Research Article

# Antioxidant Activity of Different Extracts of *Valeriana hardwickii*

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**ABSTRACT**

*Valeriana hardwickii* is a pubscent herb, measures up to 1.5 mm in height, found in the temperate Himalayas from Kashmir to Bhutan at altitudes of 1200-3600 m and in the Khasi and Jaintia hills between 1,500 and 1,800 m. The root is bitter, carminative, diuretic, expectorant, nervine and stimulant. It is used as a nerve tonic and in the treatment of conditions such as epilepsy and hysteria. It is also used in the treatment of rheumatism and low blood pressure. In the present investigation, the antioxidant activities of different polar and non polar solvent extracts viz. hexane (H), petroleum ether (PE), acetone (AC), chloroform (C), ethanolic (E) and water (W) extracts of whole plant (1 mg/ml) of *Valeriana hardwickii* were determined by standard and routine in vitro antioxidant procedures. The results confirmed that acetone extract and hexane extract of whole plant of *Valeriana hardwickii* exhibited potent antioxidant activity in comparison to that of ethanol, aqueous and petroleum ether extracts. The results thus concluded that *Valeriana hardwickii* acts as a potent antioxidant.

**Keywords:** Anti oxidant activity, Polar and non polar solvent extracts, Potent molecules, *Valeriana hardwickii*.

## INTRODUCTION

Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis of many known drugs. The Himalayas have a great wealth of medicinal plants and traditional medicinal knowledge.[1] Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. The Health advantages of diets rich in antioxidant plant compounds include lowering the risk of cardiovascular disease, certain cancers and the natural degeneration of the body associated with the aging process. Free radicals are unstable molecules formed when the body uses oxygen for energy. The instability of these molecules can damage tissues, alter DNA and change cell structure. Ultimately, free radicals start a chain reaction resulting in the reproduction of even more free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage, free radicals cause to the body. The role antioxidants have in free radical stabilization involves the antioxidants donating one of their own electrons to the free radical. This electron donation is done without the antioxidant becoming unstable or damaging to the body. This remarkable action stabilizes the free radicals as quickly as they are produced in the human body. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavanoids[2] which prevents free radical damage, reducing risk of chronic diseases. Antioxidant activity in peel and pulp of Citrus fruits, different varieties of Apple (*Pyrus malus* L.) of Kashmir (J&K) and different plants of Uttarakhand were investigated.[3-4] Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular disease.[5] Recently, Epoxy sesquithujene, a new sesquiterpene epoxide and also other fourteen other terpenoids has been characterized in the essential oil of *Valeriana hardwickii* on the basis of chemical reactions, extensive NMR data and GC-MS.[6] In *V. hardwickii*, sixty-two, thirty-one, and thirty-one components were identified representing 90, 90, and 92% of total oil in the roots, stems, and leaves, respectively. The major compounds in the root oil were camphene (12.9%), bornyl acetate (17.6%), and maaliol (10.6%), while borneol (6.2%), *trans*-anethole (32.7%), and maaliol (6.3%) were the dominant components in the stem oil and camphene (12.6%), bornyl acetate (15.0%), and hexahydrofarnesyl acetone (9.2%) were the principal components in the leaf oil.[7] Volvalerenol A, an unprecedented type of triterpenoid with a 7,12,7 tricyclic ring system, was obtained from the ethanol extracts of the roots of *Valeriana hardwickii* by comprehensive analysis of MS and NMR spectroscopic data.[8]

The volatile oil of *Valeriana hardwickii* was mainly composed of oxygenated sesquiterpenes (25.7%) including Valeracetate (11.6%), Cuparene (7.1%) and β-Acoradienol (3.5%) as the major components. Where as, α-Gurjunene (3.1%) and α- Guaiene (2.4%) were the dominant compounds among sesquiterpene hydrocarbons.[9] *Valeriana hardwickii Wall*. Rhizome exhibited dose-dependent protection against castor oil-induced diarrhoea in mice.[10] The search for newer natural antioxidants and antimicrobials especially of plant origin has ever since increased. Antimicrobial potential of some plants of Uttarakhand were investigated.[11-13] In this study, the Traditional solvent extraction (TSE) methods were used for extraction of antioxidants. The results can determine the natural antioxidants available in the plant parts having solubility in the specific solvent. Also, the extraction methods will emphasize on using the specific solvent (hexane, petroleum ether, chloroform, acetone, ethanol and water) for extracting antioxidants and polyphenolics. This study may provide insight for future extraction solvents and natural potent antioxidants which can be used as dietary supplements.

