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Exploring the antioxidant efficacy of boldine: A natural compound with broad-spectrum activity

Maharani Jaganathan[1], Kathiresan Suresh[*1], Manickam John[2], Rajeswari Vasu[1], Theerthu Azhamuthu[1], Nihal Ahamed Abulkalam Asath[1], Ravichandran Pugazhendhi[1], Pratheeba Veerapandiyan[1]

[1]*Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, Tamil Nadu, Chidambaram, India*

[2] *Central University of Punjab, Ghuddha, Bathinda, Punjab, India*

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ABSTRACT

Free radicals are unshared electrons that readily reacts with macromolecules and generate the reactive oxygen species, triggering lipid peroxidation reaction. This reaction lead to inflammation and oxidative stress, which can initiate and promote the progression of degenerative diseases, including cancer and diabetes mellitus. Antioxidants are free radical scavenging molecules that help to limit such diseases. Plant derived alkaloids boldine to possess their strong scavenging ability of free radicals. The boldine's ability to scavenge free radicals and donate electrons was tested using various assays, including Superoxide anion, hydrogen peroxide, DPPH, ABTS, hydroxyl radical, nitric oxide, and reducing power. The findings revealed that boldine effectively inhibited free radicals, as evidenced by its IC_{50} value, compared to the standard ascorbic acid. This study found that boldine has substantial free radical scavenging action, as indicated by its IC_{50} values when compared to the conventional antioxidant, ascorbic acid. Boldine and ascorbic acid had the following IC_{50} values: 33.00 µg/mL and 36.00 µg/mL in the DPPH assay, 19.83 µg/mL and 23.08 µg/mL in the ABTS●+ assay, 14.00 µg/mL and 16.80 µg/mL in the hydroxyl radical assay, 29.00 µg/mL and 33.00 µg/mL in the superoxide anion assay, 27.00 µg/mL and 33.00 µg/mL in the hydrogen peroxide assay, and 11.96 µg/mL and 16.80 µg/mL in the nitric oxide assay, respectively. In addition, the reducing power of (21.00 µg/mL) ascorbic acid and boldine (19.00 µg/mL). The above findings suggest that Boldine has great antioxidant potential and effective free radical scavenging activity, suggesting that it can be applied as a natural beneficial antioxidant.

***Corresponding author:** Kathiresan Suresh ✉ sureshrajakantham@gmail.com

INTRODUCTION

Biological systems can suffer from oxidative stress; it is caused by an imbalance of pro-oxidant synthesis and antioxidant defenses (Sies *et al.*, 1985). Free radicals generated via oxygen make up the reactive oxidative species (ROS) that can be generated by nature or pulled in from outside sources. Under usual conditions, the body's natural antioxidant defense mechanism reduces their effects. However, if the balance between antioxidants and prooxidants is disturbed (Jia *et al.*, 2014; Ashok *et al.*, 2022). Excessive oxidative stress happens via reactive oxygen species, resulting in destruction of lipids and proteins and other cellular components and cause illness (Gulcin *et al.*, 2020). Plants contain natural antioxidants that assist the body neutralize damaging free radicals. Antioxidants are highly unstable and reactive chemicals that are found naturally in biological systems. They have the ability to exist on their own and have possessing one or additional unpaired electrons they react with other molecules by donating or absorbing electrons (Sundaram Sanjay *et al.*, 2021). Excessive oxidative stress, resulting from a high level of reactive oxygen species, has been associated along with the beginning and growth of various serious illnesses, such as cancer, type 2 diabetes, problems with the brain, and cardiovascular disorders (Tiwana *et al.*, 2024). Antioxidants are recognized as crucial components of the body's defensive system against free radical damage (Alkadi *et al.*, 2020). Enzymes include superoxide dismutase (SOD), catalase, and GPx help remove ROS. Catalase degrades high quantities of H_2O_2, while GPx acts at physiological doses (Anjum *et al.*, 2020). To maintain redox balance, cells rely on enzymatic and non-enzymatic antioxidants. Reduced glutathione (GSH) scavenges hydroxyl radicals and acts as a cofactor for glutathione peroxidase (Battin *et al.*, 2009). A fat-soluble antioxidant, vitamin E protects from oxidative damage to cell membranes, whereas Vitamin C facilitates this process by removing free radicals and replacing Vitamin E (GAO *et al.*, 2012; Pekiner *et al.* 2003). Reduced the essential antioxidant glutathione, also known as GSH, that maintains cellular redox state by detoxifying singlet oxygen molecules and hydroxyl radicals are a couple of reactive oxygen species (ROS) (Juurlink *et al.,* 1997) Superoxide anions turn into peroxide of hydrogen and molecules of oxygen through the antioxidant enzyme superoxide dismutase (SOD), which is essential for cellular resistance to oxidative stress. Catalase and glutathione peroxidase (GPx) eliminate the hydrogen peroxide produced. Catalase efficiently decomposes hydrogen peroxide at greater concentrations, whereas GPx acts successfully under physiological settings by using the oxidation of reduced glutathione (GSH) to glutathione disulfide to convert hydrogen peroxide to water (Andrés *et al.*, 2022). Using a variety of enzymatic and non-enzymatic processes, the body uses an integrated antioxidant defense system to counteract the harmful consequences of lipid peroxidation (El-Beltagi *et al.*, 2013).

