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Oxidative stress mitigation by *Berberis aristata* root extract: An *in vitro* antioxidant study

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Abstract
Berberis aristata DC., traditionally known for its medicinal properties, is a rich source of bioactive phytoconstituents. Oxidative stress, caused by the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), contributes to a variety of diseases. The objective of this study was to evaluate the *in vitro* antioxidant capacity of *Berberis aristata* aqueous root extract (BAE). The antioxidant capacity of BAE was evaluated using three mechanistic assays: nitric oxide (NO) scavenging activity, superoxide anion radical scavenging activity, and reducing power activity. The effects were compared with standard antioxidants such as potassium nitrite and ascorbic acid. BAE demonstrated significant, dose-dependent antioxidant activity in all assays. In a nitric oxide scavenging assay, BAE showed an IC_{50} value of 86.143 µg/ml, indicating effective quenching of nitrosative radicals. The extract also demonstrated effective inhibition of superoxide radicals with an IC_{50} of 69.828 µg/ml, comparable to standard ascorbic acid (IC_{50} 57.942 µg/ml). The results indicate that BAE possesses strong antioxidant properties, likely due to its phenolics, flavonoids, and alkaloids such as berberine. BAE's ability to effectively scavenge free radicals and reduce oxidative stress suggests its potential as a natural therapeutic agent for managing oxidative stress-related diseases.

Keywords: *Berberis aristata*, antioxidant, nitric oxide scavenging, superoxide scavenging

Introduction
Medicinal plants have been an essential part of traditional healthcare systems worldwide. Ayurveda, one of the oldest medical traditions, uses plant-based therapies to restore physical, mental, and emotional balance, thereby promoting well-being, preventing disease, and treating existing ailments. The global demand for herbal medicines is steadily increasing, driven not only by cultural acceptance but also by their perceived safety, biocompatibility, and minimal side effects (Mitra *et al.*, 2011; Ambwani *et al*., 2018) [17, 3]. More than 80% of the population in developing countries relies on botanicals for their primary healthcare needs (Ekor, 2014) [10]. Due to their rich phytochemical diversity, medicinal plants provide numerous secondary metabolites such as alkaloids, flavonoids, terpenoids, phenolics, and lignans that contribute to their therapeutic potential (Mongalo *et al*., 2016; Pant *et al*., 2021; Pandey and Ambwani, 2022) [18, 20, 19].
Berberis aristata DC commonly known as Daruharidra, is an essential medicinal plant widely used in Ayurveda, Unani, Homeopathy, Traditional Chinese Medicine, and Modern Herbal Practice. Belonging to the Berberidaceae family, this plant grows mostly in the Himalayan region (1000-3000 m) and the Nilgiri Range (1000-2400 m) (Bhardwaj and Kaushik, 2012; Chaudhary *et al*., 2021) [4, 7]. Of the more than 70 Indian *Berberis* species, B. aristata is the most valuable because of its long-standing medicinal uses, including the management of fever, arthritis, jaundice, nephrolithiasis, and various inflammatory diseases (Malhotra, *et al*., 2021).
Phytochemical investigations indicate that *Berberis* spp. contains a variety of bioactive compounds alkaloids, flavonoids, terpenoids, sterols, phenolic acids, carotenoids, and anthocyanins with isoquinoline alkaloids being the main functional components (Bhatt *et al*., 2018) [5]. The main alkaloid, berberine, is found in the roots, stem bark, rhizomes, and leaves, followed by palmatine (Shahid *et al*., 2009) [24]. These alkaloids contribute to a variety of reported pharmacological properties, including antimicrobial, antipyretic, hepatoprotective,

antihyperglycemic, wound healing, anticancer, and strong antioxidant activity (Potdar *et al*., 2012; Rawat *et al*., 2024) [21, 22].

