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Protective role of α-tomatine against oxidative stress induced reactive oxygen species: *In vitro* radical scavenging assays

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ABSTRACT

Oxidative stress is important in the pathophysiology of several chronic diseases by inducing cellular damage via the overproduction of reactive oxygen species (ROS) and free radicals. Organic compounds are attracting much attention for their antioxidant effects, providing preferable remedies in contrast to manufactured medicines. α-tomatine, a steroidal glycoalkaloid primarily found in green tomatoes, has demonstrated several biological actions, such as anti-inflammatory and anticancer properties; nevertheless, its antioxidant capacity is less investigated. The present study examines the free radical scavenging ability of α-tomatine through a series of in vitro antioxidant assays, including DPPH, ABTS, hydroxyl, hydrogen peroxide, nitric oxide, superoxide, and reducing power tests. α-tomatine exhibited concentration-dependent antioxidant properties by efficiently neutralizing free radicals and increasing electron-donating capacity. The IC_{50} values obtained from the respective assays were 22.73 µg/mL and 57.64 µg/mL in DPPH, 28.86 µg/mL and 63.38 µg/mL in ABTS, 32.8 µg/mL and 56.75 µg/mL in superoxide, 35.6 µg/mL and 66.4 µg/mL in hydroxyl, 38.5 µg/mL and 70.7 µg/mL in nitric oxide, 37.4 µg/mL and 59.3 µg/mL in H_2O_2, and 40.2 µg/mL and 61.14 µg/mL in reducing power assays, respectively, indicating moderate antioxidant efficacy compared to the standard ascorbic acid. α-tomatine exhibited notable antioxidant potential by effectively scavenging free radicals and enhancing reducing power in a dose-dependent manner. Although its activity was lower than that of ascorbic acid, these results highlight its potential role in reduce oxidative stress and protecting against ROS-induced cellular damage.

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INTRODUCTION

Free radicals are molecules that occur spontaneously within living organisms and are necessary for cellular processes and defensive mechanisms at low levels. They are highly reactive due to their great affinity for pairing their unpaired electron(s) via interactions with other radicals or neutral substances (Iakovou and Kourti, 2022). Phytochemicals have strong antioxidant activities that neutralize free radicals and reactive oxygen species (ROS), preventing oxidative stress and cell damage (Ashraf *et al.*, 2024; Guo *et al.*, 2024; Gupta *et al.*, 2023; Khan *et al.*, 2024).

Phytochemicals are organic substances exist in plants that enhance their color, flavor, and disease resistance. They can be generally categorized into various classes based on their distinct chemical constituents and biological roles, including alkaloids, carotenoids, flavonoids, glucosinolates, and polyphenols. These various groups of phytochemicals have unique biological functions associated with a diverse range of physiological benefits, including antioxidant, anti-inflammatory, and anticancer effects (Kurutas, 2015).The predominant free radicals and reactive molecules in biological systems originate from oxygen (ROS) and nitrogen (reactive nitrogen species, RNS). ROS or RNS are generated during electron transfer reactions through the loss or gain of electrons (Phaniendra *et al.*, 2015).

Oxidative stress happens during the generation of ROS and RNS beyond the capability of natural antioxidant defenses. Reactive molecules, including superoxide anion (O_2^{-}•), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), and nitric oxide (NO•), are constantly produced in living things as outcomes from regular biological processes, especially during mitochondrial oxidative phosphorylation and numerous enzymatic processes (Chatgilialoglu, 2024; Jomova *et al.*, 2023).Under normal conditions, moderate levels of ROS are essential for signaling in immune responses, apoptosis, and gene expression. Excessive formation of ROS can lead to oxidative damage to proteins, lipids, and DNA, resulting in aging, cellular malfunction, and an increased risk various illnesses including cancer, diabetes, heart disease, and neurological disorders such as Parkinson's and Alzheimer's (Sikder *et al.*, 2025).

Biological systems use an elaborate antioxidant defense system to reduce oxidative damage, which includes enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as natural antioxidants like vitamins C and E, glutathione, and different plant-based polyphenols. When intrinsic mechanisms are insufficient, naturally occurring antioxidants, especially those derived from plant-derived compounds, are essential for sustaining redox equilibrium (Jomova *et al.*, 2024; Zulaikhah, 2017).

