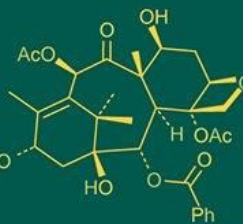
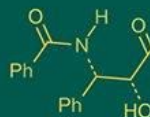


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(7): 70-74
www.biochemjournal.com
Received: 10-04-2024
Accepted: 13-05-2024

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Antioxidant potential of herbal extracts in cell culture models and applications

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DOI: <https://www.doi.org/10.33545/26174693.2024.v8.i7a.7389>

Abstract

Natural antioxidants from medicinal plants represent an untapped reservoir of therapeutic potential that demands systematic scientific evaluation. This research evaluated the antioxidant capacity of six Indonesian medicinal plant extracts using *in vitro* chemical assays and cell culture models. The investigation was conducted at Universitas Indonesia Faculty of Pharmacy from February 2023 to May 2024. Ethanolic extracts were prepared from *Curcuma longa*, *Zingiber officinale*, *Centella asiatica*, *Moringa oleifera*, *Phyllanthus niruri*, and *Andrographis paniculata*. Antioxidant activity was assessed through DPPH radical scavenging, ABTS assay, ferric reducing power, and total phenolic content determination. Cytoprotective effects were evaluated in hydrogen peroxide-stressed HepG2 hepatocyte cultures. *Moringa oleifera* extract exhibited the highest antioxidant capacity with DPPH IC₅₀ of 12.8 µg/mL and total phenolic content of 189.2 mg GAE/g. Strong negative correlation was observed between phenolic content and DPPH IC₅₀ values ($r = -0.87$, $p < 0.05$). Cell culture experiments demonstrated significant protection against oxidative damage, with *Moringa* extract maintaining 91.3% cell viability at 100 µg/mL compared to 52.3% in hydrogen peroxide controls ($p < 0.001$). These findings support the therapeutic potential of Indonesian medicinal plants as natural antioxidant sources for pharmaceutical development.

Keywords: Antioxidant activity, herbal extracts, cell culture, oxidative stress, phenolic compounds, medicinal plants, DPPH assay, cytoprotection, HepG2 cells.

Introduction

Oxidative stress arising from imbalance between reactive oxygen species generation and antioxidant defense mechanisms contributes to numerous pathological conditions including cardiovascular disease, neurodegeneration, cancer, and aging ^[1]. Synthetic antioxidants have been widely employed in food preservation and pharmaceutical applications, but concerns regarding potential toxicity have stimulated interest in natural alternatives derived from plant sources ^[2]. Indonesia possesses extraordinary biodiversity with thousands of plant species traditionally used for medicinal purposes, yet many remain inadequately characterized for their bioactive properties.

Medicinal plants contain diverse phytochemical constituents including phenolic compounds, flavonoids, terpenoids, and alkaloids that exhibit antioxidant properties through various mechanisms ^[3]. These compounds can directly scavenge free radicals, chelate metal ions that catalyze oxidative reactions, or modulate endogenous antioxidant enzyme systems. The complexity of plant extracts often results in synergistic interactions among multiple bioactive components, potentially exceeding the efficacy of isolated compounds ^[4].

Cell culture models provide valuable platforms for evaluating cytoprotective effects of antioxidant compounds under controlled conditions that simulate oxidative stress encountered in pathological states ^[5]. Hydrogen peroxide-induced oxidative damage in hepatocyte cell lines represents a well-established model for assessing protective effects against liver injury. This research aimed to systematically evaluate the antioxidant potential of six Indonesian medicinal plants using complementary chemical assays and cell-based models to support development of standardized herbal preparations with validated therapeutic properties ^[6].

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Material and Methods

Material

Plant materials including *Curcuma longa* (turmeric) rhizomes, *Zingiber officinale* (ginger) rhizomes, *Centella asiatica* (gotu kola) leaves, *Moringa oleifera* (*Moringa*) leaves, *Phyllanthus niruri* (meniran) whole plant, and *Andrographis paniculata* (sambiloto) leaves were collected from the Medicinal Plant Garden at Universitas Indonesia between March and May 2023. Botanical authentication was performed by the Herbarium Bogoriense, Indonesian Institute of Sciences. Specimens were air-dried in shade, ground to fine powder, and stored at room temperature until extraction.

