkably potent antioxidant activity. The quantities of 20 to 120 µg were seen to exhibit inhibitory percentages of antioxidant activity ranging from 26.05% to 62.49% in the methanolic extract of *F. micrantha*. Similarly, the methanolic extract of *C. wallichii* shown inhibitory percentages ranging from 25.28% to 59.75%. In the case of the aqueous extract of *F. micrantha*, the inhibitory percentages ranged from 23.54% to 60.88%. Lastly, the methanolic extract of *C. wallichii* exhibited inhibitory percentages ranging from 22.30% to 45.53%. As a result, the IC_{50} values of the leaves and root sections of *F. micrantha* and *C. wallichii* were recorded (88.02 mg/ml, 87.87 mg /ml, 91.57 mg/ml, and 109.64 mg /ml), whereas the IC_{50} value of the standard medication ascorbic acid was =77.25 mg/ml. A low IC_{50} value indicates that the antioxidant is highly effective **(Table 4, Figure 5, 6)**. As a result, both of these selected species have antioxidant activity. In previous similar studies using the DPPH and H_2O_2 scavenging assays, the *F. micrantha* extract has IC_{50} values of 43.93 mg/ml and 68.90 mg/ml, respectively.

ABTS: A diode-array spectrophotometer is used in this method to assess the loss of colour when an antioxidant is introduced to the blue-green chromophore ABTS + (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid). The antioxidant decolorizes and lowers ABTS+ to ABTS. In this study, the antioxidant capacity of the extracts based on ABTS radical scavenging was evaluated using the IC_{50} measure. The leaves and roots of *Cirisium wallichii* and *Fraxinus micrantha* displayed antioxidant activity in the ABTS technique. The methanolic extract of *F. micrantha* exhibited an inhibitory percentage of antioxidant activity ranging from 34.84% to 55.54%, while the methanolic extract of *C. wallichii* showed a range of 32.28% to 49.44%. In the case of the aqueous extract of F. micrantha, the inhibitory percentage of antioxidant activity ranged from 30.76% to 51.62%, while for *C. wallichii* methanolic extract, the range was 29.08% to 47.18%. These results were observed at concentrations ranging from 20 to 120 mg/ml. As a result, the IC_{50} values of *F. micrantha* and *C. wallichii* leaf and root sections were determined (94.22 mg/ml, 88.02 mg/ml, 120.004 mg/ml, and 120.192 mg/ml, respectively), while the IC_{50} value of the reference prescription ascorbic acid was =78.16 mg/ml. An antioxidant with a low IC_{50} value is extremely effective. As a result, both of these chosen species have antioxidants **(Table 5, Figure 7, 8)**.

Previous research has examined the antioxidant properties of various *Fraxinus* species, such as *Fraxinus ornus*, *Fraxinus oxycarpa* and *Fraxinus angustifolia* vahl **[34-38]**. Additionally, the antioxidant potential of *Cirsium* species has also been explored **[39]**

Cirsium setidens **[40]**, and Taiwanese *Cirsium* species **[41]**. However, the present study represents the first investigation into the antioxidant potential of *F. micrantha* leaves and *C. wallichii* roots in the highlands of Kumaun. A dose-dependent relationship was discovered in the plant materials, where the levels of FRSA (Free Radical Scavenging Activity) increased proportionally with the intensity of the extracts. In contrast to prior literature examining the antioxidant properties of these two botanical species, the findings of this study indicate that plants inhabiting elevated regions of the Himalayas have a notable antioxidant profile. The significant presence of phenolics, polyphenols, flavonoids, tannins, and vitamins in diverse plant components has led to the deduction that these compounds demonstrate noteworthy antioxidant properties.

Kumaun Himalayas

Enzyme Inhibitory:

Starch is broken down into simpler sugars like glucose, maltose, and dextrins by the enzyme alpha-amylase (EC 3.2.1.1). Paper chromatography, high-performance liquid chromatography and thin-layer chromatography are only some of the chromatography methods that can be used to characterize α-amylases and glucoamylases. The following formula can be used to determine the relative activity of α-amylase.

