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Research Article

Comparative phytochemical profiles and bioactive potentials of Eastern white-cedar (*Thuja occidentalis* L.) and spiny sow-thistle (*Sonchus asper* (L.) Hill subsp. *asper*)

Prachi Baliyan[1], **Supriya Kumari Sharma**[1*], **Afroz Alam**[1]

[1]Banasthali Vidyapith University, Department of Bioscience and Biotechnology, Rajasthan, India, 304022

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Abstract: The nutritional value of many native edible plants is not well understood by scientists, leading to their underutilisation. There is a great deal of promise in using weeds as medicinal plants or as sources of pharmaceutical compounds. Developing new drugs has been shown to benefit greatly from the use of medicinal plants that are pertinent to ethnomedicinal knowledge. This study was aimed at a comparative analysis of phytochemical profiling and the bioactive potential of *Thuja occidentalis* L. (Cupressaceae) and *Sonchus asper* (L.) Hill subsp. *asper* (Asteraceae). The results showed that the distilled water extract of *Thuja occidentalis* has high phenolic (2073±0.017 mg GAE/g) and flavonoid content (1598.8±0.0056 mg QE/g) while the methanolic extract has high tannin (1908±0.0098 mg/g) and alkaloid content (1192±0.095 mg/g). But the methanolic extract of *Sonchus asper* has high flavonoid (408.8±0.001 mg QE/g), phenolic (259.02±0.006 mg GAE/g), tannin (820.9±0.005 mg/g), and alkaloid content (653.87±0.002 mg/g). The methanolic extracts of both plants showed strong antioxidant potential. In the DPPH assay, the methanolic extract of *Thuja occidentalis* showed an IC_{50} of 50.01±0.02 µg/mL, and in *Sonchus asper* subsp. *asper* with IC_{50} value of 50.97±0.017 µg/mL. In the NOSA assay, the methanolic extract of both plants shows strong antioxidant potential with IC_{50} values of 50.7±0.024 µg/mL and 51.9±0.011 µg/mL, respectively. This study highlighted the presence of phytochemical compounds and antioxidant activity, which is richest in *Thuja occidentalis*. They provide a great neutraceutical value to this plant.

INTRODUCTION

The world is endowed with a profusion of therapeutic plants, and without the plant kingdom, man could not live a long, healthy life on Earth since plant products and their active ingredients were essential to sustaining the ideal way of life. Ethnopharmacological research is currently being promoted globally as a means of highlighting species that harbor compounds or advantageous products with potential applications in the nutritional, cosmetic, and medicinal domains (Rathor, 2021). Worldwide, traditional medicine based on plants is crucial to the recovery of people from a wide range of illnesses. The creation of safe and effective treatments is said to have its potential foundation in ethnomedical traditions (Agidew, 2022). A variety of phytochemicals, including flavonoids, phenols, glycosides, alkaloids, tannins, and lignins, are found naturally in plants and plant-based products. The most prevalent phytoconstituents that

*Corresponding Author: Supriya Kumari SHARMA supriya.sharma2608@gmail.com Banasthali Vidyapith University, Department of Bioscience and Biotechnology, Rajasthan, India, 304022

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provide various vegetables, fruits, and medicinal plants with their antioxidant properties are flavonoids and phenols (Phuyal *et al*., 2020). Underutilised wild Edible plants show that despite being abundant in a variety of functional characteristics, some underutilized herbs, roots, fruits, vegetables, flowers, yams, tubers, leaves, and other plant parts remain unexplored (Peduruhewa *et al*., 2021). Less common, rarely used, or region-specific foods can also be referred to as underutilized foods. They provide national security to poor people in terms of nutrition (Sah *et al*., 2024). Conversely, weeds or crop plants with the ability to restrict growth through allelopathy had a higher propensity to function as substitutes for synthetic herbicides. Certain species don't create toxic waste products despite being highly active in direct allelopathic effects.

There are 88 genera and over 1000 species of gymnosperms in the world. Other plant types known as gymnosperms possess naked seeds (Jacobsen, 2021). The variety of gymnosperms is extremely limited. Angiosperms or flowering plants are vascular plants that, as their name suggests, produce fruit or fully grown ovaries that contain seeds. These plants are more adaptable to life on land and are found throughout the world; to date, some 250000 species have been recognized. On Earth, there are roughly 30,000 different species of flowering plants. In contrast, because gymnosperms have longer leaf lifespans than angiosperms, they might have benefits in terms of C balance in low-productivity locales, while due to limitations on growth and photosynthesis imposed by the environment, angiosperms might not realize their full potential (Piper *et al*., 2019). In terms of genera, the Cupressaceae family is the largest among conifers, and in terms of species, it ranks third. With roughly 130–162 species overall, the family consists of 27–32 genera (17 monotypic) (Mao *et al*., 2012). As a member of the dicotyledonous flowering family, the Asteraceae, also known as the sunflower family (formerly composite, also called the Aster family), highlights its unique flower heads made up of numerous small flowers (called florets), encircled by bracts (Bisht & Purohit, 2010). With approximately 1,535 genera, 23,000 species spread across three subfamilies, and 17 tribes, it is the largest family of Angiosperms. Plants in this genus exhibited antioxidant capacity in vitro (Sharma & Alam, 2023).

