

**PHYTOCHEMICAL COMPOSITION AND IN-VITRO ANTIOXIDANT ACTIVITY
OF GINGER EXTRACT ENCAPSULATED IN CHITOSAN HYDROGEL**

BY

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CERTIFICATION

This is to certify that the research project have been conducted by ALIYU, Aisha Abdulmalik (2010304040) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc) degree in Biochemistry of Usmanu Danfodiyo University, Sokoto.

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Dedication

This work is lovingly dedicated to my beloved parents.

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Abstract

Medicinal plants have attracted scientific interest because they contain phytochemicals that show antioxidant, antimicrobial, anti-inflammatory, and chemoprotective properties. Ginger, the rhizome of *Zingiber officinale*, is widely consumed as a culinary spice and valued in traditional medicine. This study investigated the phytochemical composition and in vitro antioxidant activity of methanol ginger extracts (*Zingiber officinale*) and evaluated how encapsulation in a chitosan hydrogel influences its antioxidant performance. Fresh ginger rhizomes were cleaned, dried, pulverized, and extracted with methanol before encapsulation using a chitosan hydrogel prepared with glycerin, acetic acid, and sodium hydroxide. Antioxidant activity was assessed using DPPH and FRAP assays. Qualitative phytochemical screening revealed the presence of alkaloids, steroids, glycosides, and saponin glycosides. Quantitative assays showed high levels of alkaloids (233 mg/g) and saponins (216 mg/g), confirming that ginger is rich in bioactive compounds associated with antioxidant activity. The result of DPPH at 20mg/ml of loaded chitosan hydrogel and ginger extract were significantly higher when compared with ascorbic acid. Furthermore, at higher concentration 2.0mg/ml The Ferric reducing power of ginger extract was significantly higher compared to loaded chitosan hydrogel and naive groups. Overall, the results suggest that chitosan loaded with ginger extract is a promoted strategy as phytochemical delivery system.

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CHAPTER ONE

1.1 Introduction

Natural products remain central to the discovery of bioactive compounds with therapeutic potential. Among these, medicinal plants have attracted scientific interest because they contain phytochemicals that show antioxidant, antimicrobial, anti-inflammatory, and chemoprotective properties (Ogunyemi *et al.*, 2021). Ginger, the rhizome of *Zingiber officinale*, is widely consumed as a culinary spice and valued in traditional medicine. It contains an array of secondary metabolites such as gingerols, shogaols, paradols, flavonoids, tannins, alkaloids, saponins, and diverse glycosides that contribute to its pharmacological activities (Srinivasan, 2020). These constituents are known for their ability to scavenge free radicals and reduce oxidative stress, a major contributor to chronic diseases.

Oxidative stress arises when the production of reactive oxygen species outweighs the capacity of endogenous antioxidant defenses. This imbalance is implicated in cardiovascular disorders, neurodegeneration, diabetes, and cancer (Birben *et al.*, 2019). Plant antioxidants offer a natural approach to disrupting this imbalance. However, many phytochemicals are unstable, poorly soluble, and prone to degradation under environmental or physiological conditions. These challenges reduce their bioavailability and therapeutic efficiency.

Encapsulation offers a promising strategy to enhance the stability and functionality of plant-derived antioxidants. Chitosan, a biopolymer obtained from chitin, has gained attention for encapsulation due to its biocompatibility, biodegradability, mild gelation conditions, and ability to form hydrogels suitable for controlled release (Ahmed & Ikram, 2017). Hydrogels allow the entrapment of sensitive compounds in a hydrated, three-dimensional matrix that protects them from oxidation and improves their sustained release profile.

Creating chitosan hydrogels by dissolving chitosan in acetic acid, incorporating glycerin as a plasticizer, and inducing gelation with sodium hydroxide provides a simple and effective system for loading plant extracts. Encapsulating ginger extract in this matrix may enhance the stability of its phytochemicals and improve its antioxidant performance. Understanding how ginger behaves within such a hydrogel system is important for advancing its potential applications in food preservation, drug delivery, and biomedical formulations.

This study evaluates the phytochemical composition and antioxidant activity of ginger extract when encapsulated in a chitosan hydrogel. Methanol extraction is used to obtain the active constituents of ginger, followed by qualitative and quantitative phytochemical assessment. Encapsulation is then carried out and the antioxidant properties are tested using established in vitro assays. By comparing the free extract with the encapsulated form, the study seeks to determine whether hydrogel incorporation improves or alters antioxidant capacity.

1.2 Statement of the Problem

Despite the recognized antioxidant potential of ginger phytochemicals, their application is limited by low stability and susceptibility to degradation during storage or under physiological conditions (Ali *et al.*, 2008). Many of the bioactive components degrade rapidly when exposed to light, heat, or oxygen, reducing their potency. This limits their usefulness in pharmaceutical or nutraceutical formulations where controlled release and stability are crucial. Encapsulation in chitosan hydrogels may offer a viable solution, but studies evaluating how this affects the phytochemical profile and antioxidant activity remain limited, particularly within local research contexts (Dash *et al.*, 2011). There is a need for a systematic assessment to determine whether encapsulation improves the functional properties of ginger extract.

1.5 Justification of the Study

The growing interest in natural antioxidants has increased demand for delivery systems that improve their stability and effectiveness. Natural plants such as Ginger (*Zingiber officinale*) are rich in bioactive compounds such as gingerols and shogaols, which exhibit strong antioxidant activity. However, the practical application of ginger extract is limited by its poor stability and low bioavailability, as these compounds are sensitive to environmental and physiological conditions (Szymczak *et al.*, 2025; Jalali-Jivan *et al.*, 2025). To address these limitations, recent research has focused on developing efficient delivery systems that enhance the stability and controlled release of bioactive compounds. Chitosan hydrogels have emerged as promising carriers due to their biodegradability, biocompatibility, and ability to encapsulate phenolic compounds, thereby improving their functional performance (Blebea *et al.*, 2025). Despite these advantages, limited studies have explored the encapsulation of ginger extract specifically within chitosan hydrogel systems. Therefore, the study contributes to ongoing research on plant-based antioxidants, providing insight into how hydrogel encapsulation may enhance their performance.

