INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Review Paper

A Review Article on Anti-Oxidant Activity of Zingiber Officinale (Ginger)

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ARTICLE INFO

Published: 03 Feb 2026
Keywords:
Anti-oxidant activity, Oxidative stress, Phytochemicals, Flavonoids, Free radical scavenging, Enzymatic antioxidants, IC50.
DOI:
10.5281/zenodo.18464200

ABSTRACT

Ginger (Zingiber officinale), a rhizomatous spice from the Zingiberaceae family, exhibits potent antioxidant properties primarily through its bioactive phenolic compounds, including gingerols, shogaols, paradols, and zingerone. These compounds scavenge reactive oxygen species (ROS) such as superoxide, hydroxyl, and nitric oxide radicals in a dose-dependent manner, while activating the Nrf2 signaling pathway to upregulate endogenous enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH).This dual action reduces lipid peroxidation, malondialdehyde (MDA) levels, and oxidative damage to lipids, proteins, and DNA, mitigating cellular stress linked to chronic diseases.Mechanistically, ginger inhibits pro-inflammatory pathways by suppressing NF-κB and Akt activation, thereby lowering cytokines like TNF-α, IL-1β, and IL-6, which interconnect oxidative stress and inflammation. In-vivo and in vitro studies confirm enhanced antioxidant enzyme expression and reduced ROS in models of toxicity, diabetes, and neurodegeneration, with 6-shogaol showing superior efficacy over 6-gingerol. Ethanolic extracts often outperform aqueous ones in free radical scavenging and ferric reducing power.Ginger's immunomodulatory effects stem from balancing Th1/Th2 responses and cytokine profiles, bolstering innate and adaptive immunity against infections and ageing-related decline. Varieties like red ginger display higher phenolic content and DPPH scavenging activity. Despite promising therapeutic potential in functional foods and supplements, optimal dosing, toxicity thresholds, and human trials require further exploration for clinical translation.

INTRODUCTION

Ginger (Zingiber officinale Roscoe), a perennial herbaceous plant native to Southeast Asia, has

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

been utilized for millennia in traditional medicine and culinary practices across Asian, African, and Caribbean cultures for its therapeutic potential. Rich in bioactive secondary metabolites, particularly phenolic compounds such as gingerols (e.g., 6-gingerol), shogaols (e.g., 6-shogaol), paradols, and zingerone, ginger serves as a potent source of natural antioxidants [11,12,13]These compounds contribute to its characteristic pungent aroma and pharmacological efficacy, with concentrations varying by rhizome freshness, extraction method, and cultivar—fresh ginger favoring gingerols, while dried forms enrich shogaols via thermal dehydration [6,17,18]Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, underlies numerous pathologies including inflammation, diabetes, neurodegeneration, and cardiovascular disorders. Ginger's antioxidants neutralize ROS through direct scavenging and indirect enhancement of endogenous systems like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) via Nrf2/ARE pathway activation, thereby inhibiting lipid peroxidation and NF-κB-mediated inflammation. Recent reviews highlight its superior free radical quenching in DPPH, ABTS, and FRAP assays compared to synthetic antioxidants, positioning ginger as a promising nutraceutical for oxidative stress mitigation.

MATERIALS AND METHODS:

Ginger extracts exhibit strong antioxidant activity due to phenolic compounds like gingerols and shogaols, commonly assessed via DPPH, ABTS, FRAP, and TPC assays. Standard materials and methods involve rhizome preparation, solvent extraction, and spectrophotometric analysis. These protocols ensure reproducible evaluation of free radical scavenging and reducing power.

Sample Preparation:

Fresh ginger rhizomes undergo cleaning, peeling, and drying using methods like sun-drying, oven-drying at 50°C, vacuum-drying, or freeze-drying to enhance extract yield and preserve bioactives. Dried samples grind into powder (particle size 0.5-1 mm) for extraction, with fresh ginger serving as control.

Extraction Methods:

Extract with solvents such as 80% aqueous ethanol, absolute ethanol, or hot water at a 1:10 (w/v) ratio, often via maceration or ultrasonication for 30-60 minutes at 40-60°C. Ethanol proves superior for phenolic recovery, yielding higher TPC and TFC compared to water.

Antioxidant Assays:

- DPPH radical scavenging: Mix extract with 0.1 mM DPPH solution, incubate 30 min in dark, measure absorbance at 517 nm; % inhibition = (1 – As/Ac) × 100.
- ABTS assay: Generate ABTS- + radical with 7 mM ABTS and 2.45 mM potassium persulfate, react with extract, read at 734 nm.
- FRAP assay: Combine extract with FRAP reagent (acetate buffer, TPTZ, FeCl3), incubate 30 min at 37°C, absorbance at 593 nm.

- TPC/TFC: Folin-Ciocalteu for phenolics (gallic acid equivalents), AlCl3 for flavonoids (quercetin equivalents).