The Cupressaceae family tree *Thuja occidentalis*, sometimes referred to as the "tree of life" or "white cedar," is grown as a decorative tree in Brazil and Europe. It originated in Eastern North America. Members of the Cupressaceae family and the order Pineales include *Thuja* trees. Known by many names, such as Arborvitae or white cedar, *Thuja occidentalis* (Figure 1. A) is a tree species native to eastern North America that is now planted as a garden plant in many places, including Europe, India, and Brazil (Thakur *et al*., 2023). The leaves are green throughout the year, with green on the underside where resin ducts are located. The seeds are enclosed in tiny, green to brown coniferous pins that are 1-2 cm long (Naser *et al*., 2005). The leafy branchlets of T. occidentalis are traditionally used as a tea substitute, offering an aromatic flavor and being a potential source of vitamin C. The pith of young shoots and inner bark are sometimes eaten, especially in times of scarcity, cooked and potentially added to soups or used to make bread or biscuits. All of the Canadian provinces' agricultural areas are home to *Sonchus asper* subsp. *asper* (Figure 1. B) (Asteraceae), also referred to locally as prickly sow-thistle, or dudhi. However, it is primarily found in disturbed habitats in Ontario, British Columbia, and southern Quebec (Hussain *et al*., 2010). Originating in subtropical, temperate, Mediterranean, and tropical parts of Eurasia and tropical Africa, sow thistles are widespread roadside plants found practically everywhere (Mohammed, 2020). Because they spread swiftly in a variety of environments and develop quickly, sow thistles are regarded as noxious weeds (Hutchinson *et al*., 1984). It may grow in lakes, seasonally wet places, somewhat acidic to alkaline soils, and a variety of soil types, such as black sandy loam, brown clayey loam, white to grey sand, and nutrient-rich soils (Sharma & Alam, 2023).

To investigate the antioxidant activity of natural sources, such as fruits, vegetables, and plants, several prior studies have been conducted. Even though *Thuja occidentalis* and *Sonchus asper* belong to completely different plant families, bringing them together in one study has a strong

rationale. Both species have a long history of use in traditional healing practices, especially for treating infections, inflammation, and other health disorders, yet detailed scientific comparisons of their phytochemicals are still scarce. Looking at them side by side not only broadens our understanding of the diversity of bioactive compounds across unrelated plant groups, but it also helps us see whether plants with very different evolutionary backgrounds can show similar therapeutic potentials. Moreover, the contrast between a woody, long-lived species like *Thuja* and a short-lived, fast-growing herb like *Sonchus* offers an interesting perspective on how growth habit and ecology might influence the production of secondary metabolites. In this way, the comparison provides fresh insights that go beyond the study of each species alone. The purpose of this study is not to compare them taxonomically, but rather to evaluate and compare their antioxidant capacities and phytochemical profiles under uniform experimental conditions. By doing so, we aim to identify and highlight potential candidates for natural antioxidant applications, regardless of their taxonomic origin. This approach provides a broader perspective on inter-family variations in antioxidant potency, which could guide future pharmacological screening or nutraceutical development.

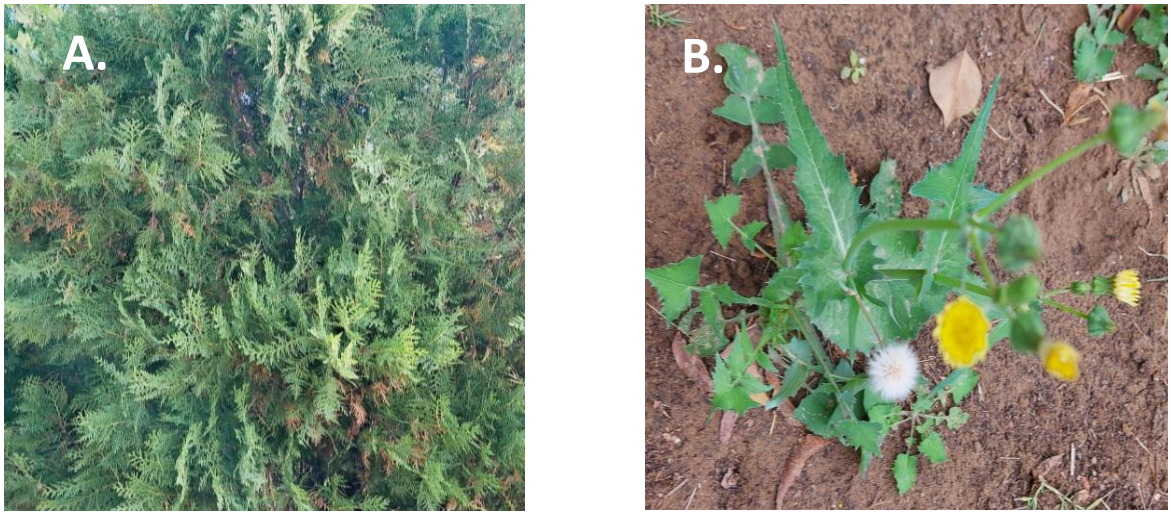


Figure 1. Picture showing (A) *Thuja occidentalis* and (B) *Sonchus asper* subsp. *asper*.