α-tomatine is a steroidal glycoalkaloid (SGA) commonly found in tomato plants, mostly develops in green tissues, such as leaves, stems, and immature fruits, while the concentration of α-tomatine reduces in mature fruits (Liu *et al.*, 2023). It has essential roles in plant defense and medical development with respect to its pharmacological characteristics and structural complex (Sonawane *et al.*, 2023). It demonstrates significant antioxidant properties, specifically by diminishing oxidative stress in scavenging experiments that evaluate ROS and the capacity for free radical neutralization(Silva-Beltrán *et al.*, 2015). It provides various therapeutic benefits, including antibacterial, antibiotic, anti-inflammatory, anticarcinogenic, anti-obesity, and anti-aging properties (Nakayasu *et al.*, 2021).

In the present study, the antioxidant efficacy of α-tomatine is evaluated using different *in vitro* free radical scavenging methods. Generally used assays include DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), superoxide radical scavenging assay, hydroxyl radical scavenging assay, nitric oxide scavenging assay, hydrogen peroxide scavenging assay and reducing power assays. These experiments offer significant insights into the capability of α-tomatine to neutralize various reactive species and, thus, its potential for reducing oxidative stress.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate ($K_2S_2O_8$), hydrogen peroxide (H_2O_2), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), NADH, sodium nitroprusside, Griess reagent, ascorbic acid, Folin–Ciocalteu reagent, potassium ferricyanide, ferric chloride, and analytical-grade solvents (methanol, ethanol, and phosphate buffers) were procured from HiMedia Laboratories (Mumbai, India). α-tomatine (≥95% purity, HPLC) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

DPPH assay

The DPPH assay was employed to evaluate the antioxidant potential of α-tomatine and its ability to scavenge ROS. Five concentrations of α-tomatine and ascorbic acid 20,40,60,80 and 100 µg/mL were prepared in suitable solvents and adjusted to a final volume of 1 mL. Each sample was mixed with an equal volume (1 mL) of DPPH methanolic solution and incubated in the dark for 30 min. The decrease in DPPH radical absorbance was measured spectrophotometrically at 517 nm, and the antioxidant activity of α-tomatine was expressed as the percentage reduction in absorbance relative to the control (Sharmila and Selvaraj, 2024).

Inhibition (%)={(Absorbance of control-Absorbance of sample)/(Absorbance of control)}× 100

ABTS assay

The antioxidant activity of α-tomatine was evaluated using the ABTS radical cation decolorization assay, with ascorbic acid serving as the reference standard. The ABTS radical cation was generated by mixing equal volumes of 7 mM ABTS stock solution and 2.45 mM potassium persulfate, followed by incubation in the dark at room temperature for 12–16 h. α-tomatine was prepared and diluted to final concentrations of 20-100 µg/mL, while ascorbic acid was diluted to equivalent concentrations as the standard. Subsequently, 300 µL of sample, standard or vehicle control was mixed with 3mL of the ABTS working solution. After incubation for 6 min in the dark at room temperature, absorbance was measured at 734 nm using a microplate reader (Kolgi *et al.*, 2021).

Inhibition (%)={(Absorbance of control-Absorbance of sample)/(Absorbance of control)}× 100

Superoxide radical scavenging activity

The superoxide radical scavenging activity of α-tomatine was assessed using the PMS–NADH–NBT assay with slight modifications (Hazra *et al.*, 2008). The reaction mixture (final volume: 1.0 mL) consisted of 50 mM phosphate buffer (pH 7.4), NBT (50 µM), NADH (150 µM), and varying concentrations of α-tomatine (20–100 µg/mL). The reaction was initiated by adding PMS (30 µM) and incubating the mixture at room temperature for 2 min. The reduction of NBT to formazan was quantified spectrophotometrically at 560 nm. Ascorbic acid served as the reference standard. Control reactions lacking the test compound and blanks without NBT were included to correct for the intrinsic absorbance of the sample.