Although it regulates the breakdown of superoxide anions into hydrogen peroxide and oxygen, superoxide dismutase (SOD) is a vital enzyme for cellular defense (Ighodaro *et al.*, 2018). Catalase and glutathione peroxidase (GPx) then eliminate the resulting hydrogen peroxide. Catalase is highly efficient at decomposing H_2O_2 when present at elevated concentrations, whereas GPx functions effectively at physiological levels, reducing H_2O_2 to water through the oxidation of reduced glutathione (GSH) to glutathione disulphide (Andrés *et al.*, 2022). The last several decades have seen a rise in focus on plant-derived therapies because of their effectiveness and relatively fewer adverse effects (Houghton *et al.*, 1995).

For thousands of years, herbal remedies have been widely used for treating an extensive spectrum of illnesses. They are generally associated with fewer side effects, greater accessibility, and lower cost. These products contain a variety of biologically active substances that serve as lead structures for the development of new drug candidates (Tran *et al.*, 2020). Dietary sources such as fruits and vegetables are enriched with high concentrations of phytochemicals, which possess strong free radical-scavenging abilities, are crucial in eliminating cellular damage bring by oxidative stress (Jideani *et al.*, 2020). Mainly isolated from the leaves and bark of the Chilean boldo plant, boldine ((S)-2,9-dihydroxy-1,10-dimethoxy-aporphine) is a benzylisoquinoline alkaloid featuring significant pharmacological qualities (Gulcin *et al.*, 2020). Boldine has proven a broad range of pharmacological acts, which includes anti-inflammatory, anti-epileptic, and neuroprotective activities (Lamba *et al.*, 2024). Recent studies further suggest its potential utility in the management of urolithiasis, primarily due to its lithotriptic properties (Gottlieb *et al.*, 2018).

Demirtaş *et al.* (2025) demonstrated that boldine has cardioprotective properties in a rat model of cardiac ischemia-reperfusion, highlighting its broad protective functions across different biological systems. These results indicate that boldine is a promising treatment candidate with multi-system protective effects. This investigation's aim was to examine its bioactive potential of boldine, with the aim of identifying its capacity to exert beneficial effects through oxidative stress modulation. The findings may contribute to the discovery of novel antioxidant compounds from natural sources for potential medical uses in the coming years.

MATERIALS AND METHODS

Chemicals

This study utilized analytical grade chemicals and reagents that include Thiobarbituric acid , or TBA, 1,1-diphenyl-2-picrylhydrazyl or DPPH, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$\bullet^+$), phenazine methosulfate (PMS), sulfanilic acid and glacial acetic acid , ferrous chloride, potassium ferric cyanide, 5-dithiobis-(2-nitrobenzoic acid) or DTNB, naphthyl ethylenediamine dihydrochloride nitro blue tetrazolium (NBT), and methosulfate-nicotinamide adenine dinucleotide, NADH, were used.