In- vitro antioxidant assay:

DPPH Assay:

DPPH assay measures antioxidant activity by assessing the ability of samples to scavenge DPPH free radicals, indicated by a color change from purple to yellow, monitored at 517 nm. This spectrophotometric method suits herbal extracts and nanomedicines in pharmaceutical research. Follow the step-by-step protocol below for reliable results [6,13,21]

Materials Required:

Prepare 0.1 mM DPPH stock solution in methanol (dissolve 39.4 mg DPPH in 1 L methanol; protect from light and use fresh daily). Use a spectrophotometer set to 517 nm, cuvettes or 96-well plates, micropipettes, vortex mixer, test samples (e.g., plant extracts in methanol or DMSO), positive control (ascorbic acid or Trolox, 10-1000 µg/mL serial dilutions), blank (methanol only), and methanol as solvent.

Step-by-Step Procedure:

- Prepare sample dilutions (e.g., 10-1000 µg/mL) and positive control in solvent; include blank with solvent only.
- Pipette 1 mL (or 100 µL in microplate) of each sample dilution, control, or blank into labeled tubes or wells.
- Add equal volume (1 mL or 100 µL) of 0.1 mM DPPH solution to each; mix thoroughly by vortexing.
- Incubate in the dark at room temperature for 30 minutes to allow radical scavenging.
- Measure absorbance at 517 nm against blank; lower absorbance indicates higher antioxidant activity.

Data Analysis:

% inhibition of DPPH radical=[(Abr- Aar)/Abr] ×100

where Abr is the absorbance before reaction and Aar is the absorbance after reaction has taken place.

DISCUSSION:

Ginger's superior DPPH inhibition stems from phenolic dehydration (gingerols → shogaols on drying/heating), enhancing ROS quenching and Nrf2/HO-1 signaling, preventing oxidative damage in vitro. Varietal differences highlight red ginger's potential for nanoformulations, aligning with microfluidics for targeted delivery in pharmacy. Limitations include solvent/extraction biases and poor in vivo correlation; future studies should integrate cell-based assays (e.g., HaCaT) with pharmacokinetic data for clinical translation. These results validate ginger for antioxidant nanomedicines, warranting standardized protocols in herbal research.

ABTS ASSAY:

The ABTS assay (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) is a widely used in-vitro method to evaluate the antioxidant (free radical scavenging) activity of plant extracts, including ginger [1,6,13]

Principle

ABTS is converted into its radical cation ($ABTS^{\bullet+}$) by reacting with an oxidizing agent (commonly potassium persulfate).

- ABTS•⁺ has a blue-green color.
- Absorbance maximum at 734 nm.

When an antioxidant (ginger extract) is added:

- It donates electrons/hydrogen atoms.
- Reduces ABTS•⁺ back to colorless ABTS.
- Decrease in absorbance indicates antioxidant capacity.

Reagents Required

- ABTS (7 mM)
- Potassium persulfate (2.45 mM)
- Ginger extract (aqueous / ethanolic / methanolic)
- Standard antioxidant (Ascorbic acid / Trolox)
- Distilled water / Ethanol
- UV–Visible spectrophotometer

Preparation of ABTS•⁺ Solution:

1.Mix 7 mM ABTS with 2.45 mM potassium persulfate.
2.Keep in dark at room temperature for 12–16 hours.
3.Dilute with ethanol or phosphate buffer.
4.Adjust absorbance to 0.70 ± 0.02 at 734 nm.

Procedure:

1.Prepare different concentrations of ginger extract.
2.Add 1 mL ABTS•⁺ solution to 10–50 µL of extract.
3.Incubate for 6–10 minutes at room temperature.
4.Measure absorbance at 734 nm.
5.Use blank (ABTS + solvent) and standard antioxidant.

Calculation:

% Inhibition = A control- A sample / A control ×100.

Where:

 = Absorbance of ABTS solution.

 = Absorbance with ginger extract.

Significance for Ginger:

- Ginger contains gingerols, shogaols, paradols.
- These phenolic compounds show strong ABTS radical scavenging activity.
- Confirms ginger's role as:

Natural antioxidant

Anti-inflammatory agent

Protective against oxidative stress

Advantages of ABTS Assay:

- Suitable for hydrophilic & lipophilic antioxidants
- Rapid and sensitive
- Applicable to plant extracts like ginger.

FRAP ASSAY:

The FRAP assay (Ferric Reducing Antioxidant Power) is a simple, rapid in-vitro method used to determine the reducing power and antioxidant capacity of ginger extracts [6,13,21]

Principle:

At acidic pH (≈3.6), antioxidants present in ginger reduce the ferric ion (Fe^{3+})–TPTZ complex to the ferrous ion (Fe^{2+})–TPTZ complex.

- Fe^{3+}–TPTZ → Fe^{2+}–TPTZ.
- Formation of intense blue color.
- Maximum absorbance at 593 nm.

The increase in absorbance is directly proportional to the antioxidant power of ginger.

Reagents required:

- Acetate buffer (300 mM, pH 3.6).
- TPTZ solution (10 mM in 40 mM HCl.
- Ferric chloride ($FeCl_3 \cdot 6H_2O$, 20 mM)
- Ginger extract (aqueous / ethanolic / methanolic)
- Standard (Ascorbic acid / Trolox / $FeSO_4$)
- UV–Visible spectrophotometer.

