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DETERMINATION OF ANTIOXIDANTS BY DPPH RADICAL SCAVENGING ACTIVITY AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *DIANTHUS BARBATUS*

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Article History	Abstract
Received: 24-06-2026 Revised: 14-07-2026 Accepted: 18-08-2026	Antioxidants play an important role in protecting biological systems from oxidative damage by neutralizing free radicals and inhibiting oxidation processes. Medicinal plants are recognized as valuable sources of naturally occurring bioactive compounds with potential antioxidant properties. The present study was undertaken to investigate the phytochemical composition and antioxidant potential of <i>Dianthus barbatus</i> leaf extracts prepared using aqueous and alcoholic solvents. Preliminary phytochemical screening of the extracts demonstrated the presence of several secondary metabolites, including alkaloids, glycosides, steroids, gums, flavonoids, saponins, reducing sugars, and tannins. The antioxidant potential of the aqueous and alcoholic extracts was evaluated using different <i>in vitro</i> antioxidant models, including ferric reducing antioxidant power (FRAP), metal-chelating activity, DPPH radical-scavenging activity, superoxide radical-scavenging activity, and hydrogen peroxide-scavenging activity. The findings indicated that both extracts exhibited antioxidant activity, although the alcoholic leaf extract demonstrated comparatively greater activity across the evaluated antioxidant assays. The observed antioxidant effects may be associated with the presence of flavonoids, tannins, and other phenolic constituents capable of donating hydrogen atoms or electrons and thereby neutralizing reactive oxygen species. The results suggest that <i>Dianthus barbatus</i> leaves may represent a promising natural source of antioxidant phytoconstituents. Further investigations involving isolation, purification, structural characterization, and individual assessment of the bioactive constituents are warranted to establish the compounds primarily responsible for the observed antioxidant activity and to explore their potential pharmacological applications.
Keywords: <i>Dianthus barbatus</i> ; phytochemicals; antioxidant activity; flavonoids; DPPH; FRAP; free radicals.	
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INTRODUCTION

Antioxidants are bioactive molecules that inhibit oxidation and protect biological systems from damage caused by reactive oxygen species (ROS) and free radicals. Oxidative stress results from an imbalance between ROS generation and endogenous antioxidant defenses and is implicated in several chronic disorders, including diabetes, cardiovascular diseases, cancer, and inflammation. Endogenous defenses include antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, whereas exogenous antioxidants are obtained mainly from dietary and medicinal plant sources. Plant-derived polyphenols, flavonoids, alkaloids, terpenoids, tannins, and related compounds have demonstrated free-radical-scavenging, metal-chelating, and lipid-peroxidation-inhibitory activities [1-2].

Several medicinal plants have been investigated for their antioxidant potential. *Rhizophora mangle*, *Diospyros malabarica*, *Asparagus racemosus*, *Auricularia auricular*, *Eucalyptus globulus*, *Acacia arabica*, *Ligustrum vulgare*, and *Terminalia chebula* have demonstrated antioxidant effects through mechanisms including DPPH and superoxide radical scavenging, hydrogen-peroxide neutralization, metal chelation, reduction of oxidative reactions, and inhibition of lipid

peroxidation. These activities are generally associated with their phenolic and flavonoid constituents [3-4].

Natural antioxidants are also important in food preservation because they can delay lipid oxidation. Although synthetic antioxidants such as BHA, BHT, and TBHQ are widely used, increasing interest in natural alternatives has encouraged investigation of plant-derived antioxidants. Tocopherols, particularly α -, β -, γ -, and δ -tocopherols and tocotrienols, are important lipid-soluble antioxidants found in vegetable oils, cereals, nuts, seeds, and green vegetables. They protect polyunsaturated fatty acids by donating hydrogen atoms to lipid radicals and thereby interrupting oxidative chain reactions. Thus, medicinal plants and naturally occurring phenolic compounds represent promising sources of antioxidant agents for pharmaceutical, nutritional, and food applications [4-5].