DPPH radical scavenging assay

We employed the technique described by Baliyan *et al.* (2022) to find out how boldine and ascorbic acid, the positive control, affected 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Concentrations ranging from 10 to 50 µg/mL were used to produce the sample solutions in 100% ethanol. A vortex mixer was used to violently shake the liquids after adding boldine at these concentrations to 2 mL of methanolic DPPH solution. Spectrophotometric measurements of absorbance were made at 517 nm right away, and they were taken every five minutes until a steady value was obtained. A DPPH solution devoid of any sample served as the control. The inhibition percentages were determined relative to the standard, ascorbic acid, with all tests executed in triplicate.

DPPH radical inhibition (%) = [(Abs control − Abs sample) / Abs control] ×100

ABTS●+ radical scavenging assay

The capacity of Boldine to scavenge ABTS$\bullet^+$ radicals was assessed using the method established by Arnao *et al.*, with a few adjustments. The reaction mixture consisted of 15 µM hydrogen peroxide, 7 mM ABTS and 50 mM saline buffering solution at pH 7.5. Boldine was tested at levels of ten to fifty µg/mL. A control sample was prepared by substituting Boldine with distilled water, and ascorbic acid served as the baseline sample. The absorbance was calculated at 734 nm with the aid of a spectrophotometer. The half-maximal inhibitory concentration (IC_{50}), indicating the dose necessary to destroy 50% of ABTS$\bullet^+$ radicals, was calculated using the appropriate formula.

% ABTS●+ scavenging = [(Abs control − Abs sample) / Abs control] ×100

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging ability of Boldine was assessed utilizing the Kunchandy and Rao procedure with small modifications. There was 100 µL of 2-deoxyribose (28 mM in 20 mM KH_2PO_4-KOH buffer, pH 7.4) in the 1.0 mL reaction mixture various concentrations of Boldine (10–50 µg/mL), Mix 100 µL of hydrogen peroxide (1.0 mM), 100 µL of ascorbic acid (1.0 mM), and 200 µL of EDTA (1.04 mM) with 200 µM $FeCl_3$ in a 1:1 volume ratio.

The mix was kept warm for an hour at a temperature of 37 Celsius. Following the incubation period, the samples were steamed at hundred degrees Celsius for twenty minutes. and then treated with 1.0 mL of 1% Thio barbituric acid and 1.0 mL of 2.8% trichloroacetic acid. The test sample was replaced with distilled water to create a blank, and its absorbance was taken at 532 nm following cooling.

% of hydroxyl radical scavenging = [(Abs control – Abs sample) / Abs control] ×100

Superoxide radical scavenging assay

The Fontana *et al.* strategy, with a few alterations, was used to examine the superoxide radical scavenging activity of boldine. Nitroblue tetrazolium (NBT) turns into purple-colored substance called formazan via superoxide radicals formed by phenazine methosulfate and nicotinamide adenine dinucleotide. The reaction mix (1.0 mL) comprised 20 mM phosphate buffer (pH 7.4), 73 µM NADH, 50 µM NBT, 15 µM PMS, and multiple amounts of Boldine (10-50 µg/mL). During a five-minute incubation at ambient temperature, an absorbance at 562 nm was recorded in comparison to the respective blank. All experiments were operated in triplicate, and the results were compared to ascorbic acid, the reference antioxidant.

% Superoxide radical scavenging = [(Abs control – Abs sample) / Abs control] ×100

Hydrogen peroxide scavenging assay

The Jayaprakash *et al.* method has been used to evaluate the hydrogen peroxide (H_2O_2) the scavenging actions of Boldine, with a few minor adjustments. Phosphate-buffered saline (PBS, pH 7.4) was used to make a 20 mM H_2O_2 solution. After being dissolved in 1 milliliter of ethanol, ascorbic acid and boldine were added to 2 milliliters of the H_2O_2 solution at dosages between 10 to 50 µg/mL. The mixture was incubated under appropriate conditions, and the scavenging activity was quantified by analyzing the reduction in absorbance at the relevant wavelength compared to a control containing no test sample. During a ten- minutes incubation at ambient temperature, absorbance at 230 nm was assessed a blank with only H_2O_2 solution and no test chemical. The subsequent formula was employed to calculate the percentage of H_2O_2 scavenging activity and IC_{50} values.

