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Research Article

Antioxidant Potential of Aqueous and Hydroalcoholic Extracts of *Gossypium hirsutum* L.

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Abstract

Plant extracts serve as a reservoir of bioactive phytochemicals applicable in various domains, with *Gossypium hirsutum* L. encompassing constituents such as terpenes, phenolic compounds, and flavonoids, all exhibiting notable antioxidant properties, as well as metal-reducing capabilities, alongside potential toxicity. These compounds are distributed throughout the seeds, capsules, goblets, leaves, stems, flowers, and roots of the plant. To assess these potential activities, this study investigated the antioxidant capacity of the extracts by neutralizing free radicals and iron ions. Aqueous and hydroalcoholic extracts from the leaves and roots (AERG, HERG, AELG, and HELG) were prepared and subsequently dried using spray drying techniques. Following this, evaluations of the antioxidant potential were conducted utilizing the DPPH method (1,1-diphenyl-2-picrylhydrazyl), while the reducing and chelating capacities of iron ions (Fe^{2+} and Fe^{3+}) were determined by measuring the intensity of the orange complex formed with 1,10-phenanthroline and free Fe^{2+} in the control supernatant and extracts. The hydroalcoholic extracts exhibited antioxidant activity comparable to that of ascorbic acid (IC_{50} 46.30 µg/mL), effectively donating electrons to stabilize free radicals, demonstrating a higher percentage of efficacy than their aqueous counterparts from *G. hirsutum*. Nevertheless, the chelation and reduction assays for the iron extracts did not demonstrate significant chelation or oxidation of iron. It was concluded that the extracts displayed a mild antioxidant action; thus, conducting further tests with various metals would be beneficial to ascertain their reducing or chelating potential.

Keywords: *Malvaceae; Chelation activity; Iron reduction; Free radical.*

Introduction

Plants synthesize a myriad of secondary metabolites, which exhibit notable biological activity. The Malvaceae family encompasses species that have demonstrated significant antioxidant properties [1, 2, 3]. *Gossypium hirsutum* L. it is a perennial species cultivated annually worldwide, important for economic and social development [4, 5, 6]. It encompasses an array of bioactive phytochemical constituents, including terpenes, phenolic compounds, flavonoids, as well as an assortment of additional compounds such as fatty acids, lipids, carbohydrates, and proteins. These constituents are intricately distributed throughout the seeds, capsules, calyx, leaves, stems, stalks, flowers, and roots of the plant [7, 8, 9, 10, 11, 11b, 12, 13, 14, 15, 16].

Among this diverse array of constituents, certain molecules effectively capture free radicals, including flavonoids and phenols, thereby enhancing antioxidant activity and rendering this plant invaluable for medicinal applications. Similarly, there exist deleterious secondary metabolites, such as gossypol and its derivatives, that warrant thorough evaluation and investigation to enhance our comprehension of the plant extracts derived from *Gossypium hirsutum* L. [17, 18].

Excessive accumulation of free radicals and reactive oxygen species precipitates oxidative stress, subsequently resulting in cellular damage. Phenolic compounds possess the ability to donate electrons, with the hydroxyl groups of the aromatic ring playing a pivotal role in their antioxidant efficacy. They mitigate oxidative stress by inhibiting free radicals, decomposing peroxides, and sequestering reactive oxygen species within biological systems. Flavonoids, characterized by their aromatic ring structure, are low molecular weight polyphenolic compounds, rendering them an excellent source of antioxidants and offering protection against lipid peroxidation. Although the endogenous cellular antioxidant defense system is operative, exogenous antioxidants, particularly natural antioxidants, play a crucial role in sustaining cellular homeostasis [19, 20, 21].

This research on the antioxidant capacity of extracts derived from *Gossypium hirsutum* L. is underpinned by the imperative to elucidate the effects of the bioactive secondary metabolites inherent in this plant, which possess considerable economic and social significance. Given that the phenolic compounds and flavonoids present in *G. hirsutum* L. exhibit the potential to neutralize free radicals and mitigate oxidative stress, it is crucial to investigate these mechanisms in depth to explore their therapeutic applications. Consequently, the study aims to assess the antioxidant capacity of *G. hirsutum* L. extracts through methodologies such as free radical scavenging, metal chelation, and iron reduction, with the objective of augmenting its medicinal benefits and ensuring safe and effective utilization.

