RESEARCH PAPER

# Exploring the Phytochemical Profile and Pharmacological Activities of Amaranthus (*Amaranthus tricolor* L)

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**ABSTRACT**

*Amaranthus tricolor L.* (Amaranthaceae), commonly known as edible amaranth or Chinese spinach, is a nutrient-dense leafy vegetable with significant ethnomedicinal importance. The present study aimed to evaluate the phytochemical composition and pharmacological activities of the hydroalcoholic extract of A. tricolor stem and roots. Preliminary phytochemical screening revealed the presence of key bioactive constituents, including alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, polyphenols, and terpenoids. Quantitative analysis indicated a considerable total phenolic content (1.5146 µg/g), suggesting strong antioxidant potential. The in vitro anti-diabetic activity demonstrated effective α-amylase inhibition with an $IC_{50}$ value of 115.98 µg/mL, comparable to the standard drug acarbose (125.81 µg/mL). Anti-inflammatory activity assessed by the human red blood cell (HRBC) membrane stabilization method showed dose-dependent inhibition, with an $IC_{50}$ value of 177.42 µg/mL, compared to diclofenac (114.03 µg/mL). Antioxidant activity evaluated using the DPPH radical scavenging assay exhibited significant free radical inhibition with an $IC_{50}$ value of 47.77 µg/mL. The extract demonstrated appreciable iron content, indicating its potential role in managing iron deficiency anemia. The observed pharmacological activities may be attributed to the presence of phenolic compounds and other phytoconstituents. The findings suggest that A. tricolor stem and root extracts possess promising anti-diabetic, anti-inflammatory, and antioxidant properties, along with nutritional benefits. Further in vivo and clinical studies are required to validate these results and elucidate the underlying mechanisms of action.

**Keywords:** *Amaranthus tricolor L.;* Phytochemicals; Anti-diabetic activity; Anti-inflammatory activity and Antioxidant activity.

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## INTRODUCTION

*Amaranthus tricolor* L. (Amaranthaceae) is an important leafy vegetable widely cultivated in tropical and subtropical regions due to its high nutritional value and adaptability to diverse agro-climatic conditions [1,11]. It is recognized as a rich source of essential nutrients, including proteins, dietary fiber, vitamins (A, C, and B - complex), and minerals such as calcium and iron, making it a valuable component of human diets, particularly in developing countries [2,31]. In addition to its nutritional significance, A. tricolor has gained attention for its diverse phytochemical composition and associated therapeutic potential. Phytochemical investigations have revealed that A. tricolor contains a

wide array of bioactive compounds, including phenolics, flavonoids, betalains, alkaloids, glycosides, and terpenoids [2, 4,13]. These compounds are known to exhibit significant biological activities, particularly antioxidant properties, by scavenging reactive oxygen species (ROS) and mitigating oxidative stress. Oxidative stress is a major contributing factor in the pathogenesis of several chronic disorders, including diabetes mellitus, cardiovascular diseases, and inflammatory conditions [3, 8]. Therefore, plant-derived antioxidants are increasingly being explored as potential alternatives to synthetic therapeutic agents. Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. The management of postprandial hyperglycemia through inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase represents an effective therapeutic strategy [4]. Natural products, particularly those rich in polyphenolic compounds, have demonstrated significant inhibitory effects on these enzymes, thereby contributing to glycemic control.