MATERIAL and METHODS

Chemicals and Reagents

HI Media, Sigma, and SRL provided all of the analytical-grade chemicals and reagents used in this study.

Plant Collection and Authentication

Fresh plant materials of *Thuja occidentalis* and *Sonchus asper* subsp. *asper* were collected from the campus of Banasthali Vidyapeeth. The species were identified and authenticated based on their morphological features using standard floristic keys and descriptions available in the Flora of India (Sharma *et al.*, 2012, Botanical Survey of India) and Flora Europaea (Tutin *et al.*, 1980). The identification was further verified against herbarium specimens, and the accepted nomenclature was cross-checked using Plants of the World Online (POWO). Voucher specimens were prepared, labelled, and deposited in the Banasthali University Herbarium (BURI) for future reference under voucher numbers: *Thuja occidentalis* (BURI-2905/2024) and *Sonchus asper* subsp. *asper* (BURI-1621/2022). This procedure follows standard authentication practices reported in earlier phytochemical studies of these taxa (Hussain *et al.*, 2010).

Extract Preparation

Before the extraction process, the samples (leaves) were cleaned and allowed to dry in the shade

for a week. Next, a grinder was used to grind each dried sample into a powder. The dried samples were weighed and placed inside the Soxhlet apparatus using a thimble. The hot Soxhlet extraction method, also known as continuous extraction, was then used to extract them individually using methanol. About 100 g of dried sample powder was weighed, and the extraction process was carried out by using 250 mL of Chloroform in a Soxhlet apparatus. To acquire the crude extracts of the components, the system was operated for 72 hours until the colored solvent began to show in the siphon. The solvent was evaporated using a rotary evaporator under reduced pressure at a bath temperature of 65 °C, which corresponds to a lower effective evaporation temperature due to vacuum conditions, minimizing thermal degradation of the bioactive compounds. Then, the water bath was used to dry the extracted materials. For additional examination, the dried extracts were placed within 20 mL sterile culture tubes and refrigerated between 2-8°C (Shraim *et al*., 2021).

Qualitative Phytochemical Analysis

Phytochemical analysis was carried out by following the standard protocol to detect the presence of alkaloids, cardiac glycosides, carbohydrates, tannins, steroids, flavonoids, phenols, saponins, fixed oils, and fats (Shaikh & Patil, 2020).

Quantitative Analysis

Spectrophotometric measurements

It was carried out based on the protocol adopted from Dadi and Yasir (2022). All of the absorbance measurements were performed using a standard quartz cuvette and a UV/Vis double-beam spectrophotometer (Systronix, Model 2022).

Preparation of standard solution

Gallic acid and quercetin, both measured at approximately 10 mg, were precisely weighed into dry, clean volumetric flasks. Then, methanol was used to dissolve them, and the volume was increased to 10 mL using the same solvent to achieve a solution concentration of 1 mg/mL. The standard solution of atropine was made by dissolving 1 mg of pure atropine in 10 mL of distilled water.

Preparation of test sample

10 mg of dehydrated hydro-methanolic extract was diluted in 10 mL of methanol to produce a stock solution containing 1mg/mL.

Determination of total flavonoid content (TFC)

The estimation of TFC in methanol extracts from all samples was conducted using a slightly modified version of the method outlined by Ahmed (Ahmed *et al*., 2015). A 500 µg/mL extract solution was made by dissolving each plant extract in methanol. Then, 3.4 mL of aqueous methanol (30% v/v) was added to 300 µL of extract that was taken in a test tube. Subsequently, each test tube was filled with 150 µL of a 0.5 M aqueous $NaNO_2$ solution and 150 µL of a 0.3 M aluminium chloride solution. About 1 mL of a 1 M NaOH solution was added after five minutes, and then the mixture was thoroughly mixed before the absorbance at 506 nm was measured using a UV-visible spectrophotometer in comparison to a blank. The same process was used to set up the blank, but an equivalent volume of methanol was used instead of the plant extract. By using the same process, a Quercetin standard curve was also produced to calculate the concentration of the total flavonoid, which was then represented as mg of quercetin equivalent (QE) per gram of extracts. Triplicates of the total flavonoids in the extracts and standards were measured (Shraim *et al*., 2021).

