QUANTITATIVE PHYTOCHEMICAL COMPOSITION, ANTIPYRETIC AND ANTIOXIDATIVE EFFECTS OF METHANOLIC LEAF AND ROOT EXTRACTS OF *Carica papaya* Linn.

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Biochemistry of Chuka University

CHUKA UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted for an award of diploma or conferment of degree in any University.

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Recommendation

This thesis has been examined, passed and submitted with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my father William Kiptarus and my mother Catherine Kiptarus, for their sacrifices towards my education.

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ABSTRACT

Antipyrexia and oxidative stress-related conditions are significant global health issues due to their widespread impacts on human health and well-being. Fever is mostly triggered by infectious stimuli and is characterized with increase in temperatures typically above the normal range for human, (36.5–37.5 °C). Currently, fever is treated with traditional methods such as active cooling and conventional medications such as Nonsteroidal Anti-inflammatory Drugs (NSAIDs) and paracetamol. It has been documented that NSAIDs have adverse effects on the cardiovascular, hepatic, renal, and gastric mucosa systems. On the other hand, oxidative stress contributes to the pathogenesis of a many chronic and degenerative disorders. Conventional antioxidants have been reported to be less effective and relatively expensive. Due to these draw backs, there is a need to research for alternative method for the management of fever and oxidative stress, including the use of herbal medicines. *Carica papaya* has been known to be used traditionally in treating fever and conditions associated with oxidative stress, but concrete evidence on its medicinal value is limited. Thus, this study evaluated phytochemical profiles, antipyretic and antioxidative effects of methanolic leaf and root extracts of *Carica papaya.* Leaves and roots of *C. papaya* were collected from Karingani ward, Chuka-Igambang'ombe sub-county, Tharaka Nithi County, Kenya. The plant materials were extracted methanol. Quantitative phytochemical screening was done using GC-MS. The *in-vivo* antipyretic activities of the extract were screened in thirty male white albino Wistar rats weighing 90-150g. Turpentine was used to induce fever in rats. Antioxidant activity of the methanol leaf and root extracts was evaluated using non-enzymatic antioxidant assays. The GC–MS analysis of methanolic root and leaf extracts of *Carica papaya* revealed the bioactive phytochemicals incluiding flavonoids (quercetin) phenolic(caffeic),terpenes and terpenoids (squalene, myrcene), vitamin C (Ascorbic acid), fatty acid (n-hexadecanoic), and alkaloids (reserpinine). The methanolic root and leaf extracts of *Carica papaya* showed dose- and time-dependent antipyretic activity. The temperature reduction of the root extract in the first hour was 1.15%, 1.77%, and 2.20% and second hour 3.83%, 4.32%, and 4.30% (50, 100, and 150 mg/kg; respectively, comparable to paracetamol (1.57%, 3.72% in the first and second hour respectively. At the third and fourth hours, reductions reached 5.25%, 5.68%, and 5.08%, and 5.20%, 5.84%, and 5.13%, respectively, statistically similar to paracetamol (4.82% in the third hour and 5.30% in the fourth hour). The leaf extract percentage reduction in the first hour was 0.73%, 1.87%, and 1.57%, (50, 100, and 150 mg/kg; respectively, comparable to paracetamol (1.57%). At the second hour, significant decreases of 2.50%, 4.22%, and 3.93% were observed, with the higher doses showing effects similar to the positive control (3.72%). By the third hour, the extract reduced by 3.77%, 4.70%, and 4.77%, and paracetamol (4.82%). At the fourth hour, the reductions were 4.25%, 5.16%, and 5.30%, and statistically comparable to the positive control (5.30%). The root extract showed greater antipyretic effects as compared the leaf extract at lower doses. At higher doses both extracts were similarly effective. *Carica papaya* indicated *in vitro* antioxidant effects in all the tested non-enzymatic assays. The results from the study indicates that *C. papaya* is a potent natural therapeutic alternative for management of fever and oxidative stress related conditions.

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LIST OF ABBREVIATION AND ACRONYMS

ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ACE-2	Angiotensin-Converting Enzyme 2
ALA	Alpha-Lipoic Acid
ANOVA	Analysis of Variance
BC	Before Christ
bwt	Body weight
CE	Common Era
CoQ10	Coenzyme Q10
COVID-19	Coronavirus Disease 2019
COX	Cyclooxygenase
CRD	Completely Randomized Design
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EPR	Electron Paramagnetic Resonance
FRAP	Ferric Reducing Antioxidant Power
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
H_2O_2	Hydrogen Peroxide
HNE	4-Hydroxy-2-nonenal
IFN	Interferon
IL-1	Interleukin-1
IL-6	Interleukin-6
LD_{50}	Lethal Dose 50
LDL	Low-Density Lipoprotein
LPS	Lipopolysaccharide
MDA	Malondialdehyde
NAC	N-acetylcysteine
NACOSTI	National Commission for Science, Technology, and Innovation
NIST	National Institute of Standards and Technology
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs

OVLT	Organum Vasculosum of the Lamina Terminalis
PGE2	Prostaglandin E2
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean
SOD	Superoxide Dismutase
TFC	Total Flavonoid Content
TNF	Tumor Necrosis Factor
TPC	Total Phenolic Content
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
UV	Ultraviolet
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Fever and oxidative stress–related conditions are major health concerns due to their impact on normal physiological functions. Fever is a symptom associated with conditions such as coughs, colds, teething in infants, post-vaccination reactions, inflammation, tissue injury, and viral or bacterial infections (Belon *et al.*, 2021). It is also caused by tissue injury, inflammation, rejection of a transplant, cancer, or other illnesses (El-Radhi, 2019). It is the most prevalent physical symptom in patients and is an important aspect of infectious and non-infectious inflammatory processes. Fever is a prevalent symptom in children, representing up to 20% of pediatric physician visits. A detailed history and physical assessment can be used to determine the cause of fever in most pediatrics aged 0 and 36 months. The most frequent focus is an upper respiratory tract viral infection (Sasongko *et al.*, 2019).

Fever is primarily caused by pyrogens which can be either exogenous or endogenous. These pyrogens stimulate the immune system and activate the release of cytokines, which in turn signal the hypothalamus to increase the body thermal set point. One of the key mediators in this process is prostaglandin E2 (PGE2) (Osilla *et al.*, 2023). An increase in prostaglandin E2 (PGE2) concentrations in the hypothalamus causes fever by changing the rate at which neurons regulate the body temperature. This results in metabolic disturbances including increased blood pressure, pulse rate, cardiac output, and respiration rate (Balli *et al.*, 2023).

Fever can be managed using non-pharmacological approaches like active cooling and pharmacological treatments such as antipyretic drugs including NSAIDs and paracetamol (Ma *et al.*, 2021). However, NSAIDs have negative effects on the cardiac system, hepatic system, renal system, and gastric mucosa (Ghlichloo & Gerriets, 2022). Sasongko *et al.* (2019) reported that a supratherapeutic dosage of paracetamol causes hepatotoxicity disseminated intravascular coagulation and persistent renal insufficiency. Additionally, Ma *et al.* (2021) reported that excessive use of active cooling can raise the metabolic rate, triggers hypothermia, and causes thermal discomfort, thus individuals with mild fever should avoid it. These adverse effects are the main reason why many people choose natural drugs as alternative treatment (Ma *et al.*, 2021).

On the other hand, ROS are formed as intermediate products in several enzymatic processes. However, an overproduction of these free radicals leads to an imbalance between ROS and endogenous antioxidants cause oxidative stress (Pizzino *et al.*, 2017; Sharifi-Rad *et al.*, 2020). Oxygen-derived free radicals contribute significantly to the damage of cellular components, which is a key factor in the development of various chronic and degenerative diseases, such as aging, atherosclerosis, neurodegenerative disorders, cancer, and cataracts. The oxidative properties of these species can cause cell injury and several physiological and pathological abnormalities (Sharma *et al.*, 2022).

Antioxidants are molecules that prevent the oxidation of other molecules. They also prevent cellular damage due to free radical formation, by terminating the reaction chain or by removing free radical intermediates and suppressing other oxidation reactions (Aribi & Hameed, 2025). The antioxidant functions are related with reduced DNA damage, decreased lipid peroxidation, retained immune function and inhibited malignant transformation of cells (Chaudhary *et al*., 2023).

In many African communities, herbalists use medicinal plants to treat illnesses, a practice driven by the high cost of conventional healthcare and the growing global preference for natural remedies over synthetic drugs (Aribi & Hameed, 2025). One of these medicinal plants that can be used to treat fever and oxidative related diseases is *Carica papaya* Linn. *Carica papaya* is a tropical fruit-bearing tree that has been appreciated worldwide due to its medicinal properties, especially in cases of fever and oxidative stress. Recent findings suggest that aqueous extracts of the plant leaves possess compounds that can have antioxidant effects (Kong *et al.*, 2021). This traditional knowledge is derived from the observations of indigenous people in tropical regions, who noted that regular consumption of prepared papaya possesses fever-reducing properties and provides protection against oxidative stress-related diseases (Sharma *et al.*, 2022).

However, scientific research into the exact impact of the methanolic leaf and root extracts of *Carica papaya* has not been fully explored (Sharma *et al.*, 2022). Although the pharmacological effects of papaya extracts have been studied, much of the study was on the effects of the fruit and seeds (Munir *et al.*, 2022). There is little information on its pharmacological activities especially on antipyretic and antioxidant properties of the leaves and roots. Furthermore, given that papaya is readily available and easily grown in tropical climates, it has the potential to be a source of affordable treatment remedies for people with limited access to conventional health care (Santana *et al.*, 2019).

Due to increasing number of fever and oxidative stress related diseases and limited scientific information available to support the therapeutic benefits of *Carica papaya* this study therefore aims at assessing phytochemical composition, evaluation of antipyretic and antioxidative properties of methanolic leaf and root extracts of *Carica papaya Linn*. the variety of Sunrise Solo. This study will use methanol because it yields high crude extracts even if they are in low concentration. A study conducted by Dagne *et al*. (2021) showed that the highest percentage yield of crude extract (72.5%), was obtained from *Carica papaya* leaves when using methanol, ethanol, and acetone as solvents. The generated data will provide a valuable framework for developing new, effective, affordable, and safe antipyretic drugs and antioxidant supplements.

1.2 Statement of Problem

Fever often leads to discomfort, fatigue, and general malaise, significantly lowering the productivity of the patients, hence the need to manage it accordingly. Nonsteroidal anti-inflammatory drugs such as ibuprofen and diclofenac, that are commonly used to manage fever, are associated with several adverse effects, including cardiovascular, hepatic, renal, and gastrointestinal complications such as nausea, vomiting, heartburn, stomach ulcers, and diarrhea. These concerns have necessitated the increase in the utilization of herbal remedies, which are generally safer, and readily accessible. Alternative herbal medicines such as *Carica papaya* remain a main resource for discovering more effective therapies for management of fever.

On the other hand, oxidative stress is known to inflict cellular damage and can play a role in the onset and advancement of many chronic ailments. The overproduction of free radicals leads to an imbalance between the free radical production and antioxidative rates thus resulting to oxidative stress which contribute to the pathogenesis of many chronic and degenerative diseases. Additionally, conventional antioxidants have been reported to be less effective and relatively expensive. The high costs of these therapies underscore the ongoing challenge of rapid advancements in pharmaceutical innovation with the financial sustainability of healthcare systems. Alternative herbal medicines such as *Carica papaya* is low-cost antioxidant agents for managing oxidative stress-related health conditions.

Carica papaya has been utilized traditionally to treat fever and oxidative stress-related conditions. However, scientific data supporting its medicinal value is scarce, particularly regarding the leaves and roots. Most existing studies focus on the fruit and seeds, thus there is a knowledge gap in understanding the full pharmacological potential of the plant.

1.3 Objective of the Study

1.3.1 General Objective

To determine the phytochemical composition, evaluate antipyretic and antioxidative effects of methanolic leaf and root extracts of *Carica papaya* Linn, the variety of Sunrise Solo.

1.3.2 Specific Objectives

i. To determine quantitative phytochemical composition of methanolic leaf and root extracts of *Carica papaya* using gas chromatography-mass spectrometry.
ii. To determine the *in vivo* antipyretic effect of methanolic extract of leaf and root extracts of *Carica papaya* in albino Wistar rats.
iii. To determine the *in vitro* antioxidative activity of methanolic leaf and root extracts of *Carica papaya* using non-enzymatic essays.

1.4 Hypotheses

H01: There are no significant phytochemicals associated with antipyretic and antioxidant activities in the methanolic leaf and root extracts of *Carica papaya*.

H02: There is no *in vivo* antipyretic effect of methanolic leaf and root extracts of *Carica papaya* in albino Wistar rats.

H03: There is no *in vitro* antioxidative effect of methanolic leaf and root extracts of *Carica papaya*.

1.5 Justification of the Study

Fever and oxidative stress related disorders are major global health concerns that significantly impact morbidity, mortality, and healthcare costs (Fanget *et al*., 2023). Managing these conditions pharmacologically while minimizing adverse effects remains challenging. Herbal medicine continues to play a vital role, particularly in underdeveloped regions, due to its accessibility and affordability; however, its increased use has not been matched by sufficient clinical evidence to support safety and efficacy (Arika *et al*., 2019). As the demand for natural remedies rises, scientific evaluation of medicinal plants with potential antipyretic and antioxidant effects has become essential (Arul Raj *et al*., 2025).

Carica papaya Linn. is a widely cultivated tropical plant traditionally utilized in the treatment of fever and oxidative stress–related illnesses, particularly among native populations in tropical regions. Despite its extensive use in folk medicine, there remains limited scientific supporting these traditional claims (Teh *et al*., 2022). Investigating the phytochemical constitution of *C. papaya* is therefore crucial to bridge the gap between indigenous knowledge and modern pharmacological research. The present study focuses on its methanolic leaf and root extracts to identify bioactive phytochemicals responsible for its medicinal properties, which may serve as a foundation for the manufacturing of safe, effective, and affordable phytotherapeutic agents. Additionally, identifying bioactive compounds from *C. papaya* could aid in the synthesis of pure compounds for use as alternative treatments in managing fever and oxidative stress–related conditions.