% Hydrogen peroxide scavenging = [(Abs control – Abs sample) / Abs control] ×100

Nitric oxide radical scavenging assay

The Nitric oxide (NO) scavenge efficacy of Boldine was measured using the Garrat method, with slight modifications. A 10 mM solution of sodium nitroprusside (2 mL) in a pH 7.4 solution of PBS was mixed to 0.5 mL of the Boldine at doses of ten to fifty µg/mL. The standard used was ascorbic acid at equivalent quantities (10-50 µg/mL). The mixes were preserved at 25°C for a total of 150 minutes. During incubation, 0.5 mL of the reaction solution was mixed with 0.5 mL of Griess reagent, prepared by mixing 1 mL of 0.33% sulfanilic acid in 20% glacial acetic acid with 1 mL of 0.1% naphthyl ethylenediamine dihydrochloride. The solution was left at ambient temperature for 30 minutes, and intensity was calculated at 540 nm via a wavelength spectrophotometer. The standard formula was used to determine the IC_{50} values and the proportion of nitric oxide scavenging:

% Nitric oxide radical scavenging = [(Abs control – Abs sample) / Abs control] ×100

Reducing power assay

The reductive ability of Boldine was assessed using Yen and Duh's approach, with some changes. Boldine was dissolved in ethanol at concentrations between 10 and 50 µg/mL. Each sample was treated utilizing 2.5 mL of 200 mM buffered phosphate (pH 6.6) and 2.5 mL of 1% potassium ferricyanide as the solvent. The combination mixtures they were kept at 50 °C for a duration of 20 minutes. Following incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added and centrifuged at 3000 × g for 10 min. An aliquot of the supernatant (5 mL) was mixed with 5 mL distilled water (1:1) and 1 mL 0.1% ferric chloride. The absorbance of the resultant solution was determined at 700 nm using a spectrophotometer. Ascorbic acid was chosen as the reference standard.

Statistical analysis

The mean ± SD is used to display the results. The statistical value of the disparities between the groups was assessed using DMRT and ANOVA. *p*-values below 0.05 were considered statistically substantial.

RESULTS

DPPH radical scavenging assay

In the current investigation, the antioxidant sample caused the DPPH radical, a stable deep violet free radical, to change colour from deep violet to yellow, signifying the donation of hydrogen atoms and the transformation of DPPH into its non-radical form. Fig. 1 shows Boldine's ability to scavenge DPPH free radicals. Boldine's IC_{50} value was 33 µg/mL, comparable to the conventional antioxidant ascorbic acid (36.8 µg/mL). Boldine's ability to scavenge DPPH increased proportionally with increasing concentrations.

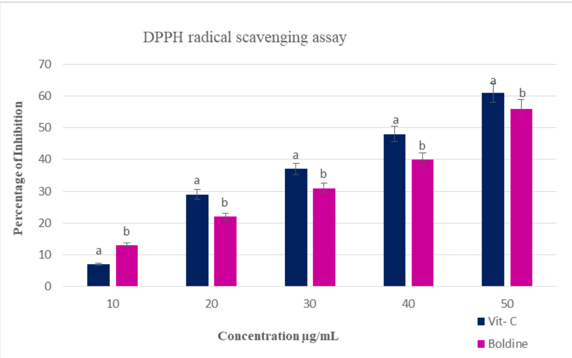

Fig. 1. DPPH radical scavenging assay

The outcome of Boldine and ascorbic acid on DPPH radical scavenging activity (n = 3) is presented. Different superscript letters at $p < 0.05$ denote statistically significant differences among increasing concentrations of the test samples.

ABTS radical scavenging assay

The $ABTS^{+}$• radical cation, which has a distinctive blue-green hue, is produced when potassium persulfate oxidizes ABTS. Antioxidants show their ability to donate electrons or hydrogen by reducing this chromophore to its colorless state. The ability of Boldine to scavenge ABTS radicals is demonstrated in Fig. 2. An IC_{50} value of 19.83 µg/mL for boldine indicated that it was effective at scavenging ABTS radicals, whereas the reference antioxidant, Vitamin - C had an IC_{50} value of 23.08 µg/mL. This implies that boldine has significant potential as an antioxidant. Additionally, Boldine demonstrated an increase in radical scavenging that was concentration-dependent. Ascorbic acid demonstrated somewhat more radical scavenging activity, while boldine's response was nearly identical, suggesting that boldine is an effective antioxidant.