Materials and Methods

Collection and Sample Description

The leaves and roots were collected from specimens in Brejinho, located in the rural area of Araripe municipality, southern Ceará state, Brazil. The geographic coordinates, south latitude and west longitude of Greenwich are: (7° 12'12.5"S 40°01'10.2"W; 7°12'23.1"S 40°00'50.7"W; 7°13'38.5"S 39°59'44.5"W). The collection took place in April 2019, from 7:30 to 9:00 AM. The plant material was cleaned, weighed, and sent to the laboratory. The exsiccate produced is deposited in the Dárdano de Andrade Lima Herbarium of the Regional University of Cariri – URCA under number 65.2019 and identified as *G. hirsutum* L.

Preparation of Extracts

According to Matos[22], aqueous and hydroethanolic extracts of the leaves and roots (EARG, EHRG, EALG, and EHLG) were prepared. Approximately 1,172.3 g of leaves and 1,265.4 g of roots were cut to increase their surface area and dried at room temperature. Subsequently, sterile distilled water or 70% alcohol was added and kept in a container protected from light and air. After 72 hours, the extracts were filtered and subjected to Spray drying (130°C, flow rate quotient 0.5 L/H, fan control 1.95, outlet temperature 84.6, airflow meter 40 L/min air, particles with one millimeter in diameter), producing crude extract with 2.273 g, 0.624 g, 8.380 g, and 5.486 g, respectively.

Antioxidant Activity Assay

DPPH Free Radical Scavenging Activity

To evaluate the antioxidant potential, the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical assay was conducted following the method described by Kamdem [23], with some modifications. Initially, 50 µL of the extracts at different concentrations (1, 50, 200, and 500 µg/mL) were mixed with 100 µL of freshly prepared DPPH solution (0.3 mM in ethanol). The plate was then kept in the dark at room temperature for 30 minutes. The reduction of DPPH radical was measured by monitoring the decrease in absorbance at 517 nm using a microplate reader (Kasuaki microplate reader). Ascorbic acid was used as a positive control at concentrations of (1, 10, 30, 50, 100, and 400 µg/mL). The percentage inhibition of DPPH radical was calculated using the equation:

$$\% \text{ Inhibition} = 100 - [(A_{sample} - A_{blank}) / A_{control}] \times 100$$

where A_sample is the absorbance of the tested sample with DPPH, A_blank is the absorbance of the assay tube without adding DPPH, and A_control is the absorbance of the DPPH solution.

Iron Ion Reducing Power in Gossypium hirsutum L. Extracts

The chelating capacity of ferrous iron ions (Fe^{2+}) and the reducing property of ferric iron ions (Fe^{3+}) in cotton extracts were determined by measuring the intensity of the orange complex formed with 1,10-phenanthroline plus iron in the supernatant of the control and extracts using the modified method of Kamdem [24]. A mixture containing saline solution (58 µL, 0.9% w/v), Tris-HCl buffer (45 µL, 0.1 M, pH 7.5), extract (27 µL, 1–480 µg/mL), and $FeSO_4$ (36 µL, 110 µM) for chelating power test or $FeCl_3$ (36 µL, 110 µM) for reducing power test, was incubated for 10 min at 37°C. Subsequently, 1,10-phenanthroline (34 µL, 0.25% w/v) was added, and the absorption of the orange complex formed was measured at 0, 10, 20, and 30 minutes. The readings were taken at 30 minutes with the addition of ascorbic acid. Subsequent readings were taken at 40, 50 minutes - from the first reading - at a wavelength of 492 nm (against blank solutions of samples) using the SpectraMax microplate reader (Molecular Devices, Orleans Drive Sunnyvale CA, USA). The same procedure was performed for the control (e.g., Fe^{3+}) without the extracts. Ascorbic acid inhibits the oxidation of Fe^{2+} to Fe^{3+} after long periods, this oxidation leads to a decrease in absorbance unrelated to the reduction of Fe^{3+} to Fe^{2+}.

Statistical Analysis

The analysis used Graph Pad Prism software (version 6.0) is Free Version. To determine the total levels of iron, the data were expressed as mean ± SEM (standard error of the mean). Data were analyzed by two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. A significant difference was considered at $p < 0.5$. IC_{50} values were estimated using nonlinear regression.

Results

Free Radical Scavenging Activity of *Gossypium hirsutum* L.

The influence of *G. hirsutum* extracts and ascorbic acid on the mitigation of free radicals is illustrated in Figure 1. Ascorbic acid demonstrated a concentration-dependent DPPH radical scavenging efficacy (Figure 1), with an IC_{50} value of 46.30 µg/mL. The extracts exhibited notable antioxidant properties across all experimental conditions. Both aqueous extracts (AELG, AERG) and hydroalcoholic extracts (HELG, HERG) revealed concentration-dependent antioxidant activity against the DPPH radical (Fig. 1).