Determination of total phenol content (TPC)

The most popular technique for quantifying phenolic content is spectrophotometric analysis. Folin–Ciocalteu colorimetric method was used to measure the total phenol content of the selected plant leaf extract spectrophotometrically. Gallic acid was used as the standard, and the

results were expressed as gallic acid equivalent (GAE) per gram of sample. Methanol was used to prepare gallic acid in different concentrations (0.01-0.1 mg/mL). Then 2 mL of Folin-Ciocalteu reagent (1:10 in deionized water) and 4 mL of a saturated solution of Na_2CO_3 (7.5% w/v) were combined with aliquots of 0.5 mL each of the test sample and each sample of the standard solution. Silver foil was placed over the tubes, and they were allowed to sit at room temperature for half an hour while being periodically shaken. Methanol as a blank was used to measure the absorbance at 650 nm. Each sample underwent three replications of analysis (Do *et al*., 2014).

Determination of total alkaloid content (TAC)

TAC was measured using the spectrophotometric technique. This technique depends on the interaction between alkaloids and bromocresol green (BCG). After dissolving the plant extract (1 mg/mL) in 2 N HCl, the mixture was filtered. 0.1 N NaOH was used to bring the phosphate buffer solution's pH to neutral. Thereafter, 1mL of this solution was put into a separation funnel, to which BCG solution (5mL) and phosphate buffer (5mL) were added. Chloroform was used to extract the complex that had formed after thoroughly shaking the mixture. In a 10 mL volumetric flask, the extract was collected and diluted to volume using chloroform. At 470 nm, the complex's absorbance in chloroform was calculated. Atropine standard solutions are compared to a blank. Triplicates were used for the entire experiment (Ajanal *et al*., 2012).

Determination of total tannin content (TTC)

The tannins were ascertained by utilising the Folin-Ciocalteu reagent (FCR). In a nutshell, 1 mL of 35% sodium carbonate Na_2CO_3 solution, 7.5 mL of distilled H_2O, 0.5 mL of FCR, and 0.1 mL of the sample extract were combined, and the mixture was diluted to 10 mL with distilled H_2O. After giving the mixture a good shake and letting it sit at room temperature for 30 min, the absorbance at 725 nm was determined using a UV/visible spectrophotometer. Water was used to prepare the blank. Tannic acid standard solutions are compared to a blank. Using the formula derived from a standard tannic acid calibration curve, the total tannin concentration was calculated as mg of tannic acid equivalent per gram (Labu *et al*., 2015).

Antioxidant assay

Samples for the DPPH and NOSA tests were suitably diluted using 50% (v/v) methanol. Rutin and ascorbic acid were utilized as standard antioxidants.

DPPH radical scavenging assay. The DPPH test method is based on the reduction of a stable free radical, DPPH. It is a stable free radical that interacts with antioxidants when a hydrogen donor is present; then it becomes paired off and is reduced to the DPPH, which causes the absorbance from the DPPH to drop. Greater decolorization results in increased reduction power, which loses its violet colour. However, the picryl group should still be present and give off a slight yellow colour. The standard approach was followed in evaluating the plant extract's ability to scavenge DPPH radicals. Approximately 2 mL of any plant extract solution was added to 2 mL of 0.1 mM DPPH (2,2-diphenylpicrylhydrazyl) solution in methanol in a typical experiment. The mixture was then incubated for 30 minutes at 37 °C in a dark environment. Methanol was used as a blank to measure the absorbance at 517 nm. The DPPH radical scavenging activity percentage was determined as follows:

Percentage (%) of DPPH radical scavenging assay = $[(A_0-A_1)/A_0] \times 100$

where A_0 was the absorbance of the control, and A_1 was the absorbance of the treated sample (Medini *et al*., 2014).

Nitric oxide (NO) radical scavenging assay. The process relies on the idea that, in an aqueous solution at physiological pH, sodium nitroprusside (SNP) spontaneously produces NO. Griess reagent can be used to calculate the nitrite ions that are produced when this oxide combines with oxygen. Then 50 µL of each concentration of the plant extracts that had been previously dissolved in DMSO and ascorbic acid (the reference component) were added to separate tubes.

The volume was then evenly increased to 150 µL with methanol. A 2.0 mL solution of 10 mM SNP in PBS (phosphate buffer saline) was added to every tube. The solutions were incubated for 150 minutes at room temperature. A similar procedure was carried out again using methanol as the control blank. Each tube, including the control, got 5 mL of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% NEDD (naphthylethylene diamine dihydrochloride) after the incubation period. Using a UV-visible spectrometer, the absorbance of the purple azo dye chromophore, which was created when nitrite ions were diazotized with sulphanilamide and then coupled with NEDD, was measured at 546 nm. To calculate the quantity of nitrite generated in the presence or absence of the plant extract, a standard curve based on $NaNO_2$ solutions with known values was used. Every experiment was conducted a minimum of three times, and the results were displayed as the mean of three separate assessments (Jayachandra *et al*., 2012). The IC_{50} value for each test compound and the standard preparation was determined as follows:

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

Statistical Analysis

Every determination was repeated in three separate assays, and the mean±standard deviation was used to describe the results.

