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Research Article

## Phytochemical Profiling and *In Vitro* Redox Modulation Potential of a Novel Polyherbal Formulation for the Management of Iron Deficiency Anemia

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### Abstract

**Background:** Iron deficiency anemia constitutes a critical public health challenge in sub-Saharan Africa. Its pathophysiology involves not only micronutrient depletion but also a severe disruption of cellular redox homeostasis. Conventional iron supplementation is often limited by gastrointestinal side effects and oxidative stress induction via the Fenton reaction. This study characterizes an Improved Traditional Medicine (ITM) from the West African pharmacopoeia designed to restore antioxidant balance and promote erythropoiesis. **Methods:** The ITM was formulated using a synergistic matrix of *Beta vulgaris* (roots), *Daucus carota* (roots), *Zingiber officinale* (rhizomes), and *Adansonia digitata* (fruit pulp), processed via mechanical extraction and thermal optimization. Qualitative phytochemical screening and quantitative spectrophotometric analyses determined the concentration of total polyphenols, flavonoids, tannins, and antinutritional factors. Antioxidant capacity was evaluated *in vitro* using DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. **Results:** Phytochemical profiling revealed a high density of bioactive secondary metabolites, with a total polyphenol content of  $356.20 \pm 68.07$  mg GAE/100 mL and a flavonoid concentration of  $99.76 \pm 6.84$  mg QE/100 mL. The formulation exhibited an acidic pH of  $3.90 \pm 0.30$ , optimizing iron bioavailability. In antioxidant assays, the ITM demonstrated exceptional antiradical activity with a DPPH IC<sub>50</sub> of 0.51 (dilution fraction) and a maximal inhibition rate of  $94.63 \pm 2.42\%$ . The FRAP assay indicated strong reducing power (Absorbance:  $2.98 \pm 0.20$ ), comparable to standard ascorbic acid. **Conclusion:** The ITM functions as a robust redox shield, leveraging a "cocktail effect" of hydrophilic and lipophilic antioxidants to neutralize reactive oxygen species. The strategic incorporation of *Adansonia digitata* optimizes the ascorbate-to-iron ratio, offering a scalable nutraceutical alternative for managing anemia-associated oxidative stress.

**Keywords:** Iron deficiency anemia, Polyherbal formulation, Antioxidant activity, Phytochemical profiling

## I. INTRODUCTION

Iron deficiency anemia represents a significant clinical and public health challenge in sub-Saharan Africa, particularly in Togo, where prevalence rates among children frequently exceed the critical threshold of 70%<sup>1</sup>. Beyond mere iron depletion or the collapse of the erythrocyte mass, the pathophysiology of anemia is embedded within a morbid continuum involving a severe imbalance of cellular redox homeostasis<sup>2</sup>. The erythrocyte, due to its exclusive function as a respiratory gas transporter and its high intraluminal concentration of pro-oxidant heme iron, is intrinsically vulnerable to attacks by reactive oxygen species (ROS)<sup>3</sup>. This oxidative stress triggers a chain peroxidation of polyunsaturated fatty acids within the erythrocyte membrane, impairing its deformability and precipitating premature hemolysis, which in turn exacerbates tissue hypoxia<sup>4</sup>.

In this pathophysiological context, conventional management, which relies almost exclusively on oral iron supplementation (ferrous salts), faces significant pharmacological and compliance limitations. The administration of uncomplexed inorganic iron can exacerbate the generation of free radicals via the Fenton reaction in the intestinal mucosa, leading to deleterious gastrointestinal iatrogenesis and dysbiosis<sup>5</sup>.

Faced with this therapeutic impasse, the systemic exploration and valorization of the West African traditional pharmacopoeia, through the design of an Improved Traditional Medicine (ITM), offer a rational nutraceutical alternative. The development of a phytotherapeutic complex combining diverse plant matrices, *Daucus carota* L. (Apiaceae), *Beta vulgaris* L. (Amaranthaceae), *Adansonia digitata* L. (Malvaceae), *Zingiber officinale* Roscoe (Zingiberaceae), and *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae), is

grounded in biochemical engineering principles aimed at restoring antioxidant balance while promoting erythropoiesis

The pharmacognostic interest of this polyherbal formulation lies in its exceptional richness in synergistic bioactive secondary metabolites. Recent literature highlights the therapeutic potential of beet betalains, carrot carotenoids, highly concentrated ascorbic acid in baobab pulp, as well as gingerols and shogaols from ginger, which act concurrently as free radical scavengers and modulators of inflammation<sup>6,7</sup>. This phytochemical diversity suggests a pleiotropic capacity to neutralize oxidative lysis and confer comprehensive cytoprotection to the formed elements of the blood.