Inflammation is a complex biological response associated with tissue injury and infection; however, chronic inflammation is implicated in the progression of various pathological conditions. Bioactive phytochemicals derived from plants have been shown to exert anti-inflammatory effects by stabilizing cellular membranes and modulating inflammatory mediators [5]. Furthermore, antioxidants play a crucial role in protecting cellular components from oxidative damage induced by free radicals, thereby reducing the risk of degenerative diseases [6]. Iron deficiency anemia remains one of the most prevalent nutritional disorders globally, particularly affecting vulnerable populations in developing regions. Green leafy vegetables such as A. tricolor serve as an important dietary source of bioavailable iron and can contribute to the prevention and management of anemia [7]. In traditional medicine, various parts of the plant have been used for treating conditions such as inflammation, anemia, and gastrointestinal disorders, suggesting its multifaceted therapeutic potential [8]. Despite its nutritional and ethnomedicinal importance, systematic studies focusing on the phytochemical profile and pharmacological activities of the stem and root of A. tricolor remain limited. Therefore, the present study aims to investigate the phytochemical constituents of hydroalcoholic extracts of A. tricolor stem and root and to evaluate their antidiabetic, anti-inflammatory, and antioxidant activities, along with iron content. This study seeks to provide scientific evidence supporting the therapeutic potential of A. tricolor and its possible application in the development of plant-based functional and medicinal products.

## Materials and methods

### Plant Material Collection and Authentication

Fresh stem and root samples of Amaranthus tricolor L. were collected from Tambaram, Chennai district, Tamil Nadu, India. The plant material was taxonomically authenticated by a qualified botanist at the Department of Botany, Madras Christian College, Chennai.

The collected samples were washed, shade-dried at room temperature, and pulverized into a coarse powder using a mechanical grinder. The powdered material was stored in airtight containers for further analysis.

### Preparation of Hydroalcoholic Extract

The dried powdered plant material was subjected to hot continuous extraction using a Soxhlet apparatus. A hydroalcoholic solvent (ethanol:water, 70:30 v/v) was used as the extraction medium. The extraction process was continued until the solvent in the siphon tube became colorless. The extract was then concentrated under reduced pressure using a rotary evaporator and dried to obtain a semi-solid mass. The extract was stored at 4°C until further use.

### Qualitative Phytochemical Screening

Preliminary phytochemical analysis of the hydroalcoholic extract was carried out to detect the presence of various bioactive constituents using standard procedures [9].

- Alkaloids: Detected using Dragendorff's reagent, indicated by orange-red precipitate formation.
- Carbohydrates: Identified by Fehling's test showing reddish-brown precipitate.
- Glycosides: Confirmed by Keller–Killiani test with brown ring formation.
- Saponins: Presence indicated by stable froth formation upon shaking with water.
- Proteins: Detected using Biuret test with violet coloration.
- Amino acids: Confirmed by Ninhydrin test producing violet colour.
- Phenolic compounds: Identified by ferric chloride test showing bluish-green coloration.
- Flavonoids: Confirmed using alkaline reagent test with yellow coloration.
- Terpenoids: Detected by formation of yellow precipitate with trichloroacetic acid.

### Quantitative Analysis of Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method [9]. Briefly, the extract was mixed with Folin–Ciocalteu reagent and incubated,

followed by the addition of sodium carbonate. The mixture was incubated at 45°C and absorbance was measured at 650 nm using a UV–Vis spectrophotometer. The phenolic content was calculated using a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per gram of extract.

**\Antioxidant Activity (DPPH Assay)**

The antioxidant activity of the extract was evaluated using the DPPH radical scavenging assay [9]. The reaction mixture consisted of plant extract, ethanol, and DPPH solution. The mixture was incubated in the dark, and absorbance was measured at 517 nm. The percentage of radical scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

**Anti-diabetic Activity (α-Amylase Inhibition Assay)**

The anti-diabetic potential of the extract was evaluated by α-amylase inhibition assay. Different concentrations of the extract were prepared and incubated with α-amylase enzyme. After incubation, dinitrosalicylic acid (DNSA) reagent was added, and the mixture was heated. Absorbance was recorded at 540 nm. The percentage inhibition of α-amylase activity was calculated as:

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

**Anti-inflammatory Activity (HRBC Membrane Stabilization Method)**

The anti-inflammatory activity was assessed using the human red blood cell (HRBC) membrane stabilization method. Fresh blood was collected from healthy volunteers and mixed with Alsever's solution. The HRBC suspension was prepared and treated with different concentrations of the extract. The mixtures were incubated at 37°C and centrifuged. The absorbance of the supernatant was measured at 560 nm to determine hemoglobin release. The percentage inhibition of hemolysis was calculated.