1.4 Aim and Objectives of the Study

1.4.1 Aim

To assess the phytochemical composition and *in vitro* antioxidant activity of ginger extract encapsulated in a chitosan hydrogel matrix.

1.4.2 Objectives

1. Determine the qualitative and quantitative phytochemicals present in the methanol extract of ginger.

2. Evaluate the antioxidant activity of the free and encapsulated extracts using DPPH and FRAP assays.

CHAPTER TWO

LITERATURE REVIEW

2.1 Theoretical Background

The study of phytochemicals and their biological properties is grounded in well-established theories that explain how plants synthesize secondary metabolites and how these compounds interact with biological systems. Phytochemicals are non-nutritive plant metabolites produced through pathways such as the shikimate, acetate-malonate, and mevalonate pathways. These pathways generate phenolics alkaloids, terpenoids, tannins, and glycosides, many of which function as defense mechanisms against environmental stressors including pathogens, ultraviolet radiation, and oxidative stress (Pandey and Rizvi, 2020). Their antioxidant properties arise from their ability to donate electrons or hydrogen atoms to neutralize free radicals.

Free radical theory provides the scientific basis for understanding antioxidant behavior. Reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide are generated during normal cellular metabolism, but their accumulation leads to oxidative stress. According to the oxidative stress theory, the imbalance between oxidants and antioxidants contributes to chronic diseases including diabetes, neurodegenerative disorders, and cancer (Birben *et al.*, 2019). Antioxidants counteract this process through radical scavenging, metal chelation, or enhancing endogenous antioxidant enzymes.

The concept of encapsulation draws from polymer science and controlled-release theory. Chitosan, a cationic polysaccharide derived from chitin, possesses amino groups that become protonated in acidic environments, allowing it to dissolve in acids such as acetic acid. When the pH is increased, chitosan chains undergo crosslinking or gelation, forming a hydrogel network capable of entrapping bioactive molecules. This network restricts diffusion and

protects encapsulated compounds from degradation. The hydrogel structure is stabilized by hydrogen bonding, hydrophobic interactions, and electrostatic forces (Ahmed and Ikram, 2017).

The behavior of encapsulated phytochemicals is influenced by diffusion theory and polymer–solute interactions. Encapsulation modifies the release profile of the active compounds and can enhance their stability and solubility. This principle forms the basis for using hydrogels in drug delivery, food preservation, and tissue engineering. The antioxidant activity of encapsulated extracts is informed by the interaction between the hydrogel matrix and radical scavenging mechanisms. The DPPH assay relies on electron donation to reduce the DPPH radical, while the FRAP assay measures the reduction of ferric ions to ferrous ions, indicating the reducing power of a compound (Benzie and Strain, 1996). Both assays offer insight into the functional performance of free versus encapsulated antioxidants.

2.2 Ginger: Botanical Profile, Phytochemical Composition, and Pharmacological Activities

Ginger (*Zingiber officinale* Roscoe) is a perennial herb belonging to the Zingiberaceae family. It is a cone-shaped floral spike that has pale yellow and purplish colour (fig 1), and it is widely cultivated across tropical and subtropical regions and has a long history of use as a culinary spice and medicinal agent. The rhizome, which is the primary part used, consists of aromatic, fleshy underground stems characterized by a sharp flavor and distinctive scent. Morphologically, the plant grows up to one meter in height, with narrow leaves and yellow-green flowers. Its adaptability and resilience have contributed to its widespread availability and continuous use in traditional medicine (Yemele *et al.*, 2020).

The phytochemical composition of ginger is diverse. Its major bioactive compounds include phenolic constituents such as gingerols, shogaols, paradols, and zingerone. Among these, 6-gingerol and 6-shogaol are considered the primary contributors to its antioxidant and anti-

inflammatory activities (Srinivasan, 2020). Ginger also contains flavonoids, tannins, alkaloids, saponins, and various glycosides, which collectively enhance its pharmacological value. The presence of both polar and non-polar compounds accounts for the efficacy of different extraction solvents, with methanol frequently reported as one of the most effective solvents for extracting phenolics and other phytochemicals (Olatunde *et al.*, 2021).



Figure 1: Ginger Rhizome (Edo *et al.*, 2025)

Several studies have examined the antioxidant properties of ginger. Phenolic compounds in ginger act as electron or hydrogen donors, neutralizing reactive oxygen species and preventing oxidative damage. These compounds also possess metal-chelating abilities, which reduce the catalytic activity of transition metals involved in radical generation. Recent investigations show that ginger extract significantly reduces oxidative stress markers and enhances antioxidant enzyme activities *in vitro* and *in vivo* (Akinyemi *et al.*, 2022). The rich phytochemical profile makes ginger a promising candidate for developing natural antioxidant formulations.

Beyond antioxidant activity, ginger exhibits anti-inflammatory, antimicrobial, antidiabetic, anticancer, and gastroprotective effects. These activities are attributed to its ability to modulate signaling pathways such as NF- κ B, COX-2, and MAPK, which play central roles in inflammation and oxidative stress (Rahman et al., 2022). Gingerols and shogaols have demonstrated strong antimicrobial activity against bacteria, fungi, and some parasites, while also showing cytotoxic and antiproliferative effects against cancer cell lines.

Recent advances in food science and pharmaceutical formulations have explored incorporating ginger extract into delivery systems that enhance its stability and bioavailability. Studies published between 2020 and 2024 highlight the benefits of encapsulating ginger in micro- and nano-sized carriers, including polymeric matrices, liposomes, and nanoparticles, which help preserve its phenolic compounds and improve its controlled release (Farooq *et al.*, 2023). These findings support the rationale for exploring hydrogel encapsulation as a means of improving the antioxidant performance of ginger extract.