RESULTS and DISCUSSION

Qualitative Analysis

Phytochemical active compounds of *Thuja occidentalis* and *Sonchus asper* subsp. *asper* were qualitatively analysed from leaves of different extracts. The results (Table 1) showed that *Thuja occidentalis* contains carbohydrates, alkaloids, phenolic compounds, flavonoids, saponins, tannins, and phytosterols. *Sonchus asper* subsp. *asper* possesses alkaloids, carbohydrates, phenolic compounds, glycosides, phytosterol, flavonoids, tannins, and fixed oils and fats.

Table 1. Phytochemical constituents in different extracts of leaf of *Thuja occidentalis* (TO) and *Sonchus asper* subsp. *asper* (SA).

Phytochemical constituents	Test	Methanol extract		Chloroform extract		Distilled water extract	
		TO	SA	TO	SA	TO	SA
Carbohydrate	Fehling's test	+	-	+	-	+	-
	Iodine test	+	-	++	+	+	-
	Molisch's test	+	++	+	+	++	+
Alkaloids	Mayer's test	+	+	++	+	+	-
	Wagner's test	++	-	-	-	++	+
	Hager's test	++	+	++	+	++	-
	Dragendroff's test	+	++	+	+	+	+
Glycosides	Borntrager's test	-	-	+	-	-	-
	Killer-Killani test	-	+	-	-	-	-
	Hydroxyanthraquinone test	-	-	-	+	-	-
Phenolic compounds	Lead acetate test	++	++	++	+	++	+
	$Fecl_3$ test	++	+	+	+	+++	+
Saponins	Foam test	++	-	+	-	++	-
Flavanoids	Alkaline reagent test	+	+	++	+	+++	+
	Lead acetate test	+++	+	+++	+	++	-
	Zinc test	-	++	++	-	++	+
Fixed oils and fats	Spot test	-	-	-	+	-	-
	Saponification test	-	-	+	-	-	-
Tannins	Gelatin test	++	+	++	+	++	+
phytosterols	Salkowski's test	+	+	-	+	++	-

+++ = highly present, ++ = moderately present, + = present, - = absent

Quantitative Analysis

Determination of total phenolic content

Determination of the phenolic content of the samples was done by TPC activity. Plant-based phenolic compounds possess redox characteristics that enable them to function as antioxidants. The absorbance values obtained at different concentrations of gallic acid were used for the construction of a calibration curve. Total phenolic content of the extracts was calculated from the regression equation of the calibration curve ($Y = 0.1258x$; $R^2 = 0.996$) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). In comparison to the methanol and chloroform extracts, the distilled water extract demonstrated a higher TPC, as shown in Figure 2, measuring roughly 2073±0.017 mg GAE/g, 1392.6±0.077 mg GAE/g for the methanolic extract, and 898±0.005 mg GAE/g (Table 2) for the chloroform extract of *Thuja occidentalis*. When it came to *Sonchus asper*, the TPC values for the methanolic extract were greater than those for the chloroform and distilled water, showing at about 259.02±0.006 mg GAE/g, 241±0.015 mg GAE/g, and 85.3±0.001 mg GAE/g, respectively. When comparing the two plants, *Thuja occidentalis* showed high TPC.

Table 2. Total phenol, flavonoid, tannin and alkaloid content of *Thuja occidentalis* and *Sonchus asper* subsp. *asper* leaf extract in different solvents.

Variables	Leaf extracts of *Thuja occidentalis* mg/g			Leaf extracts of *Sonchus asper* subsp. *asper* *mg*/g		
	M	C	DW	M	C	DW
TPC	1392.6±0.077	898±0.005	2073±0.017	259.02±0.006	241±0.015	85.3±0.001
TFC	982.02±0.001	879±0.003	1598.8±0.005	408.8±0.001	348.2±0.002	84.95±0.021
TTC	2024±0.006	1146±0.003	1908±0.009	820.9±0.005	606.9±0.001	49.07±0.301
TAC	2667±0.095	2543±0.201	1192±0.095	653.87±0.002	246±0.001	87.7±0.001

Values are presented as mean±SD. SD= Standard deviation
TPC=Total phenol content, TFC=Total flavonoid content, TTC=Total tannin content
M=Methanol, C=Chloroform, DW=Distilled water

The phenolic content of any plant is directly related to its antioxidant properties. Phenolic compounds act as reducing agents and hydrogen donors and are capable of scavenging free radicals. The presence of a considerable amount of phenolics in the different extracts of *Thuja occidentalis* may contribute significantly to the antioxidant properties. Because of these properties, this plant might have been used in several traditional herbal medications. These phenolic chemicals are safe for human health, and the food industry is using more plants high in phenolics to enhance food quality. These phenolic chemicals are safe for human health, and the food industry is using more plants high in phenolics to enhance food quality.