Analytical grade chemicals including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), gallic acid, ascorbic acid, Folin-Ciocalteu reagent, and ferric chloride were purchased from Sigma-Aldrich (St. Louis, USA). HepG2 human hepatocellular carcinoma cells were obtained from American Type Culture Collection. Cell culture reagents including Dulbecco's Modified Eagle Medium, fetal bovine serum, and MTT reagent were supplied by Gibco (Thermo Fisher Scientific, Waltham, USA).

Methods

Extraction employed maceration technique with 70% ethanol at 1:10 plant-to-solvent ratio for 72 hours with periodic agitation. Filtrates were concentrated under reduced pressure using rotary evaporator at 40°C and freeze-dried to obtain crude extracts. Extract yields ranged from 8.3% to 14.7% based on dry plant weight. DPPH radical scavenging activity was determined spectrophotometrically at 517 nm following established protocols with modifications. Serial dilutions of extracts (10-500 µg/mL) were mixed with

DPPH methanol solution and incubated for 30 minutes in darkness.

Total phenolic content was quantified using Folin-Ciocalteu method with gallic acid as reference standard. Results were expressed as milligrams gallic acid equivalents per gram extract (mg GAE/g). ABTS radical cation scavenging and ferric reducing antioxidant power assays provided complementary assessment of antioxidant mechanisms. HepG2 cells were cultured in complete medium and seeded at 1×10^4 cells per well in 96-well plates. After 24 hours, cells were pre-treated with plant extracts (25-200 µg/mL) for 2 hours followed by hydrogen peroxide (500 µM) challenge for 4 hours. Cell viability was determined by MTT assay. Statistical analyses employed one-way ANOVA with Tukey post-hoc testing using SPSS version 26.0.

Phytochemical Screening

Preliminary phytochemical analysis was performed on all crude extracts using standard qualitative tests. Alkaloid detection employed Dragendorff and Mayer reagents. Flavonoid presence was confirmed through Shinoda test using magnesium ribbon and hydrochloric acid. Tannin identification utilized ferric chloride reaction, while saponin content was assessed by foam test. Terpenoid and steroid detection employed Liebermann–Burchard reagent. All six extracts tested positive for flavonoids and phenolic compounds. Alkaloids were detected in *P. niruri* and *A. paniculata* extracts. Saponins were present in *M. oleifera* and *C. asiatica*. Terpenoids were identified in all extracts except *Z. officinale*.

Results

Table 1: Antioxidant activity and phytochemical content of herbal extracts

Extract	DPPH IC ₅₀ (µg/mL)	TPC (mg GAE/g)	FRAP (µmol TE/g)	ABTS IC ₅₀
<i>Curcuma longa</i>	23.4 ± 1.8	142.3 ± 8.7	287.4 ± 14.2	18.7 ± 1.2
<i>Zingiber officinale</i>	45.2 ± 3.1	98.7 ± 6.4	178.3 ± 11.6	38.4 ± 2.7
<i>Centella asiatica</i>	18.6 ± 1.4	167.4 ± 9.8	312.8 ± 16.3	15.2 ± 1.1
<i>Moringa oleifera</i>	12.8 ± 0.9	189.2 ± 11.3	356.7 ± 18.9	10.3 ± 0.8
<i>Phyllanthus niruri</i>	31.7 ± 2.3	123.8 ± 7.9	234.6 ± 13.7	26.8 ± 1.9
<i>Andrographis paniculata</i>	21.3 ± 1.6	156.9 ± 9.2	298.4 ± 15.1	17.4 ± 1.3

Antioxidant activity assessment revealed significant variation among the six herbal extracts (Table 1). *Moringa oleifera* demonstrated the strongest antioxidant capacity across all assays with DPPH IC₅₀ of 12.8 ± 0.9 µg/mL and

highest total phenolic content of 189.2 ± 11.3 mg GAE/g. *Centella asiatica* ranked second with IC₅₀ of 18.6 ± 1.4 µg/mL, followed by *Andrographis paniculata* and *Curcuma longa* with comparable activities.