Flavonoids are widely distributed polyphenolic compounds found throughout plant tissues and are classified into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and chalcones. More than 4,000 flavonoids have been identified, many of which exhibit significant antioxidant activity. Their antioxidant effects include scavenging free radicals and reactive oxygen species, chelating metal ions, and

inhibiting oxidative enzymes such as lipoxygenase. Catechin and quercetin are particularly recognized for their strong antioxidant properties. The antioxidant activity of flavonoids is strongly influenced by their molecular structure, especially the number and position of hydroxyl groups on the B ring. Hydroxylation at the 3' and 4' positions contributes substantially to radical-scavenging capacity, while additional hydroxyl groups, as present in myricetin and robinetin, may further enhance antioxidant activity. Thus, structural characteristics play an important role in determining the antioxidant potential of individual flavonoids [6-7].

Ascorbic acid is an important water-soluble antioxidant that functions primarily as a reducing agent and free-radical scavenger. Through its redox properties, it can donate hydrogen atoms to reactive intermediates and thereby interrupt oxidative chain reactions. Ascorbic acid also acts synergistically with tocopherols by facilitating the regeneration of oxidized tocopherol into its reduced antioxidant form. Its oxidized product, dehydroascorbic acid, can subsequently be regenerated through reducing systems involving glutathione or NADH. These properties contribute to the protective role of ascorbic acid against oxidative damage [8-9].

Carotenoids are naturally occurring, lipid-soluble pigments widely distributed in plants and other photosynthetic organisms [10]. They contribute to the characteristic red, orange, and yellow coloration of many fruits, flowers, and leaves and play an important protective role against light- and oxygen-induced cellular damage. Major carotenoid groups include carotenes, such as β -carotene, and oxygen-containing xanthophylls, such as lutein. Most carotenoids possess a 40-carbon conjugated polyene structure with extensively delocalized π -electrons. This structural feature enables them to absorb excess energy and quench singlet oxygen, thereby contributing to antioxidant defense [11-12]. Carotenoids may occur as *trans* and *cis* geometric isomers, with the *trans* configuration generally predominating naturally. Although isolated carotenoids are susceptible to oxidation and degradation in the presence of oxygen, their stability is considerably greater within biological systems [13-15].

AIM AND OBJECTIVES

The present study aims to evaluate the **in-vitro antioxidant activity of aqueous and alcoholic extracts of *Dianthus barbatus***. The study is designed to investigate the antioxidant potential of the plant extracts using different in-vitro models.

The objectives are:

- To prepare aqueous and alcoholic extracts of *Dianthus barbatus* leaves.
- To evaluate antioxidant activity by **Ferric Reducing Antioxidant Power (FRAP) assay**.
- To determine **metal-chelating activity**.
- To assess **DPPH radical-scavenging activity**.
- To evaluate **superoxide radical-scavenging activity**.
- To determine **hydrogen peroxide-scavenging activity**.

NEED FOR THE STUDY

Medicinal plants have traditionally served as important sources of therapeutic agents and continue to provide

affordable healthcare, particularly in developing and rural communities. Plant-derived compounds are known to contain several bioactive constituents with antioxidant properties.

Dianthus barbatus, commonly known as Sweet William, is an ornamental plant belonging to the family Caryophyllaceae. Previous investigations have reported various phytochemical constituents and pharmacological properties in different parts of *Dianthus* species. However, comparatively limited information is available regarding the antioxidant potential of *D. barbatus* leaves. Therefore, the present study was undertaken to evaluate the antioxidant activity of its aqueous and alcoholic extracts using multiple in-vitro antioxidant models.

PLAN OF WORK

The study was carried out in the following sequence:

1. Selection and identification of *Dianthus barbatus*.
2. Collection, cleaning, drying and pulverization of plant material.
3. Preparation of aqueous and alcoholic leaf extracts.
4. Preliminary phytochemical evaluation of the extracts.
5. In-vitro evaluation of antioxidant activity using:
 - FRAP assay
 - Metal-chelating assay
 - DPPH radical-scavenging assay
 - Superoxide radical-scavenging assay
 - Hydrogen peroxide-scavenging assay.