Oxidative stress is considered a major factor affecting animal health and productivity, especially in intensive poultry production systems. Broiler chickens, due to their high metabolic rate, environmental stress, and rapid growth requirements, are highly susceptible to oxidative damage, which can impair immunity, gut health, and overall performance. Therefore, plant-based antioxidants in food have gained attention as a natural alternative to synthetic additives to reduce oxidative stress and improve physiological resilience. Given the strong phytochemical profile and documented free-radical-scavenging activity of *Berberis aristata*, it is scientifically important to evaluate its antioxidant potential in relation to poultry health. This study investigated the antioxidant properties of *Berberis aristata* DC. Aqueous root extract (BAE) through several *in vitro* tests, with the aim of establishing its potential use as a natural antioxidant supplement to reduce oxidative stress in broiler chickens.

Materials and Methods

Preparation of *Berberis aristata* DC. aqueous root extract (BAE)

Root material of *Berberis aristata* DC was collected from Berinag (Pithoragarh, Uttarakhand) and authenticated using herbarium accession GBPUH-1207. The aqueous root extract of *Berberis aristata* DC. (BAE) was prepared as per the methods of Deb *et al*. (2018). It was shade-dried and ground into a fine powder. For aqueous extraction, 100 g of this powder was dissolved in 1 L of autoclaved distilled water in a shaking incubator with continuous stirring for 72 h. The resulting mixture was initially filtered through a muslin cloth, followed by fine filtration using Whatman No. 1 filter paper. The clear filtrate was then concentrated using a rotary evaporator at 45 °C, and the final aqueous extract (BAE) was obtained as a dry powder by lyophilization (freeze-drying). The BAE was weighed for yield calculations and stored at -20 °C for later analysis.

Evaluation of *In vitro* Antioxidative Potential of BAE

The antioxidant capacity of BAE was evaluated using biochemical assays as previously reported by Ambwani *et al*., 2024, 2025. [22, 2]

Nitric Oxide scavenging activity

Nitric oxide scavenging activity was tested using the method of Jagetia *et al*. (2004) [12]. Fresh stock solutions of potassium nitrite and BAE (1 mg/ml) were prepared using sodium nitroprusside (5 mM) in PBS (10 mM, pH 7.4), and the working concentration was maintained at 10-100 µg/ml. For each concentration, 90 µl of the reaction mixture was added to a 96-well plate, with only sodium nitroprusside used as a control. The samples were incubated for 150 min at 25 °C, after which 90 µl of Griess reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% naphthyl-ethylenediamine hydrochloride) was added. The chromophore formed by diazotization and subsequent coupling of nitrite was quantified at 546 nm using an ELISA reader. To ensure accuracy, all experiments were performed three times, with three technical replicates per experiment. The percentage of nitric oxide scavenging was calculated as:

$$\text{Percent Nitric Oxide scavenging} = \frac{Ab_c - Ab_s}{Ab_c} \times 100$$

where Ab_c represents the control absorbance and Ab_s represents the absorbance in the presence of BAE, and IC_{50} values were determined to reflect the scavenging efficiency of the extract.

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity of BAE was tested using a slightly modified method from Iqbal *et al*. (2012) [11]. Stock solutions of ascorbic acid and BAE (1 mg/ml) were prepared in double-distilled water, and a working concentration of 10-100 µg/ml was created accordingly. For each reaction, 3 ml of phosphate buffer (pH 7.4), 1 ml of NBT (50 µM), and 1 ml of NADH (73 µM) were added to the test solution, and the reaction was initiated by adding 1 ml of PMS (15 µM). After 5 min of incubation at room temperature, absorbance was measured at 560 nm against a reagent blank. The assay was performed in triplicate, and the percent scavenging activity was calculated as:

$$\text{Percent superoxide anion radical scavenging} = \frac{Ab_c - Ab_s}{Ab_c} \times 100$$

Where, Ab_c is the absorbance of the control and Ab_s is the absorbance in the presence of the BAE extract. The concentration (in µg/ml) at which 50% inhibition occurred is known as the IC_{50} value, and it is used to expressed the superoxide anion radical scavenging activity of the BAE.