CHAPTER TWO
LITERATURE REVIEW

2.1 General Information

Phytochemicals such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds are identified for their antipyretic and antioxidant activities. Fever, a common symptom of infection, results from the release of endogenous pyrogens that stimulate the production of prostaglandin E2 in the hypothalamus, raising body temperature beyond the set point (Osilla *et al.*, 2023). Antipyretic phytochemicals work by inhibiting PGE2 synthesis, similar to conventional drugs like paracetamol and ibuprofen. On the other hand, oxidative stress contributes to the pathogenesis of diseases such as cancer, diabetes, cardiovascular conditions, and neurodegenerative conditions. Antioxidants have been used to manage these conditions by counteracting the ROS through free radical scavenging and metal chelation (Aribi & Hameed, 2025). *Carica papaya* has been used traditionally in the management of fever and oxidative stress-related diseases, however its full pharmacological potential has not been fully explored.

2.2 Taxonomy of *Carica papaya*

Carica papaya is commonly known as papaya and is a plant from the kingdom Plantae in the phylum Angiosperms and in the class of eudicots. It is placed in the order Brassicales and the family Caricaceae, which is a small family of tropical plants that bear fruits. There are several species of Carica, but *Carica papaya* is the most common one (Hernández-Salinas *et al.*, 2022). Papaya is commonly referred to as papaw or pawpaw in some regions but should not be confused with the temperate *Asimina triloba* that also bears fruits called pawpaw (Chávez-Pesqueira & Núñez-Farfán, 2017).

2.2.1 Botanical Features and Phytochemical Composition of *Carica papaya*

Carica papaya trees have palmate shaped leaves clustered densely at apex of trunk measuring between 50–70 cm diameters with long dangling petioles and conspicuous parallel veins (Plate 1) (Kong *et al.*, 2021). The trees have taproots and shallow adventitious fibrous roots that sprout from lower parts of the soft-wooded stem above ground level forming props for structural support against strong winds (Singh *et al.*, 2020) (Plate 1). The papaya plants normally occur as dioecious male and female forms and contains male and female flowers on separate individual trees leading to outcrossing reproduction (Shaikh *et al.*, 2022). The female papaya trees yield the commercially pear-shaped sweet edible golden yellow fruits (Plate 1) while male trees produce flowers required for pollination in fruit production (Hariono *et al.*, 2021).

All aerial young and mature plant organs inclusive of stems, petioles, leaves, flower stalks and fruit are characterized by sparsely distributed short hollow trichomes and possession of thin green external epidermal layer (Nafiu *et al.*, 2019). Additionally, papaya leaves, stems and fruits contain an extensive central spongy layer comprising of a long stretching loosely arranged colorless thin-walled parenchyma cells along with plenty of prominent white colored cells clustered over veins (Singh *et al.*, 2020).

Plate 1: An image of *Carica papaya* (Sunrise Solo) showing the leaves, fruits, stem and roots (by the author).

2.2.2 Historical Domestication and Worldwide Distribution of Papaya

The original native home of cultivated papaya is regarded as southern Mexico and neighboring Central American countries of Guatemala, Belize and Costa Rica situated between latitudes 140N and 200N considered the center of genetic diversity (Hernández-Salinas *et al*., 2022). Papayas represent one of oldest domesticated and cultivated fruit crops in the Mesoamerica region with ancient seed remnants traced to middens around Cuello archaeological ruins in northern Belize indicating papaya consumption since 3000 BC (Chávez-Pesqueira & Núñez-Farfán, 2017). The current widespread papaya distribution results from worldwide dispersal through oceanic exploration and colonization over past five centuries after Christopher Columbus observed bountiful papaya trees fruiting on his voyages reaching Caribbean islands and Panama isthmus during 1492-1504 CE (Nafiu *et al.*, 2019).

Papaya transplanting in Mexico and Europe started in early 1500s. Thereafter it was planted in Africa, India, Indonesia, Philippines, Hawaii, Australia and rest of tropical world by late 16th century (Shaikh *et al.*, 2022). Progressive papaya cultivation presently occurs between latitudes 300N to 300S in equatorial lowlands and mid-hill regions with hot humid climate, fertile soil and mild winter droughts near coastal belts or river basins that mimic native indispensable parameters (Singh *et al.*, 2020). Major contemporary commercial producers include India, Mexico, Brazil, Indonesia, Nigeria and China with thriving export trade generating billions of dollars annually (Teh *et al.*, 2022).

2.2.3 Medicinal Importance of *Carica papaya*

All components of *Carica papaya* plant including roots, stems, bark, leaves, flowers, fruits and seeds have been used extensively in traditional medicinal among Indian, Chinese, Malay and Amerindian cultures for preventing and healing various human diseases (Shaikh *et al.*, 2022). Most medicinal applications involve crude aqueous or alcohol extractions prepared from different papaya parts (Singh *et al.*, 2020). Modern pharmacological investigations on *Carica papaya* extracts and isolated compounds confirm antioxidant, antimicrobial, anti-inflammatory, wound healing, antidiabetic, antitumor, immunomodulatory, neuroprotective, diuretic, antifertility and abortifacient properties (Anjana *et al.*, 2018).

Numerous experiments demonstrated antimicrobial activities of *Carica papaya* extracts against wound infection causing Gram positive *Staphylococcus aureus* and Gram-negative *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* bacteria as well as anti-fungal properties against *Aspergillus fumigatus*, *Candida albicans* (Hariono *et al.*, 2021). Standardized leaf extracts display strong antiviral inhibition on envelope viruses like herpes simplex, hepatitis B, dengue and chikungunya in cell culture models and infected animal demonstrating potential medicinal applications (Teh *et al.*, 2022). *Carica papaya* leaf extracts exhibit inhibitory influence on parameters linked to progression of diabetes manifestations in induced rodent subjects to standard drugs glibenclamide and metformin (Kong *et al.*, 2021).

Additionally, leaf extracts of *Carica papaya* demonstrate high phenolic contents and potent free radical scavenging capacities comparable to commercial antioxidant compounds in numerous chemical assays and cell free systems indicating ability to counter oxidative stress and lipid peroxidation (Nafiu *et al.*, 2019). Oral and topical papaya leaf product formulations accelerate wound contraction and epithelialization phases of cutaneous wound repair processes in rat and mice excision models via interactive anti-inflammatory, cell proliferation and matrix remodeling mechanisms necessary for normal healing (Singh *et al.*, 2020). *Carica papaya* unripe fruit extracts decrease solid tumor sizes and prolong survival rate in induced cancer rat models through activation of cell mediated immune responses and protective antioxidant actions similar to standard drugs urethane and cisplatin (Shaikh *et al.*, 2022).

2.2.4 The Role of *C. papaya* in the Management of Fever and Oxidative Stress

Carica papaya leaves and roots are widely used traditionally for fever reduction and contain antioxidants that could combat oxidative stress. Since fever is a stress response involving inflammatory mediators and mitochondrial dysfunction leading to increased ROS production (Sharifi-Rad *et al.*, 2020), papaya antipyretic effects may lower the oxidative burden. Compounds in papaya such as polyphenols, flavonoids, alkaloids, and vitamins function as antioxidants by inhibiting lipid peroxidation and protein/DNA damage (Chaudhary *et al.*, 2023). Given known antipyretic and antioxidant properties of papaya, further research is still needed to substantiate such claims.

2.2.5 Phytochemicals with Antipyretic Properties

Recent studies have explored the antipyretic potential of various plant extracts rich in phytochemicals. For example, research on *Litsea glutinosa* leaves demonstrated that methanolic extracts significantly reduced body temperature in yeast-induced mice, indicating notable antipyretic activity (Labu *et al.*, 2025). The study attributed these effects to the availability of bioactive phytochemicals such as flavonoids and alkaloids. Flavonoids, compounds like quercetin, kaempferol, and their glycosides (quercetin-3-O-glucoside) were likely present thus inhibited prostaglandin synthesis particularly prostaglandin E2 via cyclooxygenase (COX) suppression (Labu *et al.*, 2025). The alkaloids enhance this effect by modulating inflammatory pathways and exerting sedative properties that aid in temperature regulation (Dey *et al.*, 2020). The methanolic extracts efficacy was dose-dependent, with significant reductions in rectal temperature as compared to paracetamol in some models thus emphasizing the synergistic role of these phytochemicals in combating pyrexia through anti-inflammatory and antioxidant mechanisms (Labu *et al.*, 2025).

Similarly, an investigation into the fractions of *Chenopodium ambrosioides* revealed the presence of various bioactive compounds, including protocatechuic acid, vanillin, syringaldehyde, flavonoids, and phenolic acids (Drioua *et al.*, 2024). Protocatechuic acid, a phenolic acid, is known for its anti-inflammatory and antioxidant properties, potentially contributing to antipyretic effects by inhibiting pro-inflammatory cytokines such as TNF-α and IL-1β, which are involved in fever induction (Drioua *et al.*, 2024). Vanillin offers mild antioxidant activity that may indirectly support fever reduction by mitigating oxidative stress. Syringaldehyde, another phenolic compound, similarly scavenges free radicals, potentially stabilizing inflammation-related pathways (Wu *et al.*, 2022). Flavonoids, likely including compounds like quercetin, kaempferol, or their glycosides are recognized for suppressing prostaglandin E2 synthesis through cyclooxygenase (COX) inhibition, a direct mechanism for antipyretic action (An *et al.*,2025). Additional phenolic acids, such as caffeic acid or ferulic acid, enhance this effect with their dual antioxidant and anti-inflammatory capabilities. While the study emphasized chemical profiling, the pharmacological activities of these compounds rooted in their ability to modulate inflammation and oxidative stress strongly suggest potential antipyretic effects (Drioua *et al.*, 2024).

Research on plant extracts like *Cuscuta reflexa* has shown that flavonoids and organic acids (citric and malic acids) significantly reduce temperatures of a mice which was induced with pyrexia using brewer’s yeast. These compounds lower rectal temperature in a dose-dependent manner, with effects comparable to standard drugs like paracetamol (Bhattacharya & Roy, 2010). Similarly, investigations into *Satureja hortensis* revealed that its phenolic-rich extracts, including rosmarinic acid, exhibit antipyretic activity by suppressing pro-inflammatory cytokines like IL-6, which are implicated in fever induction (Grigore-Gurgu *et al*., 2025). Other studies have identified terpenoids, such as those in *Terminalia arjuna* bark, as potent antipyretics. These compounds reduce fever by modulating cyclooxygenase (COX) enzymes, thereby decreasing prostaglandin E2 levels (Das *et al.*, 2020).

2.2.6 Phytochemicals with Antioxidant Properties

Phytochemicals, particularly polyphenolic compounds and flavonoids, have demonstrated significant antioxidant properties by neutralizing free radicals and reducing lipid peroxidation (Hassanpour & Doroudi, 2023). A study on the nutritional composition and phytochemicals of *Abies marocana* needles revealed a high content of bioactive components contributing to notable antioxidant capacity, suggesting their potential in combating oxidative stress (Zirari *et al.*, 2024). The methanolic extract of *Abies marocana* needles was found to be rich in phenolic compounds, including high levels of total phenols (quantified as gallic acid equivalents), flavonoids, and tannins, were prominent, with flavonoids such as quercetin and kaempferol likely present based on patterns in related *Abies* species (Zirari *et al.*, 2024). Phytochemical screening also confirmed the presence of sterols, terpenes, and reducing compounds contributed to the antioxidant effects by scavenging free radicals and inhibiting oxidative processes. These phytochemicals, particularly phenols, flavonoids, and terpenes, demonstrated strong antioxidant activity in assays such as DPPH, ABTS, and FRAP (Al Jaafreh, 2024).

Research on *Melilotus albus* leaves demonstrates that methanolic and aqueous extracts possess substantial antioxidant activity due to a rich phytochemical profile, including phenolic compounds like rosmarinic acid, p-coumaric acid, and chlorogenic acid, which excel in scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation (Ed-Dahmani *et al.,*2024). Flavonoids such as quercetin, kaempferol, hyperoside, and luteolin further enhance this activity by neutralizing free radicals and boosting reducing power, while coumarins like melilotoside and coumestrol, along with tannins, contribute through metal ion chelation and oxidative pathway modulation (Jan *et al.*, 2022). Methanolic extracts typically show higher concentrations of these compounds, including terpenoids and saponins, leading to superior antioxidant effects compared to aqueous extracts, which are richer in water-soluble tannins and glycosylated flavonoids (Ed-Dahmani *et al.*, 2024).

Flavonoids from plants like *Vaccinium myrtilloides* and *Morus alba* have been shown to exhibit concentration-dependent reducing power, effectively protecting cells from oxidative damage. In *Vaccinium myrtilloides*, a blueberry species, flavonoids such as quercetin, myricetin, and anthocyanins (cyanidin-3-glucoside and delphinidin-3-glucoside) are key contributors, identified through phytochemical assays (Bayazid *et al.*, 2021). These compounds demonstrate potent antioxidant activity by donating electrons to neutralize free radicals, as evidenced in assays like Ferric Reducing Antioxidant Power (FRAP) with their efficacy increasing with concentration (Bayazid *et al.*, 2021). Similarly, *Morus alba* (white mulberry) leaves are rich in flavonoids including quercetin-3-O-glucoside, kaempferol-3-O-rhamnoside, and rutin, which exhibit strong reducing power and protect cellular structures by scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation (Chen *et al.*, 2021a). Research highlights that these flavonoids upregulate antioxidant enzymes like superoxide dismutase (SOD) and catalase, further enhancing their protective effects against oxidative stress in a dose-dependent manner, making both plants valuable for potential therapeutic applications.

2.3 Etiology, Symptoms and Prevalence of Fever

Fever is a condition which is characterized by elevated of body temperature above 37.5°C. Normal body temperature is considered to be ranging from 36.5–37.5 °C (Balli *et al.*, 2023). This physiological response is an immunological response that occurs through the body immune system and is initiated by pathogens such as bacteria or viruses (El-Radhi, 2019). Fever is usually associated with shivering, sweating, headaches, muscle aches, and malaise. This means that increased temperature will slow down the multiplication rate of the pathogens and assist the immune system in fighting the invading organisms successfully (Balli *et al.*, 2023). However, fever can become dangerous in certain cases, such as when it is excessively high, and lasts for an extended period, or occurs in young children under 5 years old and the elderly (Barbi *et al.*, 2017). The condition predominantly affects children between 6 and 60 months of age, with its prevalence estimated at approximately 2% to 5% in this population (Tarhani *et al.*, 2022). In Kenya, over 10 million cases of acute febrile illness are treated each year among children under five years of age, with the majority being clinically managed as malaria in the absence of parasitological confirmation (O'Meara *et al.*, 2015). Active management may sometimes be required, including rehydration and administration of antipyretic agents to lessen the discomfort and other complications.