2.3 Chitosan and Hydrogels: Structure, Properties, and Applications

Chitosan is a linear polysaccharide derived from the partial deacetylation of chitin, which is the second most abundant biopolymer in nature after cellulose. It is primarily obtained from the exoskeletons of crustaceans such as shrimp and crabs, although fungal sources have also been identified. Structurally, chitosan consists of β -(1 $\rightarrow$ 4)-linked D-glucosamine and N-acetyl-D-glucosamine units. The proportion of these units determines the degree of deacetylation, which in turn influences solubility, viscosity, charge distribution, and biological activity (Badawy & Rabea, 2020). Chitosan is insoluble in water at neutral pH but dissolves easily in dilute organic acids like acetic acid because the amino groups in its backbone become protonated.

Hydrogels are three-dimensional, crosslinked polymer networks capable of absorbing large amounts of water without dissolving. When chitosan is dissolved in acetic acid and subsequently neutralized with a base such as sodium hydroxide, it undergoes gelation. The gelation process results from reduced protonation of amino groups, enhanced hydrogen bonding, and increased polymer–polymer interactions that cause the chains to aggregate into a stable hydrogel matrix. Glycerin, often added as a plasticizer, improves flexibility, moisture retention, and uniformity of the hydrogel structure (Ahmed and Ikram, 2017). The resulting material is soft, highly hydrated, biocompatible, and biodegradable.

Chitosan hydrogels exhibit several functional properties that support their use in biomedical and pharmaceutical applications. They possess intrinsic antimicrobial activity due to the presence of cationic amino groups that interact with negatively charged microbial membranes (Goy *et al.*, 2016). Their biocompatibility makes them suitable for wound healing, tissue engineering, and drug delivery. The porous nature of hydrogels allows them to encapsulate bioactive molecules, including plant extracts, peptides, and small drugs. Encapsulation within chitosan hydrogels can enhance the stability of sensitive compounds, control their release, and protect them from environmental degradation.

Recent studies have explored chitosan hydrogels as delivery carriers for plant-derived antioxidants. Hydrogels loaded with phenolic extracts show improved thermal stability, reduced degradation, and enhanced antioxidant performance due to slow and sustained diffusion of active components (Rodrigues *et al.*, 2022). Encapsulation can also increase solubility of hydrophobic compounds and prevent interactions with oxygen or light. Between 2020 and 2024, research efforts have focused on improving encapsulation efficiency, optimizing crosslinking conditions, and understanding how matrix characteristics influence the release behavior of natural phytochemicals (Singh *et al.*, 2023).

In addition to biomedical applications, chitosan hydrogels are used in food preservation as edible coatings, in agriculture for controlled nutrient delivery, and in environmental science for adsorption of pollutants. Their versatility is linked to their modifiable structure, tunable porosity, and ability to form stable complexes with a wide range of molecules.

Given these properties, chitosan hydrogels present a suitable platform for encapsulating ginger extract. Their mild preparation conditions preserve heat-sensitive phytochemicals, while their structure offers protection and sustained release, potentially improving the antioxidant performance of ginger's phenolic constituents.

2.4 Phytochemicals and Biological Relevance of Plants

Phytochemicals are naturally occurring, biologically active compounds produced by plants as part of their secondary metabolic processes. Although they are not required for basic growth or development, they play essential ecological roles such as defense against pathogens, UV protection, and adaptation to environmental stress. These compounds include phenolics, flavonoids, tannins, alkaloids, saponins, terpenoids, and glycosides, each with distinct structural characteristics and biological functions (Pandey & Rizvi, 2020). Their presence in medicinal plants underpins many of the pharmacological activities attributed to traditional herbal remedies.

Phenolic compounds are among the most widely studied phytochemicals due to their strong antioxidant properties. Their antioxidant activity arises from hydroxyl groups capable of donating electrons or hydrogen atoms to neutralize free radicals. They also chelate transition metals, reducing metal-catalyzed formation of reactive oxygen species. Flavonoids, a major subgroup of phenolics, have additional benefits including modulation of oxidative stress pathways, enhancement of endogenous antioxidant enzymes, and inhibition of lipid peroxidation (Gorniak *et al.*, 2019). Tannins contribute to antioxidant activity through protein precipitation and radical scavenging mechanisms.

Alkaloids are nitrogen-containing compounds known for their antimicrobial, analgesic, and neuroactive properties. Many alkaloids also show antioxidant activity by scavenging free radicals or inhibiting enzymes involved in oxidative reactions. Saponins, characterized by their soap-like foaming properties, possess antioxidant, cholesterol-lowering, and immune-modulating effects. Glycosides, which consist of sugar moieties linked to non-sugar aglycones, contribute to diverse bioactivities depending on the aglycone type. Cardiac glycosides, for example, modulate ion transport in cardiac tissues, while phenolic glycosides exhibit antioxidant and anti-inflammatory properties (Akbar, 2020).

Recent studies between 2020 and 2024 continue to highlight the biological significance of phytochemicals in preventing and managing diseases driven by oxidative stress. Their protective effects are linked to their capacity to stabilize reactive species, regulate redox-sensitive signaling pathways, and enhance cellular resilience. Phytochemicals from ginger, in particular, have demonstrated strong antioxidant, anti-inflammatory, and anticancer effects in both *in vitro* and *in vivo* models (Akinyemi *et al.*, 2022). The interplay between these compounds often results in synergistic activity, where whole plant extracts show stronger effects than isolated constituents.

The biological relevance of phytochemicals provides justification for studying how encapsulation in delivery systems such as chitosan hydrogels can enhance their stability and performance. Because many phytochemicals are sensitive to pH, temperature, oxygen, and light, encapsulating them in polymeric matrices may protect them from degradation and prolong their functional lifespan. The improved solubility, controlled release, and enhanced stability of encapsulated phytochemicals have been reported in several recent studies focusing on plant extracts (Rodrigues *et al.*, 2022).

