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In Vitro Comprehensive Analysis of The Antioxidant Activity of Aqueous *Gladiolus psittacinus* Bulb Extract

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ABSTRACT

Gladiolus psittacinus, commonly known as Maid of the Mist, is a perennial bulb-bearing plant traditionally used for managing diabetes and oxidative stress-related disorders. Despite its ethnomedicinal relevance, its antioxidant potential remains underexplored. This study investigated the phytochemical composition and *in vitro* antioxidant activity of the aqueous bulb extract of *G. psittacinus.* Fresh bulbs were obtained from Oja Oba Market, Ado-Ekiti, Nigeria, in September 2023, air-dried, powdered, and extracted with distilled water (1:10) for 48 hours. The extract was analyzed for its phytochemical constituents and antioxidant properties using DPPH, ABTS, hydroxyl radical, and ferric reducing antioxidant power (FRAP) assays. Quantitative analyses revealed high total phenolic (50.33 ± 0.02 mg GAE/g) and flavonoid (43.22 ± 0.03 mg QE/g) contents. The extract exhibited strong, concentration-dependent radical scavenging activity against DPPH (4.82 ± 0.01–8.38 ± 0.02), ABTS (0.29 ± 0.04–0.62 ± 0.01), hydroxyl radicals (4.94 ± 0.02–10.62 ± 0.01), and FRAP (45.86 ± 0.01–94.61 ± 0.33). These findings suggest that the antioxidant capacity of *G. psittacinus* bulb extract is linked to its abundant phenolic and flavonoid compounds. The study provides scientific support for its traditional use and highlights its potential as a natural source of antioxidants for managing oxidative stress-related conditions.

Keywords: *Gladiolus psittacinus* bulb, free radicals, antioxidant activity, oxidative stress, *in vitro* analysis

Introduction

Gladiolus psittacinus (Iridaceae), locally called *Maid of the Mist* and *Baaka*[1] among the Yoruba people of Southwest Nigeria[2], is a perennial herbaceous plant widely distributed across the grasslands and savannas of sub-Saharan Africa. Its native range extends from eastern South Africa and Madagascar through tropical Africa to Western Arabia[3]. Traditionally, the bulb has been used in African ethnomedicine to manage various health conditions, including infertility, respiratory ailments, cough, mental disorders, diabetes, and asthma, as well as bacterial and fungal infections[4].

Emerging pharmacological evidence has begun to validate these traditional uses. Methanolic extracts of *G. psittacinus* have shown hypoglycemic activity in alloxan-induced diabetic rats, suggesting a possible modulatory role on glucose metabolism[5], while its neuroprotective potential has been reported in scopolamine-induced memory dysfunction models, highlighting its relevance in neurological and cognitive disorders[2]. These studies suggest the presence of potent bioactive constituents that warrant further biochemical and pharmacological evaluation.

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Medicinal plants are recognized as reservoirs of phytochemicals with therapeutic benefits against oxidative-stress-related diseases[6–7]. Oxidative stress, a physiological imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, plays a key role in the pathogenesis of chronic disorders such as diabetes, cardiovascular diseases, cancer, aging, and neurodegenerative diseases[8–9]. Overproduction of ROS damages essential biomolecules like proteins, lipids, and DNA, thereby impairing normal cell function[10]. Antioxidants are compounds that can neutralize free radicals, terminate oxidative chain reactions, and protect biomolecules from oxidative injury[11]. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are effective but have raised toxicity concerns, prompting a shift toward safer natural antioxidants[12]. Natural antioxidants from medicinal plants often exert additional benefits, including anti-inflammatory, anti-aging, and immunomodulatory properties[13–14]. Among plant-derived antioxidants, polyphenols and flavonoids are particularly important due to their redox-active hydroxyl groups and conjugated structures that facilitate electron donation[15]. These phytochemicals exhibit a broad range of biological activities, including anticancer, anti-inflammatory, antimicrobial, and neuroprotective effects[16–18]. Phenolic compounds scavenge free radicals, chelate metal ions, and modulate intracellular antioxidant enzymes, such as catalase, glutathione peroxidase, and superoxide dismutase[19]. Flavonoids, in turn, modulate cellular signaling pathways such as NF-κB, MAPK, and Nrf2, enhancing endogenous antioxidant defenses[20–22]. Although *Gladiolus psittacinus* is traditionally used in folk medicine and preliminary reports suggest that it possesses antioxidant potential, there is a lack of systematic scientific data on the antioxidant capacity of its aqueous bulb extract, particularly with respect to its total phenolic and flavonoid composition and detailed free radical scavenging mechanisms. Moreover, the flavonoid chemical framework underlying its antioxidant activity has not been clearly characterized, and comparative evaluation using multiple *in vitro* antioxidant assays remains limited. Consequently, the mechanistic basis and therapeutic relevance of *G. psittacinus* in the management of oxidative stress–related disorders are not well established.

