



IN VITRO EVALUATION OF ANTIOXIDANT PROPERTIES OF CAESALPINIA PULCHERRIMA LEAF EXTRACT

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ABSTRACT

This study investigated the in vitro antioxidant capabilities of ethanolic leaf extracts from *Caesalpinia pulcherrima*, a medicinal plant traditionally employed in managing oxidative stress-related conditions. The research evaluated two key antioxidant parameters: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and hydroxyl radical scavenging capacity. Results demonstrated concentration-dependent antioxidant effects in both assays. For DPPH radical scavenging, the extract exhibited IC₅₀ value of 0.27 mg/ml compared to 0.07 mg/ml for L-ascorbic acid (standard), indicating strong antioxidant potential. The hydroxyl radical scavenging assay revealed a concentration-dependent decrease in activity, with IC₅₀ values of 0.14 mg/ml for the extract versus 1.06 mg/ml for the standard. These findings support the traditional use of *C. pulcherrima* in treating oxidative stress-associated disorders and suggest its potential as a natural alternative to synthetic antioxidants. Further investigations on active compound isolation, characterization, and safety evaluation are recommended.

INTRODUCTION

The imbalance between reactive oxygen species production and antioxidant defense mechanisms leads to oxidative stress, a primary contributor to numerous pathological conditions including malignancies, metabolic disorders, cardiovascular complications, neurological deterioration, and inflammatory syndromes [1]. This phenomenon arises from either excessive generation of free radicals or insufficient cellular detoxification capacity [2].

Free radicals, comprising unstable oxygen and nitrogen species, originate from both external environmental sources and internal metabolic pathways. External contributors include tobacco smoke, atmospheric pollutants, electromagnetic radiation, and pharmaceutical compounds [3]. Internal generation occurs through

mitochondrial electron transport processes, enzymatic pathways such as xanthine oxidase, and pathological states including tissue inflammation and ischemia-reperfusion events [4]. Under normal physiological conditions, the body maintains oxidative homeostasis through sophisticated antioxidant systems encompassing both enzymatic components (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic molecules (bilirubin, uric acid, lactoferrin). However, pathological states overwhelm these protective mechanisms, resulting in free radical accumulation and subsequent cellular damage [5].

Current therapeutic approaches employ synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) [6]. Despite widespread application, these compounds present significant limitations including hepatotoxicity, carcinogenic potential, limited accessibility, economic constraints, and chemical instability [7].

Medicinal plants offer promising alternatives, containing diverse secondary metabolites with established pharmacological activities. Plant-derived antioxidants have

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demonstrated protective effects against various diseases, with regular consumption associated with reduced incidence of cancer, cardiovascular disease, hypertension, cognitive decline, and cerebrovascular accidents [8]. The antioxidant capacity of plants primarily derives from polyphenolic compounds and vitamins (A, C, E), which function through free radical neutralization and oxidative damage prevention [9].

Caesalpinia pulcherrima, a member of the Caesalpinaceae family, represents a potential source of natural antioxidants. Traditional practitioners utilize fruit and leaf preparations to address diarrheal conditions, dysentery, ophthalmic disorders, and diabetic complications. This investigation evaluates the antioxidant potential of *C. pulcherrima* ethanolic leaf extracts, aiming to establish scientific validation for traditional applications and identify safer, more accessible therapeutic alternatives for oxidative stress management [10].

METHODOLOGY

Plant Material Collection and Preparation

Fresh *Caesalpinia pulcherrima* leaves were collected from native habitats following comprehensive ethnobotanical surveys and consultation with traditional medicine practitioners. Plant identification utilized indigenous nomenclature and therapeutic applications documented by local herbalists. Following collection, leaf material underwent shade drying for fourteen days. Dried material was pulverized using an electric mill to obtain fine powder, which was stored in sealed, labeled containers at ambient temperature pending extraction procedures.

Extraction Procedure

Four hundred grams of powdered plant material was macerated in one liter of analytical-grade ethanol within a two-liter conical flask. The mixture was subjected to periodic agitation, sealed, and allowed to stand for 48 hours at room temperature. The resulting menstruum underwent filtration through Whatman No. 1 filter paper. Filtrates were concentrated using rotary evaporation at 50°C, followed by complete desiccation in a hot-air oven at 35°C. Concentrated extracts were transferred to airtight

containers and refrigerated at 4°C until bioassay analysis. [11]

Antioxidant Activity Assessment

DPPH Radical Scavenging Assay: The assessment employed 1,1-diphenyl-2-picrylhydrazyl methodology with modifications from established protocols. Five extract concentrations (0.0625, 0.125, 0.25, 0.5, and 1.0 mg/ml) were prepared in analytical-grade ethanol. Corresponding L-ascorbic acid concentrations served as reference standards. One milliliter of test extract was combined with 0.5 ml of 0.3 mM ethanolic DPPH solution, mixed thoroughly, and incubated in darkness at room temperature for fifteen minutes. Baseline measurements utilized extract solutions (2.5 ml) with ethanol (1 ml). Negative controls consisted of DPPH solution (2.5 ml) and ethanol (1 ml). Absorbance measurements at 517 nm were performed spectrophotometrically. Experiments were conducted in triplicate, with IC₅₀ values determined from percentage inhibition versus concentration plots.