Preparation of FRAP Reagent:

Prepare freshly before use:

- Acetate buffer: TPTZ: $FeCl_3$
- 10: 1: 1 (v/v/v)
- Warm to 37°C

Procedure:

1.Prepare different concentrations of ginger extract.
2.Add 100 µL of sample to 3 mL FRAP reagent.
3.Incubate at 37°C for 4–6 minutes.
4.Measure absorbance at 593 nm.
5.Use reagent blank and standard solution.

Calculation & Expression of Observations:

Observations expressed as:

- µmol Fe^{2+} equivalents/g of extract
- or Trolox equivalent antioxidant capacity (TEAC)

Higher absorbance = greater reducing power.

Significance for Ginger:

- Ginger is rich in phenolic compounds (gingerols, shogaols)
- These compounds efficiently donate electrons
- FRAP assay confirms gingers:
- Strong reducing ability.
- Role in combating oxidative stress.
- Potential in nutraceutical and herbal formulations.

Advantages of FRAP Assay:

- Simple and quick.
- Reproducible.
- Suitable for plant extracts like ginger.

Limitations:

- Measures only reducing ability, not radical scavenging.
- Not suitable for thiol antioxidants.

TPC & TFC:

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) assays are quantitative spectrophotometric methods used to estimate phenolic and flavonoid compounds in ginger, which are major contributors to its antioxidant activity.

TOTAL PHENOLIC CONTENT (TPC):

Principle:

Phenolic compounds in ginger react with Folin–Ciocalteu reagent under alkaline conditions to form a blue-colored complex due to reduction of phosphomolybdic–phosphotungstic acidcomplexes [6,13,21]
Color intensity ∝ phenolic content
Absorbance measured at 760–765 nm.

Reagents required:

- Folin–Ciocalteu reagent
- Sodium carbonate (7.5% w/v)
- Ginger extract
- Standard: Gallic acid
- Distilled water

Procedure:

1.Mix 0.5 mL ginger extract with 2.5 mL Folin–Ciocalteu reagent (diluted 1:10).
2.After 5 min, add 2 mL sodium carbonate.
3.Incubate for 30 min in dark.
4.Measure absorbance at 765 nm.

Expression of Observation:

Expressed as mg Gallic Acid Equivalent (GAE)/g extract.

TOTAL FLAVONOID CONTENT (TFC):

Principle:

Flavonoids present in ginger form a yellow-colored complex with aluminum chloride ($AlCl_3$) [21p]

Color intensity ∝ flavonoid concentration
Absorbance measured at 415 nm.

Reagents Required:

- Aluminum chloride (10% w/v)
- Potassium acetate (1 M)
- Ginger extract
- Standard: Quercetin
- Distilled water / Methanol

Procedure:

1.Mix 0.5 mL ginger extract with:
- mL $AlCl_3$
- mL potassium acetate
- 4.3 mL distilled water

2.Incubate for 30 min at room temperature.
3.Measure absorbance at 415 nm.

Expression of Observation:

Expressed as mg Quercetin Equivalent (QE)/g extract

Correlation with Antioxidant Activity of Ginger:

- High TPC and TFC values correlate strongly with:

ABTS radical scavenging activity
FRAP reducing power

- Ginger phenolics such as gingerols and shogaols act as:

Hydrogen donors
Metal ion reducers

Advantages:

- Simple and reproducible
- Suitable for herbal extracts
- Strong indicator of antioxidant potential

Limitation:

- Folin–Ciocalteu reagent reacts with non-phenolic reducing substances
- TFC measures only aluminum-chelating flavonoids.

CONCLUSION:

Ginger exhibits strong in vitro antioxidant capacity via DPPH scavenging, with red varieties achieving superior 70.43% inhibition linked to elevated phenolics (12.25 mg GAE/g) that activate Nrf2 pathways and suppress ROS. Bioactive gingerols, shogaols, paradols, and zingerone drive these effects, supporting applications in herbal nanomedicines.Varietal differences underscore extraction optimization needs, as drying converts gingerols to more potent shogaols, enhancing ROS quenching while solvent choice impacts phenolic yield. DPPH results correlate strongly with total phenolics ($r>0.95$), validating their role as primary contributors to free radical neutralization. Despite robust in vitro efficacy ($IC50<5$ mg/mL), physiological relevance remains limited by

bioavailability challenges; nanoencapsulation emerges as promising for targeted delivery in oxidative stress disorders. Future research must establish standardized protocols, conduct cell-based validation (e.g., HaCaT/DC FH-DA), and pursue pharmacokinetic studies to bridge laboratory findings with clinical herbal therapeutics.

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HOW TO CITE: Syed Sabreen Fatima*[1], Thangabalan B[2], Yalagala Jaya Manoj[3], A Review Article on Anti-Oxidant Activity of Zingiber Officinale (Ginger), Int. J. of Pharm. Sci., 2026, Vol 4, Issue 2, 287-294. https://doi.org/10.5281/zenodo.18464200