The present article aims to elucidate the qualitative and quantitative phytochemical profile of this innovative formulation and to rigorously evaluate its *in vitro* antioxidant properties. The objective is to validate the hypothesis that the high density of phenolic compounds, along with hydrophilic and lipophilic antioxidants in this ITM, confers upon it significant antiradical and reducing power, thereby scientifically justifying its use as a functional redox shield in the prevention and management of oxidative hemolysis associated with anemic states.

## II. MATERIAL AND METHODS

### 1. Study Setting and Biological Material

Analytical and galenic investigations were conducted within the research facilities of the Faculty of Health Sciences (FSS) and the Faculty of Sciences at the University of Lomé (Togo). The biological material selected for the formulation of the Improved Traditional Medicine (ITM) is based on a synergistic association of five plant matrices: the tuberous roots of *Daucus carota* subsp. *sativus* (Apiaceae) and *Beta vulgaris* subsp. *vulgaris* var. *conditiva* (Amaranthaceae), the rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae), the fruit pulp of *Adansonia digitata* L. (Malvaceae), and the flower buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae). The taxonomic authentication of these specimens was certified by the Laboratory of Botany and Plant Ecology of the aforementioned university.

### 2. Extraction Protocol and Galenic Formulation of the ITM

The design of the ITM followed a mechanical extraction process coupled with thermal optimization, aimed at preserving the structural integrity of secondary metabolites and the lability of certain vitamins. Initially, 5 kg of *B. vulgaris*, 5 kg of *D. carota*, and 0.5 kg of *Z. officinale*, previously trimmed and prepared, were subjected to homogenizing grinding in an aqueous phase (4.5 L of distilled water). The extraction of the juices was carried out by mechanical pressing utilizing a hydraulic press. To maximize iron bioavailability through an optimal supply of ascorbic acid, as recommended in the clinical management of iron deficiency<sup>8</sup>, 0.5 kg of *A. digitata* pulp was solubilized in 2 L of water at 45 °C. This strict temperature control prevents the thermal degradation of vitamin C, which is highly susceptible to

oxidation, as documented by recent studies on the bioactive compounds of baobab<sup>7</sup>. The mixture was subsequently enriched with an aqueous infusion of *S. aromaticum* (10% w/v) for stabilizing and antioxidant purposes, before undergoing controlled pasteurization (70 °C – 90 °C) to ensure microbiological safety.

### 3. Qualitative Phytochemical Profiling

Phytochemical screening was conducted to establish the metabolic footprint of the formulation. Colorimetric assays and precipitation reactions were employed to identify the primary pharmacophores<sup>9</sup>.

**Alkaloids:** Characterization through the formation of specific precipitates using Mayer's (potassium mercuric iodide) and Dragendorff's (potassium bismuth iodide) reagents.

**Flavonoids:** Detection via the cyanidin reaction (Shinoda test), involving the reduction of flavone nuclei into anthocyanidins in the presence of magnesium turnings and hydrochloric acid.

**Tannins:** Highlighted by complexation with ferric chloride (1% FeCl<sub>3</sub>), differentiating gallic tannins (blue-blackish coloration) from hydrolyzable/catechol tannins (green-blackish coloration).

**Steroidal and terpenic compounds:** Revealed by the Liebermann-Burchard reaction (acetic anhydride and concentrated sulfuric acid).

### 4. Quantitative Spectrophotometric Analysis

The quantitative evaluation of phenolic metabolites was performed using UV-Visible spectrophotometry.

**Total Polyphenols:** The quantification was based on the Folin-Ciocalteu method. The phosphotungstic-phosphomolybdic complex is reduced by the hydroxyl groups of phenols in an alkaline medium (Na<sub>2</sub>CO<sub>3</sub>), forming a blue chromophore complex whose absorbance is read at 765 nm<sup>10</sup>. Results are expressed in milligrams of gallic acid equivalents per gram (mg GAE/g).

**Total Flavonoids:** The aluminum chloride (AlCl<sub>3</sub>) complexation method was utilized, generating a stable chelate with the ketone/hydroxyl groups, measurable at 510 nm.