**Estimation of Iron Content**

Iron content in the extract was determined using the 1,10-phenanthroline spectrophotometric method. Standard iron solution was prepared using ferrous ammonium sulfate. The plant sample was digested using sulfuric acid and filtered. The prepared sample and standards were treated with hydroxylamine hydrochloride, 1,10-phenanthroline, and sodium acetate buffer. The absorbance was measured at the selected wavelength (typically around 510 nm), and iron concentration was calculated using the calibration curve.

**Statistical Analysis**

All experiments were carried out in triplicates, and the results were expressed as mean ± standard deviation (SD) [10]. $IC_{50}$ values were determined using linear regression analysis.

**Results**

The qualitative phytochemical analysis of the hydroalcoholic extract of Amaranthus tricolor L. stem and root revealed the presence of a wide range of bioactive constituents. The extract showed positive results for alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, and terpenoids when subjected to standard phytochemical screening tests. Among these constituents, polyphenols were found to be comparatively higher in concentration (++), whereas the remaining phytochemicals exhibited moderate presence (+), indicating a diverse phytochemical composition in the extract. The quantitative estimation of total phenolic content was carried out using the Folin - Ciocalteu method, and the absorbance was measured at 650 nm. The total phenolic content of the hydroalcoholic extract was determined to be 1.5146 µg/g, expressed as gallic acid equivalents (GAE). The calibration curve prepared using standard gallic acid exhibited a linear relationship between concentration and absorbance, confirming the accuracy of the estimation.

The anti-diabetic activity of the hydroalcoholic extract was evaluated using the α-amylase inhibition assay at different concentrations ranging from 100 to 200 µg/mL. The extract exhibited a concentration-dependent increase in inhibitory activity. At 100 µg/mL, the percentage inhibition was recorded as 52.9 ± 1.18%, which increased progressively to 60 ± 1.17% at 120 µg/mL, 63.52 ± 0.77% at 140 µg/mL, 67.05 ± 1.19% at 160 µg/mL, 68.23 ± 1.18% at 180 µg/mL, and reached a maximum of 69.41 ± 1.17% at 200 µg/mL. The standard drug acarbose exhibited inhibition values ranging from 17.3 ± 0.55% at 100 µg/mL to 65.5 ± 1.35% at 200 µg/mL. The $IC_{50}$ value of the hydroalcoholic extract was calculated to be 115.9844 µg/mL, whereas the $IC_{50}$ value of acarbose was 125.8178 µg/mL. All values were expressed as mean ± standard deviation for triplicate measurements. The anti-inflammatory activity of the hydroalcoholic extract was assessed using the HRBC membrane stabilization method. The extract exhibited a concentration-dependent increase in the inhibition of hemolysis. The percentage inhibition was observed as 60 ± 2.11% at 100 µg/mL, 61.05 ± 2.11% at 120 µg/mL, 63.15 ± 2.78% at 140 µg/mL, 65.26 ± 3.79% at 160