Percent Inhibition (%) = [Acontrol-Asample/Acontrol] x 100 where a control is the absorbance of the enzyme sample, and a sample is the absorbance of the amylose control without the addition of enzyme. Therefore, units of enzyme activity calculated with this formula are not directly proportional to substrate utilization. The alpha amylase inhibitory method was calculated in different plant parts of *Cirisium wallichii* and *Fraxinus micrantha* that demonstrated high enzyme inhibitory activity **(Table 6, Figure 9)**.

Table 3: Determination of Phenolics, Flavonoids and Tannins in selected plant samples

Name of the Sample	Amount mg/g	SD±
	Phenolics Content mg GAE/g	
Fraxinus micrantha Methanolic	**136.97**	**0.00577**
Crisium veratrum Methanolic	**59.25**	**0.00882**
Fraxinus micrantha Aqueous	**122.81**	**0.00664**
Crisium veratrum Aqueous	**63.57**	**0.00882**
	Flavonoids Content mg QE/g	
Fraxinus micrantha Methanolic	**176.52**	**0.001732**
Crisium veratrum Methanolic	**128.28**	**0.019079**
Fraxinus micrantha Aqueous	**149.49**	**0.001528**
Crisium veratrum Aqueous	**96.72**	**0.030097**
	Tannins Content mg TAE/g	
Fraxinus micrantha Methanolic	**35.40**	**0.001202**
Crisium veratrum Methanolic	**24.57**	**0.001155**
Fraxinus micrantha Aqueous	**29.67**	**0.001453**
Crisium veratrum Aqueous	**11.82**	**0.001732**