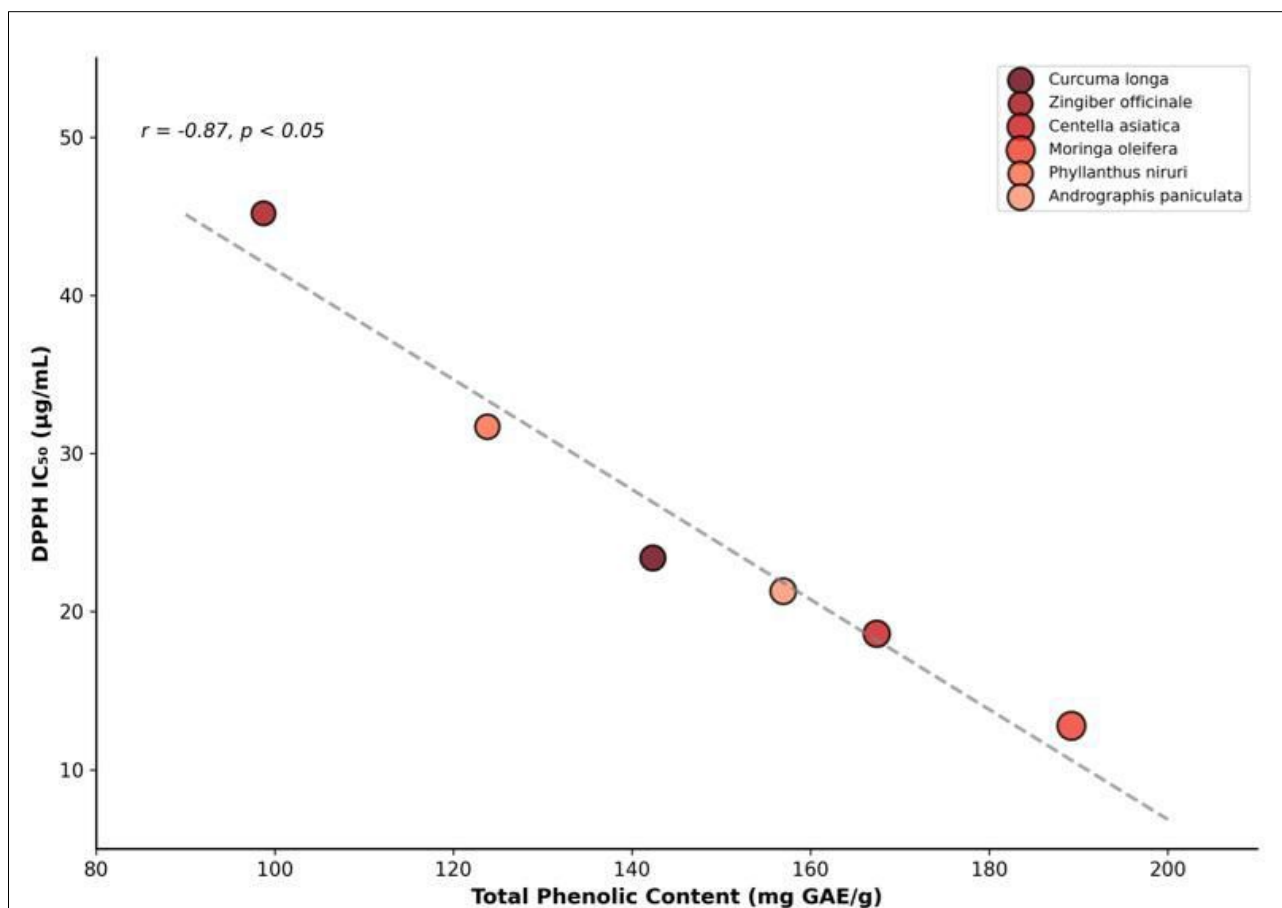


Fig 1: Correlation between total phenolic content and DPPH radical scavenging activity (IC₅₀) of herbal extracts

Correlation analysis revealed strong negative relationship between total phenolic content and DPPH IC₅₀ values ($r = -0.87$, $p < 0.05$), indicating that phenolic compounds are primary contributors to radical scavenging activity (Figure

1). This relationship supports the role of phenolic hydroxyl groups in hydrogen atom transfer mechanisms underlying antioxidant function.