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Therefore, this study aimed to determine the total phenolic and flavonoid contents of the aqueous bulb extract of *G. psittacinus* and to assess its antioxidant activity using *in vitro* assays (DPPH, ABTS, FRAP, and hydroxyl radical scavenging). The findings are intended to provide scientific validation for its traditional use and reveal its potential for pharmaceutical and nutraceutical development.

Materials and Methods

Plant Material

Fresh bulbs of *Gladiolus psittacinus* were collected in September 2023 from Oja Oba Market, Ado-Ekiti, Ekiti State, Nigeria (GPS coordinates: 7.6232° N, 5.2200° E). The plant material was identified and authenticated by Mr. Omotayo Felix, Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria. A voucher specimen (UHAE 2019049) was prepared and deposited in the University Herbarium, Ekiti State University, Ado-Ekiti, for future reference.

Preparation of Extract

The bulbs were washed, sliced, air-dried at room temperature, and pulverized using an electric grinder (Stuart Scientific, UK). Exactly 100 g of the powdered sample was macerated in 1 L of distilled water for 48 h with intermittent stirring. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated by oven-drying at 40 °C to a constant weight. The dried aqueous extract was stored in an airtight container at 4 °C until further analyses.

Chemicals and Reagents

All reagents were of analytical grade and purchased from Sigma-Aldrich (Germany) and Merck (USA). Solutions were freshly prepared using double-distilled water.

Determination of Total Phenolic Content

Total phenolics were estimated using the Folin–Ciocalteu method[18–21]. An aliquot (0.5 mL) of extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3. After incubation at 40 °C for 30 min, absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Model 6305, Jenway, UK). Results were expressed as mg gallic acid equivalent (GAE)/g extract.

Determination of Total Flavonoid Content

Flavonoids were quantified by the aluminum chloride ($AlCl_3$) colorimetric assay[18,19,21]. A 0.5 mL aliquot of extract was mixed with 0.5 mL of 2% $AlCl_3$ and incubated for 60 min at room temperature. Absorbance was read at 420 nm, and results expressed as mg quercetin equivalent (QE)/g extract.

Antioxidant Assays

Antioxidant capacity was determined using four complementary *in vitro* methods:

Ferric Reducing Antioxidant Power (FRAP): The extract (0.1 mL) was combined with phosphate buffer and potassium ferricyanide, incubated at 50 °C for 20 min, treated with trichloroacetic acid, and centrifuged. The supernatant was reacted with $FeCl_3$, and absorbance was read at 700 nm[22].

DPPH Radical Scavenging: Different concentrations (0.025–0.5 mg/mL) were mixed with 0.135 mM DPPH and incubated for 30 min in the dark. Absorbance was measured at 517 nm, using BHT and rutin as standards[23]. Scavenging activity was calculated as:

$$\% \text{ inhibition} = \frac{Acontrol - Asample}{Acontrol} \times 100$$

ABTS Radical Scavenging: $ABTS^{\bullet+}$ was produced by mixing 7 mM ABTS with 2.4 mM potassium persulfate and incubating for 12 h. The solution was diluted to an absorbance of 0.706 ± 0.001 at 734 nm before reacting with extract, and absorbance was read after 7 min[18,22].

Hydroxyl Radical Scavenging: The reaction mixture contained phosphate buffer, 1,10-phenanthroline, H_2O_2, $FeCl_3$, and extract. Absorbance was measured at 560 nm after 5 min using ascorbic acid as standard[23].

Statistical Analysis

All experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD) and analyzed by one-way ANOVA (GraphPad Prism v9.0). Significance was set at $p < 0.05$[24–25].

Results and Discussion

Medicinal plants are important sources of natural antioxidants that neutralize reactive oxygen species (ROS) and preserve cellular redox balance. In this study, the aqueous bulb extract of *Gladiolus psittacinus* demonstrated strong antioxidant capacity, as reflected by its high phenolic and flavonoid contents and its marked activity in multiple in vitro antioxidant assays.

The extract exhibited high total phenolic (50.33 ± 0.02 mg GAE/g) and flavonoid (43.22 ± 0.03 mg QE/g) contents (Table 1) confirming that *G. psittacinus* bulb is a rich reservoir of antioxidant compounds. Phenolics and flavonoids are primary antioxidants that exert their effects by donating hydrogen atoms or electrons to neutralize free radicals and terminate oxidative chain reactions[26–28]. The abundance of these compounds in the extract likely accounts for its strong antioxidant performance. Similar associations between high phenolic/flavonoid content and antioxidant activity have been reported in medicinal plants such as *Aloe ferox* and *Rosmarinus officinalis*[22,25], as well as several members of the Iridaceae family[29-32].