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials, Reagents, and Instruments

This study utilized analytical-grade reagents, high-quality laboratory materials, and calibrated instruments to ensure reproducibility and accuracy of results. All procedures were performed under standard laboratory conditions. The materials, reagents, and instruments used are presented in the tables below

Table 3.1: Materials Used in the Study

Material	Manufacturer
Fresh ginger rhizomes	Sourced from Sokoto local market
Whatman filter paper No. 1	GE Healthcare Life Sciences, UK
Measuring cylinders (10–100 mL)	Pyrex, USA
Glass beakers (50–500 mL)	Pyrex, USA
Volumetric flasks (10–250 mL)	Pyrex, USA
Test tubes and test-tube racks	Fisherbrand, USA
Cotton wool	Local laboratory supply
Distilled water	Laboratory distillation unit
Mortar and pestle	Local supply
Aluminum foil	Local supply

Table 3.2: Reagents and Chemicals

Reagent/Chemical	Grade / Concentration	Manufacturer
Methanol	Analytical grade	Fisher Scientific, UK
Chitosan powder	1 percent w/v solution prepared	Sigma-Aldrich, USA
Acetic acid (glacial)	1 percent v/v	Merck Chemicals, Germany
Glycerin	Analytical grade	BDH Laboratory Supplies, UK
Sodium hydroxide (NaOH) pellets	Analytical grade	Sigma-Aldrich, USA
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	2 percent solution in methanol	Sigma-Aldrich, USA
Ferric chloride (FeCl ₃)	Analytical grade	Sigma-Aldrich, USA
TPTZ (2,4,6-Tripyridyl-s-triazine)	Analytical grade	Sigma-Aldrich, USA
Acetate buffer (pH 3.6)	Prepared in lab	Laboratory formulation
Folin–Ciocalteu reagent	Analytical grade	Merck, Germany
Aluminium chloride solution	10 percent	BDH, UK
Lead acetate	Analytical grade	BDH, UK
Mayer’s reagent	Analytical grade	Sigma-Aldrich, USA
Ferric chloride solution (5 percent)	Analytical grade	Sigma-Aldrich, USA
FeSO ₄ standard	Analytical grade	Sigma-Aldrich, USA

Table 3.3: Instruments and Equipment

Instrument/Equipment	Model / Specification	Manufacturer
Rotary evaporator	Heidolph Advantage Hei-VAP	Heidolph Instruments, Germany
UV–Visible spectrophotometer	Cary 60 UV-Vis	Agilent Technologies, USA
Analytical balance	Mettler Toledo ME-T Series	Mettler Toledo, Switzerland
pH meter	Hanna HI 2211	Hanna Instruments, Romania
Oven (optional)	Laboratory drying oven	Thermo Scientific, USA
Water bath	Grant JB Series	Grant Instruments, UK
Centrifuge	Eppendorf 5702	Eppendorf, Germany
Refrigerator (4 °C)	–	LG Medical Cooling Unit
Magnetic stirrer with hot plate	Stuart UC152	Cole-Parmer, UK

3.2 Sample Collection and Preparation

Fresh ginger (*Zingiber officinale*) rhizomes were purchased from a local market in Sokoto, ensuring that only healthy, mature, and undamaged samples were selected. The rhizomes were transported to the department of Biochemistry and molecular biology immediately after purchase to prevent microbial decay and oxidative changes.

The clean rhizomes were then sliced into thin pieces to facilitate moisture loss and subjected to air drying at ambient laboratory temperature for several days until a constant weight was achieved. Air drying was chosen to preserve thermolabile phytochemicals that might degrade under high-temperature conditions. The dried ginger slices were subsequently pulverized into fine powder using a clean laboratory mortar and pestle. The powdered sample was stored in airtight, light-resistant containers to prevent moisture absorption and oxidative degradation before extraction.

3.3 Extraction of Ginger Phytochemicals

Extraction of phytochemicals from the powdered ginger sample was carried out using the cold maceration method. This approach was selected to preserve heat-sensitive constituents such as phenolics, flavonoids, and glycosides that may degrade under elevated temperatures. Approximately 100g of the air-dried ginger powder was measured using an analytical balance and transferred into a clean conical flask. The sample was soaked in an appropriate volume of analytical-grade methanol, ensuring that the solvent completely covered the plant material.

The mixture was agitated gently and allowed to stand for 48 hours at room temperature to enable adequate diffusion of phytochemicals into the solvent. Intermittent shaking was performed to enhance solvent penetration and extraction efficiency. After maceration, the mixture was filtered using Whatman No. 1 filter paper to obtain a clear filtrate free of solid

particles. The residue was re-soaked in fresh methanol for an additional period to maximize yield, and the filtrates were pooled together.

The combined filtrate was concentrated using a rotary evaporator operated at controlled temperature and pressure settings to remove methanol without degrading the extract. The resulting semisolid crude methanol extract was transferred into airtight amber containers and stored at 4 °C until required for further analysis.

3.4 Preparation of Chitosan Hydrogel

The chitosan hydrogel was prepared using a pH-induced gelation method involving chitosan, acetic acid, glycerin, and sodium hydroxide. A one (1) percent (w/v) chitosan solution was prepared by dissolving accurately weighed chitosan powder in 1 percent (v/v) acetic acid. The mixture was stirred continuously on a magnetic stirrer at moderate speed until a clear, homogenous solution was obtained. Glycerin, used as a plasticizer to improve hydrogel flexibility and moisture retention, was added gradually to the chitosan solution in a controlled volume ratio and mixed thoroughly to ensure uniform dispersion.

