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Phytochemical and Antioxidant Screening of *Trillium govanianum* Wall. ex. D. Don., from Western Himalayas Azad Jammu & Kashmir, Pakistan

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Abstract

Trillium govanianum is an important recently explored commercial medicinal plant. The present research deals with phytochemical screening and antioxidant studies of root, stem and leaves of *T. govanianum*. Antioxidant activity of ethanol, methanol and distilled water extract of root, stem and leaves of this plant was also examined. It was found that all parts of the plant possessed pronounced antioxidant activity. The highest activity was found for ethanol leaf extract with lowest IC50 value. Phytochemical screening revealed that plant contains tannins, saponines, terpenoids, alkaloids, glycosides, quinin and flavonoids. This is important tool for the standardization of plant materials, isolation of bioactive principles, screening of phytochemical activities, ensuring the quality of formulation and also useful to distinguish it from its related species.

Keywords: *Trillium govanianum,* Phytochemical, Antioxidant

1. Introduction

Natural bioactive compounds from plants are chemicals compounds found in various parts of plants, such as leaves, stem, fruit and seeds that have the ability to interact with biological systems in the human body (Narzary et al., 2016). Plants are incredibly diverse organisms that contain a wide array of chemical compounds known as phytoconstituents. Phytochemical compounds like alkaloids, flavonoids, Phlobatannins, Glycosides and Saponins have shown promise in enhancing therapeutic activities, particularly in the areas of anti-carcinogenic, anti-mutagenic, anti-inflammatory and antioxidant properties (Batiha et al. 2020). These compounds are synthesized by plants through various biochemical pathways and are responsible for their unique biological activities, flavors, scents and colors (Mercy et al. 2017). Plant-derived phytochemicals have been extensively investigated for their antioxidant properties, particularly in combating reactive oxygen species provides various health benefits (Sarikurkcu et al., 2017). These compounds known for their specific biological activities they can influence and modify various physiological functions to promote and improve

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human health (Niaz et al., 2020). Antioxidants are compounds found in medicinal plants, play a crucial role in combating oxidative stress and free radicals in the body, which can lead to cellular damage and contribute to various health issues (Mucha et al., 2021) Physicochemical characterization involves the evaluation of various physical and chemical properties of a substance. These properties provide valuable information about its structure, composition and behavior (Annan et al., 2013). Medicinal plants have been used for the treatment of various diseases since ancient time. The effectiveness of these plants in providing relief and cure can be attributed to the presence of secondary metabolites (Rahman et al., 2015). *Trillium govanianum* (family Melanthiaceae) is an herbaceous plant distributed in Bhutan, Nepal, India and Pakistan Between altitudinal ranges of 2400-3600 m (Sharma et al., 2018). It is commonly known as Nag Chatri (India) and Teen patra (Pakistan). The dried roots and rhizome of this plant are used traditionally for the treatment of stomach ache joint pain and sexual disorders (Mahmood and Malik, 2012). In recent years, there is a substantial increase in demand for a specific plant or substances with high diosgenin content due to its importance in the commercial preparation of sex hormones (Sharma et al., 2018). Characterizing phytochemical contents, antioxidant activity and physicochemical properties of plants is a valuable scientific effort. It not only helps in identifying potential health benefits but also open up opportunities for further production and commercialization of functional food. By contributing to food and health security efforts of a country, this study can have a significant impact on public health and well-being.

2. Materials and Method

2.1 Collection and identification of plant materials

Trillium govanianum samples were collected from different areas of District Neelum Azad Jammu & Kashmir, Pakistan. The specimen was identified with the help of flora of Pakistan, Flora of China, Flora of India and available literature. Identified sample was preserved and deposited to the Herbarium of Department of Botany, University of Azad Jammu and Kashmir Muzaffarabad.

