Estimation of total phenolic, tannins, and flavonoid contents and antioxidant activity of *Cedrus deodara* heart wood extracts

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Purpose
The present study was investigated to determine *in-vitro* antioxidant activity and total phenolic, total flavonoids, and total tannins contents of extracts of *Cedrus deodara* heart wood.

Materials and methods
Antioxidant activity of aqueous and alcoholic extract of *C. deodara* heart wood was evaluated against 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picryl hydrazyl (DPPH), and hydroxyl radical-scavenging activity models. Total flavonoids, tannins, and phenolic content of *C. deodara* were also determined.

Results and conclusion
Among both extracts, aqueous extract showed the highest total phenolic contents (23.97 μg/g of gallic acid equivalent/g of extract). In DPPH, superoxide anion, and ABTS scavenging test, the IC_{50} (μg/ml) value of aqueous and alcoholic extract was 61.89, 75.79, 87.76, 121.55, 115.29, and 122.42, respectively. Hence, the above evidences suggest that *C. deodara* heart wood is a potential source of natural antioxidant and can be used to prevent diseases associated with free radicals.

Keywords:
antioxidants, *Cedrus deodara,* flavonoids, phenolic

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Introduction

Free radicals are responsible for more than 100 chronic disorders in humans, including atherosclerosis, arthritis, jaundice, liver injury, central nervous system injury, gastritis, cancer, and AIDS [1,2]. Free radicals are generated due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, which cause depletion of the immune system antioxidants, change in gene expression, and induce abnormal proteins. The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of vegetables and fruits. Foods from plant origin usually contain natural antioxidants that can scavenge free radical [3]. Antioxidants also play important role in preventing oxidative deterioration of food and indirectly eliminating radicals from it [4]. Antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and tertiary butylhydroquinone are often used in foods to prevent oxidative degradation. Oxidative atmosphere in cell is also created by the impairment in functioning of endogenous antioxidant enzymes namely superoxide dismutase, glutathione peroxidase, and catalase. Glutathione peroxidase, catalase, superoxide dismutase, and glutathione reductase are known to be inhibited in diabetes mellitus as a result of nonenzymatic glycosylation and oxidation. Antioxidants are protective agents that inactivate reactive oxygen species, and thereby significantly delay or prevent oxidative damage [5].

Recently, there is interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical-induced tissue injury. Antioxidant factors found in plants are based upon constituent nutrients with demonstrated radical-scavenging capacities as well as upon nonvitamin or mineral substances. Hence, in addition to α-tocopherol, ascorbate, carotenoids, and zinc, plant-based medicines may contain flavonoids, polyphenols, and flavoproteins [6–8], but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds [2]. Flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory action [9]. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity [10].

The plant *Cedrus deodara* (Roxb.), belonging to the family Pinaceae (Sanskrit — Devadaru; Hindi, Marathi — Deodar; English — Cedar), is a graceful, ornamental evergreen tree growing extensively on the slopes of the Himalayas. The wood of *C. deodara*

has been used since ancient days in Ayurvedic medical practice for the treatment of inflammations and rheumatoid arthritis [11,12]. During a routine screening of Indian medicinal plants for biological activity, 50% ethanolic extract of the wood of *C. deodara* showed a significant antispasmodic activity [13]. The alcoholic extract of the stem of *C. deodara* was found to have anticancer activity against human epidermoid carcinoma of nasopharynx in tissue culture [14]. The oil of *C. deodara* wood was found to have potent disinfectant [15] and antifungal properties. The *C. deodara* contains dihydroflavonols including cedeodarin (6-methyltxifolin), cedrin (6-methyl dihydromyricetin), and cedrinoside (cedrin 3-*O*-β-d-glucopyranoside) [16]. The chloroform extract of *C. deodara* showed strong antioxidant activity on 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical, and (-)-matairesinol, (-)-nortrachelogenin, and dibenzyl butyrolactone (4,4',9-trihydroxy-3,3'-dimethoxy-9,9'-epoxylignan) are isolated from that chloroform extract [17]. In present study, we carried out a systematic record of the relative free radical-scavenging activity in *C. deodara* heart wood aqueous and alcoholic extracts. We also found the relationship of total flavonoids, total tannins, and total phenols contents with antioxidant activity. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals-induced tissue damage.