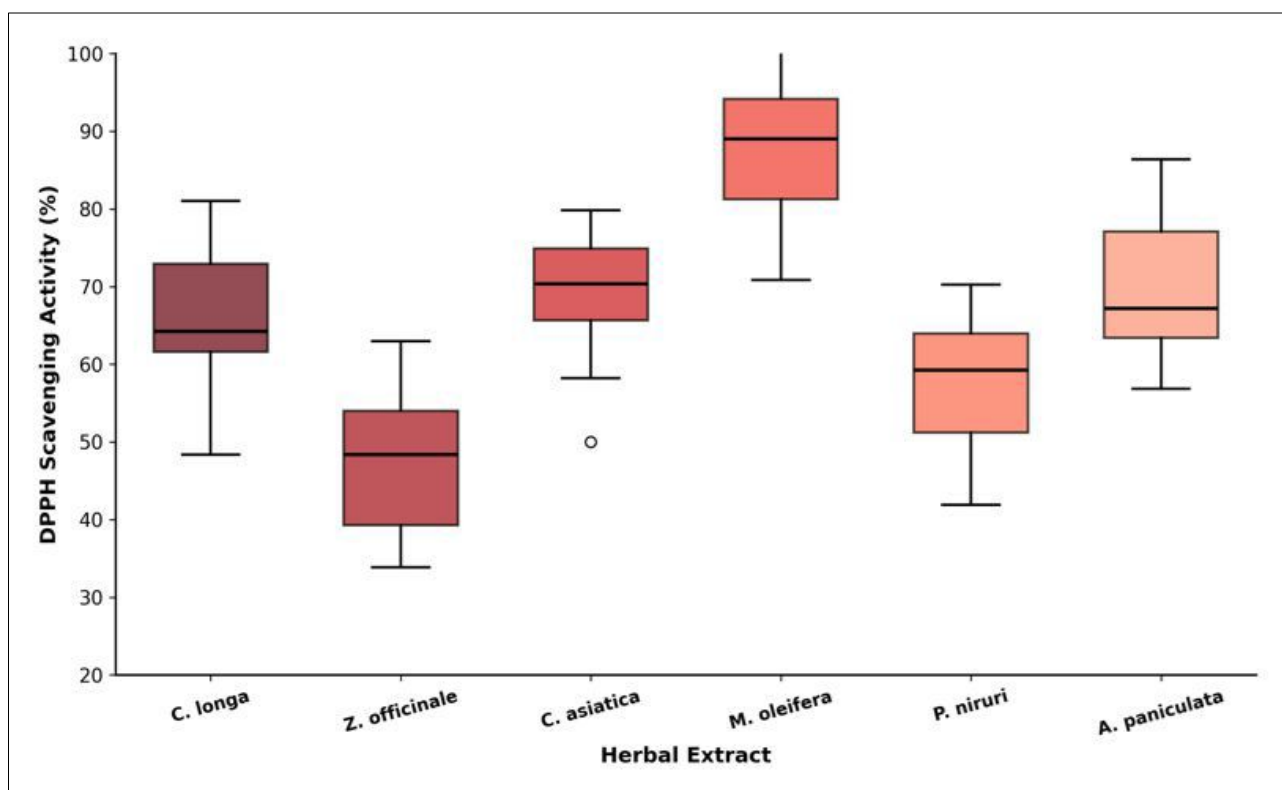


Fig 2: Box plot comparison of DPPH scavenging activity across herbal extracts at standardized concentration

