

# In -Vitro Antioxidant Activity of *Cedrus Deodara* plant Leaves by Methanolic Extraction

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DOI 10.51129/ujpah-2025-38-1(2)

Received - May 29, 2025

Revised - May 31, 2025

Accepted - June 07, 2025

Published - June 28, 2025

**Abstract** - Oxidative stress results from an inequity between the reactive oxygen species (ROS) formation or the bodies' abilities to eliminate them, leading to cellular damage of biomolecules such as DNA, lipids, and proteins. This condition contributes to several diseases including cancer, liver injury, and aging. Antioxidants, particularly those derived from plants, play a vital role in scavenging free radicals. *Cedrus deodar* (Roxb.) G. Don, a tall coniferous tree native to the Himalayan region, has been traditionally used in Ayurvedic medicine and is known for its rich phytochemical profile. In the current studies, antioxidant activity of the methanolic extraction of *Cedrus deodar* leaves was assessed using the DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical scavenging assay. The phytochemicals screening revealing the occurrence of alkaloid, flavonoid, tannin, saponin, terpenoid and phenolic compounds, and carbohydrate. The DPPH assay verified a concentration-dependent antioxidant activity, though lower than that of the standard antioxidant silymarin. At concentration of 10, 20, and 30 µg/mL, the extract showed 14.61%, 22.12%, and 25.44% inhibition respectively. These findings suggest *Cedrus deodar* possesses free radical neutralizing potential. While its antioxidant activity is moderate, the

presence of multiple bioactive compounds highlights its promise in developing plant-based therapeutic agents. Further investigations are needed to isolate specific active constituents and explore other pharmacological properties.

**Keywords:** *Cedrus deodar*, Antioxidants activity, DPPH assay, Phytochemicals, Free radical scavenging

## Introduction

Formation of reactive oxygen species (ROS) and the intracellular capacity to remove ROS are out of balance, resulting in oxidative stress. It causes significant damage to all biomolecules, including lipids, proteins, DNA, and RNA<sup>[1]</sup>. This damage can lead to the development of a variety of diseases, including cancer, oxygen toxicity, aging, atherosclerosis, lipofuscinosis, and liver injury<sup>[2,3]</sup>. They may act as antioxidants against a variety of free radical-related illnesses<sup>[4]</sup>. Plants' antioxidant activity is caused by phytochemicals that interact. Antioxidants are chemicals that inhibit oxidation or oxidative damage caused by free radicals. As a result, it has the potential to neutralize reactive oxygen species and free radicals. The existence of these phytochemicals

in plant products has also led to recent research demonstrating that they can interact with other species in the environment to obstruct the growing of bacteria or fungi. Because these chemicals inhibit infections and have little toxicity to host cells, they are expected to provide the groundwork for the creation of novel antimicrobial drugs<sup>[5]</sup>.

The towering, perennial coniferous species *Cedrus deodar* (Roxb.) G. Don exhibits branches that are expansive, slightly inclined, or gently pendulous, accompanied by foliage. This species features needle-like, azure-green leaves that are arranged in clusters on smaller branches and are organized in a spiral formation on elongated branches. The reproductive structures, or cones, are upright, presenting a green coloration prior to maturation and transitioning to a reddish-brown hue upon development. The flowering period occurs between October and November, and this species is characterized as monoecious, with individual flowers positioned at the apex of the stems. Native to elevations approximately 2,000 meters above sea level in regions of Afghanistan and the Himalayas, *Cedrus deodar* derives its nomenclature from its remarkable resilience to severe cold conditions. Presently, it is recognized as a prevalent species of afforestation tree in numerous countries within the Northern Hemisphere. Trew was the initial scholar to document *Cedrus deodar*<sup>[6,7]</sup>

This plant is also referred to as *Cedrus deodar* (Latin), deodar, Himalaya cedar (English), devdaar, diar, diyar (Hindi), devdaru, amara, devahvaya (Sanskrit), devdaar (Gujrati), deodar (Marathi), devadaru, devadaram, devataram (Malyalam), bhadradaaru, daevadaaru, gunduguragi (Kannada), burada

deodar, deodar (Urdu), than sin, than-sin (Tibetan), devadaram, tevataram, tunumaram (Tamil), and devadaru (Nepali). The Ayurvedic plant *Cedrus deodar* is purported to exhibit numerous vital, magical, and significant properties, including Virya (potency)-ushan (hot), Rasa (taste)-tickt (bitter), and Gunna (properties)-laghu (light) and snigdha (slimy)<sup>[8]</sup>. *Cedrus deodar* has been the subject of extensive investigation regarding its phytochemical attributes, resulting in the discovery of a variety of chemical constituents, including the occurrence of alkaloid, glycosides, saponin, tannin, or other phenolic compounds, all of which exhibit antioxidant properties.