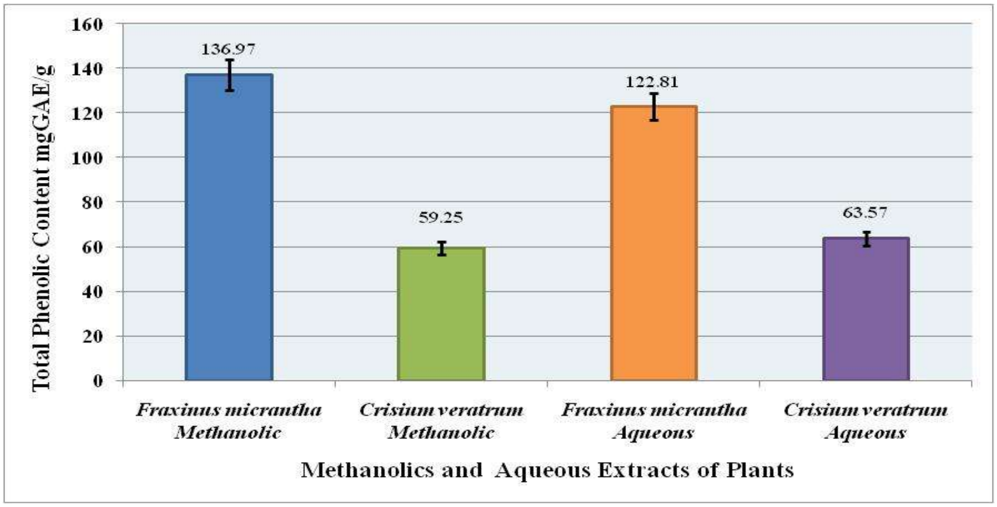

Figure 2: Phenolics content of two medicinal plants in different solvents

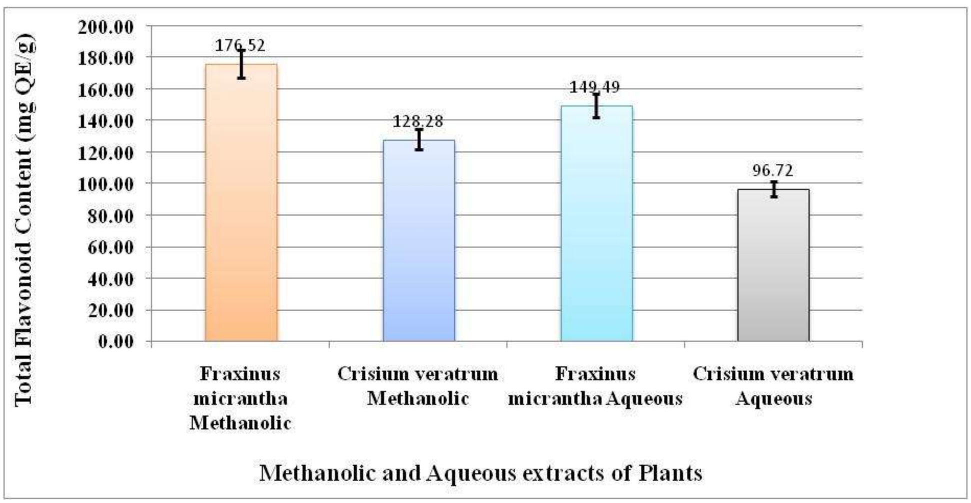

Figure 3: Flavonoids or polyphenolics compounds content of two medicinal plants in different solvents

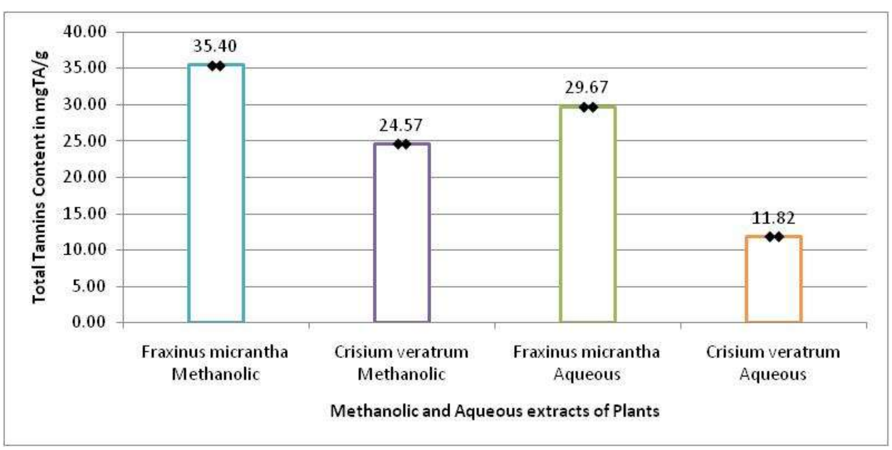

Figure 4: Total tannin content in methanolic and aqueous extracts

Table 4: Antioxidant potential of selected plants from highlands

Name of Sample	Ascorbic Acid	Fraxinus micrantha Methanolic	Cirisium wallichii Methanolic	Fraxinus micrantha Aqueous	Cirisium wallichii Aqueous
%FRSA/ Conc	34.1046 (20mg/ml)	26.0566 (20mg/ml)	25.2854 (20mg/ml)	23.5403 (20mg/ml)	22.3094 (20mg/ml)
%FRSA/ Conc	43.9477 (40mg/ml)	31.1983 (40mg/ml)	32.8475 (40mg/ml)	29.5403 (40mg/ml)	26.4967 (40mg/ml)
%FRSA/ Conc	49.7908 (60mg/ml)	39.2941 (60mg/ml)	41.5926 (60mg/ml)	37.5926 (60mg/ml)	31.4205 (60mg/ml)
%FRSA/ Conc	58.3268 (80mg/ml)	46.2593 (80mg/ml)	48.8192 (80mg/ml)	44.6449 (60mg/ml)	34.5163 (60mg/ml)
%FRSA/ Conc	65.1699 (100 mg/ml)	54.0523 (100mg/ml)	53.1373 (100mg/ml)	51.0022 (100mg/ml)	39.6558 (100mg/ml)
%FRSA/ Conc	74.7059 (120mg/ml)	62.4967 (120mg/ml)	59.7516 (120mg/ml)	60.8824 (120mg/ml)	45.5338 (120mg/ml)
IC_{50} mg/ml	71.22507	87.87346	88.02817	91.57509	109.6491