Scavenging activity (%)={(Absorbance of control-Absorbance of sample)/(Absorbance of control)}× 100

Hydroxyl radical scavenging activity

We evaluated the hydroxyl radical scavenging activity of α-tomatine using the deoxyribose degradation assay (Mathew *et al.*, 2015). The reaction mixture (1.0 mL) contained 2.8 mM deoxyribose, 100 µM $FeCl_3$, 100 µM EDTA, 100 µM ascorbic acid, and 1.0 mM H_2O_2 in 20 mM phosphate buffer (pH 7.4). α-Tomatine was tested at concentrations ranging from 20-100 µg/mL, and the reaction was initiated by the addition of H_2O_2. The mixture was incubated at 37 °C for 1 h, after which the reaction was terminated by adding 1.0 mL of 2.8% trichloroacetic acid (TCA) and 1.0 mL of 1% thiobarbituric acid (TBA). The samples were then heated in a boiling water bath for 15 min, cooled to room temperature, and the absorbance of the resulting pink chromogen was measured at 532

nm against a reagent blank. Ascorbic acid was used as the standard reference antioxidant.

Scavenging activity (%)={(Absorbance of control-Absorbance of sample)/(Absorbance of control)}× 100

Nitric oxide radical scavenging activity

The nitric oxide (NO) scavenging activity of α-tomatine was assessed using a modified sodium nitroprusside (SNP) assay. In aqueous solution at physiological pH, SNP spontaneously releases NO, which reacts with molecular oxygen to form nitrite ions that can be detected using the Griess reagent (Boora *et al.*, 2014). Antioxidants compete with oxygen to inhibit nitrite formation. The reaction mixture consisted of 10 mM SNP in 0.5 mL phosphate-buffered saline (PBS, pH 7.4) and varying concentrations of α-tomatine (20–100 µg/mL), adjusted to a final volume of 1.0 mL. The samples were incubated at 25 °C for 150 min under light exposure. Following incubation, 0.5 mL of the reaction mixture was combined with 0.5 mL of Griess reagent (1% sulfanilamide in 2.5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) and left to stand at room temperature for 10 min. The absorbance was then recorded at 540 nm.

Scavenging activity (%)={(Absorbance of control-Absorbance of sample)/(Absorbance of control)}× 100

Hydrogen peroxide assay

The hydrogen peroxide (H_2O_2) neutralization ability of α-tomatine was assessed using a previously reported method with a few modifications (Fn *et al.*, 2015). The concentration was verified using spectrophotometric measurement at 230 nm in a freshly produced solution of 40 mM H_2O_2 in a 50 mM phosphate buffer at pH 7.4. After mixing 0.6 mL of H_2O_2 solution with α-tomatine concentrations ranging from 20-100 µg/mL, the mixture was allowed to incubate for 10 minutes at room temperature. Phosphate buffer was used as a standard blank to measure the absorbance of the reaction mixture at 230 nm. The positive control was ascorbic acid. The percentage of scavenging activity was calculated using the formula:

Scavenging activity (%)={(Absorbance of control-Absorbance of sample)/(Absorbance of control)}× 100

Reducing power assay

The reducing power assay was performed following a standard protocol with minor modifications (González-Palma *et al.*, 2016). Different concentrations of α-tomatine (20-100 µg/mL) were mixed with 150 µL of 0.2 M PBS (pH 6.6) and 150 µL of 1% potassium ferricyanide, and the reaction mixture was incubated at 50 °C for 20 min.

Subsequently, 150 µL of trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min at room temperature. To 100 µL of the resulting supernatant, 150 µL of distilled water and 50 µL of 0.1% $FeCl_3$ were added. Ascorbic acid was used as the reference standard, and absorbance was recorded at 700 nm. An increase in absorbance corresponded to higher reducing power, where A Control represents the optical density of the control and A Sample denotes the optical density of the test sample.

Inhibition (%)={(Absorbance of control-Absorbance of sample)/(Absorbance of control)}× 100

Statistical analysis

Data are expressed as mean ± SD (n = 3). Statistical significance was analyzed using one-way ANOVA, followed by appropriate post-hoc tests; $p < 0.05$ was considered significant through using GraphPad Prism software (version 10.0.0; GraphPad Software, USA).

RESULTS

Assessment of antioxidant potential of α-tomatine by DPPH assay

The antioxidant potential of α-tomatine and the standard antioxidant ascorbic acid was evaluated at

concentrations of 20-100 µg/mL. At the lowest concentration (20 µg/mL), α-tomatine exhibited a scavenging activity of 28.88 ± 0.6%, whereas ascorbic acid showed 48.53 ± 0.4%, indicating higher radical neutralization by the standard. At the highest concentration (100 µg/mL), α-tomatine reached 78.54 ± 1.6% scavenging activity, while ascorbic acid achieved 91.18 ± 0.8%. Overall, α-tomatine demonstrated a concentration-dependent increase in antioxidant activity, though consistently lower than ascorbic acid. The half-maximal inhibitory concentration (IC_{50}) of α-tomatine was 57.64 µg/mL, moderately higher than that of ascorbic acid (22.73 µg/mL), confirming its comparable but less potent scavenging efficiency (Fig. 1).