2.2 Preparation of plant extracts

The Collected plant specimen was subjected to a thorough washing process to remove dirt, dust or other debris that might have accumulated on the surface of the plant during collection. After washing the plants were shade dried. Parts used from each part (Root, stem and leaves) were grinded in fine powder. For the preparation of plant extract three solvent ethanol, methanol and distilled water were used according to procedure adopted (Khan et al., 2020) with some modification. Dried powdered (root, stem, leaves) plant samples of about 100 g each were taken in separate flasks and were extracted with 100 mL of 70% ethanol, 40% methanol, and dH2O using a stirrer. The filtration was achieved using Muslin cloth, the solutions were then centrifuged, and rotary evaporator was used for sample drying through evaporation. Collected plant material was preserved in air tight plastic vials.

2.3 Phytochemical screening

2.3.1 Qualitative phytochemical screening

Presence or absence of different Phyto-constituents in the prepared plant extractof Trillium *govanianum* were tested. Chemical regents were prepared, tests were performed according to standard procedure with slight modification (Alamzeb et al., 2013; Talukdar & Chaudhary, 2010).

2.3.2 Test for saponins

2 g of the sample's powder was heated in 20 mL of dH2O. 10 mL of filtrate and 5 mL of dH2O were violently quivered. The appearance of foaming suggested the presence of saponins (Alamzeb et al., 2013).

2.3.3 Test for flavonoids

Shinoda test: A little piece of magnesium and 4 mL of extract solution were warmed along with 1.5 mL of 50% methanol solution. There were added 5–6 drops of con. HCl. Colour red was looked for in flavonoids (Talukdar & Chaudhary, 2010).

2.3.4 Test for tannins

When 3–4 drops of 10% FeCl3 were added to the diluted extract, gallic tannins displayed a blue colour, while catechol tannins caused the solution to turn green (Talukdar & Chaudhary, 2010).

2.3.5 Test for terpenoids

When 0.2 g of each sample was combined with 3 mL of concentrated H2SO4.2 mL chloroform, the mixture turned a reddish-brown colour, indicating the presence of terpenoids (Thusa & Mulmi, 2017).

2.3.6 Test for alkaloids

2 mL of extract were combined with 1 mL of Meyer's reagent. The pale-yellow precipitate was a sign that alkaloids were present **(**Talukdar & Chaudhary, 2010).

2.3.7 Test for glycosides

The 5 mL of plant extract was treated with 2 mL of glacial acetic acid and one drop of FeCl3 solution. A violet or greenish ring, which indicated the presence of cardiac glycosides, may be seen **(**Talukdar & Chaudhary, 2010).

2.3.8 Test for quinine

The extract was mixed with the ammonium thiocyanate and newly made FeSO4 solution (1 mL), and then conc. H2SO4 was gradually added. Deep red colour was a sign of quinine present (Thusa & Mulmi, 2017).

2.4 Antioxidant Activity

To determine antioxidant activity of different parts of *Trillium govanianum*, the DPPH (2, 2-diphenyl-l-picryllhydrazyl) radical scavenging assay as describe by (Alzahrani et al., 2020) was used. The DPPH solution fades its colour when received hydrogen ions from anti-oxidant, which was initially violet. A stock solution of 7 mg of DPPH was prepared in 100 ml of methanol. The DPPH solution, methanol and extracts with various concentrations (1 mg/ul. 2 mg/ul and 5 mg/ul) were added in labelled test tube for sample and blank reading, mixed well and kept it for 30 min at room temperature. Ascorbic acid was used as control. The absorbance was read against standard at 517 nm by using UV visible spectrophotometer. DPPH free radical scavenging activity was measured.

The experiments were carried out the triplicate. The percentage radicals scavenging activity was calculated by using following formula

$$\% \text{ Inhibition} = [\text{Standard-X/Standard}] \times 100$$

Where standard is the absorbance of control reaction (containing all reagents except the test compound). 50 percent inhibition (IC50) of each extract concentrations against of inhibition was calculated.