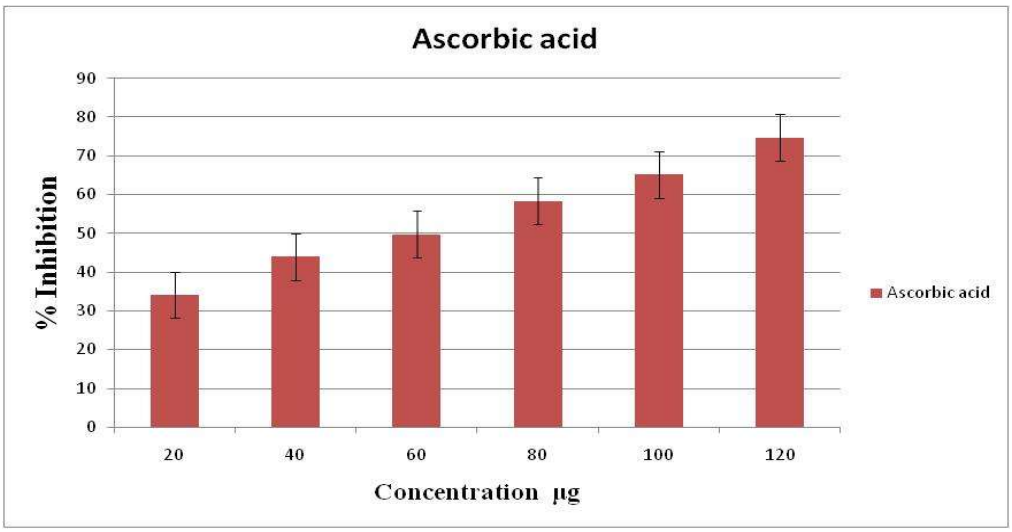

Figure 5: Standard bar graph representation of % Inhibition of Standard Ascorbic acid

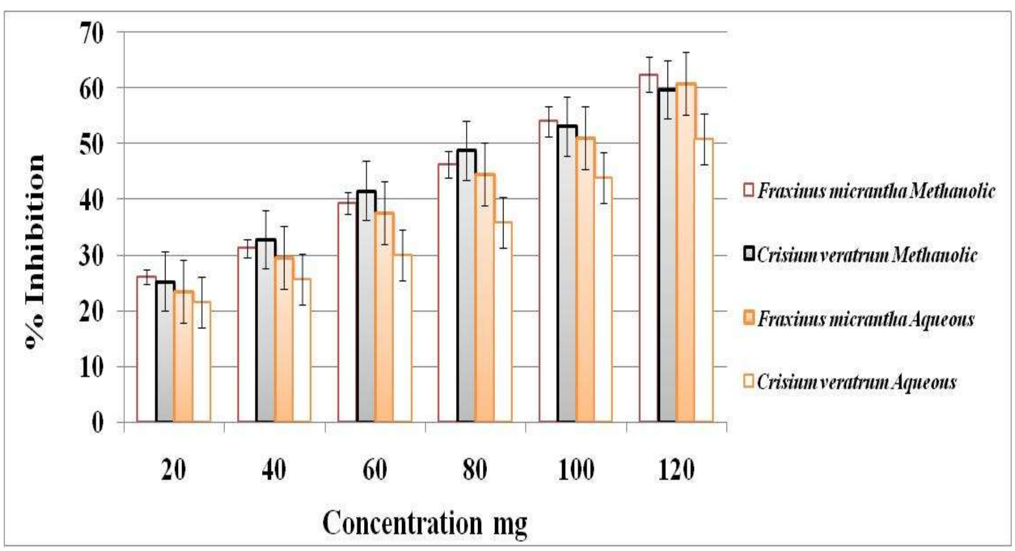

Figure 6: Antioxidant activity using DPPH assay of ethnobotanically selected plants of Kumaun Himalayas