2.3.1 Biochemistry of Fever

Fever arises when endogenous or exogenous pyrogens elevate the body's thermoregulatory set point (Osilla *et al*., 2023). These pyrogens stimulate white blood cells to release endogenous mediators that act on the anterior hypothalamus, resulting in an increase in body temperature above 37.5°C (El-Radhi, 2019). The process involving prostaglandin E2 (PGE2) begins when external pathogens such as bacteria and viruses induce the release of endogenous pyrogens, including interleukins (IL-1, IL-6), tumor necrosis factor (TNF), and interferon (IFN), which modify the hypothalamic set point through the organum vasculosum of the lamina terminalis (OVLT), thereby increasing core body temperature (Balli *et al*., 2022). These endogenous pyrogens also activate both immune and inflammatory pathways. The immune response entails leukocytosis, T-cell activation, B-cell proliferation, and enhanced leukocyte adhesion (Balli *et al*., 2023), while the inflammatory response involves elevated acute-phase reactants, accelerated muscle protein degradation, and increased collagen synthesis (Osilla *et al*., 2023). Consequently, infection or tissue injury induces the production of pro-inflammatory cytokines such as interleukin-1β and TNF-α, which promote the synthesis of PGE2 near the preoptic area of the hypothalamus, ultimately leading to an elevation in body temperature (Tegegne & Alehegn, 2023).

2.3.2 Modulation of Fever

Cytokines serve as the key endogenous pyrogens responsible for mediating the well-regulated inflammatory response to tissue damage and infection. Among these, pyrogenic cytokines such as interleukin-1β (IL-1β), tumor necrosis factor (TNF), and interleukin-6 (IL-6) act directly on the hypothalamus to initiate and regulate fever (Santacroce *et al*., 2023). Most antipyretic agents function by suppressing or inhibiting the expression of cyclooxygenase-2 (COX-2), thereby decreasing body temperature through the inhibition of prostaglandin E2 (PGE2) synthesis. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used antipyretics, and their principal mechanism involves the inhibition of the cyclooxygenase (COX) enzyme, which is essential for converting arachidonic acid into prostaglandins, thromboxanes, and prostacyclins (Ghlichloo & Gerriets, 2022).

2.3.3 Conventional Management of Fever

Fever is commonly treated using antipyretic drugs to reduce body temperature and relieve symptoms (Green *et al.*, 2021; Hussain *et al.*, 2020). Two of the most widely used antipyretics in children are paracetamol and ibuprofen (Green *et al.*, 2021). Studies have found that over 90% of parents use paracetamol to manage fever (Hussain *et al.*, 2020). Guidelines recommend that either paracetamol or ibuprofen can be safely used in children to improve discomfort manifested by fever rather than achieving euthermia (Green *et al.*, 2021).

While antipyretic use is common, significant gaps exist in optimal practices. Hussain *et al.* (2020) found issues like administering incorrect doses and using alternative routes like suppositories inconsistently. Many parents also held false beliefs such as combining antipyretics or alternating them is more effective than monotherapy. Additionally, individuals aged 16 to 45 years, the mortality risk linked to NSAID use is about 1 in 10,000, but this risk escalates nearly tenfold in those aged over 75 years. Guidelines emphasize accurate, weight-based dosing alone or in consultation with healthcare professionals as safer than unsupervised multiple drug use (Green *et al.*, 2021). Parental fever phobia and misconceptions about antipyretics have contributed to suboptimal practices (Hussain *et al.*, 2020).

Additionally, NSAIDs namely ibuprofen are known to increase susceptibility to more severe clinical manifestations associated with COVID-19 infection (de Girolamo *et al.*, 2020). Ibuprofen treatment may improve the bioavailability of angiotensin-converting enzyme (ACE-2), which can potentiate and enhance the infectious processes of coronaviruses (Fang *et al.*, 2020). Despite suggestions that patients may use paracetamol to address COVID-19 symptoms, prolonged or excessive use of the drug may also result in permanent hepatotoxicity and early gastric toxicity (Ma *et al.*, 2020).

Education on evidence-based indications for antipyretics is needed. Also, plants like *C. papaya* that have both antipyretic properties and antioxidant effects could serve as a natural alternative worth further exploration (Green *et al.*, 2021). Additional research on local understandings of treatments may facilitate cultural sensitivity in improving antipyretic guidelines and empowering informed caregiver choices. While antipyretics remain first-line therapy for fever-associated distress, their rational use requires addressing ongoing misconceptions to optimize safety and outcomes. Investigating alternative antipyretics derived from indigenous plants holds promise for developing novel evidence-based options tailored for local needs.

2.3.4 Traditional Management of Fever

Herbal management of fever encompasses a diverse array of plant-based remedies that have been traditionally used to alleviate symptoms (Hines, 2021). Willow bark, for example, contains salicin, a compound which contain antipyretic and anti-inflammatory properties, making it a natural alternative to aspirin (Lin *et al.*, 2023). Echinacea, renowned for its immune-boosting effects, is often employed to boost the body resistance to infections that can cause fever (Babich *et al.*, 2020). Ginger, with its potent anti-inflammatory and antiviral properties, is valued for its ability to soothe symptoms associated with respiratory infections, including fever (Mashhadi *et al.*, 2013). Similarly, elderflower is valued for its diaphoretic properties, which promote sweating and aid in lowering body temperature. Peppermint offers cooling relief for fever-related discomfort, while yarrow induces sweating to help dissipate heat from the body.

Catnip, lemon balm, garlic, and turmeric also contribute to herbal fever management, each with its unique blend of antimicrobial, anti-inflammatory, and immune-supportive properties (Parham *et al.*, 2020). Herbal remedies can be effective in managing fever, it is crucial to seek assistance from a healthcare professional to ensure safe and proper usage, particularly in cases of prolonged or severe fever. By integrating herbal treatments with conventional care and adopting holistic approaches to wellness, individuals can effectively navigate episodes of fever while supporting overall health (Kalariya *et al.*, 2023).

2.3.5 Approaches for Evaluating Antipyretic Agents

Screening methods are used to assess the efficacy of antipyretic agents in the treatment of fever. These techniques are usually performed on animal models by making them develop fever and then determining the effect of the tested compounds on their temperature. The yeast-induced hyperthermia test is a common method for evaluating antipyretic activity in rats. This is done by injecting a pyrogenic agent such as Brewer's yeast, which stimulates endogenous pyrogens and increases body. A 10–15% Brewer's yeast solution, dissolved in saline is injected subcutaneously, and temperature changes are recorded after 3–4 hours. If fever develops, a test compound or a control drug like paracetamol is administered orally or intraperitoneally, and temperature is monitored at set intervals to assess the antipyretic effect (Liu *et al.*, 2017). While this test is valued for its simplicity and its close resemblance to natural fever, it has some limitations, including variations in yeast response among different rat strains and the physiological stress caused by fasting, which may affect results (Xie *et al*.,2022). Despite these drawbacks, it remains a valuable tool in preclinical antipyretic research.

Secondly, Lipopolysaccharide (LPS) induced pyrexia test is widely used to study antipyretic effects as it closely mimics bacterial infection-induced fever in humans. LPS and endotoxin from gram-negative bacteria, activates cytokines such as IL-1, IL-6, and TNF-α thus triggering fever (Skrzypczak-Wiercioch & Sałat, 2022). LPS is administered intraperitoneally at a dose of 100–200 µg/kg, and body temperature is recorded at various intervals to track fever onset. Once fever is induced, a test compound or a standard antipyretic drug is administered, and temperature changes are monitored to determine its effectiveness. This test is valuable because it aligns closely with human fever mechanisms, making it a reliable model for evaluating antipyretic drugs (Lasselin *et al.*, 2020). However, LPS can cause systemic inflammation, leading to variability in fever onset and physiological responses, which may affect the accuracy of results (Wang *et al.*, 2023). Despite these challenges, it remains a widely used and effective method in antipyretic drug development.

Thirdly, the carrageenan-induced pyrexia test is another widely used method for assessing the effectiveness of antipyretic agents by provoking inflammation-induced fever. Carrageenan, a polysaccharide extracted from red seaweed, is injected subcutaneously or intraperitoneally, triggering the release of endogenous pyrogens and an increase in body temperature. This test is favored for its reliability in generating fever for antipyretic screening; however, it has some drawbacks. The carrageenan injection causes significant inflammation and localized pain, which can induce stress in the test animals and potentially impact the results (Wang *et al.*, 2023). Despite these concerns, the carrageenan-induced pyrexia test remains an important tool for evaluating new antipyretic treatments.

2.3.6 Turpentine Oil-Induced Pyrexia Test

The Turpentine oil-induced pyrexia test is an assay used in antipyretic tests to cause fever through inflammation. The turpentine oil is injected intramuscularly in rats, and this causes inflammation around the injection site and thus sets off the chain reaction to produce endogenous pyrogens which causes fever (Mworia *et al.*, 2019). The turpentine oil should be small, normally 5 mL or less, and the fever should usually be set within 4 - 6 hours. After the fever is established, the test compound is administered to the rat. The body temperature of the rat is recorded at different time intervals to assess the antipyretic activity of the compound. This study will utilize this method because the amount of turpentine that will be required to induce fever is less, additionally turpentine is cheap and readily available. Fever that is induced by using turpentine is better tolerated by animals than that induced by other exogenous pyrogens (Prasad *et al.*, 2015).

2.4 Oxidative Stress and Anti-Oxidants

Oxidative stress occurs due to an imbalance between the productions ROS and the ability of the body system to readily detoxify the reactive intermediates or easily repair the resulting damage (Chaudhary *et al.*, 2023). Reactive oxygen species are constantly being produced in the human body as a natural by-product of oxygen metabolism and have important roles in cell signaling. However, excess ROS can damage cellular lipids, proteins, and DNA and contribute to pathogenic processes through oxidative damage if not kept under control by antioxidants (Tsakni *et al.*, 2025).

2.4.1 Oxidative Stress and Reactive Oxygen Species Production

Sharifi-Rad *et al.* (2020) outlined the endogenous and exogenous sources of ROS. Endogenously, mitochondria during cellular respiration, peroxisomes, and the endoplasmic reticulum were identified as sites of ROS generation (Kıran *et al.*, 2023). Exogenously, environmental pollutants, radiation, drugs, and cigarette smoke can induce ROS formation (Sharifi-Rad *et al.*, 2020). The study by Chaudhary *et al.* (2023) describes how mitochondrial electron transport chain complexes I and III leak electrons, reducing oxygen to superoxide radicals. Superoxide is then converted to hydrogen peroxide, leading to hydroxyl radical formation via the Fenton reaction.

2.4.2 Oxidative Damage of the Biomolecules

Lipids are a major target, undergoing lipid peroxidation initiated by hydroxyl radicals removing hydrogen atoms from polyunsaturated fatty acids (Kıran *et al.*, 2023). This produces lipid radicals and peroxyl radicals, generating compounds like malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) that disrupt membrane structure (Pizzino *et al.*, 2017). Proteins are also subject to oxidation, with amino acid side chains undergoing addition or substitution reactions from ROS attack (Chaudhary *et al.*, 2023). Nucleic acids are subjected to modifications, breaks, and crosslinks thus impairing genetic integrity (Khan & Ali, 2017).

2.4.3 Role of Antioxidants in Neutralizing Free Radicals

Endogenous antioxidants include glutathione, melatonin, coenzyme Q10, uric acid, bilirubin, albumin, ferritin, and various enzymes such as superoxide dismutase, catalase, and peroxiredoxins (Pizzino *et al.*, 2017). Dietary antioxidants involving vitamins C and E, carotenoids, flavonoids, polyphenols, and organosulfur compounds enhance the body antioxidant defenses (Sharifi-Rad *et al.* (2020; Chaudhary *et al.* 2023). The studies support antioxidants directly reacting with radicals through hydrogen atom transfer, electron donation, or molecular adduction mechanisms to terminate chain reactions (Chaudhary *et al.*, 2023). Glutathione and ascorbic acid can scavenge hydroxyl radicals but may act as pro-oxidants in the presence of transition metals (Sharifi-Rad *et al.*, 2020).

2.4.4 Role of Oxidative Stress in Disease

Cancer initiation and promotion occur partly through oxidative DNA damage induced by ROS (Pizzino *et al.*, 2017). Metabolic syndrome risk rises with dysfunctional adipocytes generating excess ROS (Tsakni *et al.*, 2025). Atherosclerosis develops from the oxidation of LDL cholesterol particles (Pizzino *et al.*, 2017). Alzheimer disease exhibits elevated oxidative damage to proteins and lipids in affected brain regions (Perluigi *et al*., 2024). Pizzino *et al.* (2017) reported that rheumatoid arthritis shows ROS overproduction in inflamed joints. Other disease include malaria, neurodegeneration, preeclampsia, and chronic health conditions which are influenced by lifestyle factors (Sharifi-Rad *et al.,* 2020). Overall, oxidative stress underlies numerous pathologies.

2.4.5 Conventional Management of Oxidative Stress

Modern methods of managing oxidative stress involves decreasing the formation of ROS and increasing the antioxidants in the body with the help of drugs and changes in daily habits (Afzal *et al.*, 2023). A major approach includes using antioxidants such as vitamins, minerals, and other non-vitamins like selenium, zinc, and coenzyme Q10. These supplements have been developed to eliminate free radicals and reduce oxidative stress (Mason *et al.*, 2020). However, most of them are poorly soluble in water leading to inefficient permeability, poor bioavailability and biocompatibility (Eftekhari *et al.*, 2018).