3. Results and Discussion

Phytochemical analysis of *Trillium govanianum* was carried out and investigation determined the presence and absence of secondary metabolites. Tannins, terpenoids, Saponins, Alkaloids and Quinine were present in root, stem and leaf. Flavonoids were absent in root, stem and leaf. Chemical analysis showed that Glycosides

were present in root while absent in stem and leaf. Terpenoids and saponins are found both in root, stem and leaf. The important medicinal properties of plants are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins etc. current studies revealed that Tannins, Terpenoids and Saponins were present in root, stem and leaf. These results were in accordance with the results of Soni and Sosa (2013) which reported that tannins, saponins, flavonoids, steroids, alkaloids and terpenoids were present in *Mentha spicata, Ocimum sanctum, Spinacia oleracea, Trigonella foenum graecum, and Gmelina arborea* extracts. Similar results are reported by Khan *et al*. (2011) they found important chemicals compounds Flavonoid, Saponins, Terpenoids and Tannins in *Mentha spicata and Solanumus nigrum.* Arora (2013) also described the presence of chemicals compounds Flavonoid, Saponin, Tannin, and Terpenoid in leaves of *Solanum nigrum* and *Achyranthus aspera*, these chemicals compounds are also present in the leaf extracts of *Trillium govanianum*.

Table 1 Phytochemical screening of plants extracts of *Trillium govanianum*

Phytochemicals	Root	Stem	Leaf
Tannins	+	+	+
Flavonoids	-	-	-
Glycosides	+	-	-
Terpenoids	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Quinine	+	+	+

4. Antioxidant Activity

Antioxidant activity of Distilled water, ethanol and methanol extracts of root, stem and leaf of *Trillium govanianum was* evaluated by DPPH assay. Its rhizome, stem and leaf extracts showed competent antioxidant activity. Aqueous, ethanol and methanol extracts of rhizome exhibited an antioxidant activity with IC50 values 0.634464, 1.951179 and 0.262357 respectively. Similarly stem aqueous, ethanol and methanol extracts possess good antioxidant activity with IC50 values 0.232536, 0.407357 and 1.203429 respectively. While Leaf has also prominent free radical scavenging activity with IC50 values 0.360179, 0.108071 and 0.269786 in distilled water, ethanol and methanol respectively. Highest activity was observed for Leaf ethanol extract (IC50 0.108071) while least activity was observed in rhizome ethanol extract with IC50 value 1.951179. Antioxidants are those substances which possess free radical chain reaction breaking properties. Antioxidant neutralizes the damaging effect of free radicals and protects cell components. These radicals are natural by-product of cell metabolism. The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful (Priya et al.,2011). Root, stem and leaves of *Trillium govanianum* in aqueous, ethanol and methanol extracts showed significant antioxidant activity. Current research work showed the antioxidant activity in root, stem and leaves of *Trillium govanianum.* Highest antioxidant activity in leaves of *Trillium govanianum* was observed in ethanol extract while least activity was observed in aqueous extract, similar results were reported by Garg *et al.,* (2012) in mentha (*Cymbopogon citrtu*). They reported that methanol leaf extract of *Cymbopogon citrtu* is a more active antioxidant than extract in water. Root of *Trillium govanianum* showed highest activity in methanol extract while least activity in ethanol extract. Ethanolic stem extract of *Trillium govanianum* showed highest activity but aqueous and methanol extract has less antioxidant activity.

The antioxidant activity of plant extracts was due to presence of phenols, flavonoids and alkaloids which were present in different parts of plant as investigated in phytochemical tests. High number of phenolic compounds are extracted in polar solvents (Deb et al., 2010). Phenols contains hydroxyl group which is

responsible for high scavenging activity. Therefore, antioxidant potential of plant is directly proportional to its phenolic content (Thakur & Sidhu, 2009). Similarly, flavonoids also show antioxidant and chelating action due to pattern (structural and substitution) of hydroxyl groups (Sharififar, 2008).

Antioxidant activity of root, stem and leaves of *Trillium govanianum* showed that highest activity was observed in leaf while least activity was observed in root. Highest antioxidant activity in leaves was due to high phenolic compounds, flavonoids and alkaloids while in root amount of these compounds are low. The result of Ayoola et al., 2008) showed that ethanolic extracts in Centella *asiatica* also showed antioxidant activity.