Table 1: Presents the total phenolic and flavonoid contents of the aqueous extract of *Gladiolus psittacinus* bulb

Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
50.33±0.02	43.22±0.03

GAE; Gallic acid equivalent, QE; Quercetin equivalent. Values represent mean ± standard deviation of triplicates readings.

Table 2: Showing the aqueous extract of *Gladiolus psittacinus* bulb demonstrated notable antioxidant activity, as reflected by its SC_{50} values against DPPH, ABTS, hydroxyl radical scavenging, and ferric reducing antioxidant power assays

Parameters	*Gladiolus psittacinus* bulb	Ascorbic acid
DPPH*	24.21±0.00	6.01±0.01*
FRAP	120.23±0.01	64.50±0.01
ABTS*	7.91±0.01	1.05±0.01*
OH*	17.52±0.01	7.20±0.01*

*The radical scavenging abilities of the plant parts were determined as described and express as percentage. The SC_{50} were calculated using nonlinear regression analysis. Values represent mean ± deviation (n = 3). Mean values with the same letters within a row are not significantly difference (P > 0.05)

The DPPH radical scavenging assay revealed a concentration-dependent activity (4.82 ± 0.01–8.38 ± 0.02), indicating the extract's strong hydrogen- and electron-donating ability as shown in (Figure 1). The DPPH assay is a widely accepted method for evaluating the radical-quenching efficiency of natural antioxidants[23]. The observed activity is largely attributed to phenolic hydroxyl groups, which stabilize free radicals by forming resonance-stabilized phenoxyl species[33–35]. Comparable DPPH scavenging activities have been reported for *Crocus sativus* stigmas and *Iris versicolor* rhizomes, where hydroxylated flavonoids were identified as major contributors[36–38]. This suggests that *G. psittacinus* may effectively protect cellular lipids and proteins against oxidative damage.

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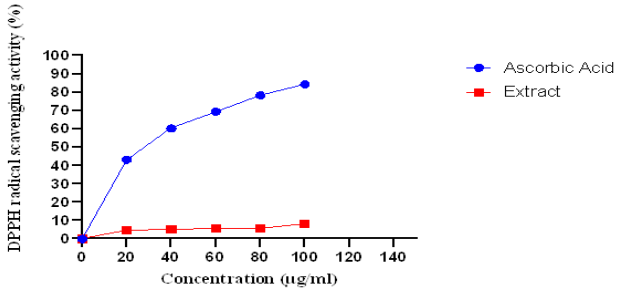

Figure 1: DPPH radical-scavenging activity of aqueous extract of *Gladiolus psittacinus* at varying concentrations

Ferric reducing antioxidant power (FRAP) analysis further demonstrated that the extract possessed strong reducing capacity, with values increasing from 45.86 ± 0.01 to 94.61 ± 0.33 as concentration increased (Figure 2). This assay reflects the electron-donating potential of antioxidants to reduce Fe^{3+} to Fe^{2+}, a key mechanism in maintaining redox homeostasis and preventing metal-catalyzed oxidative reactions[39–41]. The results align with previous studies showing that phenolic-rich extracts exhibit significant ferric-reducing power[42]. Similar findings have been reported for *Gladiolus dalenii* and *Gladiolus carneus*, where high phenolic and flavonoid contents were closely linked to strong FRAP activity[11, 43].

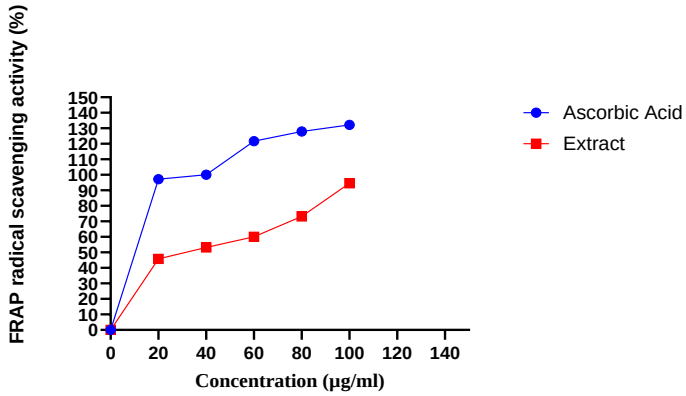

Figure 2: FRAP radical-scavenging activity (%) of aqueous extract of *Gladiolus psittacinus* compared with ascorbic acid at varying concentrations.