Reducing power antioxidant activity

The reducing power of BAE was tested using the method of Subhashini *et al*. (2011) with slight modifications. Stock solutions of the extract and ascorbic acid (1 mg/ml) were prepared, and working concentrations of 20-200 µg/ml were incubated at 50 °C for 30 min in a reaction mixture containing 2.5 ml of 0.1 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid and then centrifuged at 3000 g for 10 min. The supernatant (2.5 ml) was mixed with an equal volume of distilled water and 0.5 ml of 0.1% ferric chloride, and the formation of ferric-ferrous complexes was measured at 700 nm using a UV-visible spectrophotometer, using a distilled water blank and ascorbic acid as a reference standard. All tests were performed three times with three technical replicates to ensure reliability, and increased absorbance was interpreted as indicating higher electron-donating and reducing power potentials of the extract.

Statistical Analysis

We performed all antioxidant tests three times to ensure reliability and reusability. Therefore, results are presented as mean values with their standard deviations (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) in OPSTAT software. Microsoft Excel was used for initial data handling, and final graphical representations were created using Origin Pro.

Results

After preparing BAE (Figure 1), its antioxidant capacity was evaluated using a series of *in vitro* tests. The antioxidant capacity of BAE was assessed using different plant extract-based antioxidant tests, including nitric oxide scavenging activity, superoxide anion radical scavenging activity, and reducing power activity. These tests together examine different mechanisms of antioxidant actions.

Berberis aristata DC. plant	Aqueous root extract of *Berberis aristata* DC.

Fig 1: *Berberis aristata* DC. plant and aqueous root extract.

BAE Based Antioxidant Activity

Nitric Oxide scavenging activity

The nitric oxide scavenging activity of *Berberis aristata* aqueous extract (BAE) was tested and compared with potassium nitrite. Table 1 shows nitric oxide scavenging activity of BAE that has an IC_{50} value of 86.143 µg/ml, while the standard of potassium nitrite has an IC_{50} value of 27.719 µg/ml (Figure 2).

Table 1: *In vitro* Antioxidant potential of BAE was determined by the Nitric Oxide scavenging activity of Potassium nitrite and BAE at different concentrations (n=3).

S. No.	Mean Percent Scavenging activity	IC_{50} (µg/ml)	CD (5%)	SE(m)
1.	Mean Percent Scavenging of Potassium nitrite±SD	27.719	1.33	0.448
2.	Mean Percent Scavenging of BAE±SD	86.143	2.228	0.75

The significant nitric oxide scavenging capacity of BAE suggests the presence of bioactive compounds capable of quenching nitric oxide radicals. Nitric oxide, although a key signaling molecule, generates reactive nitrogen species that can trigger oxidative stress in pathological conditions. BAE's high scavenging percentage and low IC_{50} value suggest that its phytoconstituents probably flavonoids and phenolic compounds effectively neutralize NO radicals. This highlights BAE's potential as a strong natural antioxidant that may help reduce nitrosative stress.

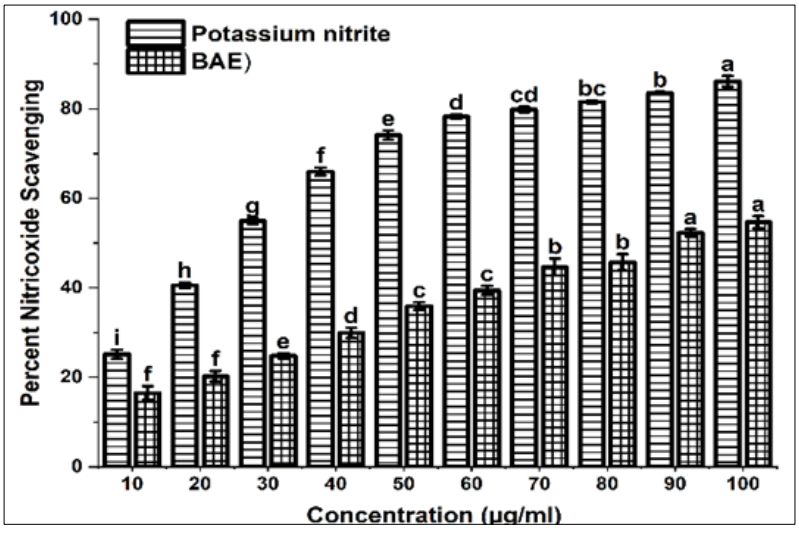

Fig 2: The *in vitro* antioxidant potential of BAE was determined by Nitric Oxide scavenging activity.

