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To investigate leaf extracts of *Verbascum thapsus* Linn. For their antioxidant potential

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Abstract
In today's times, the role of antioxidants has been continuously recognised as a critical influence on the biochemistry of living beings. Any substance that causes delay or prevents oxidative damage to a target biomolecule is often known as an antioxidant. Antioxidants prevent the oxidation of certain biomolecules such as nucleic acids, proteins, carbohydrates, fatty acids etc. by various mechanisms. The current study was undertaken to investigate the leaf extracts of *Verbascum thapsus* Linn. for their antioxidant activity. The antioxidant capacity of the different extracts i.e. methanol, acetone and aqueous was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) and reducing power tests. The plant exhibited good DPPH radical scavenging activity (57.00% at 100 µg/mL in methanol extract) but moderate reducing power potential. Thus the study provided scientific evidence to the traditional uses of this plant in the treatment of disorders caused due to oxidative stress. Therefore, the leaf extracts of this plant can be selected for further investigation to predict their exact mechanism by isolating and purifying their active constituents.

Keywords: *Verbascum thapsus*, leaf extracts, DPPH, reducing power

Introduction
Pathological conditions such as ischemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, dementia etc. are caused by accumulation of free radicals. Natural products have become the target for a great number of research studies around the globe in finding the sources of potentially safe and more effective antioxidants [1]. Herbal drugs are considered as free radical scavengers for their therapeutic applications. Antioxidants are generally applied to prevent lipid per-oxidation in the food industries [2, 3].
Reactive oxygen species (ROS) are class of highly reactive molecules mostly derived from the oxygen metabolism through various mechanisms. Reactive oxygen species are an inevitable by-product of cellular respiration causing oxidation of lipids, nucleic acids and proteins thereby ROS damage is an underlying cause of many diseases [4, 6]. These free radicals exist in the body during an imbalance between Reactive oxygen species and antioxidants. Many medicinal plants contain valuable antioxidants such as Vitamin C, Vitamin E and polyphenols [7]. Natural antioxidants enhance the antioxidant potential of the plasma and reduce the risk of certain diseases related to heart and skin. There are many synthetic antioxidants but they are associated with many side effects hence there is a need for more potent and less toxic antioxidants [8]. It has been revealed that plants rich in phytochemicals especially polyphenolic compounds such as flavonoids possess antioxidant properties [9, 10]. Cells have refined antioxidant regulatory systems to maintain proper balance between ROS and antioxidants, however, disruption in homeostasis leads to oxidative stress and tissue injury [11, 12].
Verbascum thapsus Linn. (Family: Scrophulariaceae) grows all over Europe and in temperate Asia as far as the Himalayas and in North America. The plant is found wild on stony grounds, wastelands, woodlands, clearings and roadsides. *V. thapsus* is a herbaceous annual or biennial, erect and stout weed that produces a low vegetative rosette up to 60 cm. Leaf margins are entire or obscurely crenate and alternately arranged. Stem is erect and stout having 50-180 cm range of tallness. Flowers are densely arranged usually occurring one per axil. These flowers are yellow in colour bearing five sepals, five petals, two-celled ovary and five stamens [13, 14].
V. thapsus is known to exhibit many valuable medicinal properties such as analgesic, antihistaminic, anti-inflammatory, anticancer, antioxidant, antiviral, antibacterial, cardio-depressant, estrogenic, fungicide, hypnotic, sedative etc. The leaves, roots and flowers are usually anodyne, anti-inflammatory, antiseptic, spasmolytic, astringent, demulcent, diuretic, emollient, expectorant, nervine and vulnerary [15, 16].

Because of above mentioned useful properties of this plant, we planned to find out its antioxidant potential by DPPH and Reducing power essays.

Materials and methods

Collection of Plant Material

Leaves of *V. thapsus* were plucked and collected from Nohra area of District Sirmaur, Himachal Pradesh, India. The collected plant material was brought to the Laboratory for further analysis.

Processing of Plant Material

Leaves of *V. thapsus* were washed thoroughly under tap water and then treated with 2% Mercuric chloride. After this, the leaves were cut into smaller pieces for quick drying. Cleaned leaves were shade dried for about 15-20 days. The dried plant material/leaves were crushed into fine powder with the help of pestle mortar. Finally, the fine powder was stored in an air tight container at room temperature.