## MATERIALS AND METHODS

### Plant Materials

The plant material was collected from Garhwal region of Uttarakhand, India. The plant material was identified from Botanical Survey of India, Dehradun. Voucher specimen of the plant was stored in the Dept. herbarium for future reference. The plant material was dried in the shade in an open air for 5-10 days to form the fine powder.

### Preparation of Plant extracts

Plant parts were separated, washed with distilled water, dried under shade and pulverized. The plant extracts were prepared according to the method prescribed with little modifications[14]. Briefly 20 g portions of the powdered plant material was soaked separately in different solvents i.e. petroleum ether, hexane, chloroform, acetone, ethanol and distilled water on the basis of increasing polarity for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30°C .

### Determination of *In vitro* Antioxidant activity

#### *Determination of Total Phenolic Content (TPC)*

The Total Phenolic Content of each extract obtained of each of the plant extract was determined[15] and the phenolic content was expressed as µg/g Gallic acid equivalents. In brief a 100 µl aliquot of the sample was added to 2 ml of 0.2% (w/v) $Na_2CO_3$ solution. After two minutes of incubation, 100 µl of 500ml/l Follin-Ciocalteu reagent added and the mixture was then allowed to stand for 30 minutes at $25^0$C. The absorbance was measured at 750 nm using a UV-VIS Systronics spectrophotometer. The blank consist of all reagents and solvents but no sample. The Total Phenolic Content (TPC) was determined using the standard Gallic acid calibration curve and was expressed as µg/g Gallic acid equivalents.

### Determination of Antioxidant Activity by DPPH Radical Scavenging Method

The extract solution for the DPPH test[16] was prepared by re-dissolving 0.2 g of each of the dried extract in 10 ml of the specific solvent in which the extract was prepared. The concentration of DPPH solution was 0.025 g in 1000 ml of methanol. Two ml of the DPPH solution was mixed with 40 µl of each of the plant extract solution and was transferred to a cuvette. The reaction solution was monitored at 515 nm, after an incubation period of 30 minutes at room temperature, using a UV-Visible Systronics spectrophotometer. The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation: Inhibition % = (Abst=0 min---Abst=30 min)/ Abst=0 min ×100 Where Abst=0 min was the absorbance of DPPH at zero time and Abst=30 min was the absorbance of DPPH after 30 minutes of incubation. Ascorbic acid (0.5 mM) dissolved in methanol was used as a standard to convert the inhibition capability of plant extract solution to the Ascorbic acid equivalent. $IC_{50}$ is the concentration of the sample required to scavenge 50% of DPPH free radicals.

### Superoxide Anion Radical Scavenging Activity

Superoxide Anion Radical scavenging Activity was measured according to the method[17] with some modifications. The different plant extracts were mixed with 3 ml of reaction buffer solution (pH, 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction solution was illuminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance was measured at 560 nm using Systronics UV-VIS double beam spectrophotometer. Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control.

The Superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{Ao - As}{Ao} \times 100$$

### Phytochemical screening of the extracts

The portions of the dry extracts were subjected to the phytochemical screening using the method adopted[18-19]. Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavonoids, steroids, sugars and cardiac glycosides.

### Test for alkaloids

The 0.5 g of the plant extracts were dissolved in 5 ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Dragendroff's reagent Turbidity or precipitation was taken as indicator for the presence of alkaloids.

### Test for Tannins

About 0.5 g of the sample was dissolved in 10 ml of boiling water and was filtered. Few ml of 6% $FeCl_3$ was added to the filtrate. Deep green colour appeared confirmed the presence of Tannins.

**Test for Flavonoids**

About 0.2 g of the extracts were dissolved in methanol and heated for some time. A chip of Mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was Indicator of the flavonoids.

**Test for Saponin**

About 0.5 g of the plant extracts were stirred with water in the test tube. Frothing persists on warming was taken as a evidence for the presence of saponin.

**Test for Steroids**

Salkowaski method was adopted for the detection of steroids. About 0.5 g of extracts were dissolved in 3 ml of chloroform and filtered. To the filtrate, conc. $H_2 SO_4$ was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring.