The hydroxyl radical (•OH) scavenging assay showed that the extract effectively quenched hydroxyl radicals (4.94 ± 0.02–10.62 ± 0.01) (Figure 3), with activity surpassing that of ascorbic acid. Hydroxyl radicals are among the most reactive ROS and can induce severe oxidative damage to DNA, lipids, and proteins.

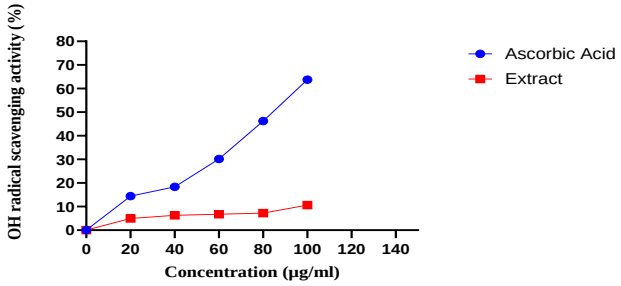

Figure 3: OH radical-scavenging activity (%) of aqueous extract of *Gladiolus psittacinus* compared with ascorbic acid at varying concentrations

The strong scavenging ability observed suggests the presence of highly potent redox-active constituents in *G. psittacinus*[42-45]. Similar hydroxyl radical scavenging activities have been reported for *Iris kashmiriana* and *Crocus sativus*, where flavonoids such as quercetin and kaempferol derivatives played key roles[26].

Figure 4 shows the ABTS radical scavenging assay, which evaluates both hydrophilic and lipophilic antioxidant components, also showed concentration-dependent activity (0.29 ± 0.04–0.62 ± 0.01), confirming the broad-spectrum antioxidant potential of the extract[46-47]. Differences observed between ABTS and DPPH scavenging activities may be attributed to variations in radical chemistry, solubility, and reaction mechanisms, as phenolic compounds interact differently with each radical system[46]. The ABTS scavenging profile of *G. psittacinus* is consistent with reports on other Iridaceae species, including *Iris germanica*, *Iris pallida*, and *Crocus sativus*, where high phenolic content strongly correlated with ABTS inhibition[27-31].

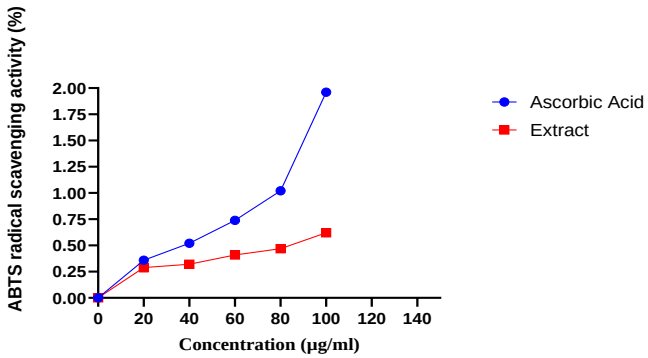

Figure 3: OH radical-scavenging activity (%) of aqueous extract of *Gladiolus psittacinus* compared with ascorbic acid at varying concentrations.

Mechanistically, the antioxidant activity of *G. psittacinus* can be attributed to multiple complementary actions of its polyphenolic constituents, including direct scavenging of ROS, chelation of transition metals involved in radical generation, and modulation of endogenous antioxidant defense systems. Flavonoids such as quercetin and rutin have been shown to activate the Nrf2/ARE signaling pathway, leading to upregulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)[33-35]. The phenolic compounds present in *G. psittacinus* may exert similar effects, thereby enhancing cellular resilience against oxidative stress.

Overall, the findings of this study demonstrate that the aqueous bulb extract of *Gladiolus psittacinus* possesses strong, concentration-dependent, and broad-spectrum antioxidant activity, primarily due to its high phenolic and flavonoid composition. The results are consistent with antioxidant trends reported for other Iridaceae members and polyphenol-rich medicinal plants such as *Aloe ferox*, *Rosmarinus officinalis*, and *Curcuma longa*[22,25,37]. These findings highlight *G. psittacinus* as a promising natural source of therapeutic antioxidants with potential applications in the management of oxidative stress–related diseases.

Conclusion

This study is the first to comprehensively assess the in vitro antioxidant potential of an aqueous extract of *Gladiolus psittacinus* bulbs. The extract demonstrated robust radical-scavenging and ferric-reducing activities that were dependent on concentration and showed a significant correlation ($p < 0.05$) with its high total phenolic and flavonoid contents. These results provide scientific backing for the traditional application of *G. psittacinus in* the treatment of conditions associated with oxidative stress. The research identifies the bulb as a significant natural source of bioactive compounds that could be used in creating antioxidant-based therapies and nutraceutical products. The results may help in creating standardized herbal products or functional foods based on *G. psittacinus* for preventing and managing diseases related to oxidative stress.

Conflict of Interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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