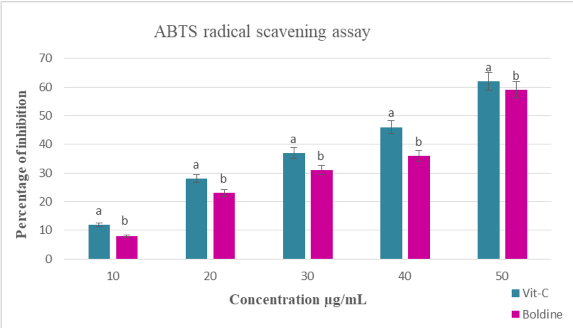

Fig. 2. ABTS radical scavening assay

ABTS scavenging potential of Boldine. The mean ± SD for three replicates (n = 3) is used to express the data. Different superscript letters denote statistically significant differences ($p < 0.05$) among the increasing concentrations of the test samples.

Hydroxyl radical scavenging activity

In this investigation, deoxyribose was damaged by hydroxyl radicals generated by the Fenton reaction, resulting in the production of malondialdehyde (MDA). A pink complex was created when the produced MDA and thiobarbituric acid (TBA) interacted. Antioxidants were shown to be able to scavenge hydroxyl radicals and shield deoxyribose from oxidative damage by reducing the intensity of this color. Boldine's capacity to scavenge hydroxyl radicals is demonstrated in Fig. 3. With an IC_{50} value of 16.08 µg/mL, boldine was found to be extremely similar to the standard antioxidant, ascorbic acid (14.18 µg/mL). Boldine showed a definite dose-dependent increase in hydroxyl radical scavenging activity, suggesting that it is strongly comparable Vitamin C in terms of antioxidant potential.

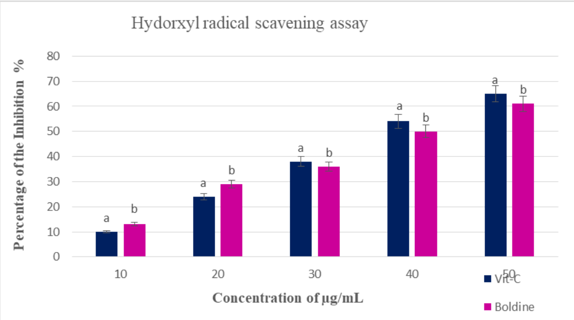

Fig. 3. Hydorxyl radical scavening assay

Data are expressed as mean ± SD for three replicates (n= 3). Different superscript letters across increasing concentrations of the test samples indicate statistically significant differences (p< 0.05).

Superoxide scavenging potential

In the present investigation, the PMS–NADH system, which breaks down nitro blue tetrazolium (NBT) to create a blue-colored formazan molecule, generated superoxide radicals. Antioxidants inhibit the degradation of NBT and cause a decrease in color intensity by scavenging the superoxide radicals that are produced. Boldine's ability to scavenge superoxide radicals is demonstrated in Fig. 4. The IC_{50} value of 29.09 µg/mL, boldine illustrates efficient scavenging. This value is marginally higher than that of the Positive control (ascorbic acid) (33.94 µg/mL), but the findings show that boldine has a similar antioxidant activity. Furthermore, boldine's potential as an efficient antioxidant was demonstrated by a pronounced dose-dependent increase in superoxide radical scavenging activity.

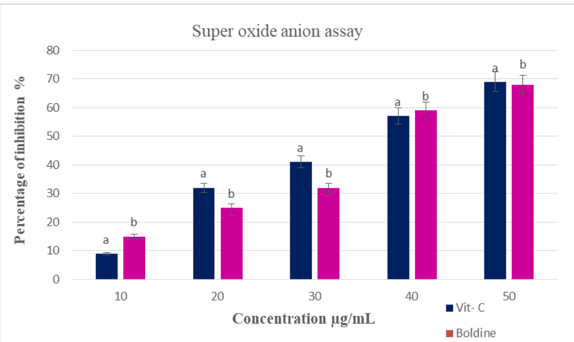

Fig. 4. Super oxide anion assay

The mean ± standard deviation (SD) of three independent replicates (n = 3) is used to express all data. When the concentrations of the test samples increase, Superscript letters indicate values that differ significantly (p< 0.05).