**Test for Cardiac glycoside**

About 0.5 g of the extracts was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% $Fecl_3$. This was under laid with conc. $H_2 SO_4$. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

**Test for reducing Sugars**

1ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extracts. The mixtures were heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

## RESULTS AND DISCUSSION

**Antioxidant activity**

*In vitro* antioxidant activity was determined by DPPH radical scavenging method and Superoxide anion radical scavenging assay. The results confirmed that acetone extract and hexane extract of whole plant of *Valeriana hardwickii* exhibited potent antioxidant activity in comparison to that of ethanol, aqueous and petroleum ether extracts. Amongst all the extracts, acetone and hexane extracts showed potent antioxidant activity as determined by the procedures. TPC in acetone extract was found to be 186 µg/ml followed by hexane extracts having 175 µg/g gallic acid equivalents. $IC_{50}$ value of acetone extract was found to be 15.60 µg/ml followed by hexane extracts viz. 18.00 µg/ml in DPPH radical scavenging method. It was found that minimum is the value of IC50, maximum is the antioxidant activity. In Superoxide anion radical scavenging method acetone extracts showed 78 % inhibition of superoxide followed by hexane extracts having 73% inhibition. Ascorbic acid was used as the standard antioxidant having $IC_{50}$ value, 78.17 µg/ml in DPPH radical scavenging method and causes 87.80 % inhibition of superoxide. The results are shown in Table 1, 2 and 3; Figure 1, 2 and 3. The results of all the three procedures are totally correlated to each other and confirm the use of plant as natural antioxidant. Presence of polyphenolics in the extract confirm their utility as potent antioxidant agent as revealed by the experimental results. Traditional Solvent Extraction (TSE) method was found to be efficient for extraction of antioxidants.[7]

**Table 1:** TPC (µg/g gallic acid equivalents) of solvent extracts of *Valeriana hardwickii*

| *Valeriana hardwickii* (Solvent Extracts) | TPC (µg/g gallic acid equivalents) |
|---|---|
| Ethanol extract (E) | 150 |
| Acetone extract (AC) | 186 |
| Water extract (W) | 145 |
| Hexane extract (H) | 175 |
| Chloroform extract (C) | 87 |
| Petroleum ether extract (PE) | 85 |

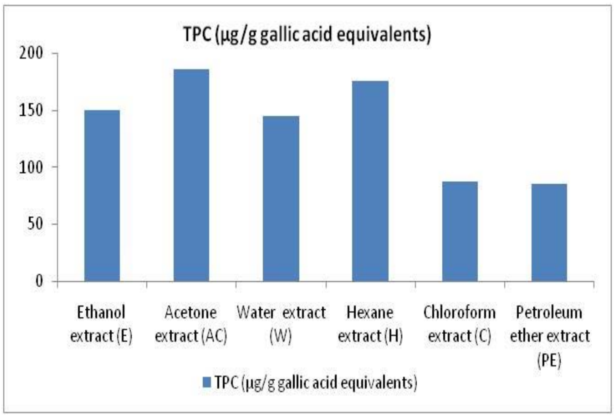


**Figure 1:** TPC (µg/g gallic acid equivalents) of solvent extracts of *Valeriana hardwickii*

**Table 2:** IC 50 values of solvent extracts of *Valeriana hardwickii* as determined by DPPH assay

| *Valeriana hardwickii* (Solvent Extracts)/ Positive Control | IC50 values |
|---|---|
| Ethanol extract (E) | 19.10 |
| Acetone extract (AC) | 15.60 |
| Water extract (W) | 20.00 |
| Hexane extract (H) | 18.00 |
| Chloroform extract (C) | 20.15 |
| Petroleum ether extract (PE) | 25.05 |
| Positive Control, Ascorbic acid | 78.17 |

**Phytochemical Screening**

Different conventional methods were followed to determine qualitatively the presence of phytochemical constituents present in the potent extracts. It was found that alkaloids and reducing sugars were found only in ethanol, aqueous and acetone extract while steroids and saponin were found only in hexane, chloroform and petroleum ether extracts.