Table 5: Antioxidant potential of selected plants from highlands

Name of Sample/ Concentration	*Ascorbic Acid*	*Fraxinus micrantha Methanolic*	*Cirisium wallichii Methanolic*	*Fraxinus micrantha Aqueous*	*Cirisium wallichii Aqueous*
% FRSA/ Conc	37.41 (20mg/ml)	34.84 (20mg/ml)	32.66 (20mg/ml)	30.76 (20mg/ml)	29.08 (20mg/ml)
% FRSA/ Conc	41.44 (40mg/ml)	37.98 (40mg/ml)	35.45 (40mg/ml)	34.56 (40mg/ml)	33.10 (40mg/ml)
% FRSA/ Conc	47.81 (60mg/ml)	42.73 (60mg/ml)	39.20 (60mg/ml)	37.97 (60mg/ml)	36.12 (60mg/)
% FRSA/ Conc	52.34 (80mg/ml)	47.09 (80mg/ml)	44.40 (80mg/ml)	42.22 (60mg/ml)	40.38 (60mg/ml)
% FRSA/ Conc	61.57 (100mg/ml)	51.34 (100mg/ml)	46.92 (100mg/ml)	46.36 (100mg/ml)	44.50 (100mg/ml)
% FRSA/ Conc	70.16 (120mg/ml)	55.54 (120mg/ml)	49.44 (120mg/ml)	51.62 (120mg/ml)	47.18 (120mg/ml)
IC50 mg/ml	78.16	94.22	88.02817	120.004	120.192

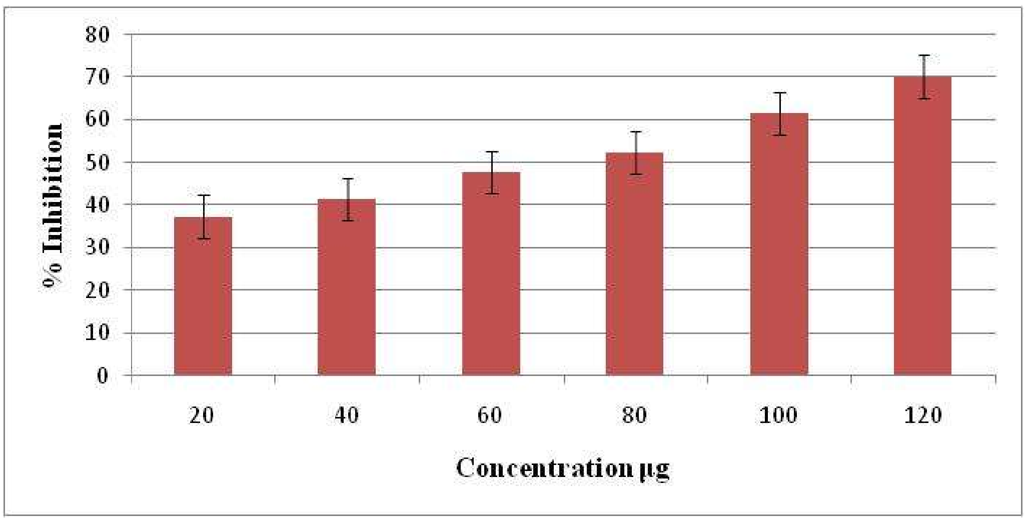

Figure 7: Standard bar graph representation of % Inhibition of Standard Ascorbic acid

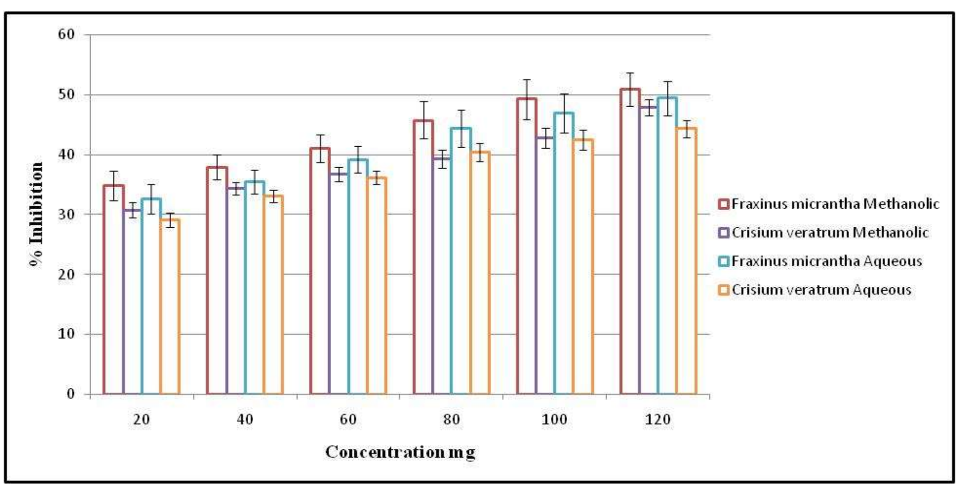

Figure 8: Antioxidant activity using ABTS assay of ethnobotanically selected plants of Kumaun Himalayas