Hydrogen peroxide (H_2O_2) radical scavenging

Antioxidants can transform hydrogen peroxide (H_2O_2), which looks as a pale-yellow solution, into oxygen and water. The ability of antioxidants to neutralize H_2O_2 and stop its transformation into dangerous hydroxyl radicals is demonstrated by this decrease in absorbance. Boldine's ability to scavenge hydrogen peroxide (H_2O_2)) is demonstrated in Fig. 5. An IC_{50} value of 27.00 µg/mL demonstrates Boldine's potent radical scavenging activity which was very close to that of ascorbic acid, the reference antioxidant (33.00 µg/mL). This showed that the two antioxidants were equally effective at neutralizing H_2O_2 in a dose-dependent manner.

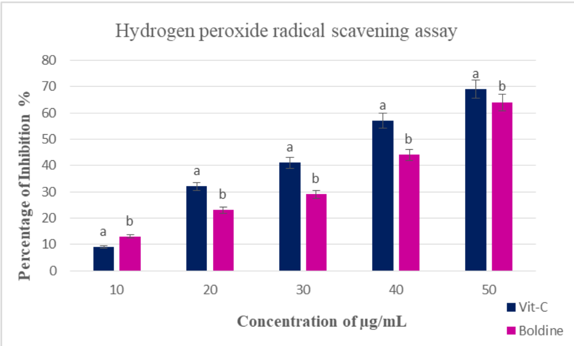

Fig. 5. Hydrogen peroxide radical scavenging assay

The mean ± SD of three separate studies (n = 3) is used to display the values. Significant changes (p< 0.05) are shown by different superscripts across concentrations.

Reducing power assay

The reducing power assay relies on antioxidants' ability to change Fe^{2+} ions into Fe^{2+} ions. A Prussian blue complex is created when the resultant Fe^{2+} ions combine with ferricyanide, and the rise in absorbance signifies increased antioxidant reduction activity. Fig. 6 depicts the decreasing power scavenging activity of Boldine. Boldine showed a dose-dependent increase in reducing power, with values comparable to ascorbic acid, the standard antioxidant. Boldine had a maximum concentration of 19.8 µg/mL, while ascorbic acid had a maximum concentration of 21.5 µg/mL, demonstrating its great electron donor capacity.

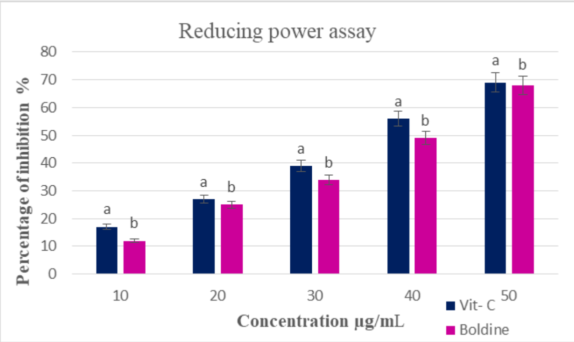

Fig. 6. Reducing power assay

The mean ± standard deviation (SD) for three separate replicates (n = 3) is how the data are displayed. Statistically significant differences are indicated by superscript letters across the test samples' various concentrations ($p < 0.05$).

Nitric oxide radical scavenging

In our study the nitric oxide scavenging assay involves the production of NO• radicals from sodium nitroprusside in aqueous solution, which react with oxygen to form nitrite ions. A pink azo dye that can be measured at 540 nm is created when the nitrite ions react with the Griess reagent. As a sign of their capacity to quench nitric oxide, antioxidants scavenge the NO• radicals, preventing this color formation. The reducing power activity of vitamin C is shown in Fig. 7.