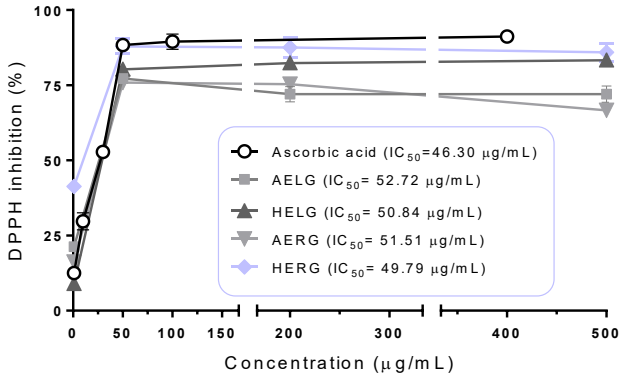

Figure 1. *Quenching of DPPH by extracts from Gossypium hirsutum. The data are expressed as means ± SEM of n = 3 independent experiments.*

Chelating and Fe^{2+} oxidant activity of *Gossypium hirsutum* extracts.

The investigation into the chelating capacity and Fe^{2+} oxidant potential of AERG revealed that at the four lowest concentrations, a modest chelating activity of the Fe^{2+} ion was observed (Figure 1A), as evidenced by a reduced absorbance relative to the control (Fe^{2+} 110 µM) and a marginal increase in absorbance following the introduction of ascorbic acid (2A). Notably, a slightly elevated absorbance value prior to the addition of AA can be discerned at the highest concentration (480 µg/mL).