Table 6: Estimation of alpha-amylase inhibitory activity

Name of Sample	Enzyme Inhibition (%)	SD±
Acarbose	66.96165	0.001202
Fraxinus micrantha Methanolic	52.80236	0.000667
Crisium veratrum Methanolic	44.24779	0.000577
Fraxinus micrantha Aqueous	45.13274	0.000577
Crisium veratrum Aqueous	27.43363	0.000577

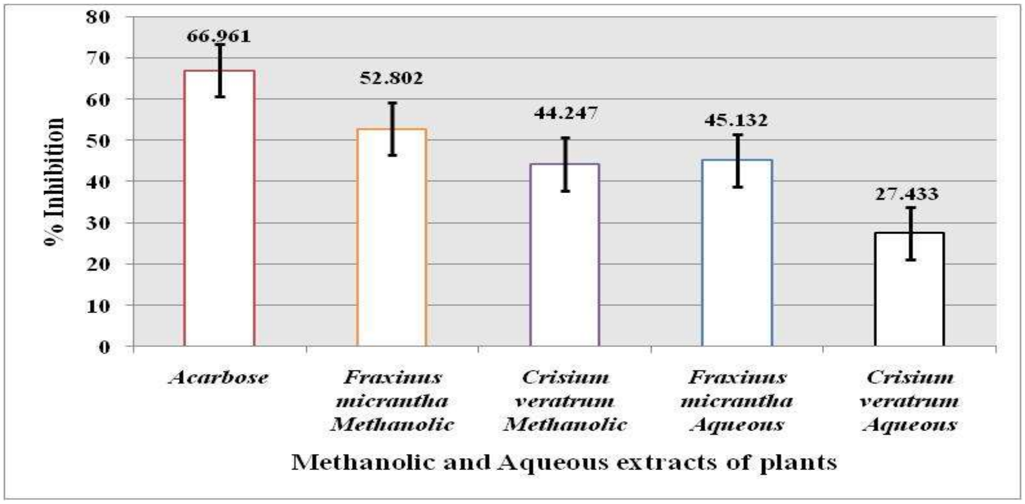

Figure 9: Enzyme inhibition activity using *In vitro* method for selected plants of Kumaun Himalayas

The study discovered notable alpha-amylase inhibitory action in several plant components of *Cirisium wallichii* and *Fraxinus micrantha*. The inhibition percentages were computed utilising the supplied methodology, providing useful insights into the possible anti-diabetic capabilities of various plant extracts. The inhibitory action reported indicates that certain substances in particular plant sections may be able to regulate alpha-amylase function, thereby impacting glucose metabolism.

The work stands out for using several chromatographic techniques to analyse alpha-amylases and glucoamylases. The methodologies offer a thorough investigation of the enzymatic components in plant extracts, leading to a detailed knowledge of their inhibitory effects on alpha-amylase activity **[42]**.

CONCLUSION:

The findings of the current study indicate that the extract derived from *Cirisium wallichii* and Fraxinus micrantha, which possess the highest concentration of flavonoid and phenolic components, demonstrated the most significant antioxidant activity. The observed high scavenging ability of the chosen plants may be attributed to the presence of hydroxyl groups within the chemical structure of their phenolic compounds. These hydroxyl groups serve as radical scavengers, hence contributing to the plants' scavenging property. Free radicals are frequently produced as byproducts of biological events or as a result of external influences. The role of free radicals in the development of numerous diseases has been well described in academic literature. The utilization of a highly effective agent that eliminates free radicals has the potential to function as a viable preventive measure for various diseases. The various extracts examined in this study demonstrated varying degrees of antioxidant activity. The extracts of *Cirisium wallichii* and *Fraxinus micrantha* demonstrated greater efficacy in scavenging DPPH and ABTS free radicals compared to ascorbic acid. The observed phenomenon could potentially be attributed to the substantial concentration of flavonoid and phenolic chemicals present in the extract derived from this particular plant.

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