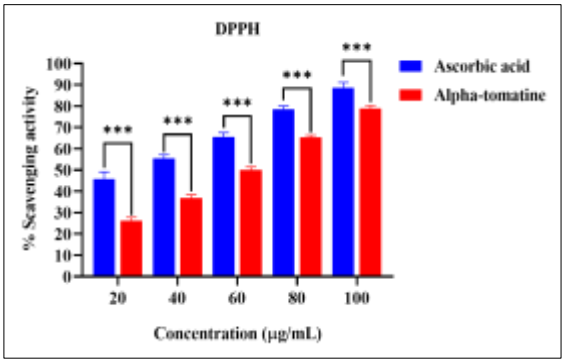

Fig. 1. DPPH free radical scavenging activity of α-tomatine compared with standard ascorbic acid

The antioxidant capacity of α-tomatine was evaluated using the DPPH radical scavenging assay. The percentage of DPPH radical scavenging activity increased in a concentration-dependent manner for both α-tomatine and ascorbic acid. Data are expressed as mean ± SD (n = 3). *$p < 0.05$ indicates significant difference compared to the control.

Assessment of ABTS radical scavenging capacity of α-tomatine

The antioxidant potential of α-tomatine was evaluated using the ABTS radical cation decolorization assay at concentrations ranging from 20-100 µg/mL. α-Tomatine displayed a dose-dependent increase in radical scavenging activity, with inhibition values of 28.16 ± 0.2% at the lowest concentration (20 µg/mL) and 72.43 ± 0.2% at the highest concentration (100 µg/mL) (Fig. 2). In comparison, ascorbic acid exhibited higher scavenging efficiency across the same concentration range, showing 45.72 ± 0.3% inhibition at 20 µg/mL and 89.64 ± 0.4% at 100 µg/mL. The IC_{50} values were 63.38 µg/mL for α-tomatine and 28.86 µg/mL for ascorbic acid, indicating that while α-tomatine demonstrates significant antioxidant potential, its activity remains lower than that of the standard reference compound.

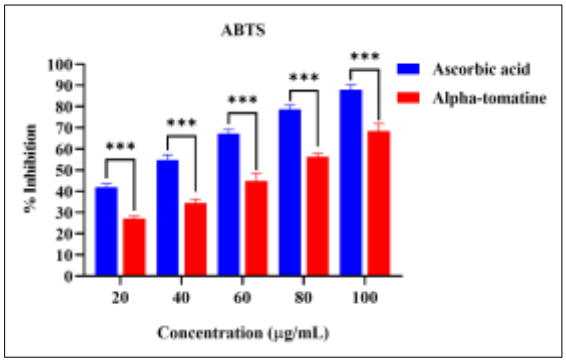

Fig. 2. ABTS radical scavenging activity of α-tomatine compared with ascorbic acid

The antioxidant capacity of α-tomatine was evaluated using the ABTS radical cation decolorization assay. α-tomatine exhibited concentration-dependent scavenging activity, though with comparatively lower efficacy than ascorbic acid. Data are presented as mean ± SD (n = 3), and statistical significance was assessed at $p < 0.05$.

Superoxide radical scavenging activity of α-tomatine

The PMS-NADH-NBT assay was employed to assess the superoxide radical scavenging activity of α-tomatine. A concentration-dependent increase in scavenging potential was observed, with α-tomatine showing 25.14 ± 0.8% inhibition at 20 µg/mL, which increased to 68.26 ± 1.2% at 100 µg/mL. In contrast, the reference standard ascorbic acid exhibited higher activity, with 39.26 ± 1.2% and 80.21 ± 0.4% inhibition at 20 and 100 µg/mL, respectively (Fig. 3). The IC_{50} value of α-tomatine was determined to be 56.75 µg/mL, whereas ascorbic acid exhibited a lower IC_{50} of 32.8 µg/mL, indicating its superior radical scavenging efficacy.