Superoxide anion radical scavenging activity

The superoxide scavenging capacity of *Berberis aristata* aqueous extract (BAE) was tested at different concentrations and compared with ascorbic acid, a standard antioxidant. As shown in Table 2, the IC_{50} value of BAE (69.828 µg/ml) was significantly higher than that of ascorbic acid (57.942 µg/ml), indicating a stronger scavenging capacity. This trend was further supported by the dose-response pattern shown in Figure 3, which confirms the concentration-dependent scavenging capacity of BAE.

Table 2: *In vitro* Antioxidant potential of BAE was determined by the Superoxide anion radical scavenging activity of Ascorbic acid and BAE at different concentrations (n=3).

S. No.	Mean Percent Scavenging activity	IC_{50} (µg/ml)	CD (5%)	SE(m)
1.	Mean Percent Scavenging of Ascorbic acid±SD	57.942	3.758	1.265
2.	Mean Percent Scavenging of BAE±SD	69.828	2.283	0.769

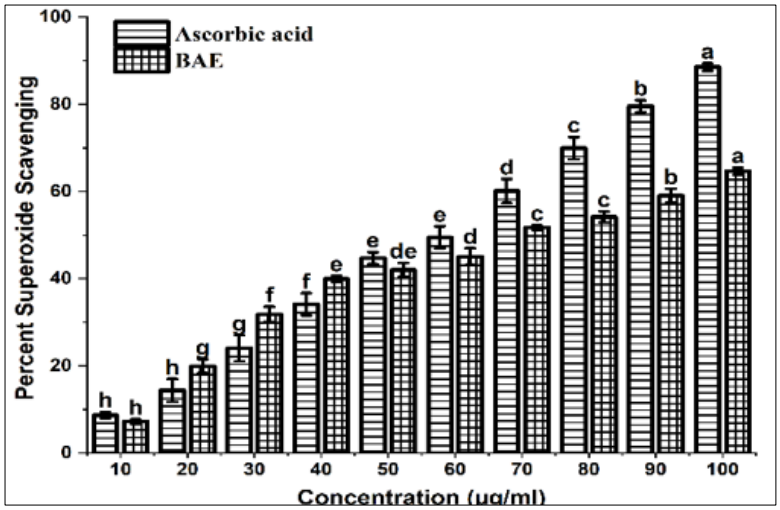

Fig 3: The *in vitro* antioxidant potential of BAE was determined by Superoxide anion radical scavenging activity.

The results indicate that BAE possesses potent superoxide radical scavenging properties. The significantly lower IC_{50} value compared to ascorbic acid indicates that the extract contains phytoconstituents capable of effectively neutralizing superoxide anions. Superoxide radicals are the main ROS that initiate oxidative chain reactions leading to cellular damage. BAE's ability to suppress these radicals demonstrates its strong electron-donating and free-radical quenching capacity. The increased activity may be due to the presence of phenolics, flavonoids, alkaloids, and other bioactive ingredients known for their redox properties. These compounds likely act by either directly scavenging superoxide radicals or by inhibiting their formation through enzyme-mediated pathways. The concentration-dependent response further supports BAE's antioxidant potency and is in line with previous reports on the antioxidant capacity of *Berberis* species.

Reducing power antioxidant activity

The reducing power of BAE was tested against ascorbic acid at concentrations ranging from 20-200 µg/ml. Both BAE and the standard showed concentration-dependent increases in absorbance. Table 3 and figure 4 shows the Ascorbic acid showed significantly higher reducing power at all concentrations (1.611±0.039 at 200 µg/ml) compared to BAE (0.406±0.011 at 200 µg/ml). Although BAE's reducing power was lower than the standard, the consistent increase in absorbance confirms its electron-donating capacity.