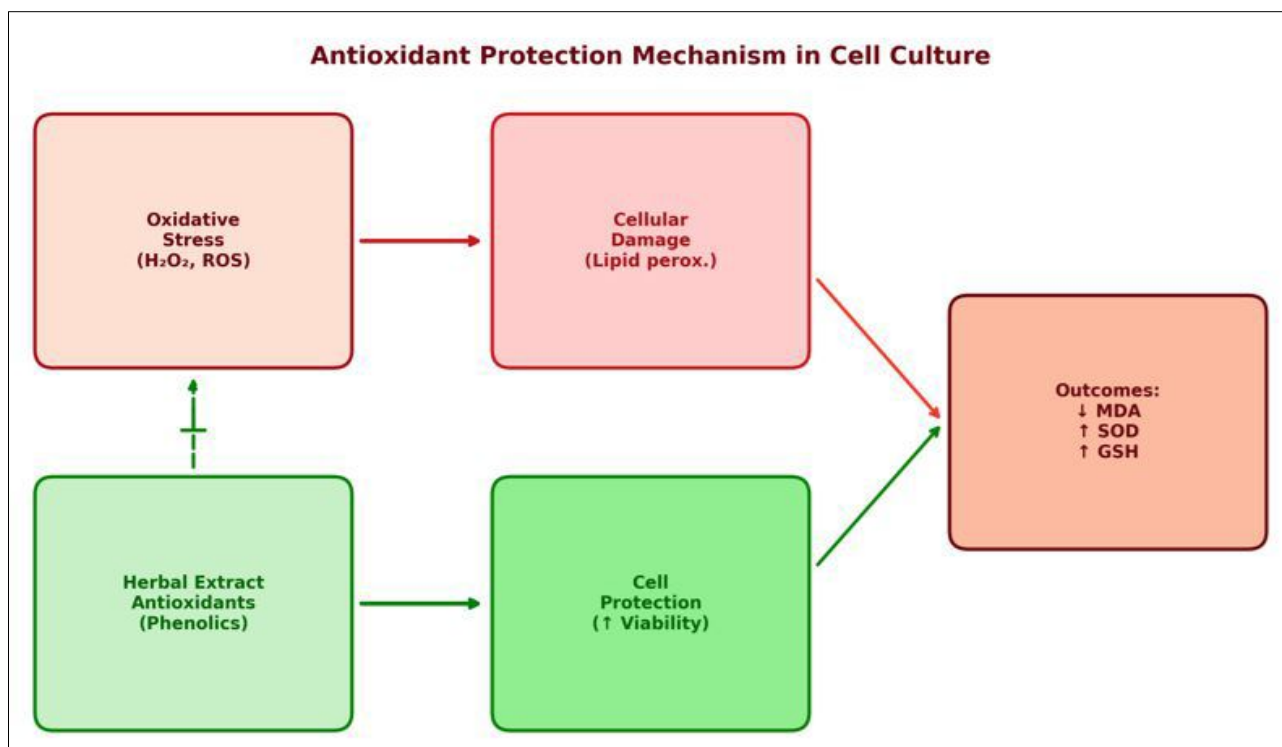


Fig 3: Schematic representation of antioxidant-mediated cytoprotection mechanism in cell culture model