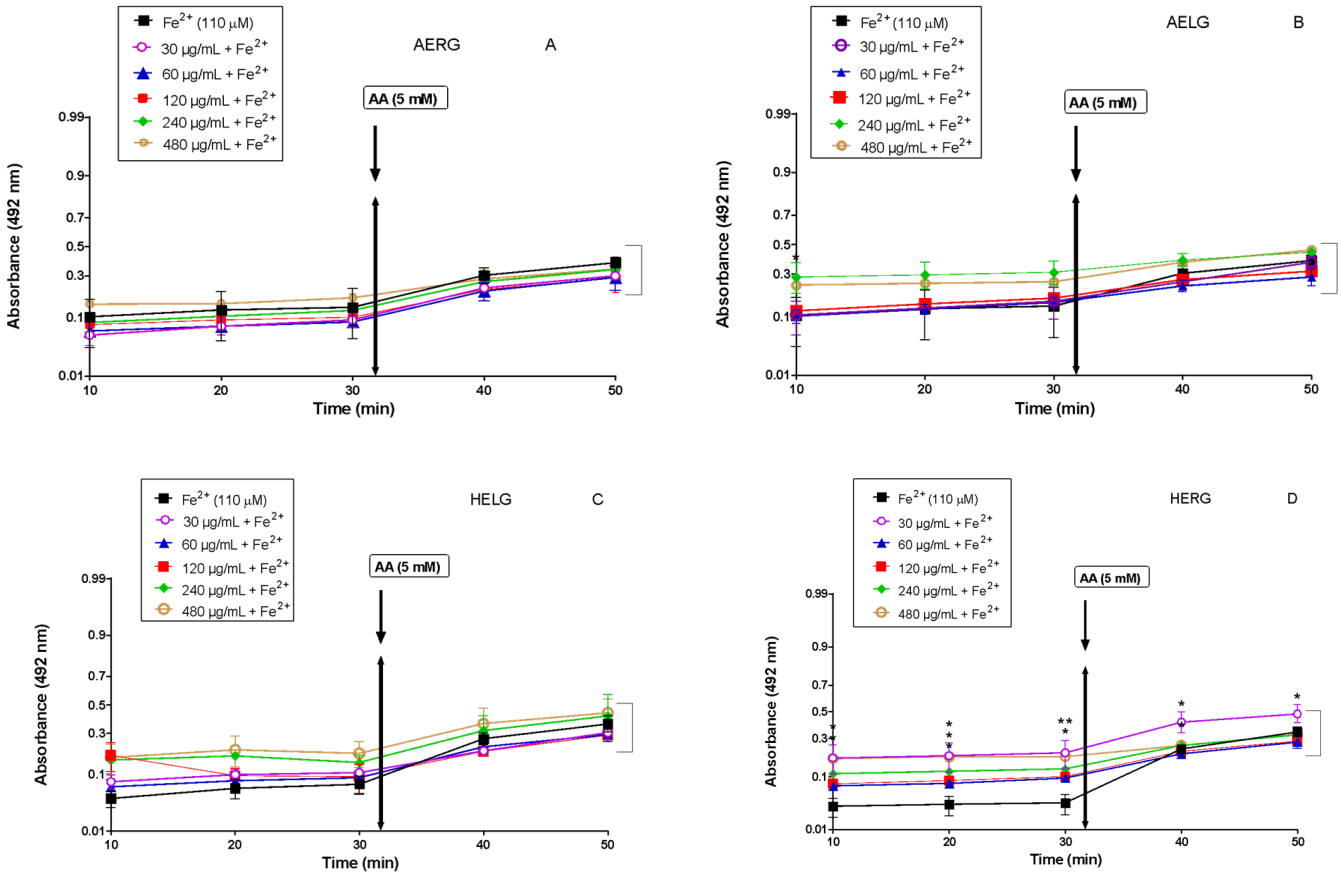

***Figure 2**. Chelating capacity of ferrous ion (Fe^{2+}) and oxidant potential of (A) AERG, (B) AELG, (C) HELG and (D) HERG.*

For AELG, elevated relative absorbance values were observed at higher concentrations (120, 240, and 480 µg/mL), as illustrated in Figure 1B, suggesting a modest reducing activity. Following the incorporation of ascorbic acid (AA), a slight augmentation in free Fe^{2+} concentration was noted, as evidenced by absorbance variations at elevated concentrations, alongside a discernible chelating effect at the two lower concentrations (30 and 60 µg/mL).

With respect to the chelating and oxidative capacity of HELG (Figure 2C), there was a notable increase in Fe^{2+} across all concentrations when compared to the control. However, at a concentration of 120 µg/mL, a reduction in the quantity of this ion was observed between the 10 and 20-minute marks, ultimately aligning with the levels found at 30 and 60 µg/mL. Furthermore, concerning ascorbic acid (AA), a modest enhancement in absorbance was detected at elevated concentrations (240 and 480 µg/mL), while exhibiting minimal chelating activity at the lower concentrations.

In contrast to the preceding extracts, HERG elicited a relative augmentation of Fe^{2+} across all tested concentrations (Figure 4D), exhibiting significantly elevated absorbance increases at both the lowest and highest concentrations (30 and 480 µg/mL) in comparison to the control (Fe^{2+} 110 µM). Shortly following the introduction of AA, diminished absorbance values were observed relative to the control, suggesting subtle chelating effects at concentrations of 60 and 120 µg/mL.

Reducing the activity of Fe^{3+} by extracts of *Gossypium hirsutum* L.

In the assay of ferric ion (Fe^{3+}) reducing activity of AELG, a modest reduction of Fe^{3+} is discernible across all evaluated concentrations (Figure 2A). Following the introduction of ascorbic acid (AA), there was a notable increase in absorbance at the three lower concentrations (30, 60, and 120 µg/mL), ultimately reaching equilibrium after 50 minutes. Conversely, the two higher concentrations (240 and 480 µg/mL) exhibited relative constancy between the 40 and 50-minute marks, along with the control (Fe^{3+} 110 µM).