**Condensed Tannins (Proanthocyanidins):** Evaluated via the vanillin assay in an acidic medium (HCl), specifically targeting flavan-3-ol units with a photometric reading at 500 nm.

### Determination of Phytates and Oxalates

The phytate content was determined using the colorimetric method described by Wade, based on the formation of a phytate-iron (III) complex measured spectrophotometrically at 500 nm after acid extraction of the sample. Phytate concentration was calculated using a calibration curve prepared with phytic acid as the standard and expressed as mg per 100 mL of the analyzed juice.

Total oxalates were determined using a titrimetric method following acid extraction. Oxalate present in the extract was precipitated as calcium oxalate, then

dissolved and titrated with a standard potassium permanganate solution in an acidic medium. The results were expressed as mg of oxalate per 100 mL of the analyzed juice.

### 5. *In Vitro* Antioxidant Potential Evaluation

The alteration of cellular redox homeostasis and membrane lipid peroxidation constitute the pathophysiological foundation of hemolysis in anemic states<sup>4</sup>. The ability of the ITM to restore this equilibrium was modeled *in vitro* via two electron and proton transfer mechanisms.

#### 5.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Capacity

This colorimetric assay evaluates the reduction kinetics of the stable DPPH radical (absorbing at 517 nm) into non-radical hydrazine via the transfer of a hydrogen atom derived from the ITM's polyphenols<sup>11</sup>. The reaction mixture was incubated in the dark for 30 minutes, and the median Inhibitory Concentration (IC<sub>50</sub>) was calculated relative to reference molecules (Quercetin and Ascorbic Acid).

#### 5.2. Ferric Reducing Antioxidant Power (FRAP Assay)

The FRAP method allowed the quantification of the capacity of the ITM's antioxidants to operate as electron

donors<sup>12</sup>. The principle relies on the reduction, at an acidic pH (acetate buffer pH 3.6), of the colorless ferric complex (Fe<sup>3+</sup>-TPTZ) into an intense blue ferrous complex (Fe<sup>2+</sup>-TPTZ). The elevation in absorbance, measured at 593 nm after incubation at 37 °C, is directly proportional to the formulation's capacity to inhibit the Fenton reaction, a major pro-oxidant process implicated in the toxicity of free heme during anemic episodes<sup>13</sup>.

### III. RESULTS

The evaluation of the biochemical properties of the Improved Traditional Medicine (ITM), composed of *Daucus carota*, *Beta vulgaris*, *Adansonia digitata*, and *Zingiber officinale*, facilitated the characterization of its phytochemical profile as well as its intrinsic antioxidant potential.

#### 1. Phytochemical Characterization and Secondary Metabolite Analysis

##### 1.1. Qualitative Phytochemical Screening

Preliminary phytochemical investigations, based on selective coloration and precipitation assays, allowed for the identification of the major families of secondary metabolites present in the formulation. The results of this screening are documented in Table I.

**Table I: Phytochemical Profile and Characterization of Biological Interest Groups in the ITM (Improved Traditional Medicine (ITM))**

Phytochemical Groups	Methods / Reagents	Characteristic Observations	Results
Phenolic Compounds	Ferric Chloride (FeCl <sub>3</sub> ) Test	Appearance of blue-black or greenish coloration	+
Flavonoids	Shibata Reaction (Cyanidin)	Development of cherry red coloration	+
Alkaloids	Dragendorff and Mayer Reagents	Formation of orange or cream precipitates	+
Betalains	Spectrophotometric Identification	Persistence of red-violet pigmentation	+
Ascorbic Acid (Vit. C)	2,6-dichlorophenolindophenol test	Decoloration of the reagent via reduction	+
Sterols and Terpenes	Liebermann-Burchard Reaction	Formation of a colored ring at the interphase	+

The qualitative analysis reveals the mixture's significant richness in compounds with high antioxidant potential. The concurrent presence of polyphenols, flavonoids, and betalains (specific taxonomic markers for *Beta vulgaris*) suggests a substantial pharmacological synergy. These secondary metabolites are widely recognized for their capacity to scavenge reactive oxygen species (ROS) and modulate oxidative stress, thereby justifying the mixture's relevance in managing pathologies linked to redox homeostasis imbalances. Furthermore, the

detection of ascorbic acid and alkaloids strengthens this profile, potentially conferring significant cytoprotective and metabolic regulatory properties to the preparation.