Gelation was induced by the controlled addition of sodium hydroxide (NaOH). The chitosan–glycerin solution was transferred into molds or beakers and NaOH solution was added dropwise along the walls of the container to avoid immediate precipitation and to promote gradual crosslinking of the polymer chains. As the pH increased, protonated amino groups in the chitosan backbone underwent deprotonation, resulting in polymer aggregation and the formation of a three-dimensional hydrogel matrix.

The hydrogel was allowed to stand until full gelation was achieved, after which it was rinsed gently with distilled water to remove residual acetic acid and sodium hydroxide. The final chitosan hydrogel was stored at low temperature in a sealed container until required for the encapsulation of ginger extract.

3.5 Encapsulation of Ginger Extract in Chitosan Hydrogel

Encapsulation of the ginger extract was performed using a post-gelation loading technique to ensure that the hydrogel structure remained intact during incorporation of the bioactive components. This method allows diffusion-driven entrapment of phytochemicals into the preformed chitosan matrix without disrupting its polymeric network.

The previously prepared chitosan hydrogel was gently blotted to remove excess surface moisture and then cut into uniform sections to standardize loading efficiency. A measured volume of the crude methanol ginger extract was prepared at the desired concentration and placed into a clean container. The hydrogel segments were then immersed completely in the extract solution, ensuring full contact between the hydrogel surface and the extract.

The system was allowed to stand for 1 hour under gentle agitation to facilitate diffusion of phytochemicals into the hydrogel matrix. During this period, the phenolic and other polar constituents migrated into the pores of the hydrogel through capillary absorption and polymer–solute interactions. After loading, the hydrogel was removed from the extract solution, lightly rinsed with distilled water to eliminate loosely bound external extract, and stored at 4 °C in airtight containers to preserve stability.

The encapsulated hydrogel samples were subsequently used for antioxidant assays (DPPH and FRAP) and were compared with the free ginger extract and naïve chitosan to evaluate the effect of encapsulation on antioxidant activity.

3.6 Qualitative Phytochemical Screening

Qualitative phytochemical screening was performed on the methanol extract of ginger using standard procedures outlined by Harborne (1998) and Trease and Evans (2002). These assays detect the major classes of secondary metabolites based on characteristic color changes or precipitate formation produced when the extract interacts with specific reagents. Each test is

based on reproducible chemical reactions that allow identification of the phytochemical groups present in the sample. The procedures used for each parameter are described below.

3.6.1 Test for Alkaloids

Alkaloids were examined using classical precipitation reactions involving Mayer's reagent (potassium mercuric iodide) and Wagner's reagent (iodine–potassium iodide). Approximately 2 mL of the ginger extract was acidified with dilute hydrochloric acid to liberate the alkaloid salts and heated gently. The mixture was filtered and divided into two portions. Addition of Mayer's reagent to the first portion produced a creamy or pale-yellow precipitate in the presence of alkaloids due to the formation of insoluble alkaloid–mercuric iodide complexes. The second portion was treated with Wagner's reagent, and the formation of a reddish-brown precipitate confirmed the presence of alkaloids. These reactions occur because alkaloids, being basic nitrogenous compounds, readily form ionic complexes with heavy metal reagents.

3.6.2 Test for Tannins

Tannins were detected using the ferric chloride test, which relies on the ability of tannins to complex with ferric ions. A small portion of the extract was diluted with distilled water and filtered to remove insoluble materials. A few drops of 5 percent ferric chloride solution were added. Hydrolysable tannins produced a blue–black coloration, while condensed tannins yielded a greenish or brownish coloration. The reaction is attributed to the formation of iron–phenolate complexes, which exhibit strong color changes due to electronic transitions within the aromatic ring. This test is widely used because tannins possess multiple phenolic groups capable of binding metal ions.

3.6.3 Test for Saponins

Saponins were evaluated using the frothing test, which assesses the surfactant properties of these glycosides. Two milliliters of the extract were diluted with 10 mL of distilled water in a

test tube and shaken vigorously for several minutes. Persistent froth, lasting for at least two minutes and up to fifteen, indicated the presence of saponins. In some cases, a few drops of olive oil were added, and the formation of emulsion further supported a positive result. Saponins naturally decrease surface tension due to their amphiphilic structure, forming stable foams when agitated in aqueous solution.

3.6.4 Test for Glycosides

General glycosides were detected by assessing the liberation of reducing sugars after hydrolysis. The extract was treated with dilute hydrochloric acid and heated in a water bath to hydrolyze glycosidic bonds. The hydrolysate was then neutralized and tested with Fehling's or Benedict's solution. A brick-red precipitate of cuprous oxide indicated the presence of glycosides capable of releasing reducing sugars. This method is based on the principle that glycosides consist of a sugar moiety attached to a non-sugar aglycone; upon hydrolysis, the sugar component behaves as a reducing agent in alkaline copper solutions.

3.6.5 Test for Cardiac Glycosides

Cardiac glycosides were screened using the Keller–Killiani test, which detects deoxysugar components within their structure. The extract was mixed with glacial acetic acid containing traces of ferric chloride, followed by careful layering of concentrated sulfuric acid along the test tube walls. A distinct reddish-brown ring at the interface indicated a positive reaction, while the appearance of a bluish-green color in the acetic acid layer further confirmed the presence of cardenolides. The color changes are attributed to the formation of ferric–deoxysugar complexes and dehydration reactions characteristic of cardiac glycosides.

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3.7 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was conducted to determine the concentrations of major bioactive constituents present in the methanol extract of ginger. These analyses were carried

out using standard spectrophotometric methods widely employed in natural product research (Harborne, 1998; Singleton and Rossi, 1965). The assays targeted flavonoids, saponins, alkaloids, tannins, glycosides, and total phenolics. Absorbance readings were taken using a UV–Visible spectrophotometer, and concentrations were calculated with reference to appropriate calibration standards. Each determination was performed in triplicate, and results were expressed as mean $\pm$ standard deviation.