Table 3: *In vitro* Antioxidant potential of BAE was determined by the Reducing power antioxidant activity of Ascorbic acid and BAE at different concentrations (n=3).

Concentration (µg/ml)	Reducing Power antioxidant activity	
	Ascorbic acid	BAE
20	0.489±0.012	0.085±0.004
40	0.725±0.028	0.108±0.006
60	0.924±0.014	0.143±0.003
80	1.05±0.025	0.177±0.005
100	1.123±0.038	0.196±0.004
120	1.296±0.036	0.249±0.011
140	1.37±0.054	0.286±0.005
160	1.444±0.045	0.328±0.006
180	1.554±0.016	0.365±0.005
200	1.611±0.039	0.406±0.011
C.D. (5%)	0.057	0.011
SE(m)	0.019	0.004

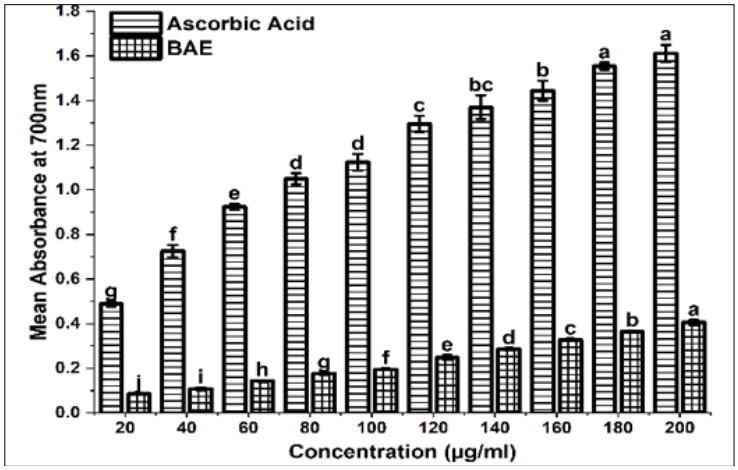

Fig 4: The *in vitro* antioxidant potential of BAE was determined by Reducing power antioxidant activity.

The reducing power assay measures the ability of antioxidants to donate electrons and reduce ferric ions to their ferrous form. BAE showed decent but concentration-dependent reducing power, suggesting that its phytochemicals possess electron-transfer capabilities. Although weaker than ascorbic acid, the consistently increasing absorbance indicates the presence of phenolic and flavonoid compounds that contribute to its reducing capacity. This supports the idea that BAE may act as a reductant under conditions of oxidative stress, complementing its free radical scavenging mechanism observed in other assays.

Discussion

BAE exhibited strong nitric oxide scavenging activity, as evidenced by a significant decrease in Griess reagent absorbance with increasing concentration. This suggests that the extract effectively neutralizes NO radicals generated by sodium nitroprusside. The activity observed in our study is consistent with previous findings on *Berberis* species, which consistently demonstrate high NO-scavenging capacities. Joshi *et al*. (2020) [14] reported that *B. aristata* exhibited one of the highest NO-scavenging effects among medicinal plants tested, while Jain *et al*. (2023) [13] demonstrated that berberine alone produced over 90% inhibition, with an IC_{50} comparable to that of ascorbic acid. These studies support berberine's role as a major contributor to BAE's NO-quenching capacity. Similarly, Sahu and Podili (2023) [23] demonstrated dose-dependent NO inhibition by *Berberis* extract, and also reported that its alkaloids and phenolics act as effective electron donors. Chaudhary and Lokhande (2023) [6] also reported effective NO scavenging by *Berberis* root extract, especially in the methanol fraction, although considerable activity remained in the aqueous extract—similar to our results for BAE.