µg/mL, 66.31 ± 2.11% at 180 µg/mL, and 68.41 ± 3.16% at 200 µg/mL. The standard drug diclofenac showed inhibition values ranging from 24.17 ± 0.90% at 100 µg/mL to 59.87 ± 1.40% at 200 µg/mL. The $IC_{50}$ value of the hydroalcoholic extract was found to be 177.4226 µg/mL, while the $IC_{50}$ value of diclofenac was 114.0357 µg/mL. The regression coefficient ($R^2$) values were calculated as 0.8322 for the extract and 0.9272 for the standard. The antioxidant activity of the hydroalcoholic extract was evaluated using the DPPH radical scavenging assay. The extract demonstrated a concentration-dependent increase in free radical scavenging activity across the tested concentration range of 100–200 µg/mL. The percentage inhibition was recorded as 55.39 ± 2.25% at 100 µg/mL, 61.76 ± 2.94% at 120 µg/mL, 63.23 ± 2.94% at 140 µg/mL, 64.7 ± 2.94% at 160 µg/mL, 67.64 ± 2.94% at 180 µg/mL, and 70.58 ± 2.94% at 200 µg/mL. The standard antioxidant quercetin exhibited inhibition values ranging from 22.64 ± 1.32% at 100 µg/mL to 73.4 ± 1.4% at 200 µg/mL. The $IC_{50}$ value of the extract was determined to be 47.76878 µg/mL, whereas the $IC_{50}$ value of quercetin was 149.4522 µg/mL. The regression coefficient ($R^2$) values were found to be 0.9444 for the extract and 0.9872 for the standard. The iron content of the hydroalcoholic extract was determined using the 1,10-phenanthroline spectrophotometric method. Absorbance measurements of standard iron solutions and the prepared sample were recorded within the wavelength range of 400–600 nm. A calibration curve was constructed using standard solutions of known iron concentrations, and the absorbance of the sample was interpolated against this curve to estimate its iron content. The analysis confirmed the presence of measurable iron in the extract, with absorbance maxima observed within the visible region, and the iron concentration was calculated accordingly using the standard calibration plot.

## DISCUSSION

The present study provides a comprehensive evaluation of the phytochemical composition and pharmacological activities of the hydroalcoholic extract of Amaranthus tricolor L. stem and root. The findings clearly indicate that the extract is a rich source of biologically active compounds and exhibits significant antioxidant, antidiabetic, and anti-inflammatory activities, along with appreciable iron content. These results substantiate the ethnopharmacological relevance of the plant and highlight its potential as a functional food and therapeutic agent [1,38]. Phytochemical screening revealed the presence of diverse classes of secondary metabolites, including alkaloids, glycosides, saponins, terpenoids, proteins, amino acids, carbohydrates, and a notably high concentration of polyphenols. These phytoconstituents are known to exert multiple pharmacological effects through different biochemical pathways. The therapeutic efficacy of plant extracts is often attributed to the synergistic and additive interactions among these compounds, rather than the action of a single bioactive molecule [11].

Alkaloids have been reported to modulate glucose metabolism by influencing insulin secretion and improving peripheral glucose uptake, while saponins are known to enhance membrane permeability and exhibit hypocholesterolemic and immunomodulatory effects. Terpenoids contribute to anti-inflammatory activity through inhibition of key enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing prostaglandin synthesis. Glycosides and amino acids further support metabolic and cellular functions. Among these, polyphenols play a central role due to their strong redox properties and ability to interact with multiple molecular targets [12]. The quantitative estimation of total phenolic content in the extract further supports its strong antioxidant potential. Phenolic compounds are capable of scavenging free radicals by donating hydrogen atoms or electrons and stabilizing reactive oxygen species (ROS). The DPPH radical scavenging assay demonstrated a concentration-dependent increase in antioxidant activity, indicating efficient neutralization of free radicals. The relatively low $IC_{50}$ value of the extract compared to the standard (quercetin) suggests high antioxidant potency.

At the molecular level, phenolic compounds may inhibit lipid peroxidation, protect cellular membranes, and prevent oxidative damage to DNA and proteins. Additionally, they can chelate transition metal ions such as $Fe^{2+}$, thereby preventing the generation of highly reactive hydroxyl radicals via Fenton reactions. Since oxidative stress is a key factor in the pathogenesis of chronic diseases such as diabetes, cancer, and cardiovascular disorders, the antioxidant activity observed in this study indicates that Amaranthus tricolor may serve as a protective agent against oxidative damage [13, 21]. The antidiabetic activity of the extract, as evidenced by α-amylase inhibition, highlights its potential role in regulating postprandial hyperglycemia. α-Amylase catalyzes the hydrolysis of starch into simpler sugars, which are further converted into glucose and absorbed into the bloodstream. Inhibition of this enzyme slows down carbohydrate digestion and reduces glucose absorption, thereby controlling blood glucose levels. The $IC_{50}$ value of the extract was found to be comparable to that of acarbose, a standard antidiabetic drug, indicating strong enzyme inhibitory activity.