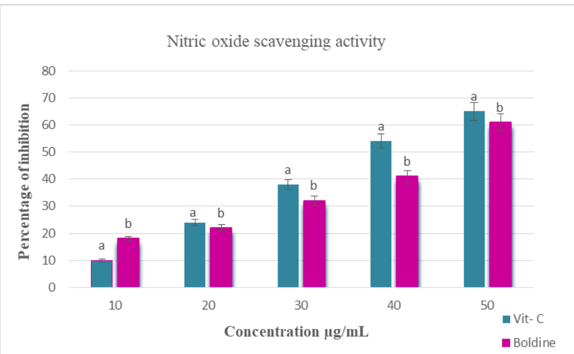

Fig. 7. Nitric oxide radical scavenging

Boldine's and ascorbic acid's (n = 3) relationship with nitric oxide scavenging. Values with prominent superscripts indicate Statistically significant variations between rising test sample concentrations are indicated by significant differences at $p < 0.05$.

The test sample demonstrated a dose-dependent increase in reducing power, with levels comparable to ascorbic acid, the standard antioxidant. At the highest tested concentration, the reducing power of ascorbic acid was higher, while at lower concentrations the values were 16.8 µg/mL and 11.96 µg/mL, respectively. Different superscript letters at $p < 0.05$ denote statistically significant variations between increasing concentrations of the test samples.

Inhibitory concentration (IC_{50}) of boldine in different radical scavenging assays

The IC_{50} values (µg/mL) of Boldine and Vitamin C were evaluated using DPPH, ABTS•$^{+}$, hydroxyl radical (HD), superoxide (SO), hydrogen peroxide (H_2O_2), reducing power (RP), and nitric oxide (NO) scavenging assays. Boldine exhibited IC_{50} values comparable to Vitamin C, indicating a similar antioxidant potential across the tested assays. Each experiment was conducted in triplicate, and the reported values correspond to the mean. The IC_{50} values for Boldine and the reference antioxidant, ascorbic acid (Vit-C), spanning several experiments are shown in Fig. 8.

Fig. 8. IC 50 values of different radical tested

DISCUSSION

Boldine exhibited dose-dependent cytotoxicity in HepG-2 cells, which was mediated via telomerase suppression and apoptosis, showing its potential as a treatment agent for hepatocellular cancer (Kazemi Noureini *et al*., 2015). Our previous study indicate the

Boldine exhibits potent anticancer activity against human oral carcinoma cells by triggering oxidative stress, resulting in mitochondrial dysfunction, and triggering apoptosis through blocking the Notch signaling pathway. Additionally, its strong antioxidant properties contribute to regulating redox homeostasis, further supporting its significant anticancer potential and highlighting its therapeutic efficacy against oral cancer (Jaganathan *et al.*, 2025). Mohammadjavad Paydar *et al.*, demonstrated that Boldine has cytotoxic and chemotherapeutic actions against breast cancer in cell lines and experimental animal model indicating its ability to reduce tumor cell proliferation, cause apoptosis, and modulate key molecular pathways associated with cancer progression. The present study used a variety of in vitro tests to evaluate the scavenging capacity of boldine against free radicals. Consequently, researchers worldwide are exploring medicinal plants for strong antioxidant agents. The antioxidative capacity of plant-derived substances is usually assessed utilizing the efficiency of boldine to eliminate various free radicals was evaluated in vitro using a number of well-known assays, such as DPPH, ABTS, superoxide and hydroxyl radicals, reducing power, hydrogen peroxide, and nitric oxide (Asif *et al.*, 2015; Thatoi *et al.*, 2014). The antioxidant efficacy of Boldine can be evaluated by its ability to reduce DPPH, a violet-colored radical, to a yellow-colored product Revealing the significant DPPH radical–scavenging efficiency via donates electron to the neutralization of oxidizing radicals exhibited a dose-dependent pattern resembling that of ascorbic acid. By evaluating a compound's ability to quench the $ABTS^{+}\bullet$ radical was evaluated by measuring the ABTS radical-scavenging activity. This assay is frequently used to assess a compound's total antioxidant capacity (TAC) (Akotkar *et al.*, 2023). In this study also Boldine effectively scavenged ABTS radicals, exhibiting a clear dose-dependent effect compared to ascorbic acid. Hydroxyl radical scavenging (•OH) is among the most effective reactive free radicals, sufficient to initiate lipid peroxidation and causing significant damage to DNA and proteins.