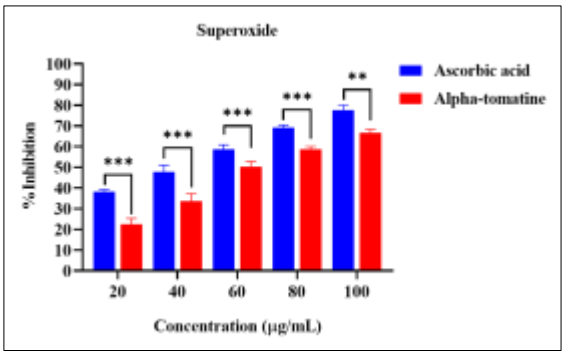

Fig. 3. Superoxide radical scavenging activity of α-tomatine and standard ascorbic acid

The superoxide anion scavenging potential of α-tomatine was evaluated using the PMS–NADH–NBT system, and the results were compared with the standard antioxidant ascorbic acid. Data are presented as mean ± SD (n = 3). Statistical significance between treatment groups was determined ($p < 0.05$).

Evaluation of hydroxyl radical inhibition by α-tomatine

The hydroxyl radical scavenging activity of α-tomatine was assessed at concentrations ranging from 20-100 µg/mL using the Fenton reaction–induced deoxyribose degradation assay. A clear dose-dependent increase in radical inhibition was observed. At the lowest concentration (20 µg/mL), α-tomatine exhibited 26.48 ± 0.4% inhibition, which progressively increased with concentration, reaching a maximum of 72.27 ± 0.8% at 100 µg/mL, thereby indicating substantial hydroxyl radical neutralizing capacity. In comparison, the reference antioxidant ascorbic acid demonstrated greater efficacy, producing 38.23 ± 0.4% inhibition at 20 µg/mL and achieving 88.45 ± 1.2% at 100 µg/mL (Fig. 4).

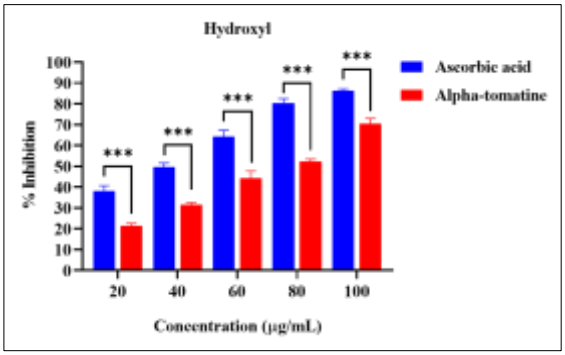

Fig. 4. The hydroxyl radical scavenging capacity of α-tomatine was evaluated in comparison with the standard antioxidant ascorbic acid using the deoxyribose degradation method. Data are presented as mean ± SD of triplicate experiments, and statistical significance was determined using one-way ANOVA followed by post-hoc analysis ($p < 0.05$).

The calculated IC_{50} values were 66.4 µg/mL for α-tomatine and 35.6 µg/mL for ascorbic acid, suggesting that although α-tomatine possesses significant hydroxyl radical scavenging potential, its antioxidant potency is comparatively lower than that of the standard.

Determination of nitric oxide quenching effect of α-tomatine

The nitric oxide scavenging capacity of α-tomatine was assessed using the Griess reaction method and compared with ascorbic acid, a well-established antioxidant standard. A dose-dependent increase in inhibition was observed for both test and standard compounds within the concentration range of 20–100 µg/mL. At 20 µg/mL, α-tomatine exhibited 21.72 ± 1.0% scavenging activity, which gradually increased to 72.36 ± 1.0% at 100 µg/mL. In contrast, ascorbic acid demonstrated significantly higher activity, with 40.4 ± 0.6% inhibition at 20 µg/mL and reaching 88.6 ± 0.8% at 100 µg/mL (Fig. 5). Interpolation analysis revealed an IC_{50} value of 70.7 µg/mL for α-tomatine, compared with 38.5 µg/mL for ascorbic acid, confirming the greater potency of the standard antioxidant.