Determination of total flavonoid content

Similar trends were also observed between the TFC and TPC values. Total flavonoid content of the extracts was calculated from the regression equation of the calibration curve ($Y = 0.0946x$; $R^2 = 0.991$) and expressed as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). The TFC values also showed similar trends to those of TPC values. The highest TFC value was obtained for distilled water extract, followed by methanolic extract, and the lowest was for chloroform extract of the plant *Thuja occidentalis*, as shown in Figure 2. The TFC values (Table 2) were as follows: 1598.8±0.005 mg QE/g for distilled water extract, 982.02±0.001 mg QE/g for methanolic extract, and 879±0.003 mg QE/g for chloroform extract. The same trend also follows in *Sonchus asper* as in TPC. In comparison to both chloroform and distilled water, the TFC for the methanolic extract was greater at 408.8±0.001 mg QE/g, 348.2±0.002 mg QE/g for the chloroform extract, and 84.95±0.021 mg QE/g for the distilled water extract. Generally, *Thuja occidentalis* also exhibited higher TFC as compared to *Sonchus asper*. The concentration of phenols and flavonoids also depends on the polarity of the solvents used for extraction.

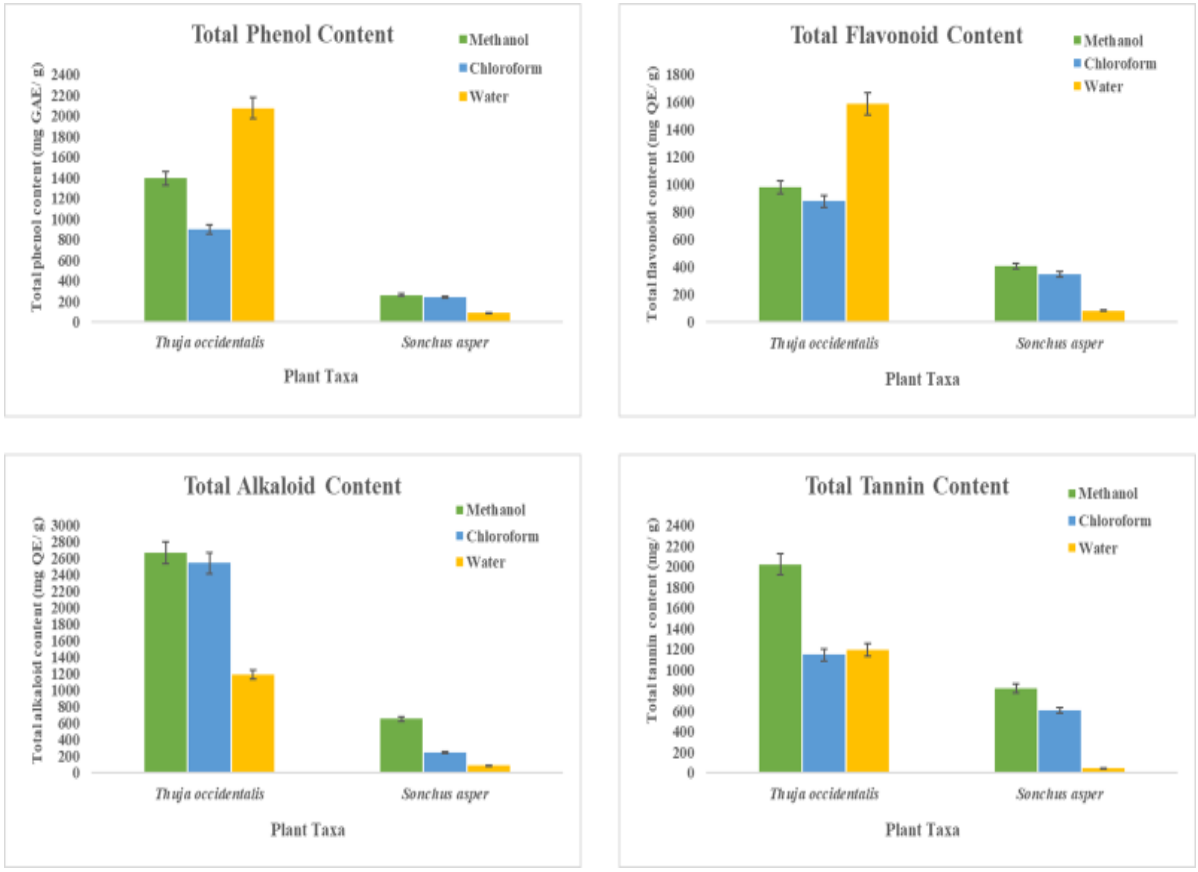


Figure 2. Graph showing a comparison of Total phenol content, Total flavonoid content, Total alkaloid content and Total tannin content of *Thuja occidentalis* and *Sonchus asper* leaf extract in different solvents.