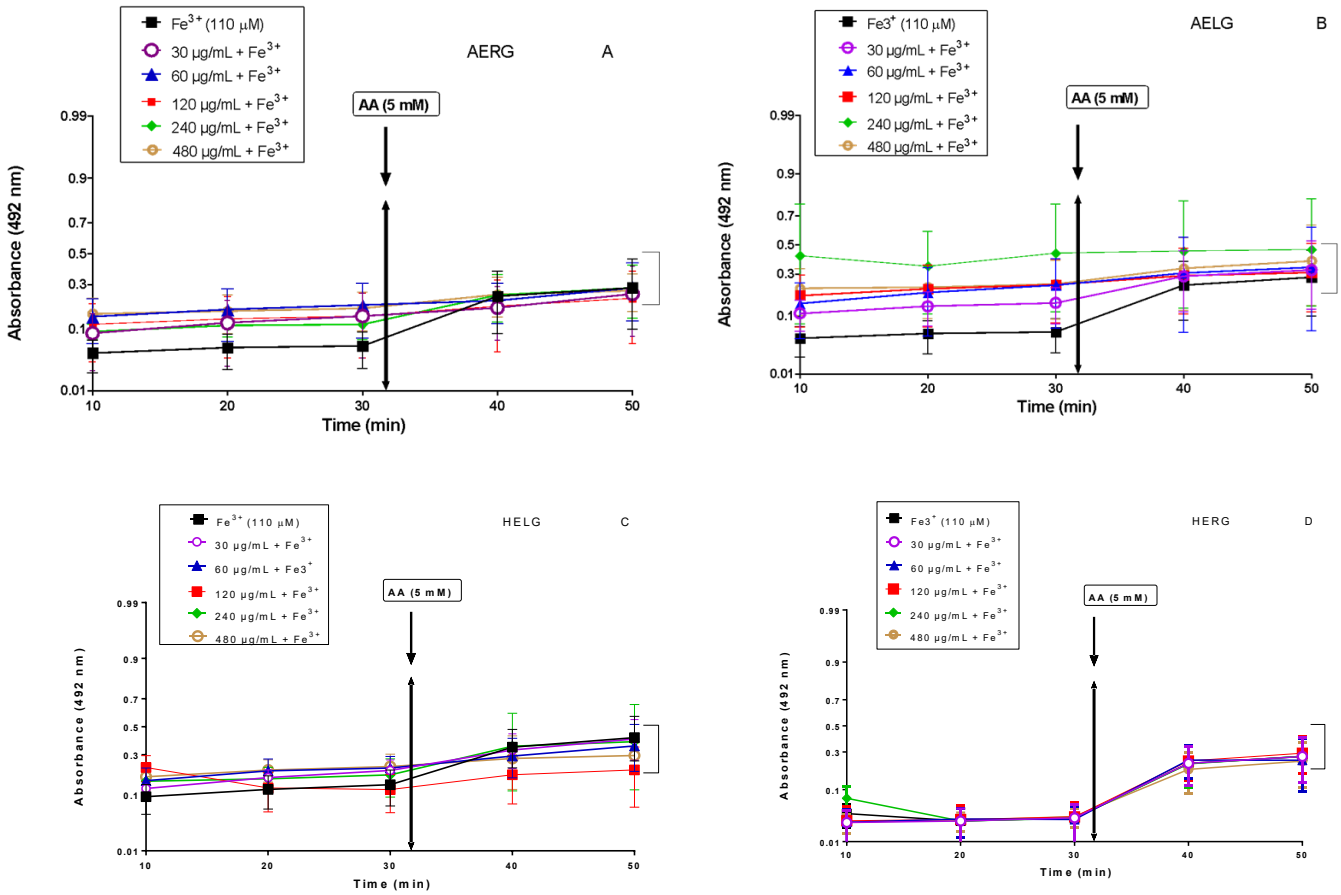

Figure 3. *Ferric ion (Fe^{3+}) reducing (A) AERG, (B) AELG, (C) HELG, and (D) HERG.*

Concerning the reducing activity of AELG, we note that all concentrations employed in the assay exhibited a marked reduction of Fe^{3+}, albeit not to a statistically significant degree (Figure 3B). Following the introduction of ascorbic acid (AA), there was a modest enhancement in absorbance across four of the concentrations tested (30, 60, 120, and 480 µg/mL), alongside a stabilization of the 240 µg/mL concentration from the 30-minute mark onwards, suggesting an almost complete reduction of the Fe^{3+} present.

At the initial time point (10 minutes), it is evident that all concentrations of HELG exhibited a marginally diminished reducing capacity (Figure 3C), with the exception of 120 µg/mL, which begins to exhibit a decline in absorbance by the 30-minute mark. Shortly after the introduction of AA, a subtle relative decrease in Fe^{2+} becomes apparent across all concentrations, contrasting with the initial conditions.

In the investigation of the reducing activity of HERG, it is noteworthy that at the three highest concentrations (120, 240, and 480 µg/mL) at the initial time point (10 minutes), a subtle reducing activity is observed (Figure 3D). However, even prior to reaching the 20-minute mark, these concentrations remained comparable to the control and other concentrations (30 and 60 µg/mL) until the conclusion of the experiment. The lack of reducing activity may suggest that the extract has undergone deactivation, potentially due to the formation of complexes with Fe^{3+} ions.

Discussion

The DPPH radical scavenging activity is extensively employed to evaluate the antioxidant properties of natural extracts, given that numerous neurodegenerative diseases, malignancies, and afflictions of the respiratory and gastrointestinal tracts are precipitated by Reactive Oxygen Species (ROS). Elevated levels of ROS in healthy cells contribute to the damage of DNA, lipids, and proteins, culminating in mutagenesis and cellular transformation [25,26].