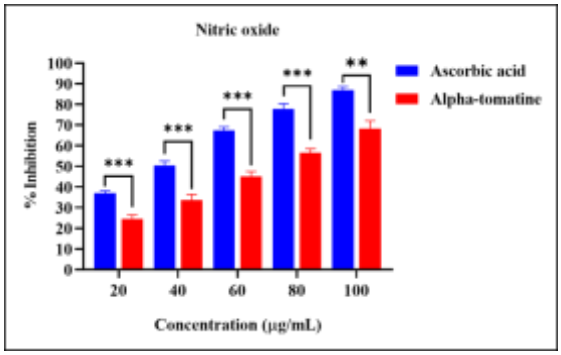

Fig. 5. Nitric oxide radical scavenging activity of α-tomatine and ascorbic acid

The nitric oxide (NO) radical scavenging potential of α-tomatine was evaluated and compared with the

standard antioxidant, ascorbic acid. Data are presented as mean ± SEM (n = 3). $p < 0.05$ was considered statistically significant when compared with the control.

Evaluation of α-tomatine for hydrogen peroxide neutralization

The hydrogen peroxide scavenging ability of α-tomatine was evaluated and compared with that of the standard antioxidant, ascorbic acid. Both α-tomatine and ascorbic acid demonstrated a concentration-dependent increase in scavenging activity within the range of 20–100 µg/mL. At 20 µg/mL, α-tomatine exhibited 26.48 ± 0.4% scavenging activity, which increased to 82.47 ± 1.4% at 100 µg/mL. In contrast, ascorbic acid showed a more pronounced effect, with 40.56 ± 0.6% inhibition at 20 µg/mL and 92.40 ± 1.2% at 100 µg/mL (Fig. 6). The IC_{50} value of α-tomatine was determined 59.3 µg/mL, whereas ascorbic acid exhibited an IC_{50} of 37.4 µg/mL, suggesting that α-tomatine possesses moderate H_2O_2 neutralizing potential compared to the standard. These findings indicate that α-tomatine can effectively attenuate hydrogen peroxide-induced oxidative stress in a dose-dependent manner.

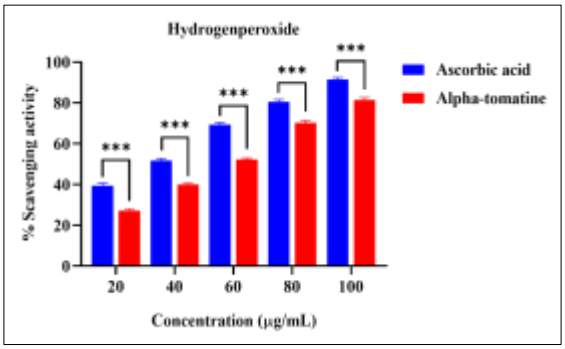

Fig. 6. Hydrogen peroxide (H_2O_2) scavenging activity of α-tomatine compared with standard ascorbic acid

Data are expressed as mean ± SD (n = 3). Statistical significance was analyzed using one-way ANOVA, followed by appropriate post-hoc tests; $p < 0.05$ was considered significant.

Evaluation of reducing power of α-tomatine

The reducing ability of α-tomatine was evidenced by its capacity to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), as measured by absorbance at 700 nm. The assay revealed a concentration-dependent increase in reducing potential between 20–100 µg/mL. At 20 µg/mL, α-tomatine showed an absorbance of 0.21 ± 0.01, which progressively increased to 0.82 ± 0.02 at 100 µg/mL. In comparison, the standard antioxidant ascorbic acid demonstrated greater activity, with absorbance values rising from 0.34 ± 0.01 at 20 µg/mL to 1.16 ± 0.03 at 100 µg/mL (Fig. 7). The IC_{50} values of α-tomatine and ascorbic acid was found to be 61.14 and 40.2 µg/mL respectively. Although less potent than ascorbic acid, α-tomatine exhibited notable electron-donating capacity, suggesting its potential role in preventing free radical chain reactions. The IC_{50} values of antioxidant activity of α-tomatine determined by various *in vitro* free radical scavenging assays in comparison with the standard antioxidant ascorbic acid is summarized in Table 1.

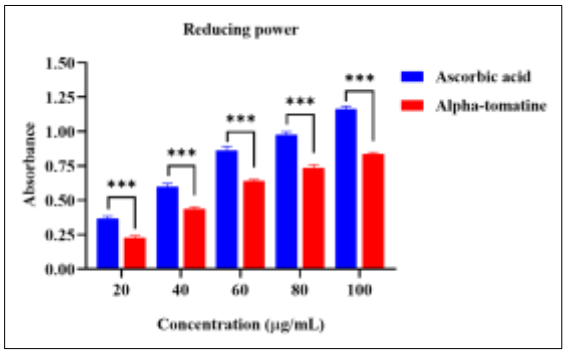

Fig. 7. Reducing power assay of α-tomatine compared with ascorbic acid

Ascorbic acid was used as the standard antioxidant for comparison. Data are expressed as mean ± SEM (n = 3), and statistical significance was considered at $p < 0.05$.