The large evergreen species, *Cedrus deodar* can achieve a height of up to 60 meters (refer to Figure-3A). The terminal sections of the horizontal branches and branchlets exhibit a slender and pendulous morphology. The acicular, glaucous green leaves, measuring between 2.5 to 5 cm in length, resemble needles, as illustrated in Figure-3B, and are predominantly arranged in dense fascicles, interspersed with a limited number of solitary leaves<sup>[9]</sup>. The bark displays both diagonal and vertical fissures and is characterized by a coloration that may be described as greyish or reddish-brown. Notably, although the male and female cones develop on distinct branches, the species is classified as monoecious. Female cones are borne singularly at the apex of the dwarf shoots and possess a barrel-shaped morphology. Their dimensions range from 2.5 to 4.5 cm in length and exhibit a cylindrical form. The fruit takes on an oval shape, measuring between 3 to 6 inches in length, is brown in colour, and features a hard or dry exterior. The bisexual flowers commence their blooming period during the autumn season<sup>[10]</sup>.



**Figure- 1** Leaves, fruits and flower of Deodar

## **Material and Methods**

### **Chemicals**

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Methanol was bought from Shaila enterprise. Analytical grade reagents were utilized in all other cases.

### **Plant material collection and authentication**

#### **Identification and Collection of the Plant-**

The leaves part of the *Cedrus Deodar* plant has been collected from the local area of Mussoorie, Uttarakhand and were air dried in the shade.

**Authentication of the Plant-**The herbal plant that is *Cedrus Deodara* is used in the study was authenticated by Botanical Survey of India, Dehradun, Uttarakhand. **Authentication no-** BSI/NRC Herb (Ident.) /2025-26/92

### **Preparation of plant extract**

**Plant extraction-** Collected plants material was washed with the help of water and dried under the dark shadow and ground to coarse fine powder in electrical crusher. Powder materials were then extracted completely in Soxhlet apparatus, using methanol. The extract

was furthermore concentrated to the semisolid mass and stored in air tight containers in a refrigerator till future use.

### **In-vitro Antioxidant activities**

**Antioxidant Assay-** The antioxidant activity of the plants extraction were determined by 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay methods. Entirely, the assay were carried out in triplicate.

**DPPH Radical Scavenging Assay-** Free radicals scavenging capacity of the hydro-methanolic extract of *Capsicum Chinense* leaves parts were determined by using DPPH. DPPH solutions (0.004% w/v) was prepared in 95% ethanol. Methanolic extracts of given plants were mixed with the ninety five percent ethanol and water respectively to prepare stock solutions (10 milligram /100ml). From this stock solution, 1ml, 2ml, and 3ml of solutions were taken in 3 test tubes respectively and by sequential dilutions with the similar solvents, the last volume of every test tubes was made up to 10 ml whose concentration was then 10  $\mu\text{g/ml}$ , 20  $\mu\text{g/ml}$ , 30  $\mu\text{g/ml}$  respectively for all extracts. Freshly prepared DPPH solutions (0.004% w/v) was added in each of these test

tubes or later 10 minutes absorbance was taken at 517 nm using in a spectrophotometer (Double beam UV-visible spectrophotometer). Silymarin was used as a reference standard drug and dissolved in a distilled water to make the stock solution with the similar concentrations. A control sample was prepared containing the same volume without any extraction or references standard. % scavenging of the DPPH free radicals was measured using the following equations.

$$\% \text{ DPPH radicals – scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test Sample}) \times 100}{(\text{Absorbance of control})}$$

## Results and Discussion

The phytochemical testing of hydro-methanolic extraction of leave of *Capsicum Chinense* shows the presence of alkaloid, flavonoid, saponin, tannin, phenol, terpenoid and carbohydrates.

**Table-1 Result of Preliminary phytochemical investigation of *Cedrus Deodar***

TESTS	HAE
<b>Tests for Alkaloid</b>	
1. Mayer's Tests	++
2. Wagner's Tests	++
3. Hager's Tests	++
4. Dragendroff's tests	++
<b>Tests for Saponins</b>	
1. Foam test	+
<b>Test for Flavonoids</b>	
1. Alkaline reagent tests	+
2. Lead Acetate tests	+
<b>Tests for Tannins</b>	
1. Gelatin + extract	+
<b>Tests for Phenolic compound</b>	
1. Ferric chloride solution	+
<b>Tests for Terpenoids</b>	
1. Salkowski test	+
<b>Tests for Carbohydrates</b>	
1. Molisch test	+

+ represents presence; ++ represents present in more concentrations; - represents absence.