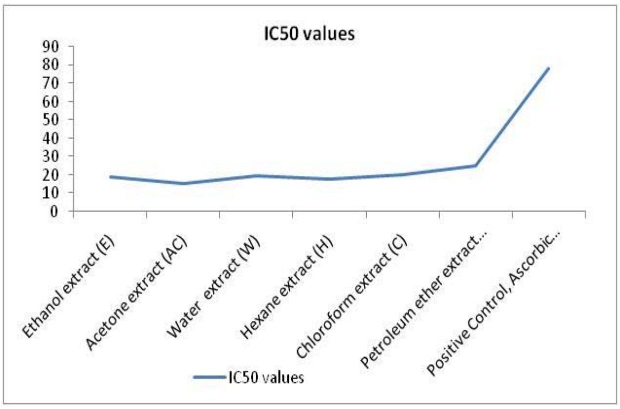


**Figure 2:** IC50 values of solvent extracts of *Valeriana hardwickii* as determined by DPPH assay

**Table 3:** Percent inhibition of superoxide free radicals of solvent extracts of *Valeriana hardwickii* as determined by Superoxide anion radical scavenging activity

| ***Valeriana hardwickii* (Solvent Extracts)/ Positive Control** | **Percent inhibition of Superoxide free radicals** |
|---|---|
| Ethanol extract (E) | 68.0 |
| Acetone extract (AC) | 78.0 |
| Water extract (W) | 63.0 |
| Hexane extract (H) | 73.0 |
| Chloroform extract (C) | 28.0 |
| Petroleum ether extract (PE) | 25.0 |
| Positive Control, Ascorbic acid | 87.80 |

**Table 4:** Phytochemical screening of solvent extracts of *Valeriana hardwickii*

| ***Valeriana hardwickii* (Solvent Extracts)** | **Phytochemicals** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Alkaloids** | **Tannins** | **Flavanoids** | **Saponin** | **Steroids** | **Cardiac glycosides** | **Reducing Sugars** |
| Ethanol extract (E) | + | _ | _ | _ | _ | _ | + |
| Acetone extract (AC) | + | _ | _ | _ | _ | _ | + |
| Water extract (W) | + | _ | _ | _ | _ | _ | + |
| Hexane extract (H) | _ | _ | _ | + | + | _ | _ |
| Chloroform extract (C) | _ | _ | _ | + | + | _ | _ |

+: Presence; -: Absence

The results also confirmed the absence of tannins, flavonoids and cardiac glycosides in all the extracts. The results are indicated in Table 4. The study thus highlighted the importance of pharmacological importance and scientific investigation of plants from North West Himalaya Garhwal region through forward bio-prospection to uncover bioactive phytochemicals of interest and thus validates traditional medicine.

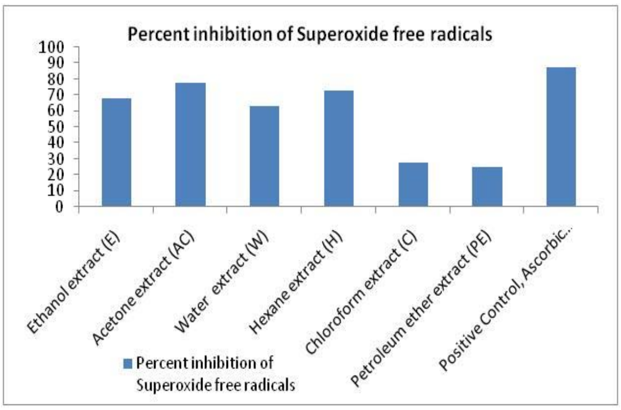


**Figure 3:** Percent inhibition of superoxide free radicals of solvent extracts of *Valeriana hardwickii* as determined by Superoxide anion radical scavenging activity

## CONCLUSION

Amongst all the extracts, acetone and hexane extracts showed potent antioxidant activity as determined by the different procedures. TPC in acetone extract was found to be 186 µg/ml followed by hexane extracts having 175 µg/g gallic acid equivalents. $IC_{50}$ value of acetone extract was found to be 15.60 µg/ml followed by hexane extracts viz. 18.00 µg/ml in DPPH radical scavenging method. In Superoxide anion radical scavenging method acetone extracts showed 78 % inhibition of superoxide followed by hexane extracts having 73 % inhibition. Anti oxidation activity of different plant extracts lead to the formulation of some antioxidants. It was found that alkaloids and reducing sugars were found only in ethanol, aqueous and acetone extract while steroids and saponin were found only in hexane, chloroform and petroleum ether extracts. Presence of these compounds in the different extracts confirm their utility as potent antioxidant agent as revealed by the experimental results. Further work is needed for the isolation and characterization of the active compounds which are responsible for anti oxidation activity.

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