Determination of total alkaloid content

Alkaloids are nitrogenous chemicals that are commonly utilised as stimulants, medications, narcotics, and poisons. They also help protect plants from diseases and herbivores. Total alkaloid content of the extracts was calculated from the regression equation of the calibration curve (Y = 0.1754x; R^2 = 0.995). Atropine standard solutions are compared to a blank. The TAC content was found to be highest in the methanolic extract of *Thuja occidentalis* (Figure 2), whose value (Table 2) was 2667±0.095 mg/g. The moderate content was found in the chloroform extract, whose value was 2543±0.201 mg/g. The lowest content was found in distilled water extract, whose value was 1192±0.095 mg/g. Similarly, in *Sonchus asper*, the methanolic content has the highest TAC content, followed by chloroform, and the lowest in distilled water. Their TAC content values were 653.87±0.002 mg/g of methanolic extract, 246±0.001 mg/g of chloroform extract, and 87.7±0.001 mg/g of distilled water extract. In comparison to both, *Thuja occidentalis* has higher TAC content than *Sonchus asper*.

Determination of total tannin content

Total tannin content of the extracts was calculated from the regression equation of the calibration curve (Y = 0.0285x; R^2 = 0.994). Tannic acid standard solutions are compared to a blank. The highest value of TTC (Figure 2) was obtained in the methanolic extract of *Thuja occidentalis*, and the value was 2024±0.006 mg/g (Table 2). The moderate value was 1908±0.009 mg/g, which was observed in distilled water extract, and the lowest in chloroform extract, whose value was 1146±0.003 mg/g. Similarly, in *Sonchus asper*, the highest value of TTC was obtained in the methanolic extract, whose value was 820.9±0.005 mg/g. The moderate value was obtained in chloroform extract, and the value was 606.9±0.001 mg/g. The lowest value was 49.07±0.301 mg/g, which was observed in the distilled water extract. The high TTC content was observed in different extracts of *Thuja occidentalis* leaf in comparison to *Sonchus*

asper. Many tannin constituents have been linked to anti-mutagenic and anti-carcinogenic properties. Tannins' anti-oxidative properties may be connected to their antimutagenic and anticarcinogenic potential. This characteristic plays a critical role in inhibiting oxidative damage to cells, such as lipid peroxidation. In food processing, tannins' antibacterial qualities can be utilised to extend the shelf life of specific foods.

Antioxidant assay

DPPH radical scavenging assay. Antioxidants found in plants naturally help to lessen or even completely eradicate the harmful effects of oxidative stress. Among many other tests, the DPPH assay is a handy tool for figuring out how antioxidant-potent plants are. By using the DPPH test, the antioxidant activity of *Thuja occidentalis* and *Sonchus asper* was assessed, as shown in Table 3. Their reducing power was calculated based on the concentration at which they provided 50% inhibition (IC_{50}) values, or, to put it another way, the quantity needed to scavenge 50% of the DPPH free radicals. Ascorbic acid was used as the standard, and the different extracts showed variable antioxidant properties. The IC_{50} value of ascorbic acid was 19.06 µg/mL. A higher IC_{50} value indicates lower radical scavenging activity or reduced antioxidant capability. When compared to chloroform and distilled water (Figure 3), the methanolic extracts from *Thuja occidentalis* and *Sonchus asper* exhibited the best antioxidant capacity. The IC_{50} value of the methanolic extract was close to that of the standard, i.e., 50.01µg/mL and 50.97 µg/mL, respectively, for *Thuja occidentalis* and *Sonchus asper* leaf extract. The chloroform extract shows moderate antioxidant activity with the IC_{50} value of 64.84 µg/mL and 57.04 µg/mL, respectively, for *Thuja occidentalis* and *Sonchus asper* leaf extract. The distilled water extract shows the lowest antioxidant activity with the IC_{50} value of 66.69 µg/mL and 74.78 µg/mL, respectively, for *Thuja occidentalis* and *Sonchus asper* leaf extract. As a result, *Thuja occidentalis* shows higher antioxidant potential than Sonchus *asper*.

Table 3. IC_{50} value of leaf extracts of *Thuja occidentalis* and *Sonchus asper* in different solvents.

Variables	Standard compound	Leaf extracts of *Thuja occidentalis* µg/mL			Leaf extracts of *Sonchus asper* subsp. *asper* µg/mL		
		M	C	DW	M	C	DW
DPPH	19.06±0.06	50.01±0.02	64.84±0.016	66.69±0.023	50.97±0.017	57.04±0.025	74.78±0.005
NOSA	10.85±0.02	50.7±0.024	57.95±0.017	62.87±0.023	51.9±0.011	58.68±0.010	78.4±0.005

DPPH= 2,2-diphenylpicrylhydrazine assay, NOSA= nitric oxide radical scavenging assay
M= methanol, C= chloroform, DW= distilled water