Hydroxyl Radical Scavenging Assay: The evaluation followed established methodology with minor adaptations. Reaction mixtures contained 2.4 ml phosphate buffer (pH 7.8), 0.3 ml ferric chloride solution (10 mM), 0.3 ml EDTA (10 mM), 0.3 ml hydrogen peroxide (2 mM), 0.3 ml deoxyribose (10 mM), and varying extract concentrations. Following incubation at 37°C for one hour, 1.0 ml thiobarbituric acid (1%) and 1.0 ml trichloroacetic acid (2.8%) were added. Mixtures were heated at 100°C for twenty minutes, cooled, and absorbance measured at 532 nm. L-ascorbic acid served as positive control, with IC₅₀ values calculated from concentration-response relationships.

RESULTS

The antioxidant evaluation of *Caesalpinia pulcherrima* ethanolic leaf extract demonstrated concentration-dependent activity in both DPPH and hydroxyl radical scavenging assays. Results are presented in Tables 1 and 2.

Table 1: DPPH Radical Scavenging Activity of *C. pulcherrima* Ethanolic Extract.

Concentration (mg/ml)	Standard (L-Ascorbic acid) % (Mean ± SEM)	<i>C. pulcherrima</i> ethanolic extract (Mean ± SEM)
0.0625	47.82 ± 0.44	34.15 ± 0.58
0.125	63.19 ± 0.19	37.11 ± 0.77
0.25	74.01 ± 1.021	52.65 ± 0.44
0.5	82.34 ± 0.29	62.31 ± 0.74
1.0	88.21 ± 0.74	68.26 ± 0.31
IC ₅₀	0.07	0.27

Values are expressed as mean ± SEM



Table 2: Hydroxyl Radical Scavenging Activity of *C. pulcherrima* Ethanolic Extract.

Concentration (mg/ml)	Standard (L-Ascorbic acid) % (Mean ± SEM)	<i>C. pulcherrima</i> ethanolic extract (Mean ± SEM)
0.0625	88.51 ± 1.31	67.99 ± 0.34
0.125	81.52 ± 0.66	57.77 ± 0.38
0.25	74.22 ± 2.02	47.77 ± 0.35
0.5	61.37 ± 0.38	41.11 ± 0.55
1.0	51.65 ± 0.67	31.98 ± 0.21
IC₅₀	1.06	0.14

Values are expressed as mean ± SEM

DISCUSSION

Oxidative stress manifests when reactive oxygen and nitrogen species production surpasses antioxidant defense capacity. In healthy organisms, controlled generation of these reactive species occurs through normal aerobic metabolism [13]. However, pathological oxidative stress triggers excessive production, resulting in lipid, protein, and nucleic acid modifications. Such molecular damage contributes to aging processes and various pathological conditions including atherosclerotic disease, oncogenesis, ischemia-reperfusion injury, and neurodegenerative disorders [14]. Contemporary synthetic antioxidants (propyl gallate, BHA, BHT) demonstrate limitations including hepatic toxicity, malignant transformation potential, and restricted efficacy in experimental models and clinical applications [15]. Consequently, increasing interest focuses on naturally-derived plant antioxidants, which offer enhanced safety profiles, accessibility, and economic viability. This investigation evaluated phytochemical content and antioxidant properties of *C. pulcherrima* ethanolic extracts. Current research consensus indicates that comprehensive antioxidant assessment requires multiple experimental methodologies rather than single-model evaluation. Various in vitro techniques including hydroxyl scavenging, lipid peroxidation inhibition, oxygen radical absorbance capacity, nitric oxide scavenging, ferric reducing power, and DPPH scavenging provide complementary antioxidant activity data [16]. The DPPH methodology evaluates antioxidant capacity through free radical reduction, characterized by colorimetric transition from blue to yellow (quantified at 517 nm). Extract classification as strong antioxidant (IC₅₀ <50 mg/ml) confirms significant radical scavenging

capability. Hydroxyl radicals cause enzymatic denaturation through thiol group oxidation, generated via Fenton reaction mechanisms. Extract capacity for hydroxyl radical scavenging indicates potential in vivo antioxidant efficacy [17].

The observed concentration-dependent decrease in hydrogen peroxide scavenging may result from reactive center saturation at elevated extract concentrations, reducing activity compared to dilute concentrations facilitating rapid reactions. This pattern contrasts with some investigations reporting concentration-dependent activity increases.

Traditional applications of studied plants address oxidative stress-associated diseases. The demonstrated antioxidant effects in experimental models suggest therapeutic value derives from oxidative stress remediation. These findings validate traditional medicinal applications of *C. pulcherrima* in managing oxidative stress-related disorders, supporting ethnopharmacological knowledge systems.

CONCLUSION

The ethanolic leaf extract of *Caesalpinia pulcherrima* demonstrates substantial antioxidant capacity and contains significant antioxidant-associated phytochemicals. These findings provide scientific validation for traditional therapeutic applications and suggest potential development as natural antioxidant alternatives. Future research should focus on isolating and characterizing active principles, conducting toxicological safety assessments, and evaluating clinical efficacy. Further investigations into structure-activity relationships and bioavailability optimization would facilitate therapeutic development and clinical translation of these promising natural compounds.

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