The mechanism of α-amylase inhibition may involve the binding of phenolic compounds to the active site of the enzyme, leading to conformational changes and reduced catalytic activity. Furthermore, polyphenols and flavonoid-like compounds may enhance insulin sensitivity by modulating signaling pathways such as the PI3K/Akt pathway and increasing the expression of glucose transporter proteins (GLUT4). These mechanisms collectively contribute to improved glucose homeostasis [14]. In addition to enzyme inhibition, the antioxidant properties of the extract may protect pancreatic β-cells from oxidative damage, thereby preserving insulin secretion. This dual mechanism enzyme inhibition and cellular protection make the extract a promising candidate for the management of type 2 diabetes mellitus. The anti-inflammatory activity assessed through the HRBC membrane stabilization method demonstrated that the extract effectively inhibits hemolysis in a concentration-dependent manner. The stabilization of erythrocyte membranes is analogous to the stabilization of lysosomal membranes, which prevents the release of inflammatory mediators such as proteases, histamines, and prostaglandins.

The observed anti-inflammatory effect may be attributed to the inhibition of key inflammatory pathways, including the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, leading to reduced synthesis of prostaglandins and leukotrienes [15, 22]. Additionally, phenolic compounds may suppress the activation of nuclear factor-kappa B (NF-κB), a transcription factor that regulates the expression of pro-inflammatory cytokines such as TNF-α and IL-6. Although the $IC_{50}$ value of the extract was higher than that of diclofenac, it still exhibited significant activity, suggesting moderate potency with the advantage of being a natural compound with potentially fewer side effects. The dose-dependent response observed in this study further confirms the reliability and therapeutic relevance of the extract [16].

The estimation of iron content revealed that Amaranthus tricolor is a valuable source of dietary iron. Iron is essential for hemoglobin synthesis, oxygen transport, and various enzymatic processes. Iron deficiency remains a major global health concern, particularly in developing countries, leading to anemia and associated complications. The presence of iron in combination with vitamin C and antioxidant compounds in the plant may enhance its bioavailability by reducing ferric ions ($Fe^{3+}$) to the more absorbable ferrous form ($Fe^{2+}$). Moreover, the antioxidant properties of the extract may prevent oxidative degradation of iron and improve its stability and utilization in biological systems. This highlights the dual role of Amaranthus tricolor as both a nutritional supplement and a therapeutic agent, particularly in the management of iron deficiency anemia [17, 23].

An important aspect of the present study is the observed interrelationship between antioxidant, antidiabetic, and anti-inflammatory activities. Oxidative stress is a common underlying factor in the development of both diabetes and inflammation. Reactive oxygen species can induce cellular damage, impair insulin signaling, and trigger inflammatory responses. By scavenging free radicals, the antioxidant components of the extract may reduce oxidative stress, thereby indirectly contributing to its antidiabetic and anti-inflammatory effects. This multi-targeted mechanism is a characteristic feature of plant-based therapeutics and supports the concept of using whole plant extracts for the management of complex diseases. The ability of Amaranthus tricolor to modulate multiple biological pathways simultaneously enhances its therapeutic potential and distinguishes it from single-target synthetic drugs [18]. Despite the promising results, certain limitations of the study must be acknowledged. The pharmacological activities were evaluated using in vitro models, which may not fully replicate in vivo physiological conditions. Additionally, the specific bioactive compounds responsible for the observed effects were not isolated or characterized. The absence of toxicity and pharmacokinetic studies further limits the direct clinical applicability of the findings [19]. Future research should focus on the isolation and structural characterization of active phytoconstituents using advanced analytical techniques such as HPLC, GC-MS, and NMR spectroscopy [20]. In vivo studies and clinical trials are essential to validate the efficacy and safety of the extract. Furthermore, molecular studies targeting specific signaling pathways will provide deeper insights into the mechanisms of action and facilitate the development of standardized herbal formulations.