Vitamin C (Ascorbic acid) is capable of neutralizing ROS and regenerating other antioxidants, such as vitamin E. A meta-analysis study by Saz-Lara *et al.* (2022) revealed that vitamin C supplementation enhance endothelial function in patients with chronic diseases, indicating potential vascular benefits. High-dose intravenous vitamin C has demonstrated potential in reducing inflammation and oxidative stress markers, thereby mitigating disease progression in the early stages of COVID-19 pneumonia. This effect may be attributed to its ability to enhance inflammatory regulation, immune response, and coagulation function (Zhao *et al.*, 2021).

Secondly, Vitamin E (α-Tocopherol) is a lipid-soluble antioxidant that protects polyunsaturated fatty acids within cell membranes from peroxidation. Jiang *et al.*

(2014) discussed the neuroprotective role of vitamin E, particularly in Alzheimer disease, reduced biomarkers of oxidative stress and delayed cognitive decline in some populations. However, the main concerns are the high-dose supplementation, which may increase all-cause mortality in certain groups (Grigore-Gurgu *et al.*, 2025).

Thirdly, Selenium is essential for glutathione peroxidase activity, a key enzyme in detoxifying peroxides. Souza *et al.* (2025) emphasized the importance of selenium in thyroid function and immunity. Selenium is an essential component of selenoproteins, which exhibit diverse biological functions, including antioxidant and anti-inflammatory activities, as well as the synthesis of active thyroid hormones. Additionally, individuals with mild cognitive impairment reported that selenium supplementation improved cognitive function and reduced oxidative markers (Pereira *et al.*, 2022).

Ubiquinone (CoQ10) functions in mitochondrial respiration and scavenges free radicals. A systematic review found that CoQ10 supplementation improves oxidative stress and inflammation markers in patients with metabolic syndrome (Dludla*et al.*, 2020). It has been shown to be promising in reducing cardiovascular and neurodegenerative diseases, often in combination with other therapies (Dludla*et al.*, 2020).

N-acetylcysteine (NAC) acts as a precursor to glutathione, the most abundant endogenous antioxidant found in the body. NAC has been studied extensively for its role in psychiatric disorders and chronic lung diseases. Dean *et al.* (2011) found that NAC significantly reduces oxidative and inflammatory markers in schizophrenia and bipolar disorder. It has shown therapeutic potential in the management of schizophrenia by addressing both oxidative stress and glutamatergic dysfunction, indicating that the phenotype of the disorder that may arise from interactions among multiple neurotransmitter pathways.

Alpha-Lipoic Acid (ALA) is both water- and fat-soluble and plays a crucial role in regenerating other antioxidants, such as glutathione and vitamins C and E. It serves as an essential cofactor in mitochondrial respiration and functions as a potent antioxidant. ALA also exhibits multiple metabolic benefits, including anti-obesity, glucose-lowering, insulin-sensitizing, and lipid-lowering effects. Moreover, it has been shown to significantly enhance insulin sensitivity and reduce oxidative stress markers in patients with type 2 diabetes (Ghelani *et al.*, 2017).

Pharmacological agents also have a role; for example, statins reduce cholesterol and have some antioxidant properties, whereas other drugs may be antioxidants or can modulate the endogenous antioxidant systems (Fracassi *et al.*, 2019). Lifestyle changes are also a part of the treatment process, which includes changes in diet, emphasizing diets rich in antioxidants like fruits and vegetables, nuts, and whole grain products. The Mediterranean diet is particularly recommended because of the high content of antioxidants and healthy fats that may decrease oxidative stress (Altawili *et al.*, 2023). Physical exercise is also recommended as it stimulates the body endurance capacity for antioxidants and the synthesis of endogenous antioxidants that would help in combating oxidative stress (Meng & Su, 2024).

2.4.6 Traditional Management of Oxidative Stress

Oxidative stress management in traditional medicine involves using natural products and treatments grounded in cultural practices and medicines. Herbal medicine are applied based on their antioxidant compounds. Green tea is rich in catechins, which play a crucial role in protecting against oxidative DNA damage and lipid peroxidation by scavenging free radicals and enhancing the activity of antioxidant enzymes. Among its numerous antioxidant compounds, catechins are the most significant and include epigallocatechin, epicatechin, epigallocatechin-3-gallate, and epicatechin-3-gallate (Dehzad *et al*., 2025). Additionally, tea flavonoids mitigate oxidative stress by activating various signaling pathways, such as protein kinase Cδ/acidic sphingomyelinase and protein kinase B/endothelial nitric oxide synthase.

Turmeric contains the active compound curcumin, which is used in Ayurveda and traditional Chinese medicine to reduce oxidative stress (Tian *et al*., 2025). It scavenges free radicals, inhibits lipid peroxidation, and boosts antioxidant enzymes like superoxide dismutase and catalase. Curcumin exhibits anti-inflammatory, antioxidant, anti-diabetic, and anti-atherosclerotic properties, which contribute to the improvement of metabolic parameters and the alleviation of symptoms associated with polycystic ovarian syndrome, non-alcoholic fatty liver disease, and cardiovascular disorders (Qiu *et al.*, 2023). Thus, it is crucial in reducing oxidative markers in metabolic syndrome and inflammation-related disorders. There are also dietary practices that go hand in hand with traditional cultures, including consuming foods that contain antioxidants. Some examples include using spices for culinary purposes, like ginger and garlic, which are attributed to their antioxidant properties (Hussain *et al.,* 2022). Medalcho *et al.* (2025) reported that spices used for popular Ethiopian spicy hot red pepper powder production is promising sources of antioxidants with positive health effects; this is due to the presence of free radical scavengers or inhibitors possibly acting as primary antioxidants.

Mind-body techniques such as meditation, yoga, and Tai Chi address stress reduction and wellness. These techniques are assumed to decrease oxidative stress by ameliorating psychological stress levels and promoting a healthy lifestyle (Sharifi-Rad *et al.*, 2020). Regular yoga and meditation practice has been shown to reduce oxidative stress by modulating cortisol and inflammatory cytokines. Syarif *et al.* (2025) reported significant reductions in ROS and increase in endogenous antioxidants (like glutathione) in yoga practitioners. Glutathione is an essential substrate for the antioxidant defense system. It is used by glutathione peroxidase as a donor of hydrogen atoms to reduce hydrogen peroxide into water, thus a defense mechanism that decreases oxidative stress.

Detoxification is another important aspect of the traditional system of management where techniques like fasting, a cleansing diet, and the use of certain herbs responsible for detoxification are directed toward eradication of toxic stuff from the body and facilitating the natural processes of the body (Hodges & Minich, 2015). Furthermore, traditional concepts focus on maintaining a healthy lifestyle by having enough sleep, engaging in moderate exercise, and managing stress are believed to integrate the body antioxidant capacity (Radulescu *et al.*, 2024).

2.4.7 *In vitro* Assays for Evaluating of Antioxidant Activity

The DPPH radical scavenging assay is a widely used technique for evaluating antioxidant activity. It is based on the reduction of the purple DPPH radical to yellow diphenylpicrylhydrazine upon receiving an electron or hydrogen atom from an antioxidant. The resulting change in absorbance at 517 nm is then measured spectrophotometrically to quantify the antioxidant potential. This method is relatively simple, quick, and suitable for preliminary testing (Teshome *et al.*, 2022).

The ABTS radical cation decolorization assay evaluates antioxidants due to their ability to reduce the blue-green ABTS•+ radical cation, with absorbance measured at 734 nm. It can assess both hydrophilic and lipophilic antioxidants and is applicable to various samples, including biological fluids (Ilyasov *et al.*, 2020; Munteanu & Apetrei, 2021). Thirdly, the Ferric Reducing Antioxidant Power (FRAP) assay quantifies antioxidant activity based on the reduction of Fe^{3+} to Fe^{2+}, which forms a colored complex with TPTZ, measured at 593 nm. This method is reliable, easy to perform, and applicable to plasma and other body fluids, making it common in biomedical research. The hydrogen peroxide scavenging assay measures an antioxidant ability to decompose H_2O_2 into water and oxygen. A decrease in absorbance at 230 nm reflects higher free radical scavenging capacity, providing insights into potential health applications and disease prevention (Pleh *et al.*, 2021).

Lipid peroxidation assays determine the capacity of antioxidants to inhibit oxidative degradation of lipids, often measured by the formation of thiobarbituric acid reactive substances at 532 nm, indicating protection against cell membrane damage (Muthoni *et al.*, 2020). Total flavonoid content is estimated using the aluminum chloride colorimetric method, where flavonoids form a yellow complex measurable at 415 nm, serving as an indicator of antioxidant potential (Munteanu & Apetrei, 2021). Total phenolic content is assessed using the Folin–Ciocalteu reagent, which reacts with phenolic compounds to produce a blue color measured at 765 nm, reflecting overall antioxidant capacity (Baliyan *et al*., 2022). Finally, the hydroxyl radical scavenging assay evaluates the ability of antioxidants to neutralize hydroxyl radicals generated through the Fenton reaction, with reduced degradation of substrates measured spectrophotometrically, indicating effective free radical scavenging (Muthoni *et al*., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The plant sample was obtained from Karingani ward, Chuka-Igambang'ombe sub-county, Tharaka Nithi County, Kenya, at coordinates -0.319178° S and 37.620659 ° E. This region is located on the windward side of Mount Kenya and has a highland equatorial climate with bimodal rainfall (1,000–1,400 mm annually) and temperatures ranging from 18°C to 27°C.

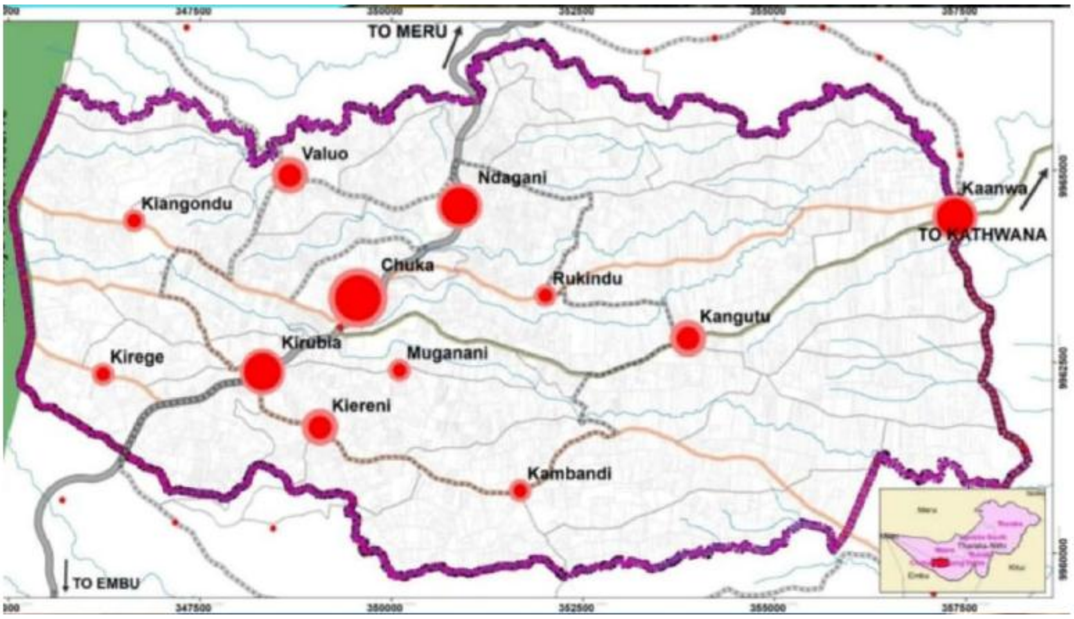

Plate 2: Map of Karingani ward showing the sampling sites

3.2 Research Design

A completely randomized design (CRD**)** was used for this study. The experiment was conducted in three phases: phytochemical profiling of methanolic leaf and root extracts was done using GC-MS with three replicates per extract type. The *in vivo* antipyretic testing was done in male albino Wistar rats with five replicates per treatment group, and *In vitro* antioxidant was done using DPPH radical scavenging assay, ferric reducing power assay, hydroxyl Radical (-OH) scavenging activity, lipid peroxidation activity, total phenolic and flavonoids content with three replicates per extract type. All samples and treatments were randomly assigned to ensure uniform experimental conditions and reduced bias.

3.3 Collection and Preparation of Sample Materials

The fresh leaves and roots of *C. papaya* the variety of Sunrise Solo were collected from Karingani ward, Chuka -Igambang'ombe sub-county, Tharaka-Nithi County, Kenya. The samples were sorted to eliminate any damaged, diseased, or extraneous parts, followed by cleaning to remove debris and soil. It was packed in sterile, labeled khaki bags and transported to the Biochemistry and Biomedical Laboratory at Chuka University for processing and analysis.

3.4 Processing and Extraction of the Plant Material

The leaves and roots were dried at room temperature and away from direct sunlight to preserve thermolabile phytochemicals. It was then ground into powder using a heavy duty blender (Appendix 2); the plant powder was stored in dry plastic bags at room temperature, away from direct sunlight until extraction. For extraction, 250 g of each sample was soaked in 1.5 liter of methanol for 24 h to extract the active compounds (Appendix 3). The extracts were decanted and filtered using Whatman No. 1 filter paper into a new dry clean conical flask (Appendix 4). The filtrate was vacuum dried by using a rotary evaporator at 40°C to give a solid residue (Arika *et al.*, 2019). The extracts were stored at -20°C.

3.5 Quantitative Phytochemical Screening

A phytochemical screening of the plant extracts was done to determine the class of secondary metabolites present which includes, total phenols, flavonoids, alkaloids, tannins and saponins using gas chromatography-mass spectrometry (GC-MS). One milligram of each of the extracts was dissolved in one milliliter of dichloromethane (sigma Aldrich gc-grade) and swirled for thirty seconds and then sonicated for fifteen minutes in an ultra-bath and thereafter centrifuged at fourteen thousand revolutions per minute for five minutes. The moisture was removed by passing the supernatants through anhydrous sodium sulphate. The resultant stock preparations of the extracts were used to make technical samples which was diluted to a final concentration of 100 ng/µl. Each extract sample was prepared in triplicates.