Materials and methods

Chemicals

DPPH, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nitro blue tetrazolium, dimethyl sulfoxide (DMSO), ascorbic acid, and EDTA were purchased from Sigma Chemicals (St Louis, Missouri, USA).

Plant material

The heart wood of *C. deodara*, obtained from a commercial supplier, was identified and authenticated from Government Agriculture College, Indore (Madhya Pradesh, India), and a voucher specimen has been submitted to Pharmacognosy Department of the college (voucher specimen no. Scope/Ph.Cog/07-09/02).

Plant extract

A total of 100 g air-dried heart wood was coarsely powdered and refluxed for 6 h using distilled water. The residue was removed by filtration and the aqueous extract was concentrated under vacuum by freeze dryer (Freeze Mobile EL; Virtis, Gardiner, New York, USA) to give brownish material 3.5%. Alcoholic extract was prepared in the same way as aqueous extract, with the exception of the use of 70% ethanol as a solvent instead of using distilled water. The extract yield was 5% w/w.

Determination of total flavonoids

One gram of powder drug was extracted with 100 ml methanol (90%) using hot decoction method for 1 h followed by filtration; from this filtrate, 1 ml was placed in 10 ml of volumetric flask and 3 ml of methanol and 0.3 ml of $NaNO_2$ (1 : 20) were added in flask. Thereafter, 3 ml of 2% aluminum chloride was added after 5 min. After 6 min, 2 ml of NaOH was added and the final volume was made up to 10 ml with methanol. The solution was mixed well in flask and the absorbance was measured against a blank at 510 nm. Total flavonoids amounts were expressed as catechin equivalents per dry matter. All samples were analyzed thrice and results were averaged [18,19].

Determination of total tannins

Drug powder was refluxed in 100 ml 70% aqueous acetone for 2 h. The filtrate was concentrated up to 25 ml. Concentrated solution was partitioned with ether (three times) and then with *n*-butyl alcohol previously saturated with water. The *n*-butyl alcohol portion was combined, dried over bath under constant weight [20].

Total tannins content was calculated by following formula:

$$\%\ \text{w/w total tannins content} = \left(\frac{\text{Weight of } n \text{ butanol fraction in g}}{\text{Weight of sample taken in g}}\right) \times 100$$

Total phenols determination

Total phenols were determined by Folin–Ciocalteu reagent [21]. A dilute extract of each plant extract (0.5 ml of 1 : 10 g/ml) or gallic acid (standard phenolic compound) was mixed with Folin–Ciocalteu reagent (5 ml, 1 : 10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 mol/l). The mixtures were allowed to stand for 15 min and the total phenols were determined colorimetrically at 765 nm. The standard curve was prepared using different aliquots of gallic acid. Total phenol values are expressed in terms of gallic acid equivalent (μg/g of dry mass), which is a common reference compound.

In-vitro antioxidant activity

1,1-Diphenyl-2-picryl hydrazyl assay

DPPH radical-scavenging activity was determined according to the method of Anandjiwala [21]. In brief, 15 mg of DPPH was dissolved in 10 ml of methanol. It was protected from light by covering the test tubes with aluminum foil. The DPPH solution (75 µl) was taken and the final volume was adjusted to 3 ml with methanol; absorbance was taken after 30 min at 517 nm for control reading. Different concentration of extracts, 75 µl of DPPH, and methanol were added to adjust the volume up to 3 ml; contents were mixed and incubated at 20°C for 30 min and absorbance was measured:

$$\% \text{ Scavenging} = \frac{A_1 - A_2}{A_1} \times 100$$

where A_1 is absorbance of control without extract and A_2 is absorbance of extracts.

Superoxide anion scavenging activity (dimethyl sulfoxide assay)

Superoxide anion scavenging activity was determined according to the method of Srinivasan *et al*. [22]. To the reaction mixture containing 0.2 ml of nitro blue tetrazolium and 0.6 ml of the various concentrations of extracts, 2 ml of alkaline DMSO was added to give a final volume of 2.8 ml. For the control, methanol or water was used instead of the test compound. The absorbance was measured at 734 nm. The experiment was performed in triplicate:

$$\% \text{ Scavenging} = \frac{A_1 - A_2}{A_1} \times 100$$

where A_1 is the absorbance of control without extract, A_2 the absorbance of extracts.