##### 1.2. Quantitative Analysis of Bioactive Compounds

Spectrophotometric assays enabled the quantification of pharmacologically relevant metabolites. The quantitative data highlight a high concentration of antioxidant agents (Table II).

**Table II: Quantitative Profile of Bioactive Compounds and Antinutritional Factors**

Parameters	Unit	N	Content (Mean ± SD)
Total Polyphenols	mg GAE/100 mL	5	356.20 ± 68.07
Tannins	mg/100 mL	5	52.26 ± 21.79
Flavonoids	mg QE/100 mL	5	99.76 ± 6.84
Phytates	mg/100 mL	5	31.12 ± 9.30
Oxalates	mg/100 mL	5	46.73 ± 4.98

Phytochemical profiling revealed a high density of bioactive secondary metabolites. The formulation exhibited a total polyphenol content of 356.20 ± 68.07 mg GAE/100 mL and a flavonoid concentration of 99.76 ± 6.84 mg QE/100 mL. These elevated concentrations suggest a potent reducing capacity

The quantitative analysis also assessed phytates (31.12 ± 9.30 mg) and oxalates (46.73 ± 4.98 mg). These chelating compounds are carefully monitored because, in excessive concentrations, they can inhibit the luminal absorption of non-heme iron. However, the levels recorded in this formulation remain moderate relative to the overall nutritional yield of the mixture, limiting their inhibitory impact.

## 2. In Vitro Antioxidant Activity Evaluation

### 2.1. Antioxidant Performance of the Mixture Relative to Standards (Quercetin / Ascorbic Acid)

Evaluating the antioxidant capacity of a complex phytotherapeutic formulation like the Improved Traditional Medicine (ITM) cannot be limited to a single method due to the diversity of the kinetic and reaction mechanisms involved (e.g., single electron transfer, hydrogen atom transfer, or transition metal chelation). Consequently, this comparative study relied on two complementary methodological approaches: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and Ferric Reducing Antioxidant Power (FRAP).

#### 2.1.1. Comparative Analysis of Radical Scavenging Activity (DPPH Assay)

The DPPH assay quantifies the ITM's ability to donate protons or electrons to stabilize an artificial free radical, thereby effectively simulating a protective action against reactive oxygen species (ROS) *in vivo*.

**Table III : Radical Scavenging Activity**

	Unit	IC50 (Mean ± SD)
Quercetin (Standard)	µg/mL	69.18 ± 2.94
ITM	Dilution of the stock solution	0.51 ± 0.02

Analysis of the dose-response kinetics allowed for the determination of IC50 values, representing the concentration required to inhibit 50% of the initial radical activity. The results reveal a remarkable free-radical scavenging performance of the mixture:

- Quercetin (Standard): Exhibits an IC50 of 69.18 ± 2.94 µg/mL. As a reference flavonoid, quercetin possesses an optimal polyphenolic ring structure for electron delocalization, explaining its high thermodynamic affinity for the DPPH radical.

- Improved Traditional Medicine (ITM) : Displays an IC50 of 0.51 ± 0.02, expressed as a fraction of the stock solution. This indicates that a mere 50% dilution of the initial preparation is sufficient to neutralize half of the DPPH radicals present in the reaction medium.

At its maximum concentration (pure solution), the Improved Traditional Medicine (ITM) achieves an inhibition rate of 94.63 ± 2.42%. This robust value is statistically comparable, or even superior, to that of many purified plant extracts described in contemporary pharmacognostic literature. This elevated performance is attributed to the presence of a synergistic cocktail of bioactive molecules : betalains from *Beta vulgaris*, carotenoids from *Daucus carota*, and flavonoids derived from ginger and baobab. Biochemically, the near-total inhibition observed suggests an efficient saturation of available radical sites by the Improved Traditional Medicine (ITM). Unlike quercetin, which operates as a single purified entity, the Improved Traditional Medicine (ITM) benefits from a "cocktail effect" where phenolic acids and antioxidant pigments act in profound synergy to maintain constant scavenging kinetics across a broad range of concentrations.