Gas Chromatography-Mass Spectrometry instrument (7890/5975 Agilent Technologies, Inc., Beijing, China in the University of Kenyatta) was used to analyses each sample. The instrument is furnished with a low bleed capillary column HP-5 MS (5% phenyl methyl siloxane) that is 30 metres long with a film having a thickness of 0.25- and 0.25-mm diameter. An electron ionization system and helium 99.999% carrier gas with a constant flow of 1.25 ml per minute in split mode was used to detect the GC-MS. One µl injector volume was added by the injector. The line transferring the mass was regulated at 250 and 200°C, respectively. The oven temperature was automated to begin at 35°C for five minutes, it was raised at 10°C per minute for 10.5 minutes until it reached to 280°C, then it was further increased at 50°C per minute for 29.9 minutes until it reaches 285°C. The total run time minutes was 70. The parameters used in the mass spectrometry was as follows: scan speed; 1666 µ/sec, ion source temperature; 230°C, energy for ionization; 70eV, interface temperature; 250°C, scan range; 40-550 m/z, and relative detector gain mode and solvent cut time of 3.3 minutes (Madhu *et al.*, 2016).

The GC-MS analysis identified compounds depending on their general of fragmentation and the results referenced to a spectra library published by National Institute of Standards and Technology (NIST). A spectra match identity of more than 80% was mandated to identify phytochemicals using NIST library. Carbon 5-Carbon 32 hydrocarbons range was used to determine the retention indices. The compound name, its chemical group and the molecular weight of the plant extract constituents was determined. Each compound relative concentration was expressed as percent with the normalized peak-area.

3.6 Experimental Animals for Testing Antipyretic Activity

The study used thirty male white albino Wistar rats aged between 12 weeks old and weighing 90-120g (Arika *et al.*, 2019). The rats were housed in standard polypropylene cages and maintained under controlled laboratory conditions, with an ambient temperature of 23 ± 2°C and relative humidity of 55 ± 5%, following a 12-hour light and 12-hour dark cycle. All animals had free access to standard rodent pellets and tap water ad libitum (Alyas, 2020). The ethical guidelines and procedures of handling the laboratory animals were followed (Kiani, 2022).

3.7 In *vivo* Evaluation of Antipyretic Activities

The test was carried out according to the procedure outlined by Gaichu *et al.* (2017). The accuracy of the digital thermometer was compared to that of a mercury thermometer. The rectal temperature was taken using thermometer model YB-009 by inserting about 3 cm of the thermistor probe into the rectum (Appendix 5). The probe was well-lubricated. For fever induction, Turpentine 20% was used at a dose of 20 ml/kg body weight and was administered intraperitoneally after measuring the initial basal rectal temperature. The animals were left for one hour. The elevation in fever magnitude after one hour of intraperitoneal turpentine injection was considered as 100% fever response. An increase of 0.8°C in rectal temperature was considered pyretic and was used in the study. The rectal temperature was measured after an hour for 4 h. The rectal temperature percentage change was calculated using the following formula based on the temperature before and after treatment

$$\text{The Rectal Temperature \% change } = \frac{B - Cn}{B} \times 100\%$$

B: Temperature of the rectum one hour after injection of turpentine

Cn: Rectal temperature after drug and extract administration

3.8 Experimental Design

A total of 30 healthy male albino Wistar rats were randomly assigned into six groups (n = 5 per group) using a completely randomized design (CRD). Randomization minimized bias and ensure equal distribution of biological variability across the groups (Arika *et al.*, 2019). Group I contained normal control albino Wistar rats; they were induced with fever but given 10% DMSO only (Table 1). These rats did not receive any treatment. Group II were the negative control albino Wistar rats which were induced with fever and treated with the vehicle solvent (10% DMSO) (Table 1). Group III were the positive control albino Wistar rats, they were induced with fever and then treated using paracetamol (500 mg to 1 g every 4 to 6 hours) (Table 1). Group IV-VI were the experimental albino Wistar rats, induced with fever and given methanolic leaf and root extracts of *Carica papaya* (50, 100, and 150 mg/kg body weight) (Table 1).

Table 1: Treatment Protocol for Evaluation of Antipyretic Activity of Methanolic Leaf and Root Extract of *C. papaya* in Wistar Albino Rats

Group	Status	Treatment
I	Normal control	10% DMSO
II	Negative control	Turpentine + DMSO
III	Positive control	Turpentine + Paracetamol
IV	Experimental group A	Turpentine +50 mg/kg bwt extract
V	Experimental group B	Turpentine +100 mg/kg bwt extract
VI	Experimental group C	Turpentine +150 mg/kg bwt extract

The preparation of plant extracts was done on the day of the experiment. A DMSO solvent was used to dissolve each treatment. Turpentine, paracetamol solution, solvent, and sample solutions were administered intraperitoneally.

3.9 *In vitro* Determination of Antioxidant Activity

The antioxidant activities of methanolic leaf and extracts of *Carica papaya* was determined using a DPPH radical scavenging assay, ferric reducing power assay, hydroxyl Radical (-OH) scavenging activity, lipid peroxidation activity, total phenolic and flavonoids content.

3.9.1 Determination of Antioxidant Activity using DPPH Radical Scavenging Assay

The antioxidant activities of the methanolic leaf and root extracts of *Carica papaya* were evaluated using the DPPH radical scavenging assay as outlined by Tsakni *et al*. (2025), with slight modifications. The extracts were prepared in methanol at concentrations of 1000, 750, 500, 250, 125, and 62.5 mg/mL. For each assay, the reaction mixture contained 1 mL of extract, 3 mL of methanol, and 0.5 mL of 0.1 mM methanolic DPPH solution. The mixtures were thoroughly mixed and incubated in the dark at room temperature for 30 minutes. Absorbance readings were then taken at 517 nm using a spectrophotometer. A solution containing methanol and DPPH served as the blank, while a mixture of methanol, DPPH, and ascorbic acid was used as the positive control. All experiments were conducted in triplicate. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\% \text{ of DPPH Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0- the absorbance of control reaction (blank)

A_1- the absorbance of the extracts and Ascorbic acid.

3.9.2 Determination of Antioxidant Activity using Ferric Reducing Power Assay

The antioxidant activities of methanolic leaf and root extracts of *Carica papaya* were determined using the ferric reducing antioxidant power (FRAP) assay, as described by González-Palma *et al.* (2016), with minor modifications. A reaction mixture containing 1 mL of extract, 2.5 mL of phosphate buffer (200 mM, pH 6.6), and 2.5 mL of potassium ferricyanide (30 mM) at different extract concentrations (50–250 µg/mL) were incubated at 50°C for 20 minutes. Thereafter, 2.5 mL of trichloroacetic acid (600 mM) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. A 2.5 mL aliquot of the supernatant was collected and mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (6 mM). The absorbance of the resulting solution was measured at 700 nm. The blank contained all reactants except the extract, and ascorbic acid was used as a standard. All tests were performed in triplicate.

3.9.3 Determination Antioxidant Activity using Hydroxyl Radical (^{-}OH) Scavenging Activity

The hydroxyl radical scavenging activity was determined following the method described by Arika *et al*. (2019), with slight modifications. A 40 mM hydrogen peroxide (H_2O_2) solution was prepared in phosphate buffer (pH 7.4). Separate sets of test tubes were used for *Carica papaya* leaf and root extracts, each tested at concentrations of 50, 100, 150, 200, and 300 µg/mL. Hydrogen peroxide solution was added to the extracts, and the mixtures were incubated for 10 minutes at room temperature. The absorbance was then measured at 230 nm against a blank containing phosphate buffer without hydrogen peroxide. Ascorbic acid served as the positive control. All experiments were carried out in triplicate, and the hydroxyl radical scavenging activity was calculated using the following formula:

$$\% \text{ of Hydroxyl Radical Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0- the absorbance of control reaction (blank)

A_1 -the absorbance of the extracts and gallic acid.

3.9.4 Determination of Antioxidant Activity Using Total Phenolic Contents

The total phenolic content of the extracts was measured using the Folin-Ciocalteu method, adapted from Muthoni *et al.* (2020) with minor modifications. Five test tubes were prepared for *Carica papaya* leaf and root extracts. Each extract was tested at concentrations of 50, 100, 150, 200, 250, and 300 mg/mL. In each tube, 1 mL of the extract was mixed with 2 mL of Folin-Ciocalteu reagent, previously diluted with distilled water in a 1:10 (v/v) ratio, followed by the addition of 1 mL of 20% sodium carbonate (Na_2CO_3). The mixture was shaken for 20 seconds and incubated at 40°C for 30 minutes. Absorbance was measured at 765 nm. Gallic acid was used to generate the standard curve, and the total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of extract. All test was performed in triplicate.

3.9.5 Determination of Antioxidant Activity Using Total Flavonoid Contents

The total flavonoid content of the extracts was determined using the method described by Muthoni *et al.* (2020) with few modifications. Five test tubes were prepared for *Carica papaya* leaf and root extracts, and each extract was tested at concentrations of 50, 100, 150, 200, 250, and 300 mg/mL. In each tube, 0.3 mL of the extract was mixed with 3.4 mL of 30% methanol, 0.15 mL of 0.5 M $NaNO_2$, and 0.15 mL of 0.3 M $AlCl_3 \cdot 6H_2O$. After 5 minutes, 1 mL of 1 M NaOH was added, mixed thoroughly, and the absorbance was measured at 510 nm against a reagent blank. The standard curve for total flavonoids was prepared using quercetin solutions ranging from 0 to 100 mg/L. Total flavonoid content was expressed as milligrams of quercetin equivalents per gram of extract. All test were performed in triplicate.

3.9.6 Determination of Antioxidant Activity Using Lipid Peroxidation Activity

The malondialdehyde (MDA) assay was conducted following the procedure described by Arika *et al*. (2019), with slight modifications. Separate sets of test tubes were prepared for the leaf and root extracts, each tested at concentrations of 25, 50, 100, 150, 200, and 250 µg/mL. The reaction mixture, with a total volume of 1.0 mL, consisted of the plant extract and 2.0 mL of TCA-TBA-HCl reagent (15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid, and 0.25 N hydrochloric acid). The mixtures were heated in a water bath at 90°C for 10 minutes, then cooled and centrifuged at 10,000 rpm for 10 minutes to remove the precipitate, yielding a light pink supernatant indicative of MDA formation. Ascorbic acid served as the reference standard. The concentration of malondialdehyde in each sample was determined by measuring the absorbance of the supernatant at 532 nm against a reference blank. All assays were performed in triplicate. The percentage inhibition of lipid peroxidation was calculated using the following equation:

$$\%\ \text{Lipid Peroxidation} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0- the absorbance of control (blank)

A_1 is the absorbance of extracts and ascorbic acid.

3.10 Statistical Analysis

The data on phytochemicals analysis were expressed as percent with the normalized peak-area then presented in tables and graphs. The data obtained from the antipyretic and antioxidative activity tests was recorded and tabulated in a spreadsheet and the results subjected to descriptive statistics. Analysis of the data was done using *Minitab* statistical software version 22. The results were expressed as mean ± standard error of mean for analysis. Differences in rectal temperature across the six groups and between extract and standard values were analyzed using One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for pair-wise mean separations and comparisons. The statistical significance was considered at $P \leq 0.05$. The data were presented in graphs and tables.

3.11 Ethical Consideration

This study followed the ethical guidelines concerning the use of animal models in research. Prior to initiating the study, ethical approval was obtained from the Chuka University Ethical Review Committee (Appendix VIII) and a research permit was secured from the National Commission for Science, Technology, and Innovation (NACOSTI) (Appendix IX). All procedures involving rat aligned with internationally accepted standards for animal care to ensure humane treatment and minimize animal suffering. These principles guided the efforts to minimize animal suffering, reduce the number of animals used, and refine procedures to improve animal welfare. Research integrity was upheld by ensuring accurate documentation of methods and findings, proper citation of prior work, and strict adherence to anti-plagiarism standards

CHAPTER FOUR

RESULTS

4.1 Phytochemical Composition of Methanolic leaf and root extracts of *C. papaya*

4.1.1 GC–MS Analysis of Methanolic Leaf Extract of *Carica papaya*

The GC–MS analysis of methanolic leaf extract of *Carica papaya* revealed the presence of 26 phytochemicals (Table 2). Based on peak area percentage, Limonene (18.9%) was the most abundant compound, followed by Linalool (12.6%), Camphene (8.4%), β-Pinene (6.3%), Myrcene (6.3%), p-Cymene (4.2%), Rosmarinic acid derivative (4.2%), Myricetin (4.2%), Caffeic acid (4.2%), α-Tocopherol derivative (4.2%), and Terpinolene (4.2%). Other notable compounds included Eugenol (2.1%), Ferulic acid (2.1%), Quercetin derivative (2.1%), Chlorogenic acid derivative (2.1%), Thymol (2.1%), Carvacrol (2.1%), α-Pinene (2.1%), and Longifolene (2.1%), alongside minor constituents such as ascorbic acid derivative (1.1%) and gallic acid derivative (0.2%) (Table 2; Figure 1).

The GC–MS chromatogram of methanolic leaf extract of *Carica papaya* revealed the presence of various compounds with distinct peaks at different retention times (Figure 1). The retention time, name of compound, molecular formula, molecular weight, peak area (%), and chemical classification of the identified constituents are presented in Table 2.