### Phytochemical screening

Preliminary phytochemicals screening of the *Cedrus Deodar* leaves extraction shows that the plants is rich in various active ingredients (2<sup>nd</sup> ry plant metabolite). The results of phytochemicals screening exposed strongest to moderates occurrence of alkaloids,

flavonoids, saponins and carbohydrates (Table-1).

### *In-Vitro* Antioxidant Activity

**DPPH radical scavenging activity**  
DPPH radicals scavenging strength of *Cedrus Deodar* leaves extract at different

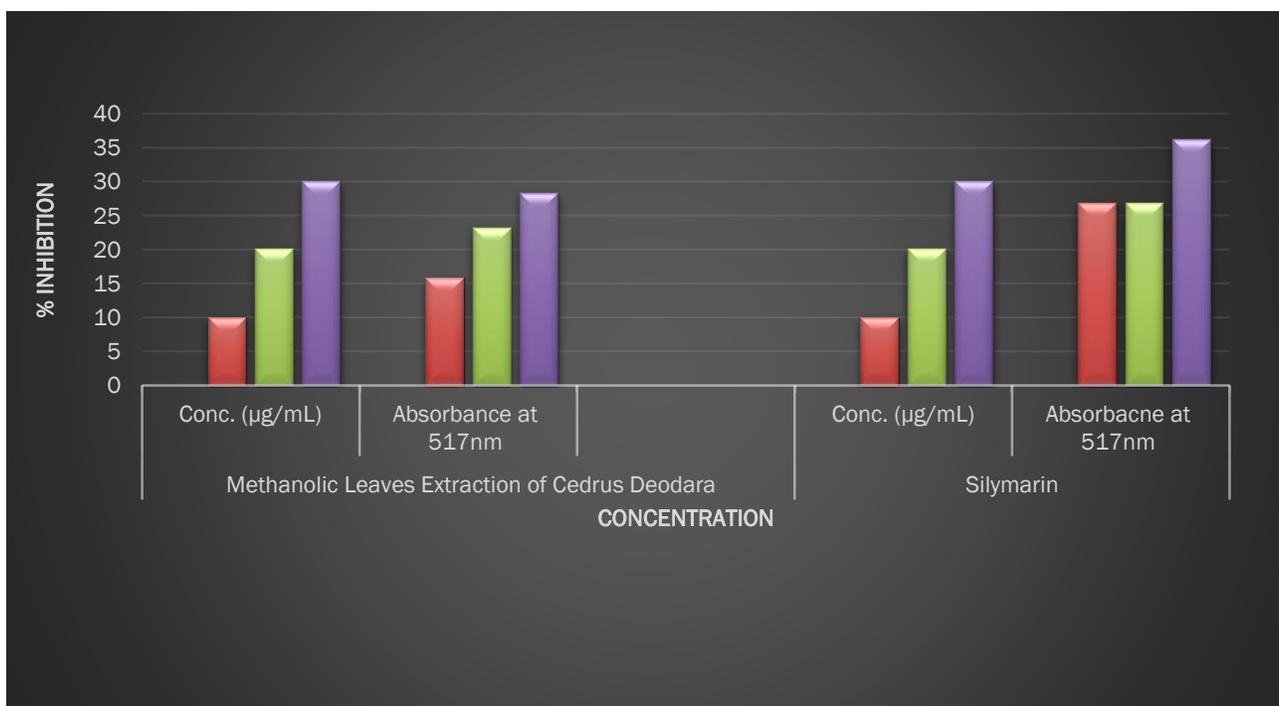
two concentrations were investigated in this present study together with standard antioxidants (Silymarin) at the similar concentration. *Cedrus Deodar* leaves extract (methanolic extracts) presented significant scavenging effects on DPPH free radicals in concentration dependents manner. When it is

compared with the standard antioxidant uses in the experiments, the extracts showed relatively lesser DPPH free radical scavenging potentials.

**Table-2 Antioxidant activities of methanolic extracts of leaves parts of *Cedrus Deodar* by DPPH method**

S. NO.	Methanolic Leaves Extraction of <i>Cedrus Deodara</i>		Silymarin	
	Concentration(µg/mL)	Percentage Inhibition	Concentration (µg/mL)	Percentage Inhibition
01	10	14.61	10	22.45
02	20	22.12	20	24.83
03	30	25.44	30	35.87