Antioxidant Activity Test

DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of leaf extracts was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Blois [17]. Briefly, to 1 mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant/test extract, 1 mL of DPPH (0.1 Mm in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1 mL methanol and 1 mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

Graphs were plotted against percent inhibition v/s conc. of leaf extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was calculated using the following equation given below:

$$IC_{50} = \frac{50 - Y - Intercept}{\text{Slope}}$$

Reducing Power Assay

The reducing power was determined according to the method described by Oyaizu[18] with slight modifications. Different concentrations of plant leaf extract (20, 40, 60, 80 and 100 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50°C for about 20 minutes. A portion (2.5 mL) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for atleast 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power thereby. Ascorbic acid was used as a standard. Phosphate buffer of pH 6.6 was used as blank solution. Higher absorbance of the reaction mixture showed greater reductive potential. Experiment was performed in triplicate at each concentration to determine percent reducing power. The % reducing power (antioxidant activity) was calculated by using the formula:

$$\text{\% Reducing power} = \left(\frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

Graphs were plotted against percent inhibition v/s conc. of plant leaf extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value for each extract and ascorbic acid was evaluated separately.

Results and discussions

Antioxidant activity analysis

In the present study, leaf extracts of *V. thapsus* in three different solvents (methanol, acetone and aqueous) were tested for their free radical scavenging ability by using DPPH assay and it was observed that the plant extracts showed good potency for scavenging free radicals as shown in Table 1.1. The extracts were tested on a concentration range (20-100 µg/mL) and it was found that the activity altogether increased with increase in concentration of plant leaf extracts as shown in Fig. 1.1. Methanol leaf extract showed highest (57.00%) DPPH scavenging activity at a concentration of 100 µg/mL. In all cases, methanol extracts proved to be better antioxidants than the corresponding acetone and aqueous/water extracts. A pattern of increased antioxidant activity with increasing polarity has been reported.

Reducing power experiment is a good reflector of antioxidant activity of the plant's extracts. The reducing capacity of compounds serves as an important indicator of their potent antioxidant activity. The plant having high reducing power generally reported to carry higher antioxidant potential too.

We investigated the reducing capacity of *V. thapsus* by measuring Fe^{3+}-Fe^{2+} conversion as given in Table 1.2 and Fig. 1.2. In this experiment, Ferric ions reduced to ferrous ions with the colour of the reaction mixture changing from yellow to bluish green. Reducing power potential of extracts increased with the dose, however, plant leaf extracts exhibited low reducing power than that of standard ascorbic acid. The methanol extract exhibited more reductive ability than the acetone and aqueous extracts, which was capable for neutralizing the free radicals. *V. thapsus* showed 15.00%, 12.60% and 12.05% reducing power for methanol, acetone and aqueous extracts respectively at 100 µg/mL. The results of the present study are in accordance with the earlier reports where organic extracts exhibited more activity than aqueous extract. Although traditional healers used primarily water but plant extracts prepared in organic solvents have been found to give more consistent activity when compared with aqueous extract [19]. The higher antioxidant activity of methanol and acetone extracts can be attributed to the presence of higher amount of polyphenols as compared to aqueous extracts. This may be due to the better solubility of their active components/metabolites in organic solvents [20]. Chemical constituents of *V. thapsus* include polysaccharides; iridoid glycosides including harpagoside, harpagide and aucubin (mainly in the leaf); flavonoids including 3'- methylquercitin, hesperidin and verbascoside; saponins and volatile oils [21]. In *V. thapsus* flower, four saponins of fairly similar structure

have been reported and named thapsuins A, B, hydroxythapsuins A and B, 6-hydroxyluteolin-7-glucoside, 3'-methylquercetin and 7, 4'-dihydroxyflavone 4'-rhamnoside. Phytosterols (β-sitosterol and ergosterol peroxide) and oleanolic acid have also been reported in this plant through various mechanisms [22].

Table 1: Free radical (DPPH) scavenging activity (%) of *V. thapsus* at different concentrations

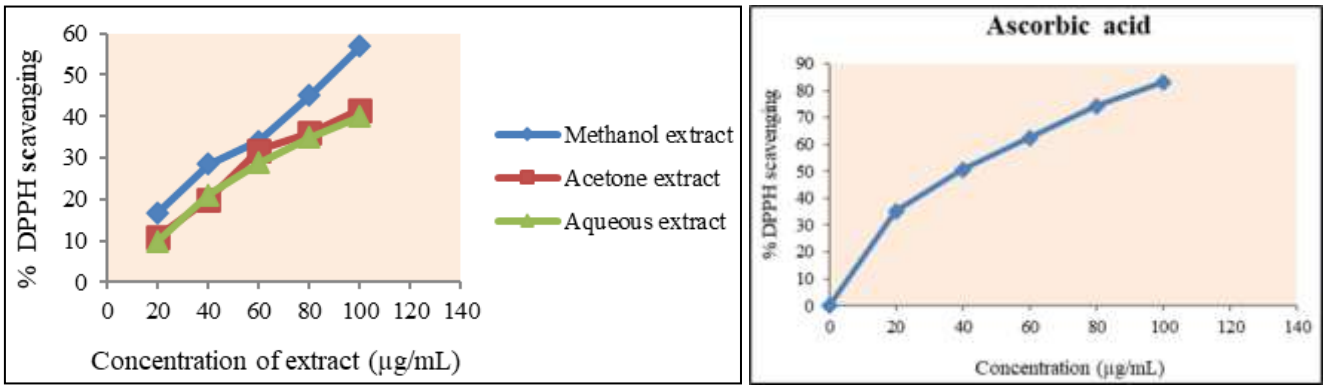
Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	16.80±2.33	11.00±0.00	10.00±1.45	35.24±0.50
40	28.45±2.00	20.00±0.00	21.15±2.00	50.54±0.42
60	34.00±0.05	31.86±2.25	28.78±0.54	62.35±1.20
80	45.00±0.00	36.00±0.00	35.00±0.00	74.14±0.00
100	57.00±0.00	41.65±0.54	40.05±2.20	83.26±2.20
IC_{50} (µg/mL)	89.25	118.65	125.52	41.44