Boldine has been reported to directly scavenge hydroxyl radicals in chemical and biological systems, thereby reducing radical-mediated chain reaction. (Lamba *et al.*, 2024). Our results also corroborate these observations. Superoxide anion ($O_2\bullet^-$) is the first free radical generated in biological systems, serving as a precursor for the creation of additional radicals generated from oxygen. Studies have revealed that Boldine has the potential to directly mitigate free radical damage by reducing hydrogen peroxide and hydroxyl radicals in mitochondrial settings and neutralizing superoxide radicals with increasing concentrations (O'Brien *et al.*, 2006).

The reducing power assay serves as a reliable method to evaluate the electron-donating potential of antioxidants (Kumar *et al.*, 2018). Boldine showed a marked dose-dependent rise in reducing activity, closely comparable to ascorbic acid, which highlights its effectiveness as a reductant in neutralizing free radicals. Recent findings further support its potent redox behaviour, revealing that Boldine not only decreases oxidant levels but also safeguards biomolecules from oxidative alteration (Lau *et al.*, 2015). According to Radi *et al.* (2018), Nitric oxide radical is a reactive free radical that can interacting with oxygen to create peroxynitrite, a strong oxidant that can harm biomolecules such as proteins, lipids, and DNA. In vitro investigations have shown that Boldine effectively scavenges NO• in a concentration-dependent way, limiting the production of downstream reactive species (Lamba *et al.,* 2024). This action demonstrates Boldine's ability to directly neutralize nitric oxide radicals and contributes to its overall free radical-quenching potential, bolstering its position as a natural antioxidant in nitrosative stress reduction (Kurutas *et al.*, 2015).

Boldine evidenced a strong, concentration-dependent radical quenching impact in the 2,2-diphenyl-1-picrylhydrazyl and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) evaluations comparable to the standard antioxidant ascorbic acid, confirming its effectiveness as an electron or hydrogen donor (O'Brien *et al.*, 2016). Its activity against superoxide anions exhibited inhibition of one of the primary free radicals responsible for the generation of more reactive species, whereas its hydroxyl radical scavenging capacity revealed protection against one of the most aggressive radicals known to damage cellular components (Ozougwu *et al.*, 2016).

Boldine was also efficient in decreasing nitric oxide levels, which helps to avoid the development of damaging peroxynitrite and decreases nitrosative stress. Its performance in the reducing power assay demonstrates its strength as an antioxidant (Lau *et al.*, 2013.) The current research shows that Boldine has significant antioxidant activity in a variety of radical scavenging assays. The IC_{50} values were consistently lower or comparable to ascorbic acid, indicating a significant free radical neutralizing capacity. Boldine demonstrated broad-spectrum antioxidant activity is effectively scavenged hydroxyl (14.00 µg/mL vs. 16.80 µg/mL), superoxide (29.00 µg/mL vs. 33.00 µg/mL), hydrogen peroxide (27.00 µg/mL vs. 33.00 µg/mL), and nitric oxide (11.96 µg/mL) radicals. Boldine's ability to donate electrons was validated by the reducing power assay (19.00 µg/mL vs. 21.00 µg/mL for ascorbic acid), highlighting its function in stopping radical chain reactions. Notably, boldine retained comparable action even at lower doses, indicating its efficacy in physiological circumstances. These findings suggest that boldine can protect cellular macromolecules like lipids, proteins, and nucleic acids from oxidative damage while also maintaining redox equilibrium. Boldine's electron-donating and radical-stabilizing capabilities, as found in prior investigations on alkaloid antioxidants, are most likely responsible for its high activity. Overall, the study found that boldine is a powerful natural antioxidant with broad-spectrum activity and potential therapeutic benefits for oxidative stress-related disorders. Additional in vivo investigations are recommended to censure its therapeutic efficacy and safety in biological systems.

CONCLUSION

The scavenging activity of boldine against a Superoxide, DPPH, ABTS, hydroxyl radicals, and nitric oxide are among the numerous free radicals that as well as its notable reducing power, reveal the substance's substantial antioxidant potential, according to the current study. Its activity was concentration-dependent and often comparable to that of ascorbic acid, a well-known benchmark. These data imply that Boldine acts as an efficient electron donor and radical quencher, reducing oxidative and nitrosative stress. Overall, Boldine appears to be a promising natural antioxidant with potential applications in managing and preventing damage caused by free radicals.

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