**Graphical representation I shows the % inhibition of DPPH radicals by extraction of *Capsicum Chinense***

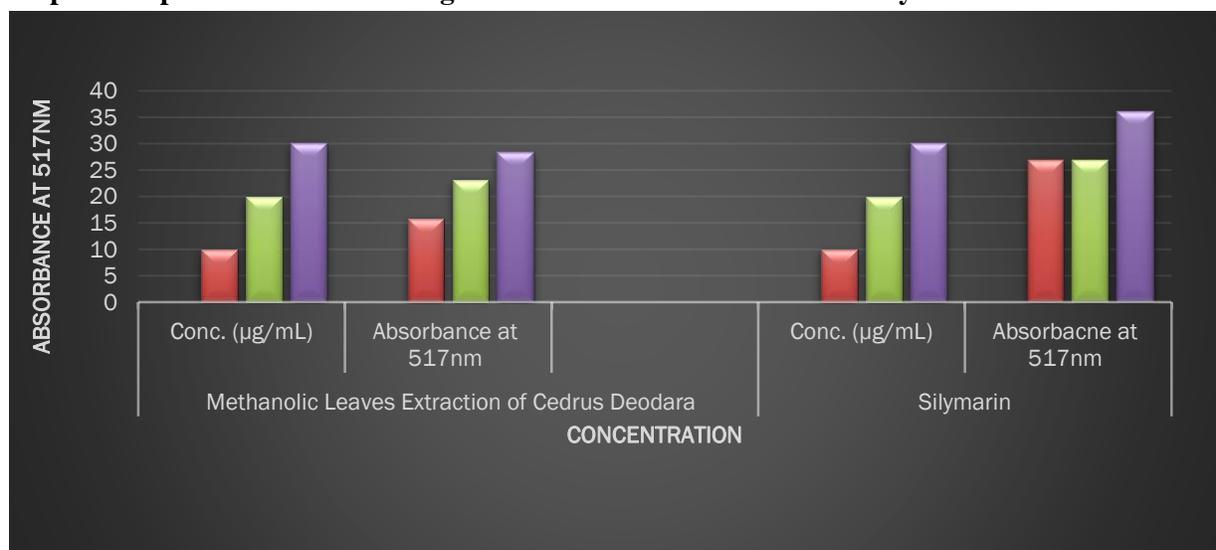


**Table-3 Antioxidant activities of methanolic extraction of leaves part of *Cedrus Deodar* by DPPH method.**

S.NO.	Methanolic Extraction of Cedrus Deodara	Silymarin
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	Concentration ( $\mu\text{g/mL}$ )	Absorbance at 517nm	Concentration ( $\mu\text{g/mL}$ )	Absorbance at 517nm
<b>01</b>	10	15.81	10	26.81
<b>02</b>	20	23.17	20	26.78
<b>03</b>	30	28.31	30	36.14

**Graphical representation II showing the absorbance of DPPH radicals by extracts of *Cedrus Deodar***



At normal temperature, DPPH is a purple stable free radicals with a distinctive absorbance at 517 nm. An antioxidant called 1,1-diphenyl-2-picrylhydrazine readily stifles nitrogen free radical of DPPH. Purple color decolorization is stoichiometric and depends on the number of electrons acquired<sup>[11]</sup>. Leaves extracts from *Cedrus deodar* demonstrated a substantial, concentration-dependent scavenging activity on the free radical DPPH. In contrast to the conventional antioxidants employed in the study, the extract exhibited comparatively reduced ability to scavenge free radical's DPPH. As a result, in vulnerable biological and food systems, *Cedrus deodar* leaf extracts may be able to stop reactive radical species from causing damage to biomolecules like DNA, proteins,

polyunsaturated fatty acids (PUFA), and carbohydrates. The high reactive species recognized as hydroxyl radical ( $\text{HO}\cdot$ ) is produced in biologicals system and targets DNA nucleotides, breaking DNA strands and causing cancer and mutagenesis. By removing the hydrogen atom from membrane lipids' polyunsaturated fatty acid, it starts the lipid peroxidation process. It has the ability to harm practically all of the molecules in living cell<sup>[12]</sup>. The leaves parts extract of *Cedrus deodar* demonstrated the capacity to neutralize free radicals produced, and it also demonstrated concentration-dependent hydroxyl radical scavenging that was equivalent to that of the reference standard (silymarin) at the same dosages<sup>[13]</sup>.

## Conclusion

The current investigation showed that *Cedrus deodar* leaves portions contain a variety of secondary metabolites. These phytochemicals may be a significant source of pharmacological compounds, meaning that the plant species may have enormous potential uses as a treatment for a range of chronic illnesses. The species' crude extract exhibits encouraging antioxidant potential as well, supporting the traditional use of this plant with scientific evidence. More research is required to produce innovative antioxidant medications.

### Acknowledgement

We acknowledge with thanks for the facilities provided by Siddhartha Institute of Pharmacy.

### Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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