2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) assay

Antioxidant activity of the samples was measured through an assay described by Siddhuraju and Manian [23], with slight modification. ABTS was produced by reacting 7 mmol/l $ABTS^+$ aqueous solution with 2.4 mmol/l potassium persulfate in the dark for 16 h at room temperature. Before assay, this solution was diluted in methanol (about 1: 89 v/v) at 30°C; absorbance was measured at 734 nm. The stock solution of the sample extracts was diluted so that after introduction of 0.5 ml of different aliquots into the assay, they produced inhibitions between 20 and 80% of the blank absorbance. After the addition of 1 ml of diluted ABTS solution to 0.5 ml of different concentrations of sample in methanol, absorbance was measured at 30°C, 30 min after the initial mixing. Appropriate solvent blank was also run in each assay. Triplicate determinations were made at each dilution. The measurement was run in triplicate and percentage inhibitions were calculated:

$$\% \text{ Scavenging} = \frac{A_1 - A_2}{A_1} \times 100$$

where A_1 is the absorbance of control without extract and A_2 the absorbance of extracts.

Result and Discussion

Flavonoids content (mg of RU/100 g) in powder of heart wood was determined against standard curve of rutin (r^2 = 0.9998). The results indicate that it contains high amount of flavonoids and tannins (Table 1). Flavonoids and tannins are key responsible phytoconstituents for antioxidant activity of the plants. Total phenolic compound of both aqueous and alcoholic extracts expressed as gallic acid equivalent (µg/g) of extract ranged from 17 to 23 µg/g (Table 2). The aqueous extract has the highest phenolics content followed by alcoholic extract.

Antioxidant activities of both extracts were determined through DPPH, DMSO, and ABTS methods. Aqueous extract showed maximum activity in all the above-mentioned methods (Figs 1–3).

The role of an antioxidant is to remove free radicals. One mechanism through which this is achieved involves donating hydrogen to a free radical, and hence its reduction to an unreactive species. Addition of hydrogen removes the odd electron feature, which is responsible for radical reactivity. DPPH is a stable free radical. *In-vitro* study was carried out on this radical based on the measurement of the scavenging ability of antioxidants toward the stable radical DPPH [21]. This radical reacts with suitable reducing agents, the electrons become paired off, and the solution loses color stoichiometrically depending on the number of electrons taken up [24]. From the present results, it may be concluded that both extracts are able to reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles,

Table 1 Estimation of total flavonoids, tannins, and phenolic contents in heart wood of *Cedrus deodara*

Phytochemicals	Results
Total flavonoids (µg/g)	19.49 ± 1.46
Total tannins (% w/w)	1.72 ± 0.04

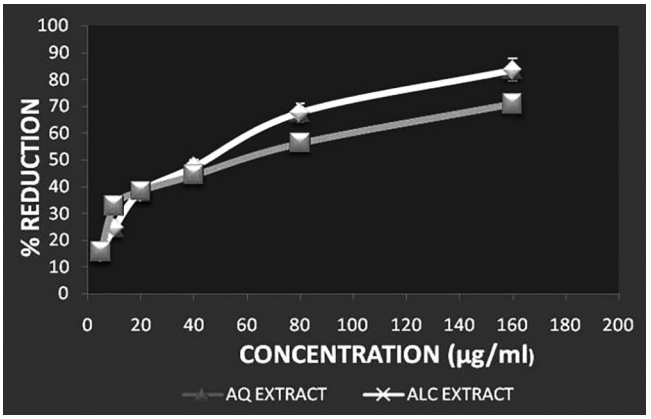
Values are represented as mean ± SEM (n = 3).

Table 2 Total phenolic contents in heart wood of *Cedrus deodara*

Total phenolic compounds	Results
Aqueous extract (µg/g)	23.97
Alcoholic extract (µg/g)	17.71

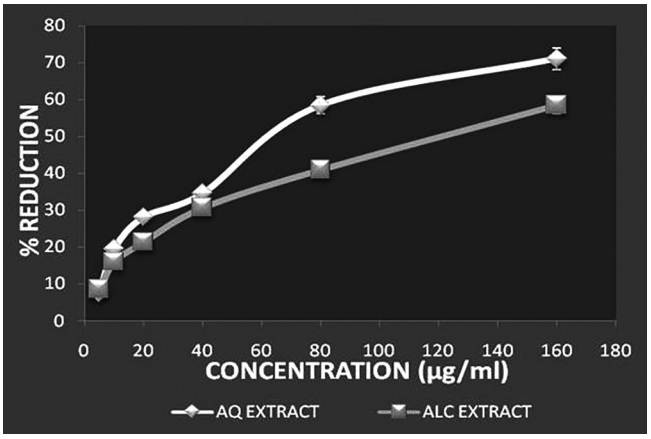
Values are represented as SEM (n = 3).