3.7.2 Determination of Saponin Content

Saponin content was quantified using the spectrophotometric method based on vanillin–sulfuric acid color formation. This reaction produces a reddish-brown chromophore proportional to saponin concentration. A known volume of the extract was mixed with vanillin reagent and sulfuric acid, followed by incubation in a water bath. The absorbance of the resulting solution was measured at the appropriate wavelength. Diosgenin or similar standards are often employed for calibration. Saponin values were recorded in three common reporting units: g percent, g/g, and mg/g. The sensitivity of this assay makes it suitable for quantifying saponins even in low concentrations.

3.7.3 Determination of Alkaloid Content

Alkaloid concentration was estimated using the gravimetric or spectrophotometric method involving acid-base extraction. The method typically relies on transforming alkaloids into soluble salts under acidic conditions, followed by precipitation using alkaline reagents. In spectrophotometric determinations, the absorbance of the resulting alkaloid complexes is measured at characteristic wavelengths. The concentration values—expressed in g percent, g/g, and mg/g—reflect the abundance of alkaloids within the methanol extract. Alkaloids in ginger contribute to its bioactive profile, including antioxidant and anti-inflammatory actions.

3.7.4 Determination of Tannin Content

Tannin concentration was measured using the Folin–Denis spectrophotometric method or the ferric chloride method. In the Folin–Denis procedure, tannins react with phosphotungstic–phosphomolybdic acid reagent to form a blue-colored complex whose intensity correlates with tannin concentration. Alternatively, the ferric chloride reaction forms blue–black complexes with hydrolysable tannins. Absorbance readings were taken and tannin concentration calculated using standards such as tannic acid. Results were expressed in mg and mg/g. Tannins, as polyphenolic compounds, play an important role in antioxidant activity.

3.7.5 Determination of Glycoside Content

Glycoside content was assessed through acid hydrolysis followed by spectrophotometric estimation of the released sugars. The extract was hydrolyzed using dilute hydrochloric acid to break glycosidic bonds, releasing the sugar components. The hydrolysate was then treated with alkaline copper sulfate solutions (e.g., Fehling’s or Benedict’s reagent), and the resulting absorbance was measured. Results were expressed in mg and mg/g. Although glycoside levels in ginger are typically low, their identification contributes to the phytochemical fingerprint of the extract.

3.8 Antioxidant Activity Assays (DPPH and FRAP)

The antioxidant activity of the ginger extract, the naïve chitosan hydrogel, and the chitosan–ginger hydrogel complex was evaluated using two complementary in vitro assays: the DPPH free radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay. Both assays were performed using standard spectrophotometric methods described in prior antioxidant studies (Brand-Williams et al., 1995; Benzie & Strain, 1996). All measurements were taken in triplicate, and mean values with standard deviations were recorded to ensure reproducibility.

3.8.1 DPPH Free Radical Scavenging Assay

Principle

DPPH is a stable free radical that absorbs light at 517 nm, giving it a deep purple colour.

When an antioxidant is added to the DPPH solution, it donates an electron to the radical to neutralize it. As the DPPH radical is scavenged, the absorbance at 517nm decreases, and the colour change is directly proportional to the antioxidant activity of the substance.

Procedure

A quantity of 0.02mg of each sample (ginger extract, naïve chitosan, and chitosan–ginger hydrogel extract) and 20ml of methanol was added to each of these test tubes, of which replicate of different concentration was prepared from it and mixed with the 1ml of DPPH reagent in clean test tubes. The mixtures were incubated in the dark at room temperature for 30 minutes to allow complete reaction between DPPH radicals and antioxidant components present in the samples.

Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The decrease in absorbance indicated the extent of radical scavenging. Percent inhibition was calculated using the formula below:

Ascorbic acid served as the reference antioxidant.

3.8.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measures the reducing power of antioxidants based on their ability to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the presence of TPTZ (2,4,6-tripyridyl-s-triazine). The Fe^{2+} –TPTZ complex forms a blue-colored chromophore that exhibits maximum absorbance at 593 nm, allowing quantification of antioxidant capacity.

The FRAP reagent was freshly prepared by mixing acetate buffer (pH 3.6), TPTZ solution, and ferric chloride in the appropriate ratios. 0.02mg of samples were dissolved in 20ml of methanol and sample concentrations of 0.4, 0.8, 1.2, 1.6, and 2.0 mg/mL were prepared for ginger extract, naïve chitosan, and the chitosan–ginger hydrogel to permit comparison across a range of antioxidant potentials.

Each 2ml of sample solution was mixed with 2.5ml of FRAP reagent and incubated at 37 °C for 30 minutes to allow complete reduction of Fe^{3+} . Absorbance was read at 593 nm. Higher absorbance values indicated stronger reducing power.

FRAP values were quantified using a standard calibration curve prepared from plotted from the standard solutions.

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CHAPTER FOUR:

RESULTS, DISCUSSION, CONCLUSION, AND RECOMMENDATION

4.1 Results

4.1.1 Qualitative Phytochemical Composition of Ginger Extract

The result of phytochemical screening is presented in Table 4.1. The result indicated the presence of saponins, tannins, alkaloids, glycosides, cardiac glycosides, steroids, and saponin glycosides.

Table 4.1: Qualitative Phytochemical Constituents of Ginger Extract

Phytochemical Parameter	Observation
Saponins	++
Tannins	+
Alkaloids	+
Glycosides	++
Cardiac glycosides	+
Steroids	+
Saponin glycosides	++

Key for Interpretation: +++ = High, ++ = Moderate, + = Low

4.2 Quantitative Phytochemical Results

Table 4.2: Quantitative Phytochemical Composition of Ginger Extract (Standard Units)

The results indicate that the ginger extract is rich in alkaloids and saponins, each exceeding 150 mg/g, demonstrating a high concentration of bioactive metabolites.