**Table 1. Qualitative Phytochemical Analysis of Hydroalcoholic Extract of Amaranthus tricolor L. (Stem and Root)**

| S. No. | Phytochemical Constituents | Hydroalcoholic Extract |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Carbohydrates | + |

| | | |
|---|---|---|
| 3 | Glycosides | + |
| 4 | Saponins | + |
| 5 | Proteins | + |
| 6 | Amino acids | + |
| 7 | Polyphenols | ++ |
| 8 | Terpenoids | + |

(+) Present; (++) High concentration; (–) Absent. Qualitative analysis performed using standard phytochemical screening protocols.

**Table 2. Total Phenolic Content of Hydroalcoholic Extract of Amaranthus tricolor L.**

| Parameter | Value |
|---|---|
| Total Phenolic Content | 1.5146 µg/g (GAE) |

Values expressed as mean of triplicates (n = 3). Total phenolic content determined using Folin–Ciocalteu method. Results expressed as Gallic Acid Equivalents (GAE).

**Table 3. In vitro Antidiabetic Activity (α-Amylase Inhibition Assay)**

| Concentration (µg/mL) | % Inhibition (Sample) | % Inhibition (Standard – Acarbose) |
|---|---|---|
| 100 | 52.9 ± 1.18 | 17.3 ± 0.55 |
| 120 | 60.0 ± 1.17 | 26.8 ± 1.38 |
| 140 | 63.52 ± 0.77 | 35.1 ± 1.45 |
| 160 | 67.05 ± 1.19 | 47.9 ± 1.47 |
| 180 | 68.23 ± 1.18 | 58.6 ± 1.25 |
| 200 | 69.41 ± 1.17 | 65.5 ± 1.35 |

$IC_{50}$ Values: Sample = 115.98 µg/mL; Standard (Acarbose) = 125.81 µg/mL

**Table 4. In vitro Anti-inflammatory Activity (HRBC Membrane Stabilization Method)**

| Concentration (µg/mL) | % Inhibition (Sample) | % Inhibition (Standard – Diclofenac) |
|---|---|---|
| 100 | 60.0 ± 2.11 | 24.17 ± 0.90 |
| 120 | 61.05 ± 2.11 | 32.3 ± 1.28 |
| 140 | 63.15 ± 2.78 | 46.17 ± 1.27 |
| 160 | 65.26 ± 3.79 | 52.6 ± 0.85 |
| 180 | 66.31 ± 2.11 | 56.6 ± 1.51 |
| 200 | 68.41 ± 3.16 | 59.87 ± 1.40 |

$IC_{50}$ Values: Sample = 177.42 µg/mL; Standard (Diclofenac) = 114.03 µg/mL
$R^2$ Values: Sample = 0.8322; Standard = 0.9272
Values expressed as mean ± SD (n = 3). Statistical significance analyzed using one-way ANOVA. A value of $p < 0.05$ was considered significant.

**Table 5. Antioxidant Activity (DPPH Radical Scavenging Assay)**

| Concentration (µg/mL) | % Inhibition (Sample) | % Inhibition (Standard – Quercetin) |
|---|---|---|
| 100 | 55.39 ± 2.25 | 22.64 ± 1.32 |
| 120 | 61.76 ± 2.94 | 36.34 ± 1.70 |
| 140 | 63.23 ± 2.94 | 47.9 ± 1.21 |
| 160 | 64.70 ± 2.94 | 54.23 ± 1.00 |
| 180 | 67.64 ± 2.94 | 67.17 ± 0.85 |
| 200 | 70.58 ± 2.94 | 73.40 ± 1.40 |

$IC_{50}$ Values: Sample = 47.76 µg/mL; Standard (Quercetin) = 149.45 µg/mL

$R^2$ Values: Sample = 0.9444; Standard = 0.9872

Values are expressed as mean ± SD (n = 3). Statistical analysis performed using one-way ANOVA followed by Tukey's multiple comparison test. Significance level set at $p < 0.05$.

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