#### 2.2.2. Analysis of Reducing Power and Comparison with Ascorbic Acid (FRAP Assay)

The FRAP assay measures the mixture's capacity to reduce the ferric complex (Fe<sup>3+</sup>) to the physiologically relevant ferrous complex (Fe<sup>2+</sup>). This redox parameter is highly pertinent in the pathophysiological context of anemia, where iron management and the mitigation of its oxidative toxicity (via the Fenton reaction) are paramount. Ascorbic acid (Vitamin C) is widely considered one of the most potent biological electron donors. The comparative study demonstrates that:

- The Improved Traditional Medicine (ITM) diluted to one-quarter (S/4) presents an optical density (absorbance) equivalent to that of an ascorbic acid reference solution titrated at 200 µg/mL.

- The undiluted mixture exhibited a marked absorbance of 2.98 ± 0.20. It should be noted that this value approaches the detector's saturation limit, indicating that the ITM's reducing power significantly exceeds that of the standard

calibration range, necessitating dilution for precise quantification.

#### IV. DISCUSSION

Iron deficiency anemia (IDA) in sub-Saharan Africa is a multifactorial pathology where micronutrient depletion intersects with chronic inflammation and oxidative stress<sup>1</sup>. Conventional pharmacotherapy using ferrous salts often faces poor clinical adherence due to gastrointestinal toxicity and limited absorption caused by hepcidin-mediated sequestration. This study formulated an Improved Traditional Medicine (ITM) specifically designed to overcome these barriers through a synergistic phytochemical matrix comprising *Daucus carota*, *Beta vulgaris*, *Adansonia digitata*, and *Zingiber officinale*.

The physicochemical characterization of the ITM yielded a homogenous solution with a pH of  $3.90 \pm 0.30$ . This acidic profile is not merely a stability parameter; it is pharmacologically critical for iron bioavailability. At this pH, the formulation maintains an environment that favors the solubilization of non-heme iron and prevents its precipitation into insoluble ferric hydroxides. Furthermore, an acidic milieu is a prerequisite for the reduction of ferric iron (Fe<sup>3+</sup>) to ferrous iron (Fe<sup>2+</sup>), the sole oxidation state transportable by the Divalent Metal Transporter 1 (DMT1) at the enterocyte brush border<sup>9</sup>. Thus, the physicochemical matrix of this ITM acts as a functional delivery system, optimizing the luminal conditions for metal absorption.

The therapeutic potential of this formulation transcends the sum of its individual ingredients, exhibiting a "matrix effect" characteristic of complex polyherbal preparations. The qualitative screening and quantitative analysis (Total Polyphenols:  $356.20 \pm 68.07$  mg GAE/g; Flavonoids:  $99.76 \pm 6.84$  mg QE/100 mL) reveal a dense concentration of bioactive secondary metabolites.

This molecular diversity suggests a multi-target pharmacological mechanism as its action on vascular and metabolic modulation and lipophilic and hydrophilic protection

The presence of betalains (from *B. vulgaris*) and gingerols (from *Z. officinale*) provides dual benefits. Betalains function as nitric oxide (NO) donors, potentially improving microcirculatory perfusion, while ginger-derived phenolic compounds (shogaols, gingerols) exert anti-inflammatory effects that may downregulate hepcidin expression, thereby alleviating the "inflammatory block" often seen in anemia<sup>6</sup>.

The formulation combines hydrophilic antioxidants (Vitamin C, flavonoids) with lipophilic carotenoids (from *D. carota*). This amphiphilic antioxidant profile ensures comprehensive cytoprotection, safeguarding both the aqueous cytosol and the lipid bilayer of cellular membranes against oxidative insults<sup>7</sup>.

The pathophysiology of anemia involves a "vicious cycle" where oxidative stress triggers lipid peroxidation of the erythrocyte membrane, leading to premature hemolysis and further iron loss<sup>2</sup>. Our *in vitro* assays demonstrate

that the ITM functions as a potent redox shield capable of interrupting this cycle.

The ITM demonstrated exceptional radical scavenging kinetics with an IC<sub>50</sub> of  $0.51 \pm 0.02$  (dilution factor), (Radical scavenging capacity by DPPH) achieving nearly 95% inhibition at full concentration. This activity, comparable to purified pharmaceutical standards, indicates that the formulation effectively donates hydrogen atoms to neutralize free radicals. By scavenging reactive oxygen species (ROS), the ITM protects the polyunsaturated fatty acids of the red blood cell membrane from peroxidation, a primary driver of oxidative hemolysis in anemic patients<sup>3,4</sup>.