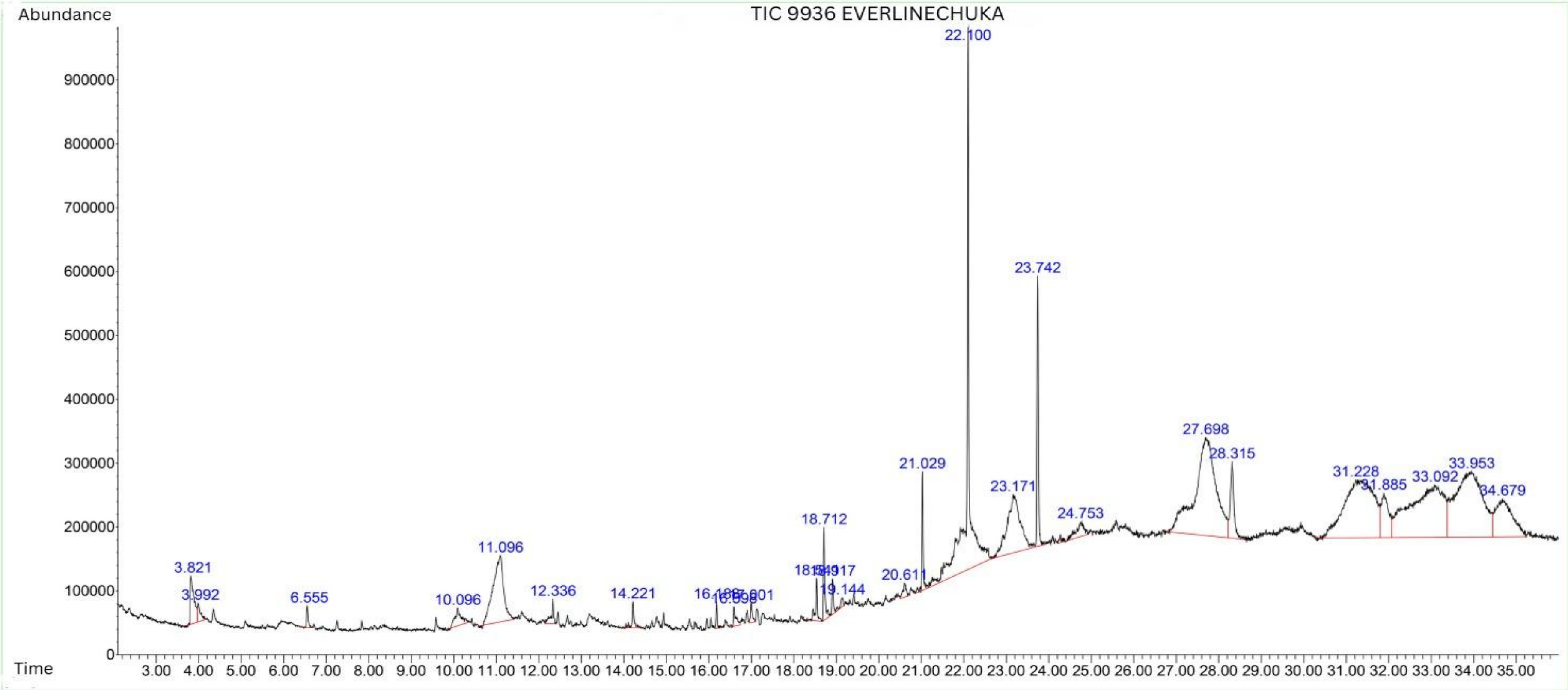

Figure 1: GC-MS chromatogram of *Carica papaya* methanolic leaf extract

Table 2:.Phytochemicals identified in methanolic leaf extract of *C. papaya* by GC-MS

Retention Time (min)	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)	Classification
3.821	Ascorbic Acid (derivative)	$C_6H_8O_6$	176.12	1.1	Vitamin C
3.992	Catechol	$C_6H_6O_2$	110.11	2.1	Phenolic
6.555	Gallic Acid (derivative)	$C_7H_6O_5$	170.12	0.2	Phenolic
10.096	Caffeic Acid	$C_9H_8O_4$	180.16	4.2	Phenolic
10.099	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324.40	2.1	Phenolic
10.131	Caffeic Acid	$C_9H_8O_4$	180.16	2.1	Phenolic
12.336	Chlorogenic Acid (derivative)	$C_{16}H_{18}O_9$	354.31	2.1	Phenolic
14.221	Ferulic Acid	$C_{10}H_{10}O_4$	194.18	2.1	Phenolic
16.896	Quercetin (derivative)	$C_{15}H_{10}O_7$	302.24	2.1	Flavonoid
18.712	Rosmarinic Acid (derivative)	$C_{18}H_{16}O_8$	360.31	4.2	Phenolic
18.917	Eugenol	$C_{10}H_{12}O_2$	164.20	2.1	Phenolic
19.144	Carvacrol	$C_{10}H_{14}O$	150.22	2.1	Phenolic Terpenoid
19.901	Thymol	$C_{10}H_{14}O$	150.22	2.1	Phenolic Terpenoid
20.611	Cinnamic Acid	$C_9H_8O_2$	148.16	2.1	Phenolic
21.029	α-Tocopherol (derivative)	$C_{29}H_{50}O_2$	430.71	4.2	Vitamin E
22.100	Limonene	$C_{10}H_{16}$	136.23	18.9	Terpene
23.171	Linalool	$C_{10}H_{18}O$	154.25	2.1	Terpene Alcohol

23.742	Linalool	$C_{10}H_{18}O$	154.25	12.6	Terpene Alcohol
24.753	α-Pinene	$C_{10}H_{16}$	136.23	2.1	Terpene
27.698	Camphene	$C_{10}H_{16}$	136.23	8.4	Terpene
28.315	β-Pinene	$C_{10}H_{16}$	136.23	6.3	Terpene
31.228	Myrcene	$C_{10}H_{16}$	136.23	6.3	Terpene
31.885	Myricetin	$C_{15}H_{10}O_8$	318.28	4.2	Flavanoid
33.092	p-Cymene	$C_{10}H_{14}$	134.22	4.2	Aromatic Terpene
33.953	Terpinolene	$C_{10}H_{16}$	136.23	4.2	Terpene
34.679	Longifolene	$C_{15}H_{24}$	204.35	2.1	Sesquiterpene

4.1.2 GC–MS Analysis of Methanolic Root Extract of *Carica papaya*

The GC–MS analysis of methanolic root extract of *Carica papaya* revealed the presence of 26 phytochemicals (Table 3). Based on peak area percentage, Squalene (27.28%) was the most abundant compound, followed by 3-Rhamnosyl-glucosyl quercetin (12.71%), Phytol (10.16%), Hexadecanoic acid, methyl ester (6.14%), Cyclooctene, 3-ethenyl- (5.74%), and n-Hexadecanoic acid (4.40%). Other notable constituents included Carbonic acid, decyl tetradecyl ester (3.04%), 9,12-Octadecadienoic acid (Z,Z)- (2.16%), Isoquercitrin (2.09%), 9,12-Octadecadienoic acid, methyl ester (2.08%), 4,8,12,16-Tetramethylheptadecan-4-olide (2.00%), Heptadecanoic acid, 16-methyl-, methyl ester (1.98%), and Reserpinine (1.76%). Minor compounds such as Quercetin (2.20%), Kaempferol glycosides (2.15%, 1.04%), Myricetin derivative (1.34%), and Caffeic acid (1.12%) were also detected in lower proportions (Table 3; Figure 2).

The GC–MS chromatogram of methanolic root extract of *C. papaya* revealed the presence of multiple bioactive compounds with distinct peaks at different retention times (Figure 2). The retention time, name of compound, molecular formula, molecular weight, peak area (%), and chemical classification of the identified constituents of are presented in Table 3.

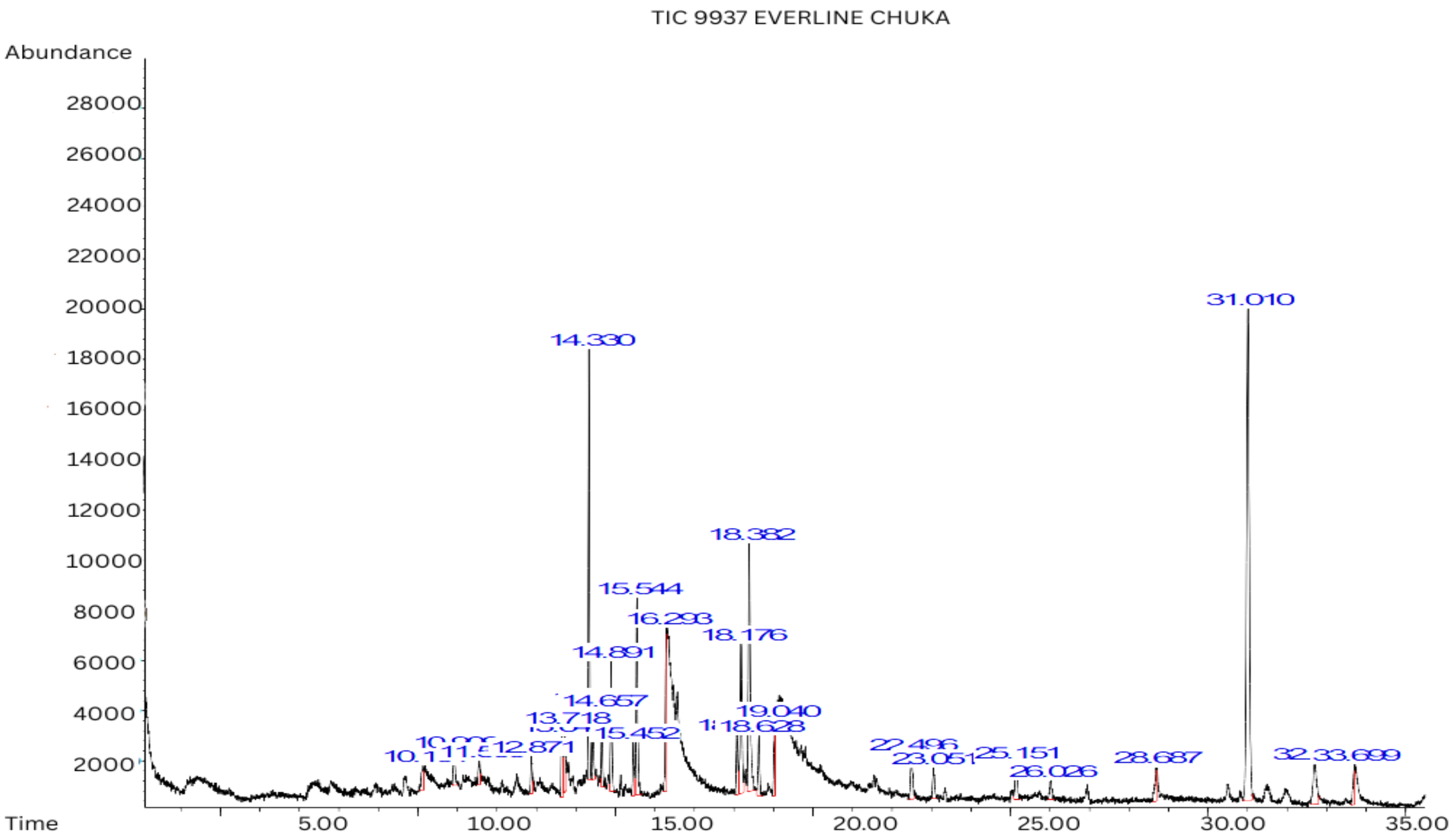

Figure 2: GC-MS chromatogram of *Carica papaya* methanolic root extract

Table 3: Phytochemicals identified in methanolic root extract of *C. papaya* by GC-MS

Retention time	Name of compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Classification
10.131	Caffeic Acid	$C_9H_8O_4$	180.159	1.12	Phenolic
10.909	3-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324.40	1.19	Phenolic derivative
11.555	cis-2,5-Dimethoxy-4-ethoxy-β-methylstyrene	$C_{13}H_{18}O_3$	222.28	0.85	Phenolic (Methoxystyrene)
12.871	4-Heptafluorobutyryloxyhexadecane	$C_{20}H_{33}F_7O_2$	438.50	1.13	Fatty acid ester
13.644	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196.24	2.53	Phenolic (Benzofuranone)
13.718	Quercetin	$C_{15}H_{10}O_7$	302.24	2.20	Flavonoid
14.330	3-Rhamnosyl-Glucosyl Quercetin	$C_{27}H_{30}O_{14}$	578.523	12.71	Flavonoid
14.433	Kaempferol-3-O-glucoside (Astragalin)	$C_{21}H_{20}O_{11}$	448.38	2.15	Flavonoid
14.657	Kaempferol derivative (glycoside)	$C_{21}H_{20}O_{11}$	448.38	1.04	Flavonoid
14.891	Isoquercitrin (Quercetin-3-O-glucoside)	$C_{21}H_{20}O_{12}$	464.379	2.09	Flavonoid
15.452	Trans-Farnesol	$C_{15}H_{26}O$	222.37	1.74	Sesquiterpene alcohol
15.544	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	6.14	Fatty acid ester
16.293	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.00	4.40	Fatty acid
18.078	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	2.08	Fatty acid ester
18.176	Cyclooctene, 3-ethenyl-	$C_{10}H_{16}$	136.23	5.74	Cycloalkene
18.382	Phytol	$C_{20}H_{40}O$	296.50	10.16	Diterpene alcohol
18.628	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298.50	1.98	Fatty acid ester
19.040	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44	2.16	Fatty acid
22.496	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324.50	2.00	Lactone
23.051	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trienyl propionate	$C_{18}H_{30}O_2$	278.43	1.15	Terpenoid ester
25.151	Reserpinine	$C_{33}H_{40}N_2O_9$	608.68	1.76	Alkaloid
26.026	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278.34	0.87	Phthalate ester
28.687	Ethanol, 2-(dodecyloxy)-	$C_{14}H_{30}O_2$	230.39	1.15	Alcohol derivative
31.010	Squalene	$C_{30}H_{50}$	410.70	27.28	Triterpene
32.698	Carbonic acid, decyl tetradecyl ester	$C_{25}H_{50}O_3$	398.66	3.04	Fatty acid ester
33.699	Myricetin derivative	$C_{15}H_{10}O_8$	318.28	1.34	Flavonoid

4.2 Antipyretic Activity on Turpentine-Induced Rats

4.2.1 Effects of Methanolic Root Extracts of *Carica papaya* on Turpentine-Induced Pyrexia in Rats

Treatment of rats with methanolic root extracts of *Carica papaya* demonstrated antipyretic properties against turpentine-induced pyrexia. The antipyretic effects at higher doses (100 mg/kg and 150 mg/kg) were comparable to those of the standard drug, paracetamol, with no statistically significant difference observed ($p > 0 .05$), but significantly greater than the negative control group ($p < 0 .05$) (Table 4; Figure 3).

In the first hour after treatment, none of the groups treated with methanolic root extracts of *Carica papaya* at dose levels of 50, 100, or 150 mg/kg body weight reduced the elevated rectal temperature to normal (Table 4). The group treated with 50 mg/kg exhibited a mild reduction of 1.15%, while the 100 mg/kg and 150 mg/kg groups showed greater reductions of 1.77% and 2.20%, respectively. However, these differences were not statistically significant ($p < 0.05$). The positive control group (turpentine + paracetamol) showed a reduction of 1.57%, which was statistically significant compared to the negative control ($p < 0.05$), but lower than that observed in the 100 and150 mg/kg experimental groups. The negative control group (turpentine + DMSO) maintained an elevated temperature with only a marginal change (–0.26%). Although the treatment groups exhibited a downward trend in rectal temperature, at this hour the reductions were not statistically significant ($p < 0.05$; Table 4; Figure 3).