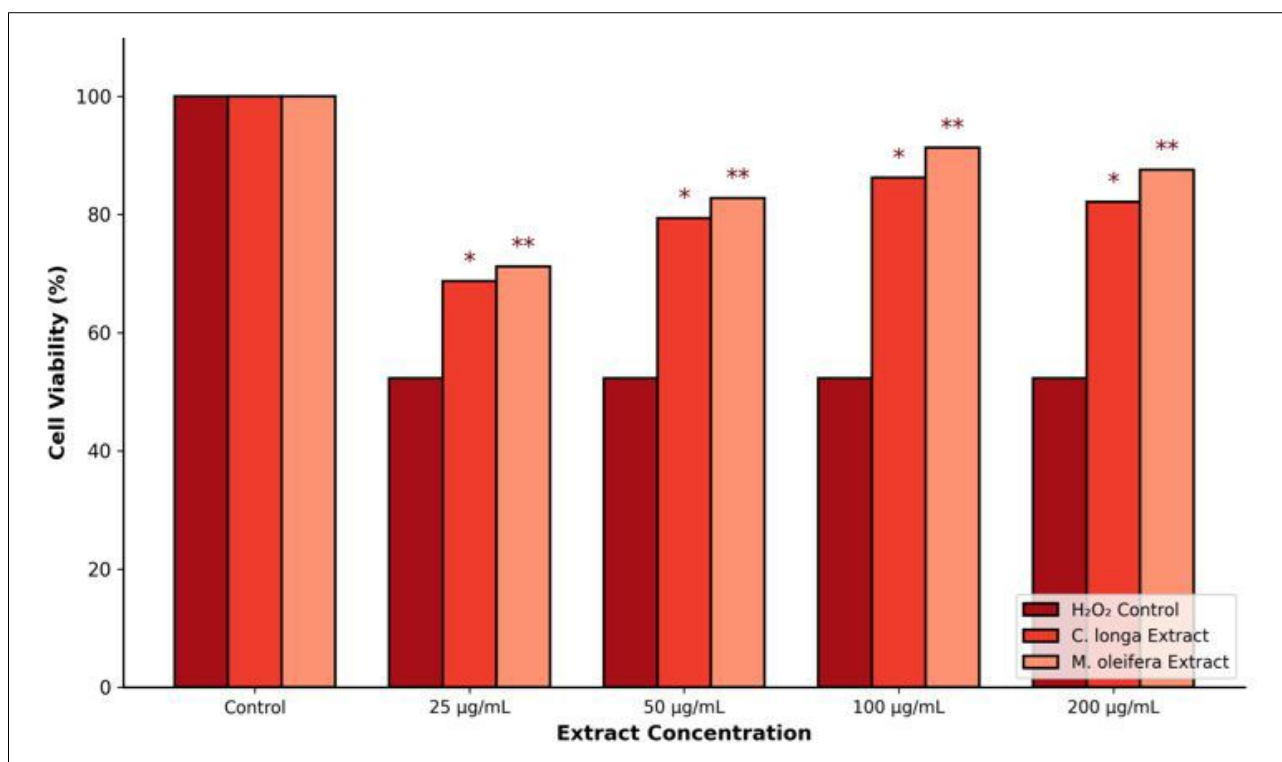


Fig 4: Cytoprotective effects of herbal extracts on hydrogen peroxide-induced oxidative damage in HepG2 cells (* $p < 0.05$, ** $p < 0.01$ vs H₂O₂ control)

Comprehensive Interpretation

Cell culture experiments demonstrated concentration-dependent cytoprotective effects of herbal extracts against hydrogen peroxide-induced oxidative damage (Figure 4). Pretreatment with *Moringa oleifera* extract at 100 µg/mL maintained cell viability at 91.3% compared to 52.3% in cells treated with hydrogen peroxide alone ($p < 0.001$). *Curcuma longa* extract showed comparable protection with 86.2% viability at the same concentration. The protective effects plateaued or slightly decreased at 200 µg/mL,

suggesting optimal concentration ranges for therapeutic application.

Discussion

The findings of this investigation demonstrate substantial antioxidant potential among Indonesian medicinal plants with *Moringa oleifera* exhibiting superior activity across multiple assay systems. The strong correlation between phenolic content and radical scavenging capacity aligns with established structure-activity relationships for plant

polyphenols^[7-9]. The presence of quercetin, kaempferol, and chlorogenic acid derivatives in *Moringa* leaves, as reported in phytochemical literature, likely contributes to the observed effects.

The cytoprotective effects demonstrated in HepG2 cell culture provide evidence for biological relevance beyond chemical assays alone^[10]. The dose-dependent protection against hydrogen peroxide damage suggests that phenolic antioxidants can access intracellular compartments and neutralize reactive species generated during oxidative stress. The slight decrease in protection at highest concentrations may reflect pro-oxidant effects that can occur when antioxidant compounds exceed optimal concentrations^[11].

These results support traditional use of these plants in Indonesian herbal medicine and provide scientific foundation for standardized extract development. The variation in antioxidant profiles among species offers opportunities for formulating combination preparations with complementary mechanisms^[12-14].

Limitations

Several limitations should be considered when interpreting these findings. The research employed crude ethanolic extracts, and the specific bioactive compounds responsible for observed effects were not isolated or characterized. The cell culture model, while useful for screening, does not account for pharmacokinetic factors including absorption, metabolism, and tissue distribution that influence *in vivo* efficacy. Additionally, variations in plant material due to geographic origin, harvest season, and processing conditions may affect reproducibility of results.

Conclusion

This research systematically evaluated the antioxidant potential of six Indonesian medicinal plant extracts using complementary chemical and biological assays. *Moringa oleifera* demonstrated the highest antioxidant capacity with DPPH IC₅₀ of 12.8 µg/mL and total phenolic content of 189.2 mg GAE/g. Strong correlation between phenolic content and radical scavenging activity confirmed phenolic compounds as primary contributors to antioxidant function. Cell culture experiments provided evidence for biologically relevant cytoprotective effects, with *Moringa* extract maintaining 91.3% cell viability under oxidative stress conditions. The findings support development of standardized herbal preparations from these indigenous plants for therapeutic applications targeting oxidative stress-related conditions.

Future investigations should focus on bioactivity-guided fractionation to identify specific compounds responsible for observed effects and evaluate pharmacokinetic properties relevant to clinical translation. The establishment of quality control parameters based on phenolic content and antioxidant activity will facilitate standardization of herbal products derived from these medicinal plants.

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