PLANT PROFILE

Botanical name: *Dianthus barbatus*

Kingdom: Plantae

Order: Caryophyllales

Family: Caryophyllaceae

Genus: *Dianthus*

Species: *D. barbatus*

Common name: Sweet William

Dianthus barbatus is a herbaceous flowering plant native mainly to southern and southeastern Europe and parts of northeastern Asia. It generally grows to approximately 13–92 cm in height and bears narrow, tapered leaves. The flowers occur in dense clusters and may range from white and pink to red, purple, or variegated colours. The plant is widely cultivated as an ornamental species and its flowers are attractive to bees and butterflies.

MATERIALS AND METHODS

Leaves of *Dianthus barbatus* were collected, authenticated, cleaned, shade-dried and coarsely powdered. For the aqueous extract, 20 g of powdered leaves was mixed with 200 mL distilled water and heated at approximately 80–100°C for 4–5 hours. The mixture was filtered and concentrated. Similarly, 20 g of plant material was extracted with 200 mL alcohol at approximately 50–60°C for 4–5 hours, filtered and concentrated. The resulting extracts were stored until further analysis.

Preliminary phytochemical screening was performed for carbohydrates, gums and mucilages, proteins, amino acids, fixed oils and fats, alkaloids, glycosides and phytosterols.

In-Vitro Antioxidant Assays

Antioxidant activity was assessed using multiple models because individual assays measure different mechanisms of antioxidant action. **FRAP assay** determines the reducing ability of the extracts by measuring the reduction of Fe^{3+} -TPTZ to Fe^{2+} -TPTZ at 593 nm. **Metal-chelating activity** was evaluated by measuring inhibition of the ferrous ion-ferrozine complex at 562 nm.

DPPH radical-scavenging activity was determined by measuring the reduction of DPPH radical absorbance at 517 nm. **Superoxide radical-scavenging activity** was evaluated using the PMS-NADH-NBT system, with absorbance measured at 560 nm. **Hydrogen peroxide-scavenging activity** was determined by incubating the extracts with H_2O_2 and measuring the resulting absorbance at 620 nm [16-17].

Quercetin and BHT were used as standard antioxidants. All experiments were performed in triplicate. Percentage inhibition was calculated using:

$$\text{Scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 represents the absorbance of the control and A_1 represents the absorbance of the test sample. IC_{50} values were determined where applicable from concentration-response curves.

RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical screening of *Dianthus barbatus* leaf extracts revealed the presence of several bioactive constituents. The aqueous extract showed alkaloids, gums, flavonoids, reducing sugars and tannins, whereas the alcoholic extract contained glycosides, flavonoids, reducing sugars and tannins. Steroids and saponins were not detected in either extract. The presence of flavonoids and tannins may contribute to the antioxidant activity observed in subsequent assays.

Table 01. Preliminary phytochemical screening of *Dianthus barbatus* leaf extracts

S. No.	Phytoconstituents	Aqueous extract	Alcoholic extract
1	Alkaloids	+	–
2	Glycosides	–	+
3	Steroids	–	–
4	Gums	+	–
5	Flavonoids	+	+
6	Saponins	–	–
7	Reducing sugars	+	+
8	Tannins	+	+

+ = Present; – = Absent.

In-vitro Antioxidant Activity

Antioxidant activity was evaluated using five complementary assays because antioxidant mechanisms differ among test systems. Overall, both aqueous and alcoholic extracts demonstrated antioxidant potential, although their activity varied according to the assay [17].

Ferric Reducing Antioxidant Power (FRAP)

Both extracts exhibited concentration-dependent ferric reducing activity. The aqueous extract (AQDB) showed an IC_{50} of $1.88 \pm 0.037 \mu\text{g/mL}$, while the alcoholic extract (ALDB) showed

$2.34 \pm 0.065 \mu\text{g/mL}$. The extracts showed lower reducing activity than quercetin and BHT, indicating moderate electron-donating capacity. The activity may be associated with phenolic and flavonoid constituents capable of donating electrons and reducing ferric ions.