By the second hour, all groups treated with the extract demonstrated a decrease in rectal temperature. The 50 mg/kg, 100 mg/kg, and 150 mg/kg doses resulted in reductions of 3.83%, 4.32%, and 4.30%, respectively ($p < 0 .05$). These values were comparable to that of the positive control group (3.72%, $p < 0.05$). Even though the antipyretic effect of the extract was evident, there was no statistically significant difference between the extract-treated groups and the reference drug ($p < 0.05$).

In the third hour, the reduction in temperature became more noticeable across all groups. The extract-treated rats showed rectal temperature reductions of 5.25% (50 mg/kg), 5.68% (100 mg/kg), and 5.08% (150 mg/kg) ($p < 0.05$). The positive control group also showed a reduction of 4.82%. At this hour, there were no statistically significant differences among the three extract-treated groups and the positive control group ($p > 0 .05$), indicating that the methanolic root extract at all doses provided comparable antipyretic effects to paracetamol.

At the fourth hour post-treatment, the methanolic root extracts of *Carica papaya* maintained the antipyretic effects. The reductions in rectal temperature were 5.20% (50 mg/kg), 5.84% (100 mg/kg), and 5.13% (150 mg/kg) ($p < 0 .05$). These values were statistically similar to that observed in the paracetamol group (5.30%, $p < 0.05$), and considerably greater than the negative control group, which showed only a 0.53% decrease ($p < 0.05$). Notably, the 100 mg/kg dose group achieved the highest temperature reduction, though this difference was not statistically significant when compared with the other extract doses and the positive control group ($p < 0.05$; Table 4; Figure 3).

Table 4: Effects of Intraperitoneal Methanolic Root Extracts of *Carica papaya* on Rectal Temperature (°C) in Turpentine-Induced Pyretic Rats

Group	Treatment	0hr	1hr	2hrs	3hrs	4hrs
Normal Control	None	36.0 ± 0.09^{b} (0.00)	35.94 ± 0.07^{c} (0.17)	35.9 ± 0.08^{c} (0.27)	35.94 ± 0.07^{c} (0.16)	35.9 ± 0.08^{c} (0.28)
Negative Control	Turpentine +10%DMSO	38.16 ± 0.09^{a} (0.00)	38.26 ± 0.10^{a} (-0.26)	38.16 ± 0.06^{a} (-0.00)	38.2 ± 0.09^{a} (-0.11)	38.36 ± 0.08^{a} (-0.53)
Positive Control	Tuperntine +Paracetamol	38.14 ± 0.01^{a} (0.00)	37.54 ± 0.11^{b} (1.57)	36.72 ± 0.10^{b} (3.72)	36.3 ± 0.07^{b} (4.82)	36.12 ± 0.05^{bc} (5.30)
Methanolic Root Extracts	50 mg/kg bwt	38.12 ± 0.04^{a} (0.00)	37.68 ± 0.06^{b} (1.15)	36.66 ± 0.07^{b} (3.83)	36.12 ± 0.06^{bc} (5.25)	36.14 ± 0.04^{bc} (5.20)
	100mg/kg bwt	38.38 ± 0.04^{a} (0.00)	37.7 ± 0.06^{b} (1.77)	36.72 ± 0.1^{b} (4.32)	36.2 ± 0.04^{bc} (5.68)	36.14 ± 0.05^{bc} (5.84)
	150 mg/kg bwt	38.18 ± 0.07^{a} (0.00)	37.34 ± 0.09^{b} (2.20)	36.54 ± 0.05^{b} (4.30)	36.24 ± 0.02^{b} (5.08)	36.22 ± 0.06^{b} (5.13)

Values are presented as Mean ± SEM for five animals per group. Means sharing the same superscript letter do not differ significantly, as determined by one-way ANOVA followed by Tukey's post hoc test ($p > 0.05$). Figures in parentheses represent the percentage reduction in pyrexia.

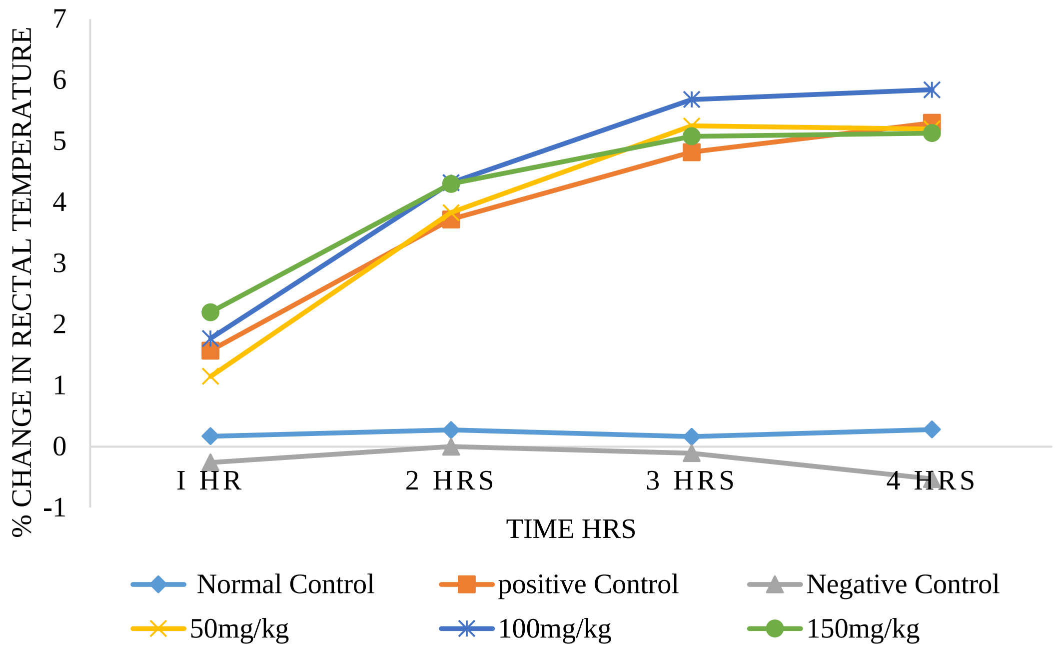

Figure 3: The percent change in rectal temperature by methanolic root extracts of *Carica papaya* in turpentine induced pyretic rats

4.2.2 Effects of Methanolic Leaf Extracts of *Carica papaya* on Turpentine-Induced Pyrexia in Rats

Similarly, methanolic leaf extracts of *C. papaya* showed antipyretic activity against turpentine induced fever in male Wistar rats (Table 5). In the first hour after treatment, the methanolic leaf extracts of *Carica papaya* at all doses (50, 100, and 150 mg/kg body weight) showed a varying effect on turpentine-induced pyrexia. The 50 mg/kg dose group showed a modest reduction in rectal temperature by 0.73%, while the 100 mg/kg and 150 mg/kg groups exhibited higher reductions of 1.87% and 1.57%, respectively ($p < 0.05$). The positive control group (paracetamol) showed a 1.57% reduction, while the negative control group demonstrated a slight increase (–0.26%). Despite the observed temperature decrease, there was no statistically significant differences between the extract-treated groups and the paracetamol-treated group at this hour ($p >$ 0 .05; Table 5; Figure 4).

At the second hour, all the extract-treated groups exhibited further reductions in rectal temperature. The 50, 100, and 150 mg/kg groups reduced temperature by 2.5%, 4.22%, and 3.93%, respectively. These reductions were statistically significant when compared to the negative control group ($p < 0.05$), which showed no significant change (–0.00%). The antipyretic effects at 100 mg/kg and 150 mg/kg were comparable to the positive control (3.72%, $p < 0.05$), indicating that the extract and the reference drug had a similar therapeutic effect (Table 5; Figure 4).

In the third hour, all treated groups exhibited a substantial antipyretic effect. The 50, 100, and 150 mg/kg doses lowered rectal temperatures by 3.77%, 4.70%, and 4.77%, respectively ($p < 0.05$). These effects closely corresponded the positive control group (4.82%, $p < 0.05$). Although the 100 mg/kg group showed slightly less reduction than the 150 mg/kg group, the difference was not statistically significant ($p < 0.05$). This suggests a consistent and constant antipyretic response across the two higher doses of the extract.

At the fourth hour, the extract-treated groups maintained strong antipyretic effects. The reductions were 4.25% for the 50 mg/kg, 5.16% for the 100 mg/kg, and 5.30% for the 150 mg/kg. Notably, the antipyretic effect of the 150 mg/kg group corresponded that of the paracetamol-treated positive control (5.30%, $p < 0.05$), indicating equivalent efficacy. The 100 mg/kg group also showed similar effectiveness ($p > 0.05$). The negative control group showed only a 0.53% decrease in rectal temperature, which was significantly lower than all treatment groups ($p < 0.05$; Table 5; Figure 4). In general, treatment with methanolic leaf extracts of *Carica papaya* produced a dose-dependent antipyretic effect against turpentine-induced pyrexia, with higher doses (100 and 150 mg/kg) showing comparable efficacy to the standard antipyretic drug, paracetamol ($p < 0.05$).

Table 5: Effects of Intraperitoneal Methanolic Leaf Extracts of *Carica papaya* on Rectal Temperature (°C) in Turpentine-Induced Pyretic Rats

Group	Treatment	0hr	1hr	2hrs	3Hrs	4hrs
Normal Control	None	36.0 ± 0.09^{b} (0.00)	35.14 ± 0.07^{c} (0.17)	35.9 ± 0.08^{c} (0.27)	35.94 ± 0.07^{d} (0.16)	35.9 ± 0.08^{d} (0.28)
Negative Control	Turpentine +10%DMSO	38.16 ± 0.09^{a} (0.00)	38.26 ± 0.10^{a} (-0.26)	38.16 ± 0.06^{a} (-0.00)	38.2 ± 0.09^{a} (-0.11)	38.36 ± 0.08^{a} (-0.53)
Positive Control	Tuperntine +Paracetamol	38.14 ± 0.01^{a} (0.00)	37.54 ± 0.11^{b} (1.57)	36.72 ± 0.10^{b} (3.72)	36.3 ± 0.07^{c} (4.82)	36.12 ± 0.05^{cd} (5.30)
Methanolic Leaf Extracts	50 mg/kg bwt	38.1 ± 0.13^{a} (0.00)	37.82 ± 0.04^{b} (0.73)	37.14 ± 0.17^{b} (2.5)	36.66 ± 0.05^{b} (3.77)	36.48 ± 0.07^{b} (4.25)
	100mg/kg bwt	38.26 ± 0.19^{a} (0.00)	37.54 ± 0.13^{b} (1.87)	36.64 ± 0.10^{b} (4.22)	36.46 ± 0.08^{bc} (4.70)	36.28 ± 0.09^{bc} (5.16)
	150 mg/kg bwt	38.12 ± 0.11^{a} (0.00)	37.52 ± 0.09^{b} (1.57)	36.62 ± 0.11^{b} (3.93)	36.3 ± 0.04^{c} (4.77)	36.1 ± 0.05^{cd} (5.30)

Values are presented as Mean ± SEM for five animals per group. Means sharing the same superscript letter do not differ significantly, as determined by one-way ANOVA followed by Tukey's post hoc test ($p > 0.05$). Figures in parentheses represent the percentage reduction in pyrexia.

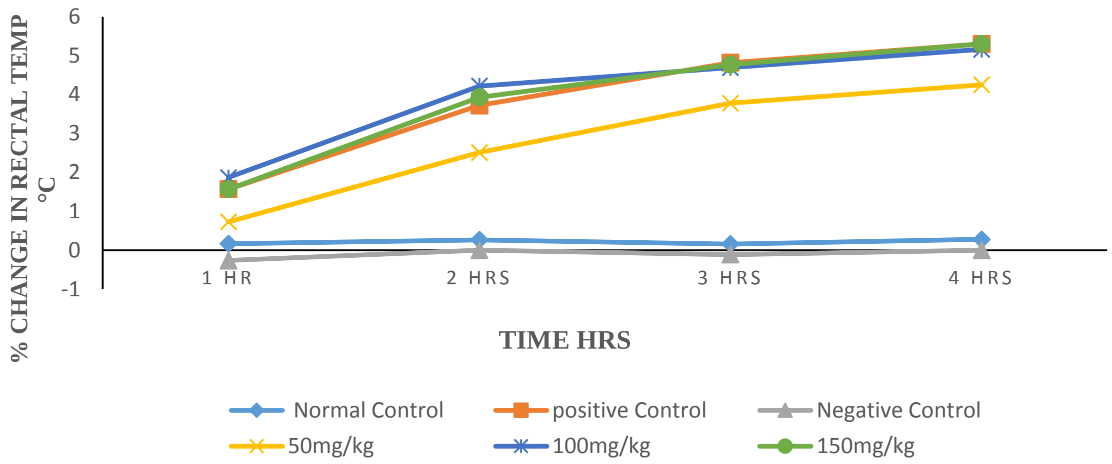

Figure 4: The percent change in rectal temperature by methanolic leaf extracts of *Carica papaya* in turpentine induced pyretic rats

4.2.3 Time- and Dose-Dependent Comparison between Root and Leaf Extracts of *Carica papaya* on Rectal Temperature Reduction

At 1 hour, the reduction in rectal temperature was relatively low across all doses. The root extract at 150 mg/kg showed the highest temperature reduction (2.20%), followed by the leaf extract at 100 mg/kg (1.87%) (Figure 5). However, the differences between root and leaf extracts at all concentrations were not statistically significant ($p < 0.05$).

At 2 hours, the root extract at 50 mg/kg (3.83%) produced a considerably greater temperature reduction compared to the leaf extract (2.51%) at the same dose ($p < 0.05$; Figure 5). However, at 100 mg/kg, the root and leaf extracts produced similar temperature reduction of 4.32% and 4.30% respectively. At 150mg/kg the root and leaf produced almost similar temperature reduction of 4.22%, and 3.93%, respectively (Figure 5), however these differences were not statistically significant ($p < 0.05$).