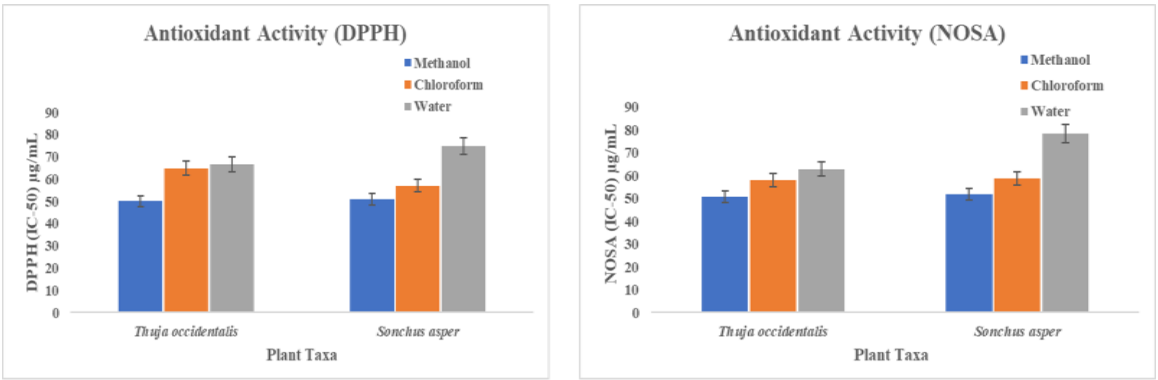


Figure 3. Graph showing a comparison of the antioxidant activity of *Thuja occidentalis* and *Sonchus asper* in different solvents by DPPH assay and NOSA assay.

Nitric oxide radical scavenging assay. In aerobic environments, nitric oxide is an extremely unstable molecule. Through the intermediates N_3O_4, N_2O_4, and NO_2, it interacts with O_2 to form the stable products NO_3 and NO_2. The Griess reagent (GR) is used to estimate it. The quantity of nitrous acid lowers when a test compound—a scavenger—is present. The degree of scavenging is reflected in the magnitude of the drop. Ascorbic acid was used as the standard,

and the different extracts showed variable antioxidant properties. The IC_{50} value of ascorbic acid was 10.85 µg/mL. The percentage inhibition of methanol, chloroform, and distilled water extracts of *Thuja occidentalis* of three parallel readings (Table 3) showed IC_{50} values of 50.7, 57.95, and 62.87 µg/mL, respectively. The methanolic extract of this plant shows greater antioxidant activity, and its values are close to those of the standard. The percentage inhibition of methanol, chloroform, and distilled water extracts of *Sonchus asper* subsp. *asper* of three parallel readings showed IC_{50} values of 51.9, 58.68, and 78.4 µg/mL, respectively. The greater antioxidant activity in this plant was shown by the methanolic extract in Figure 3.

The radical scavenging activity of different extracts of *Thuja occidentalis* and *Sonchus asper* may be due to the presence of polyphenols, flavonoids, and phenolic compounds, and most of the antioxidant activity of plants is due to the phenols. Natural antioxidants present in plants are responsible for inhibiting or preventing the harmful consequences of oxidative stress. Apart from antioxidant properties, flavonoids and other phenolics also exhibit several biological activities, such as antimicrobial, antiviral, and anticancer. These biological and pharmacological activities are usually associated with their ability to bind proteins and free radical scavenging properties. Antioxidants are tremendously important substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups. A comparison was made between edible and weed plants to show that the nutraceutical properties are present in much greater amounts in edible plants than in weed plants. These properties are beneficial for human health, and weed plants may possess harmful compounds that are harmful to human health.

CONCLUSION

The present study has shown that *Thuja occidentalis* and *Sonchus asper* subsp. *asper* leaf are rich in flavonoid, phenolic, tannin, and alkaloid compounds, and so provide a biochemical foundation for their traditional therapeutic use. In addition, compared to *Sonchus asper* subsp. *asper*, *Thuja occidentalis* has the largest content of alkaloids, tannins, phenols, and flavonoids. *Thuja occidentalis* is rich in antioxidant compounds as compared to *Sonchus asper* subsp. *asper*, boosting their usage as sources for the creation of food supplements with antioxidant qualities or as food additives to increase the antioxidant content of food products. It is a promising source of bioactive chemicals that can be used to treat a variety of ailments and make lucrative, nutritious drugs. Further studies are necessary to isolate and characterise the bioactive principles.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent by e-mail upon request.

Informed Consent

Written informed consent was obtained from the participants who participated in this study.

Authorship Contribution Statement

AA contributed to the research concept and design. **PB** and **SKS** were responsible for data collection and assembly, as well as data analysis and interpretation. **PB** and **SKS** also drafted the manuscript, while **AA** provided critical revisions. **PB**, **SKS**, and **AA** gave final approval for the article.

Orcid

Prachi Baliyan https://orcid.org/0009-0001-0827-5370
Supriya Kumari Sharma https://orcid.org/0000-0003-1488-1729
Afroz Alam https://orcid.org/0000-0001-8575-4677

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