Metal-Chelating Activity

Both extracts demonstrated ferrous-ion chelating activity. AQDB showed an IC_{50} of $25.18 \mu\text{g/mL}$, whereas ALDB showed $31.61 \mu\text{g/mL}$. Although the extracts were less effective than EDTA, quercetin and BHT, their chelating activity indicates a potential role in reducing metal-catalysed oxidative reactions [18].

DPPH Radical-Scavenging Activity

The extracts exhibited concentration-dependent DPPH radical-scavenging activity. AQDB showed an IC_{50} of $1.22 \pm 0.052 \mu\text{g/mL}$, while ALDB showed $2.20 \pm 0.000 \mu\text{g/mL}$. The relatively low IC_{50} values indicate appreciable free-radical-scavenging capacity, possibly due to flavonoids and other phenolic compounds present in the extracts [19].

Superoxide Radical-Scavenging Activity

Both extracts showed concentration-dependent inhibition of superoxide radicals. AQDB exhibited an IC_{50} of $0.34 \pm 0.008 \mu\text{g/mL}$, while ALDB showed $0.312 \pm 0.001 \mu\text{g/mL}$. The alcoholic extract therefore showed slightly greater activity than the aqueous extract. However, both extracts were less potent than the standard antioxidants [20].

Hydrogen Peroxide-Scavenging Activity

The extracts demonstrated concentration-dependent hydrogen peroxide-scavenging activity. AQDB showed an IC_{50} of $0.072 \pm 0.015 \mu\text{g/mL}$, whereas ALDB showed $0.597 \pm 0.042 \mu\text{g/mL}$. The aqueous extract was considerably more active than the alcoholic extract and approached the activity of the reference antioxidants. These findings suggest that *D. barbatus* leaf constituents may contribute to the removal of hydrogen peroxide and thereby reduce the possibility of hydroxyl-radical formation [22].

Table 02: IC_{50} values of *Dianthus barbatus* extracts and standard antioxidants in different antioxidant assays

Assay	Quercetin	BHT	AQDB	ALDB
FRAP	13.59 ± 0.098	3.10 ± 0.025	1.88 ± 0.037	2.34 ± 0.065
Metal chelating	143	89	25.18	31.61
DPPH	4.20 ± 0.024	2.16 ± 0.075	1.22 ± 0.052	2.20 ± 0.000
Superoxide	0.013 ± 0.001	0.010 ± 0.009	0.340 ± 0.008	0.312 ± 0.001
H_2O_2	0.040 ± 0.003	0.063 ± 0.009	0.072 ± 0.015	0.597 ± 0.042

Values are expressed as IC_{50} and represent mean $\pm$ SD ($n = 3$), where applicable. AQDB = aqueous extract of *Dianthus barbatus*; ALDB = alcoholic extract of *Dianthus barbatus*.

The findings demonstrate that *Dianthus barbatus* leaf extracts possess measurable antioxidant activity through different mechanisms, including electron donation, free-radical scavenging and metal chelation. The aqueous extract generally showed stronger activity in FRAP, DPPH and hydrogen

peroxide assays, whereas the alcoholic extract showed slightly greater superoxide-scavenging activity [23]. The antioxidant potential may be associated with the flavonoids, tannins and other phenolic constituents detected during phytochemical screening [24-25]. Further studies involving quantitative phytochemical analysis, isolation of active constituents and in-vivo evaluation are required to establish the therapeutic relevance of these findings.

CONCLUSION

The present study demonstrated that both aqueous and alcoholic extracts of *Dianthus barbatus* possess significant antioxidant activity, as evidenced by FRAP, metal-chelating, DPPH, superoxide and hydrogen peroxide-scavenging assays. The observed activity may be attributed mainly to the presence of phenolic and flavonoid compounds capable of donating hydrogen or electrons and neutralizing free radicals. These findings suggest that *Dianthus barbatus* may serve as a promising natural source of antioxidants with potential applications in pharmaceutical and food systems. Further in-vivo studies and isolation of active constituents are required to confirm its therapeutic potential.

AUTHOR CONTRIBUTIONS

Both authors contributed to equally.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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