Table 1. Various IC_{50} values of antioxidant activity of α-tomatine in various *in vitro* assays

Antioxidant assay	α-tomatine IC_{50} (µg/mL)	Ascorbic acid IC_{50} (µg/mL)
DPPH radical scavenging assay	57.64	22.73
ABTS radical scavenging assay	63.38	28.86
Superoxide radical scavenging assay	56.75	32.80
Hydroxyl radical scavenging assay	66.40	35.60
Nitric oxide scavenging assay	70.70	38.50
Hydrogen peroxide scavenging assay	59.30	37.40
Reducing power assay	61.14	40.20

DISCUSSION

Phytochemicals constitute a varied array of bioactive compounds that exist in fruits, vegetables, herbs, and spices, which are essential for reducing oxidative stress and preventing several chronic and degenerative diseases (Zhang *et al.*, 2015). The basic principle involves their ability to neutralize free radicals, regulate cellular antioxidant defenses, and interfere with fundamental biochemical pathways associated with inflammation and tissue injury (Forni *et al.*, 2019).

The DPPH assay is a widely used method for evaluating the antioxidant activity of phytochemical compounds. This method is rapid, easy, cost-effective, and extensively utilized to assess the capacity of substances to function as free radical scavengers or hydrogen donors, as well as to determine the antioxidant activity of food sources (Abd Rahman *et al.*, 2018; Waqas *et al.*, 2013). Previous research has found comparable antioxidant characteristics in plant-based glycoalkaloids and saponins in the reduction of oxidative damage through radical scavenging and metal-chelating mechanisms (Lee *et al.*, 2016; Milner *et al.*, 2011). In the present study α-tomatine showed an IC_{50} value (57.64 µg/mL) was higher than that of ascorbic acid (22.73 µg/mL), indicating lower potency. However, the progressive rise in scavenging ability highlights α-tomatine as a promising natural antioxidant.

In ABTS assay, the ABTS radical cation ($ABTS^{+}$•), a stable blue-green chromophore, is generated by oxidizing ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) with an agent such as potassium persulfate. Because of its high sensitivity, cost-effectiveness, and suitability for evaluating both hydrophilic and lipophilic antioxidants, the ABTS assay is considered a versatile and reliable tool in antioxidant research (Zampini *et al.*, 2010). In this study, The ABTS assay demonstrated that α-tomatine exhibits an IC_{50} value (63.38 µg/mL) indicates lower potency than ascorbic acid, α-tomatine still showed substantial free-radical neutralizing ability, supporting its role as a promising natural antioxidant candidate. Research on glycoalkaloids extracted from *Solanum nigrum* reveals moderate but significant ABTS radical inhibition, consistent with their established antioxidant characteristics that enhance the plant's therapeutic efficacy (Herqash *et al.*, 2024). In addition, saponins derived from *Polygonum hydropiper* have similar ABTS scavenging capabilities, highlighting that these glycosidic substances function through electron or hydrogen transfer mechanisms (Aouadi *et al.*, 2024).

The superoxide radical (O_2^-) is an essential and highly reactive oxygen species (ROS) in biological processes. The ability of steroidal glycoalkaloids to scavenge superoxide radicals has been shown to be an important antioxidant mechanism for reducing oxidative damage in these compounds. It neutralizes superoxide anions mostly by donating electrons or hydrogen atoms, breaking the radical chain events that cause cellular damage. Prior research on *Solanum nigrum* L. and its related species has indicated that glycoalkaloids demonstrate significant superoxide radical scavenging activity in a dose-dependent way. The antioxidant property is attributed to their unique steroidal fundamental structure, which undergoes structural alterations that facilitate effective involvement in redox processes (Q. Zhang *et al.*, 2024). In the current study, α-tomatine exhibited moderate superoxide scavenging activity with an IC_{50} value of 56.75 µg/mL, which was higher than that of ascorbic acid (IC_{50} = 32.8 µg/mL), indicating its comparatively lower antioxidant potency.