Within the purview of our investigation, the evaluated IC50 values indicate that the hydroalcoholic extracts of both leaves and roots (HELG, HERG) demonstrated superior DPPH radical scavenging activity when juxtaposed with their aqueous counterparts. Notably, HERG exhibited antioxidant efficacy comparable to that of the positive control, ascorbic acid, with an IC50 of 46.30 µg/mL.The elevated antioxidant capacity of hydroalcoholic extracts derived from leaves and roots, in comparison to their aqueous counterparts, can be ascribed to their superior concentrations of tannins and flavonoids. *Gossypium hirsutum* is capable of accumulating terpenes in all active photosynthetic tissues of the plant, storing them within subepidermal glands as a defense mechanism against insect predation. Among the various classes of terpenes, this species synthesizes monoterpenes, sesquiterpenes (C15), and terpene aldehydes, including hemigossypolone (C15) and heliocides H1 to H4 (C25)[27].

Studies elucidate the antioxidant potential of *G. hirsutum*[28,29,30], attributing the antioxidative efficacy of the ethanolic extract, with an IC50 of (70.97 ± 1.53), to the presence of phenolic compounds, predominantly flavonoids [31]. Both leaf and root extracts demonstrated significant antioxidant capacity in scavenging free radicals, as evidenced by the DPPH assay, thereby positioning them as promising sources of bioactive molecules.

Plants such as kaempferol and quercetin predominantly exist in their glycosidic derivative forms. The species *Adansonia digitata* L. (Malvaceae), in its methanolic extracts derived from dried fruit pulp and leaf powder, exhibited quercetin and kaempferol derivatives, which demonstrated notable antioxidant activity in DPPH and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical assays [32, 33]. In a similar vein, *Gossypium hirsutum* revealed the presence of kaempferol and quercetin derivative compounds in both aqueous and hydroalcoholic leaf extracts [34].

The extracts of *Gossypium hirsutum* exhibited a modest effect on iron metal in the chelation (Fe2+) and iron reduction (Fe3+) assays, revealing no significant chelating or reducing capacity. Nevertheless, secondary metabolites derived from the flavan-3-ol subclass in *G. hirsutum*, including (+)-catechin, (-)-epigallocatechin, (+)-gallocatechin, and (-)-epicatechin [34], confer resistance to fungal invasion and demonstrate chelating activity for ions such as iron and other essential metals [35, 36]. The aqueous extract (3.77 ± 0.01 µmol EDTAEs/g dry plant) of *G. hirsutum* stems exhibited superior chelating efficacy compared to the ethyl acetate extract (0.31 ± 0.01 µmol EDTAEs/g dry plant) and methanol extract (1.75 ± 0.02 µmol EDTAEs/g dry plant) in the study conducted by [29] .

Conclusion

In conclusion, the aqueous and hydroalcoholic extracts of *Gossypium hirsutum* demonstrated significant antioxidant potential by donating electrons to stabilize the DPPH free radical. This study particularly highlighted the HELG and HERG extracts, which revealed results comparable to ascorbic acid and achieved higher inhibition percentages than their aqueous counterparts. The pronounced antioxidant activity of the HELG and HERG extracts underscores their potential as effective natural antioxidants, possibly warranting further investigation into their bioactive compounds and mechanisms of action.

However, in the chelation and iron reduction assays, the cotton extracts did not yield significant results; their chelating and reducing activities were considered rather mild, indicating limited efficacy in these specific tests. This raises important questions about the inherent properties of these extracts and suggests that their potential as chelators or reducers may not be fully realized within the confines of this study. Consequently, pursuing further testing with other metals could provide deeper insights into their chelating and reducing capabilities, thereby expanding our understanding of their overall applicability in various biochemical contexts.

Conflict of Interest

The authors declare no conflict of interest.

Author's Contribution

1) M.F.B.M.B.; L.F.L.; H.D.M.C. - Study conception.

2) L.F.L.; A.R.C.; C.V.B.O.; A.E.D.; N.S.M.; J.F.S.S.; A.L.B. - data acquisition, data analysis, and interpretation;

3) L.F.L.; A.B.S.; N.S.F.; C.N.F.L.; D.G.S.F.; S.D.G.S. - Article drafting or revision aimed at making the material intellectually relevant;

4) L.F.L.; M.F.B.M.B.; H.D.M.C.; A.E.D. Final approval of the manuscript for submission.

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