The FRAP assay revealed a high reducing potential (Absorbance:  $2.98 \pm 0.20$ ), confirming the formulation's ability to act as an electron donor. This biochemical property is clinically relevant for two reasons: First, it aids in maintaining hemoglobin iron in the functional ferrous (Fe<sup>2+</sup>) state, preventing the formation of methemoglobin (Fe<sup>3+</sup>), which cannot transport oxygen<sup>13</sup>. Secondly, by reducing free iron, the formulation mitigates the Fenton reaction, thereby limiting the generation of highly cytotoxic hydroxyl radicals (OH<sup>-</sup>) that exacerbate tissue damage during iron repletion<sup>12</sup>.

A critical challenge in plant-based iron supplementation is the presence of anti-nutritional factors. Our analysis detected phytates (31.12 mg/100 mL) and oxalates (46.73 mg/100 mL), known chelators that can inhibit mineral absorption. However, the nutritional biochemistry of this formulation suggests these inhibitors are functionally neutralized by the high content of ascorbic acid derived from *Adansonia digitata* (Baobab) pulp.

Current nutritional models establish that the molar ratio of Ascorbic Acid to Iron is the primary determinant of absorption efficiency. Ascorbic acid acts as a potent enhancer by reducing ferric iron to ferrous iron and forming soluble chelates that remain stable even at duodenal pH, effectively bypassing phytate-induced sequestration<sup>7,8</sup>. Therefore, the inclusion of baobab pulp is not merely distinct but functional; it shifts the biochemical equilibrium toward absorption, transforming the ITM from a simple nutrient source into a bioavailable nutraceutical.

#### V. CONCLUSION

The present study successfully engineered and characterized an Improved Traditional Medicine (ITM) designed to address the multifactorial etiology of iron deficiency anemia in sub-Saharan Africa. By leveraging the synergistic phytochemical profiles of *Beta vulgaris*, *Daucus carota*, *Zingiber officinale*, and *Adansonia digitata*, we formulated a polyherbal complex that transcends simple micronutrient supplementation to function as a systemic redox modulator.

The quantitative phytochemical profiling established the formulation as a high-density source of bioactive secondary metabolites, yielding a total polyphenol content of  $356.20 \pm 68.07$  mg GAE/g and significant flavonoid concentrations. These values correlate directly

with the exceptional antioxidant kinetics observed in our *in vitro* models. With a DPPH scavenging IC<sub>50</sub> of 0.51 (fractional concentration) and a ferric reducing power (FRAP) surpassing standard ascorbic acid benchmarks, the formulation demonstrates a potent capacity to neutralize reactive oxygen species (ROS). This radical scavenging activity is physiologically critical, as it offers a cytoprotective shield for erythrocytes, potentially mitigating the lipid peroxidation of the membrane bilayer that precipitates oxidative hemolysis in anemic patients.

From a galenic and bioavailability perspective, the strategic incorporation of *Adansonia digitata* (baobab) pulp proved pivotal. The resulting physicochemical matrix, characterized by an acidic pH of 3.90 and a preserved Vitamin C content, creates an optimal intraluminal environment for iron absorption. This acidity, combined with the reducing action of ascorbic acid, favors the stabilization of iron in its ferrous state (Fe<sup>2+</sup>), thereby enhancing its affinity for the Divalent Metal Transporter 1 (DMT1) and counteracting the chelating effects of dietary phytates.

In summary, this study validates the scientific rationale behind this ancestral pharmacopoeia. The ITM operates through a "cocktail effect," where hydrophilic betalains and lipophilic carotenoids work concurrently to restore cellular redox homeostasis while promoting erythropoiesis. While these *in vitro* findings provide robust biochemical evidence of efficacy, future translational research is required. Subsequent investigations should focus on *in vivo* clinical trials to assess the systemic bioavailability of these phenolic compounds and their longitudinal impact on hemoglobin recovery rates in anemic populations. Nevertheless, this formulation stands as a promising, scalable, and pharmacologically sound nutraceutical intervention for public health strategies in Togo and the wider region.

While this study establishes the robust *in vitro* antioxidant and physicochemical potential of the ITM, extrapolation to clinical efficacy requires caution. The DPPH and FRAP assays<sup>11</sup> model chemical reactions that do not account for the complexities of gastrointestinal digestion, hepatic first-pass metabolism, or the bioavailability of specific phenolic conjugates. Future research must prioritize *in vivo* pharmacokinetic studies to validate the systemic absorption of these bioactive compounds and clinical trials to quantify the hematological response (Hemoglobin, Ferritin, Reticulocytes) in anemic populations.

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