The hydroxyl radical (·OH) is one of the most reactive ROS, mainly generated through the Fenton reaction between hydrogen peroxide and transition metal ions like Fe^{2+}. Due to its short lifespan and strong oxidative potential, ·OH rapidly damages lipids, proteins, and DNA, contributing to aging and various diseases. Therefore, evaluating ·OH scavenging capacity is essential to determine the antioxidant potential of bioactive compounds and their ability to protect cells from oxidative stress–induced damage

(Ahemad *et al.*, 2021; Munteanu and Apetrei, 2021). In our investigations, α-tomatine demonstrated hydroxyl radical scavenging activity, though with lower potency than the standard antioxidant. Its IC_{50} value was 66.4 µg/mL, compared to 35.6 µg/mL for ascorbic acid, indicating that higher concentrations of α-tomatine are required to achieve comparable antioxidant effects.

Nitric oxide (NO) is an important signaling molecule that supports functions such as vasodilation, neurotransmission, and immune defense. However, excess NO can generate reactive nitrogen species, leading to oxidative stress and cellular damage. Compounds that can lower NO levels help prevent the formation of harmful intermediates. In this study, nitric oxide scavenging was evaluated using sodium nitroprusside to induce NO production, followed by the Griess reaction to measure nitrite formation. A decrease in nitrite levels confirms the ability of the test compound to neutralize NO and protect against nitrogen-mediated oxidative damage (Hottinger *et al.*, 2014). The nitric oxide scavenging assay showed that α-tomatine exhibited a IC_{50} value (70.7 µg/mL) was higher than that of ascorbic acid (38.5 µg/mL). This suggests that although α-tomatine exhibits notable nitric oxide (NO) scavenging activity, its potency is comparatively lower than that of the standard antioxidant.

Consistent with these findings, previous studies have reported that a novel synthetic flavonoid, a hydroxy thiophene derivative, demonstrated dose-dependent nitric oxide radical scavenging activity, thereby confirming its antioxidant potential (Bhixavatimath *et al.*, 2020).

Hydrogen peroxide serves as an important indicator of antioxidant potential, as it can easily diffuse across cellular membranes and promote oxidative damage through the generation of highly reactive hydroxyl radicals via the Fenton reaction. The glycoalkaloid structure, consisting of sterol backbones linked to sugar moieties, likely facilitates electron or hydrogen donation, enabling the conversion of H_2O_2 into less reactive species. Consistent with previous reports, glycoalkaloid-containing extracts demonstrate notable, dose-dependent H_2O_2 neutralization, a response that is often associated with elevated phenolic and flavonoid content, further supporting their role in mitigating oxidative stress (Asif *et al.*, 2024; Humbare *et al.*, 2021). In our investigation hydrogen peroxide scavenging assay revealed that α-tomatine exhibits IC_{50} value (59.3 µg/mL) was higher than that of ascorbic acid (37.4 µg/mL), indicating moderate H_2O_2 neutralizing potential. The electron-donating capacity of the glycoalkaloids interrupts the hydrogen peroxide induced oxidative chain reactions, making these compounds promising natural antioxidants.

In this assay, the reducing capacity of the sample was evaluated based on its ability to donate electrons and convert Fe^{3+} to Fe^{2+}, resulting in the formation of a Prussian blue complex detected spectrophotometrically. An increase in absorbance corresponds to greater reducing power, reflecting enhanced antioxidant potential. Strong reducing activity is widely recognized as a key mechanism in antioxidant defense, protecting biomolecules from oxidative damage (Choi *et al.*, 2020; Hechaichi *et al.*, 2023). Experimental results showed that α-tomatine exhibited a distinct concentration-dependent enhancement in reducing ability, reflecting its strong electron-donating potential. Although its activity was lower than that of ascorbic acid, α-tomatine still demonstrated notable antioxidant capacity, highlighting its potential role in mitigating oxidative stress.

CONCLUSION

The findings from the free radical scavenging assays demonstrate that α-tomatine possesses significant antioxidant potential, effectively neutralizing reactive oxygen species in a concentration-dependent manner. Although its activity was comparatively lower than the standard antioxidant ascorbic acid, α-tomatine

exhibited strong radical-quenching and electron-donating capabilities, reflecting its role in mitigating oxidative stress. These results suggest that α-tomatine may serve as a promising natural antioxidant compound with potential applications in preventing oxidative-stress-mediated cellular damage and related pathological conditions.

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