Phytochemical Parameter	Mean $\pm$ SD
Saponins (mg/g)	0.08 $\pm$ 0.0001
Alkaloids (mg/g)	216 $\pm$ 2.6000
Tannins (mg/g)	233 $\pm$ 0.3000
Glycosides (mg/g)	0.00 $\pm$ 0.0001

4.3 Antioxidant Activity Results (DPPH and FRAP)

The antioxidant activity of the ginger extract, naïve chitosan hydrogel, and the chitosan ginger hydrogel complex was evaluated using two standard in vitro assays: the DPPH free radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay.

DPPH Radical Scavenging Activity Results (%)

The results of the DPPH scavenging activity of the ginger extract, chitosan ginger, and the naïve chitosan are shown below. The ginger extract and the loaded hydrogel showed statistical significance ($P < 0.05$) when compared with the naïve chitosan and the standard ascorbic acid.

Table 4.3.1: DPPH Radical Scavenging Activity (%)

Sample	% Inhibition
Ginger Extract	97.09 ± 0.4600a
Naïve Chitosan	94.61 ± 0.1774c
Chitosan + Ginger	96.25 ± 0.2247b
Ascorbic Acid (Standard)	94.91 ± 0.1168c

Values with different letters indicate statistical significance ($p < 0.05$) using one-way ANOVA, followed by Tukey-Kramer post hoc tests.

4.3.2 Ferric Reducing Antioxidant Power (FRAP) Results

The results of FRAP assays for the ginger extract, chitosan ginger, and the naïve chitosan is across different concentrations are shown in Figure 2. At both lower and higher concentrations of the test, the ginger extract has ($P<0.05$) significant higher FRAP values compared to the naïve chitosan and the chitosan loaded with ginger.

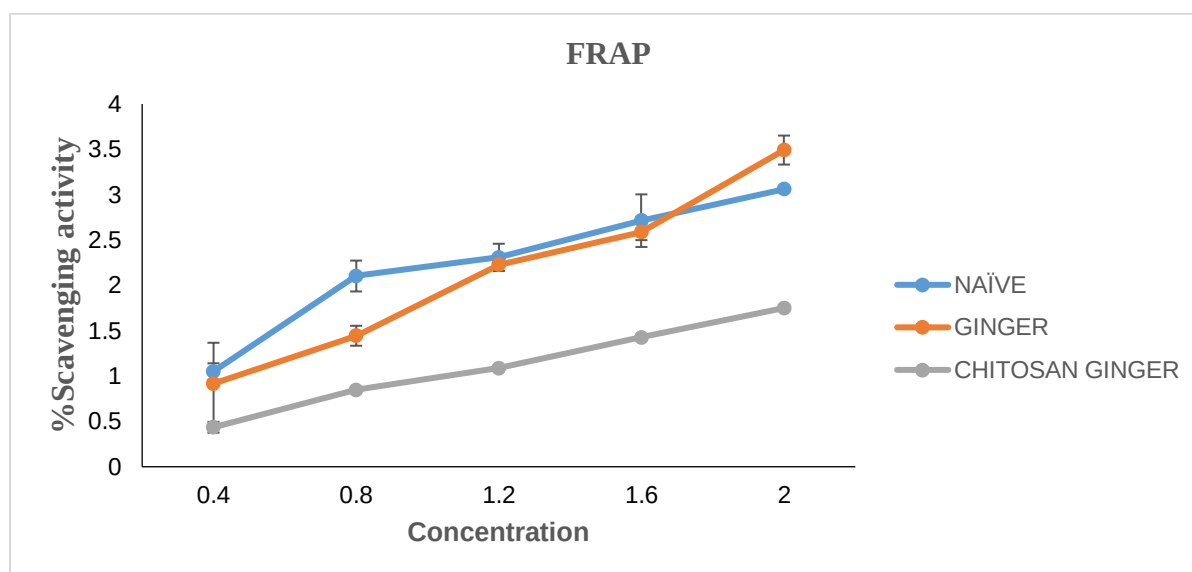


Figure 2: FRAP scavenging activity

4.4 Discussion

The results align with and extend existing literature on the phytochemical richness and antioxidant potential of *Zingiber officinale*. The high levels of phenolics, alkaloids, and saponins observed in the ginger extract support earlier reports by Olatunde *et al*, (2021), who similarly noted that methanol extracts yield a broad spectrum of antioxidant-rich phytochemicals and ginger has high concentration of bioactive compounds attributed to its nutritional and therapeutic effects. The strong presence of phenolics, which serve as potent electron donors and radical scavengers, is consistent with the elevated antioxidant activity demonstrated in this study.

The highly significant antioxidant activities of the free ginger extract is comparable to findings by Akinyemi *et al*, (2022), who reported over 90 percent radical-scavenging activity in methanolic ginger extracts. This suggests that ginger's antioxidant potential is strongly conserved across different geographic sources and preparation methods.

The lower values of antioxidant activity in the loaded hydrogel when compared with the ginger extract, as seen in Figure 2, have also been observed in other encapsulation studies. Rodrigues *et al.*, (2022) reported that encapsulating phenolic-rich extracts often results in reduced immediate antioxidant activity, which is likely due to slow or poor drug release of the extract from the hydrogel or due to restricted diffusion of the antioxidant compounds in the chitosan hydrogel imposed by the polymeric matrix. However, they noted that encapsulation enhances stability, protects bioactive compounds from oxidation, and sustains antioxidant release over longer periods. The similarity between this pattern and the results of the present work supports the conclusion that encapsulation into chitosan hydrogels preserves functional compounds while moderating their instantaneous activity.

Additionally, the moderate but notable antioxidant activity of naïve chitosan observed in this study confirms earlier reports that chitosan itself possesses intrinsic antioxidant properties. Goy *et al.*, (2016) and Singh *et al.*, (2023) proposed that the amino groups in chitosan can donate electrons or chelate pro-oxidant metal ions, contributing to observable reducing power. This explains why the naïve hydrogel exhibited measurable DPPH inhibition and FRAP activity independent of ginger.