Values are given as mean ± SD

Table 2: Antioxidant activity percentage (%) of *V. thapsus* by reducing power method at different concentrations

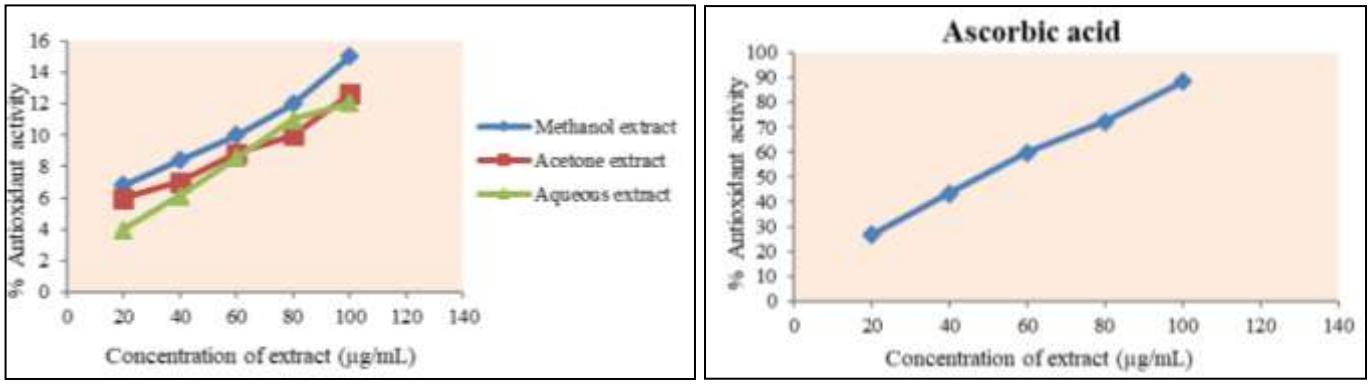
Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	6.80±2.33	6.00±0.20	4.00±1.80	26.55±2.25
40	8.40±1.00	7.00±0.00	6.15±2.05	43.44±0.45
60	10.00±0.00	8.86±2.50	8.56±0.55	59.90±1.20
80	12.00±0.75	10.00±0.00	11.00±0.00	72.15±0.54
100	15.00±0.54	12.60±0.50	12.05±1.20	88.30±1.50
IC_{50} (µg/mL)	455.6	567.50	460.89	49.40

Values are given as mean ± SD



A **B**

Fig 1: Percent scavenging (DPPH) activity of plant extracts at concentration range of 10-100 µg/mL (A) *V. thapsus* and (B) Standard curve of Ascorbic acid



A **B**

Fig. 1.2 Antioxidant activity percentage (reducing power) of different plant extracts at concentration range of 10-100 µg/mL (A) *V. thapsus*; (B) Standard curve of Ascorbic acid

Conclusions

It was concluded from the above experimental observations that the plant *Verbascum thapsus* Linn. Showed significant antioxidant activity at different concentrations. The antioxidant activity of the methanol, acetone and aqueous extracts was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) and reducing power essays. In both essays, methanol leaf extract was found to be more effective followed by acetone extract which was followed by aqueous leaf extract. This study suggests that the plant extracts possess potent antioxidant activity, which might be helpful in preventing or slowing down the progress of various oxidative stress-related diseases/disorders. Further investigations on the isolation and identification of antioxidant component (s) in

leaf extracts of this plant may lead to chemical entities with their potential for clinical use. Therefore, our study directs future research in separating the bioactive compounds from leaves responsible for antioxidant potential.

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Conflict of interest
The authors hereby declare there is no conflict of interest that would prejudice the impartiality of this scientific work.

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