Figure 1



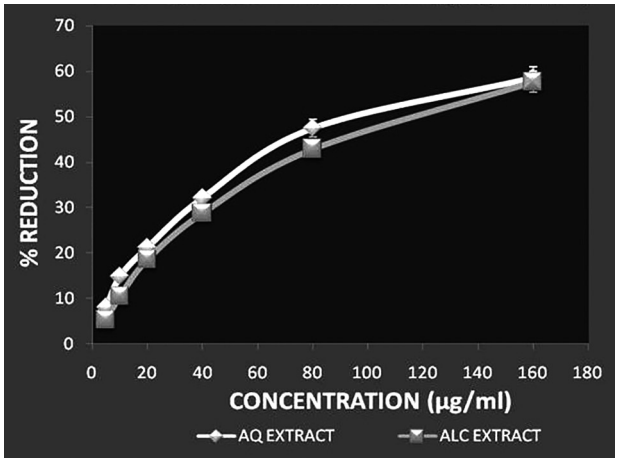
Effect of alcoholic and aqueous extracts of *Cedrus deodara* in DPPH radical-scavenging activity. IC_{50} (µg/ml): 61.89 (aqueous extract), 75.79 (alcoholic extract). Values are represented as mean ± SEM (n = 3). DPPH, 1,1-Diphenyl-2-picryl hydrazyl.

Figure 2



Effect of alcoholic and aqueous extracts of *Cedrus deodara* in superoxide radical-scavenging activity. IC_{50} (µg/ml): 87.76 (aqueous extract), 121.55 (alcoholic extract). Values are represented as mean ± SEM (n = 3).

Figure 3



Effect of alcoholic and aqueous extracts of *Cedrus deodara* in ABTS radical cation-scavenging activity. IC_{50} (µg/ml): 115.29 (aqueous extract), 122.42 (alcoholic extract). Values are represented as mean ± SEM (n = 3). ABTS, 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid).

but the extent of antioxidant activity is significantly high in the aqueous extract (Fig. 1).

ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^+$, which has a long wavelength absorption spectrum [25]. It is useful for testing fruit extracts because generally they do not absorb light at 734 nm [26]. The ABTS chemistry involves direct generation of ABTS radical monocation without involvement of any intermediary radical [27]. It is a decolorization assay; thus, the radical cation is performed before the addition of antioxidant test system, rather than the generation of the radical taking place continuously in presence of the antioxidant [28–30]. The results revealed that antioxidant activity of the extracts is either by inhibiting or scavenging the $ABTS^+$ radical (Fig. 2).

Scavenging of OH^- is an important antioxidant activity because of very high reactivity of the OH^- radical, which enables it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides. Although OH^- formation can occur in several ways, by far the most important mechanism *in vitro* is the Fenton reaction, where a transition metal is involved as a pro-oxidant in the catalyzed decomposition of superoxide and hydrogen peroxide [29]. Both extracts showed OH^- scavenging phenomenon in the assay, but aqueous extract has the highest degree of scavenging activity (Fig. 3).

Conclusion

The result of the present study showed that the aqueous extract of *C. deodara,* which contains the highest amount of tannin, flavonoids, and phenolic compounds, exhibited the greatest antioxidant activity. The data suggested that extract possesses direct and potent antioxidant activities through multiple mechanisms. Especially, the DPPH radical-scavenging activity and the reducing power of *C. deodara* were potent, which is an efficient organic electron and hydrogen donor.

The potential of *C. deodara* to scavenge the free radicals proves that it could be helpful in treatment of ailments generated due to free radicals in an effective way. *C. deodara* holds future promises as nutraceuticals in treatment of various free radicals-dependent ailments.

Acknowledgements

Conflicts of interest

None declared.

References

1 Kumpulainen JT, Salonen JT, Natural antioxidants and anticarcinogens in nutrition, health and disease, Volume 5. UK. The Royal Society of Chemistry 1999; 178–187.