Moreover, the retention of high DPPH inhibition (96.25%) and FRAP values in the encapsulated extract agrees with studies by Ahmed and Ikram (2017), who demonstrated that chitosan-based hydrogels are effective carriers for maintaining the biological activity of encapsulated plant extracts. The present findings, therefore, reaffirm chitosan's suitability as a biocompatible encapsulation matrix capable of maintaining antioxidant integrity.

4.5 Conclusion

The study concludes that methanol-extracted ginger is a potent source of antioxidant phytochemicals, with significant radical scavenging and reducing capabilities attributable to its abundant alkaloids, saponins, and phenolic compounds. Encapsulation of ginger extract into a chitosan hydrogel matrix effectively preserved these bioactive components, maintaining relatively strong antioxidant activity. These findings affirm the potential of chitosan hydrogels as natural, biocompatible carriers for plant-based antioxidants, suitable for applications in food preservation, nutraceutical delivery, and biomedical formulations requiring controlled release or enhanced stability.

4.6 Recommendations

Based on the findings of this work, it is recommended that:

- Future studies explore the long-term stability of ginger-loaded chitosan hydrogels under varying storage conditions to understand how encapsulation influences degradation over time. It would be beneficial to assess the release kinetics of phenolic compounds from the hydrogel matrix to determine whether the reduced antioxidant values observed in vitro translate into sustained activity.
- Scaling the encapsulation process for food or pharmaceutical applications should be considered to evaluate industrial feasibility, and the incorporation of crosslinking enhancers or nanoparticle reinforcements may further improve encapsulation efficiency.
- Additional in vivo or cell-based assays would provide deeper insight into the biological relevance of the encapsulated extract and determine whether chitosan hydrogels improve bioavailability compared to free ginger extract.

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APPENDICES

RAW DATA

TABLE 4.4: Absorbance for samples in DPPH assays

Sample	Absorbance*(517nm)					
Ginger Extract	85.66	85.74	85.89	86.8	86.88	86.65
Naïve Chitosan	89.39	89.47	90.01	89.63	89.95	89.70
Chitosan + Ginger	90.85	90.93	90.99	90.92	91.08	91.23
Ascorbic Acid (Standard)	63.23	62.62	62.31			

Absorbance of control = 0.911

RAW VALUES FOR FRAP

Table 4.5 : Ginger extracts raw values

Concentration(mg/ml)	Calculated values					
0.4	0.812	0.809	0.904	1.234	1.237	1.231
0.8	1.347	1.350	1.338	1.555	1.537	1.534
1.2	2.299	2.296	2.293	2.168	2.156	2.159
1.6	2.513	2.667	2.664	2.667	2.510	2.507
2.0	3.345	3.348	3.351	3.628	3.640	3.637

Table4.6: Chitosan ginger raw values

Concentration(mg/ml)	Calculated values					
0.4	0.390	0.396	0.381	0.515	0.503	0.467
0.8	0.898	0.853	0.851	0.824	0.827	0.830
1.2	1.077	1.068	1.071	1.088	1.112	1.109
1.6	1.445	1.463	1.430	1.418	1.398	1.395
2.0	1.722	1.731	1.728	1.769	1.775	1.772

Table 7: Naïve chitosan values

Concentration(mg/ml)	Calculated values					
0.4	1.142	1.136	1.127	0.975	0.964	0.958
0.8	2.099	2.093	2.079	2.013	2.325	2.004
1.2	2.308	2.287	2.296	2.325	2.316	2.319
1.6	2.977	2.971	2.983	2.456	2.444	2.447
2.0	3.045	3.036	3.030	3.087	3.081	3.045

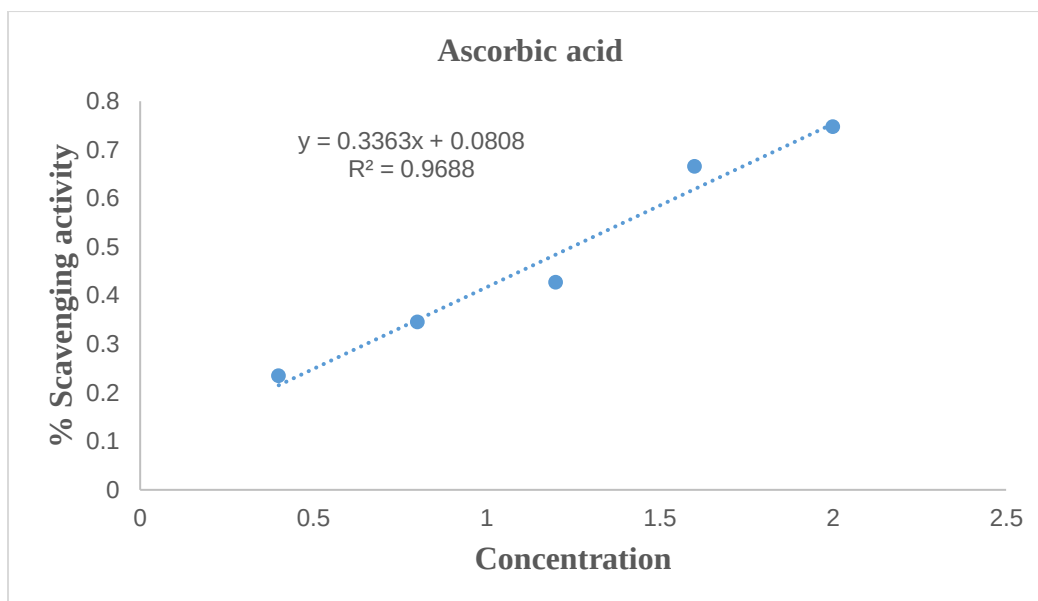


Figure 3: (FRAP) Scavenging activity of standard ascorbic acid.