The results of the current study showed a clear dose dependent increase in superoxide radical scavenging activity, indicating the potent interaction of BAE with superoxide species. Similar trends have been observed in previous studies on *Berberis* spp. and other medicinal plants. Sharmila *et al.* (2020) reported very high superoxide scavenging activity of *B. aristata* aril extract, with inhibition values exceeding 99% even at concentrations as low as 20-120 µg/mL and an IC_{50} of 192.38 µg/mL. These results demonstrate the strong antioxidant nature of phytochemicals from *Berberis*, especially berberine and its related alkaloids. Comparatively, studies on mixed plant extracts also show varying degrees of superoxide inhibition. Joshi *et al.* (2020) [14] found that methanolic extracts of botanicals such as *Withania somnifera*, Curcuma longa, *Zingiber officinale*, and *Boswellia serrata* showed significant activity, with inhibition ranging from 54% to 92% at 50 mg/mL. Specifically, *C. longa* and a mixed herbal formulation showed the highest scavenging capacity (96.1-98.5%), which was similar to or slightly lower than the maximum activity observed in our BAE samples. Therefore, the improved performance of BAEs in this study suggests higher concentrations or stronger reactivity of antioxidant components such as alkaloids, flavonoids, and phenolic acids.

Further support for the strong scavenging capacity of *Berberis* components is provided by Jain *et al.* (2023) [13], who reported an IC_{50} of 145.6 µg/mL for purified berberine. Although purified berberine showed slightly lower activity than ascorbic acid (IC_{50}: 96.4 µg/mL), its efficiency highlights the importance of berberine as one of the main contributors to the antioxidant activity of *Berberis* extracts. The IC_{50} values obtained in our study are consistent with these reports, confirming that BAE contains potent radical-quenching components that can compete well with standard antioxidants. Sahu and Podili (2023) [23] observed that *Bauhinia monandra* extract (BME) showed decent activity (46.93% inhibition at 150 µg/mL) with an EC_{50} of 154.27 µg/mL, outperforming catechin in some respects. When these results are considered alongside the nearly complete superoxide inhibition shown by BAE in this work, the superior efficacy of the *Berberis*-based preparations becomes clear.

The reducing power assay is an important measure of antioxidant capacity because it reflects the ability of bioactive compounds to donate electrons, thereby neutralizing free radicals and terminating propagating oxidative chain reactions. According to Kaushik *et al.* (2010) [15], the efficiency of converting ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions is a reliable marker of antioxidant potency. Similar results have been reported for similar plant extracts. *Terminalia arjuna* bark extract (TAE) also showed a significant increase in reducing potential depending on the concentration, which is consistent with Shahriar *et al.* (2012). *Trigonella foenum-graecum* extract (TFE) showed a mild but significant reducing power due to its polyphenols and associated phytochemicals, which is consistent with previous findings (Dhull *et al*., 2020). This activity may be due to the phytoconstituents of BAE, particularly phenolics,

flavonoids, and alkaloids such as berberine, which readily react with free radicals to form more stable, non-reactive products, thereby reducing oxidative stress.

Conclusion

In this study, a water root extract of *Berberis aristata* DC. (BAE) was successfully prepared and its antioxidant capacity was evaluated using multi-mechanistic *in vitro* assays. The results revealed that BAE possesses significant antioxidant activity, evidenced by its ability to scavenge free radicals and donate electrons. Specifically, BAE exhibited strong nitric oxide (NO) scavenging activity with an IC_{50} of 86.143 µg/ml, indicating its effectiveness in reducing nitrosative stress. Furthermore, the extract displayed strong superoxide anion radical scavenging capacity, indicating its high ability to neutralize superoxide radicals, which are the main initiators of oxidative chain reactions. Although the reducing power of BAE was lower than that of standard ascorbic acid, it showed a clear concentration-dependent increase, confirming the extract's ability to act as an electron donor to stabilize reactive species. The observed antioxidant activity is likely due to the synergistic effect of bioactive phytoconstituents present in the root, including alkaloids (such as berberine), flavonoids, and phenolics. These compounds appear to act through both direct radical quenching and electron donation mechanisms. Overall, these results support the traditional medicinal use of *Berberis aristata* and suggest that BAE is a promising source of natural antioxidants for the management of oxidative stress-related diseases.

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Declaration of Competing Interest

None

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