5. Conclusion

It is concluded that *Trillium govanianum* plant contains almost all-important types of phytochemical constituents and possesses antioxidant potential at various concentrations. As a result, extracts from methanol and water fractions had extremely strong antioxidant activity. Extracted fraction has the ability to act as an antioxidant and may be useful in halting or reversing the effects of various oxidative stressors. To isolate and purify the main phenolic compound and other bioactive compounds for additional bioactivity tests that are mediated by free radicals, further research and analysis are highly advised. The identification of the antioxidant component in the plant may result in chemical entities with greater potential for clinical use.

Table 2 Antioxidant activity of *T. govanianum*

Plant parts	Solvents	Percentage scavenging activity			IC 50
		20µl	40µl	80µl	
Root	Water	102.60	117.70	141.15	0.634464
	Ethanol	115.62	51.04	211.97	1.951179
	Methanol	84.89	339.58	150.52	0.262357
Stem	Water	113.02	170.31	137.50	0.232536
	Ethanol	237.50	94.79	231.77	0.407357
	Methanol	79.16	62.50	143.22	1.203429
Leaves	Water	114.85	105.85	133.22	0.360179
	Ethanol	102.66	40.10	96.2	0.108071
	Methanol	241.66	249.47	258.33	0.269786

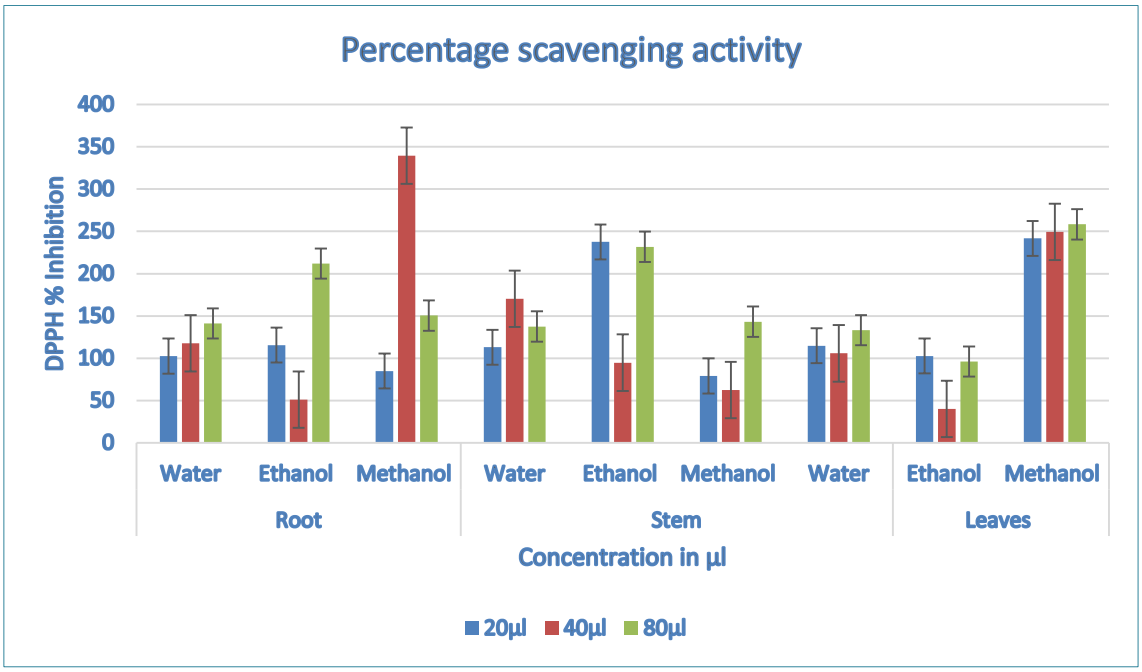

Fig. 1 Antioxidant activity of *Trillium govanianum*

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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