2 Cook NC, Samman, S, Flavonoids chemistry, metabolism, cardioprotective effects, and dietary sources. Nutr Biochem 1996; 7:66–76.

3 Nohl H, Involvement of free radicals in ageing: a consequence or cause of senescence. Br Med Bull 1993; 49:653–667.

4 Vagi E, Rapavi E, Hadolin M, Peredi KV, Balazs A, Blazovics A, Phenolic and triterpenoid antioxidants from Origanum majorana L. herb and extracts obtained with different solvents. J Agric Food Chem 2005; 53:17–21.

5 Joseph LE, Ira DG, Betty AM, Gerold MG, Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction. Diabetes 2003; 52:11–18.

6 Chu Y, Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agri 2000; 80:561–566.

7 Koleva II, Van-Beek TA, Linssen JPH, Groot A, Evstatieva LN, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal 2002; 13:8–17.

8 Shashi A, Sanjay KJ, Amita V, Mayank K, Alok M, Monika S, Herbal antioxidant in clinical practice: a review. Asian Pac J Trop Biomed 2014; 4:78–84.

9 Frankel E. International conference on food factors. Chemistry and Cancer Prevention. Hamamatsu, Japan Abstracts; 1995. Nutritional benefits of flavonoids; pp. C6–2.

10 Gryglewski RJ, Korbut R, Robak J, On the mechanism of antithrombotic action of flavonoids. Biochem Pharmacol 1987; 36:317–321.

11 Nadkarni M, Indian materia medica. Volume 1. New Delhi, India. Popular Book Depot 1954; 295–296.

12 Shinde UA, Phadke AS, Anti-inflammatory and analgesic activity of Cedrus deodara (Roxb.) Loud. Wood oil. J Ethnopharmacol 1999; 65:21–27.

13 Kar K, Spasmolytic constituents of *Cedrus deodara* pharmacological evaluation of himachalol. J Pharm Sci 1975; 64:258–262.

14 Singh SK, Shanmugavel M Chemically standardized isolates from *Cedrus deodara* stem wood having anticancer activity. Planta Med 2007; 73:519–526.

15 Parihar P, Antibacterial potential of *Cedrus deodara*. Adv Plant Sci 2003; 16:479–482.

16 Adinarayana D, Seshadri TR, Chemical investigation of the stem-bark of *Cedrus deodara*: isolation of a new dihydroflavonol deodarin. Tetrahedron 1965; 21:3727–3730.

17 Tiwari AK, Srinivas PV, Kumar SP, Rao JM, Free radical scavenging active components from *Cedrus deodara*. J Agric Food Chem 2001; 49:4642–4645.

18 Zhishen J, Mencheng T The determination of flavonoid content in mulberry and their scavenging effect on superoxide radicals. Food Chem 1999; 64:555–559.

19 Barua DN, Houghton EA, Methods for the volumetric estimation of tea tannin in green-leaf and black tea. A new alkaline permanganate method. Biochem J 1940; 34:1524–1531.

20 Pourmorad F, Hosseinimehr SJ, Shahabimajd N, Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol 2006; 5:1142–1145.

21 Anandjiwala S, Antioxidant activity of stem bark of Thespepsia populnea. J Nat Remedies 2007; 7:135–141.

22 Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B, Free radical scavenging activity of *Ipomoea obscura* (L.) Ker-Gawl. J Nat Remedies 2007; 10:184–188.

23 Siddhuraju P, Manian S, The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chem 2007; 105:950–958.

24 Klein SM, Cohen G, Cederbaum AI, Production of formaldehyde during metabolism of dimethylsulphoxide by hydroxyl radical generating system. Biochemistry 1981; 20:6006–6012.

25 Mao L, Zheng X, Feique MB, *In-vitro* and *in-vivo* antioxidant activities of daylily flowers and the involvement of phenolic compounds. Asia Pac J Clin Nutr 2007; 16:196–199.

26 Kaleem M, Asif MA, Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin induced diabetic rats. Singapore Med J 2006; 47:670–675.

27 Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcuminoids. Int J Pharmacog 1990; 58:237–240.

28 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolourisation assay. Free Radic Biol Med 1999: 26:1231–1237.

29 Stohs SJ, Bagchi D, Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 1995; 18:321–336.

30 Souad EG, Faten MAE, Emad MH, Emad S, Doha H, Antioxidant activity of phenolic compounds from different grape wastes. J Food Process Technol 2014; 5:2–7.