At 3 hours, the root extract at 100 mg/kg was 5.68%, this exhibited the highest temperature reduction, considerably greater than the corresponding leaf extract which was 4.69% ($p < 0.05$; Figure 4.3). A statistically significant difference was also observed at 50 mg/kg between root (5.25%) and leaf (3.78%) extracts ($p < 0.05$; Figure 5). However, at 150 mg/kg, the difference between root and leaf extracts was minimal (5.08% vs. 4.77%) and not statistically significant ($p < 0.05$; Figure 5).

At 4 hours, a substantial difference was noted at 50 mg/kg, where the root extract (5.20%) was more effective than the leaf extract (4.25%) ($p < 0.05$). For 100 mg/kg and 150 mg/kg doses, root and leaf extracts produced comparable reductions (root: 5.84% and 5.13%; leaf: 5.16% and 5.30%) (Figure 5), with no significant differences observed ($p < 0.05$)

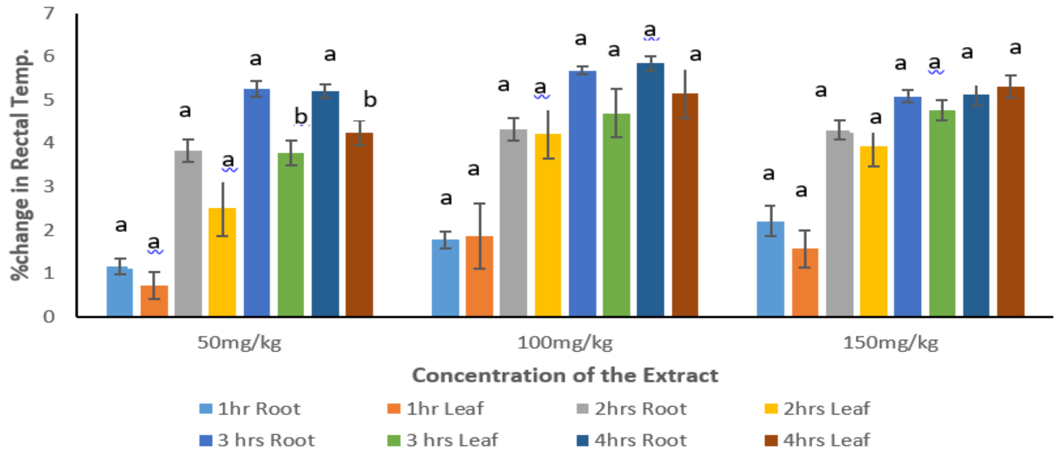

Figure 5: Comparison of percent change in rectal temperature of methanolic leaf and root extracts of *Carica papaya* at various hours of the test period.

4.3 *In vitro* Antioxidant Activities on Non-Enzymatic Essays

4.3.1 *In vitro* DPPH Radical Scavenging Activity of Methanolic Leaf and Root Extracts of *C. papaya*

The methanolic leaf and root extracts of *Carica papaya* exhibited a concentration-dependent increase in DPPH radical scavenging activity, with the lowest concentrations showing minimal activity and the highest concentrations demonstrating the greatest antioxidant potential (Figure 6; Appendix 6.1). At higher concentrations (1000 and 750 mg/ml), the DPPH radical scavenging activity of the root, leaf, and ascorbic acid differed significantly from each other ($p < 0.05$). This suggests that all three samples had statistically distinct antioxidant potencies at these concentrations. However, at the lower concentrations (500, 250, 125, and 62.5 mg/ml), there was no statistically significant difference between the root and leaf extracts ($p > 0.005$), indicating comparable radical scavenging effects at this range. In contrast the ascorbic remained significantly different from both plant extracts at all concentrations tested. These statistical patterns were consistent with the IC_{50} values: root extract (640.6 mg/ml), leaf extract (572.8 mg/ml), and ascorbic acid (312.7 mg/ml) (Appendix 6.1).

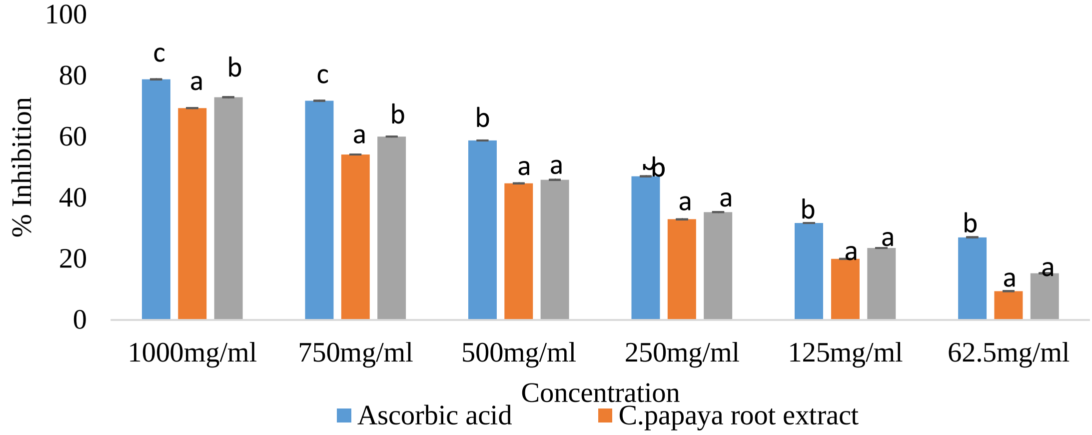

Figure 6*: In vitro* DPPH radical scavenging activity of methanolic root and leaf extracts of *C. papaya*.

4.3.2 *In vitro* Ferric Reducing Antioxidant Power (FRAP) Methanolic Leaf and Root Extracts of *C. papaya*

The leaf and root extracts of *Carica papaya* exhibited a concentration-dependent increase in ferric reducing power. The ferric reducing activity varied significantly among the tested concentrations, with the highest concentration demonstrating the strongest activity (Figure 7; Appendix 6.2). Overall, the root extract displayed significantly greater reducing power than the leaf extract at all concentrations tested, while ascorbic acid consistently showed the strongest activity ($p < 0.05$). At higher concentration (250, 200, 150 and 100 µg/ml) radical scavenging activity of the root, leaf, and ascorbic acid differed significantly from each other ($p < 0.05$), but at lower concentrations (50 and 25 µg/ml), there was there was no statistically significant difference between the root and leaf extracts ($p > 0.005$), indicating comparable radical scavenging effects at this range. Based on absorbance trends, the root extract demonstrated greater efficacy, with an estimated IC_{50} of approximately 219.1 µg/ml for the root, compared to 251.4 µg/ml for the leaf extract. Ascorbic acid showed the strongest reducing power with an IC_{50} of 147.5µg/ml (Appendix 6.2).

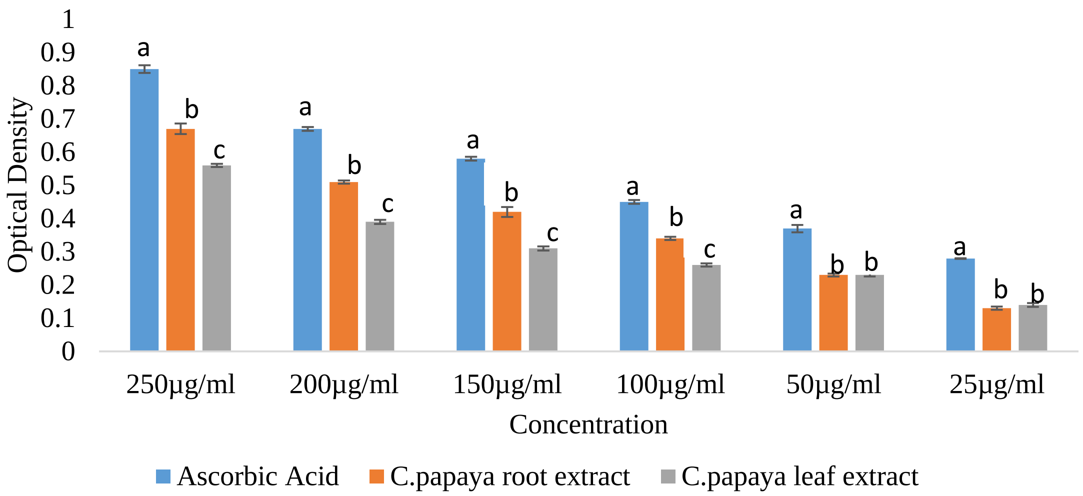

Figure 7: *In vitro* Ferric Reducing Antioxidant Power (FRAP) of methanolic root and leaf extracts of *C. papaya.*

4.3.3 *In vitro* Lipid Peroxidation Inhibition Activity of Methanolic Leaf and Root Extracts of *C. papaya*

The methanolic leaf and root extracts of *C. papaya* demonstrated a concentration-dependent increase in the inhibition of lipid peroxidation (Figure 8; Appendix 6.3). Across all concentrations tested, the root extract showed significantly greater inhibitory activity than the leaf extract ($p < 0.05$), while ascorbic acid consistently exhibited the strongest effect ($p < 0.05$). Furthermore, the lipid peroxidation radical scavenging activities of the root extract, leaf extract, and ascorbic acid differed significantly from each other at all concentrations ($p < 0.05$), suggesting that each sample has a distinct antioxidant capacity. The IC_{50} values supported these observations: root extract (125 µg/ml) was more potent than leaf extract 162.5 µg/ml, although both were less active than ascorbic acid, which had the lowest IC_{50} of 56.3µg/ml (Appendix 6.3).

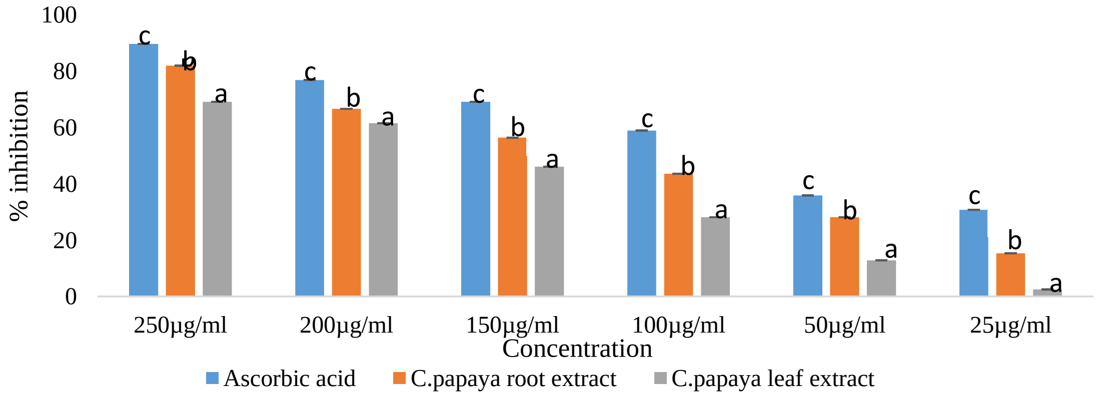

Figure 8: *In vitro* lipid peroxidation inhibition activity of methanolic root and leaf extracts of *C. papaya.*

4.3.4 *In vitro* Total Flavonoids Content of Methanolic Leaf and Root Extracts of *C.papaya*

The methanolic root and leaf extract demonstrated a concentration-dependent increase in flavonoid content (Figure 9; Appendix 6.4). The leaf extract had significantly higher flavonoid levels than the root extract ($p < 0.05$), while both were statistically lower than the standard compound, quercetin ($p < 0.05$). The higher concentration of 300 mg/ml and lower concentration of 50 mg/ml showed no statistical significant difference between the root and the leaf extracts. The IC_{50} values indicated stronger flavonoid content in the leaf extract 250 mg/ml compared to the root 270 mg/ml, with quercetin showing the highest flavonoid activity160 mg/ml (Appendix 6.4).

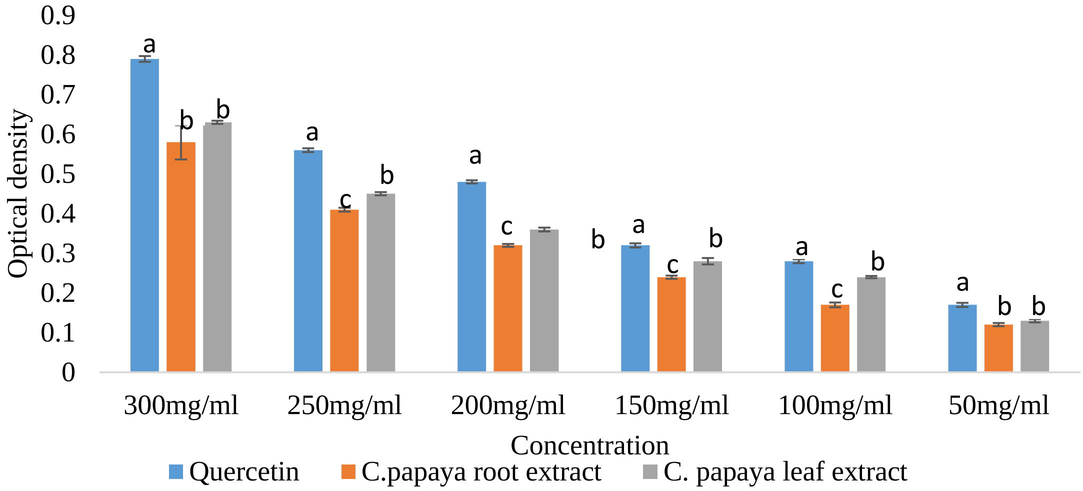

Figure 9: *In vitro* Total flavonoids content of methanolic leaf and root extracts of *C.papaya*

4.3.5 *In vitro* Total Phenolic Content of Methanolic Leaf and Root Extracts of *C.papaya*

The total phenolic content of both extracts increased in a concentration-dependent manner (Figure 10; Appendix 6.5). At higher concentration (300 mg/ml) there was no significant difference observed between the root and leaf extracts **(**$p > 0.005$**).** However, at other concentrations (250, 200,150, 100, and 50 mg/ml), there was statistically significant difference between the root and leaf extracts. In contrast the gallic acid remained significantly different from both plant extracts at all concentrations tested. The IC_{50} values of the gallic acid was 80.55 mg/ml, 125.0 mg/ml for the root extract and 162.5 mg/ml for the leaf extract (Appendix 6.5).

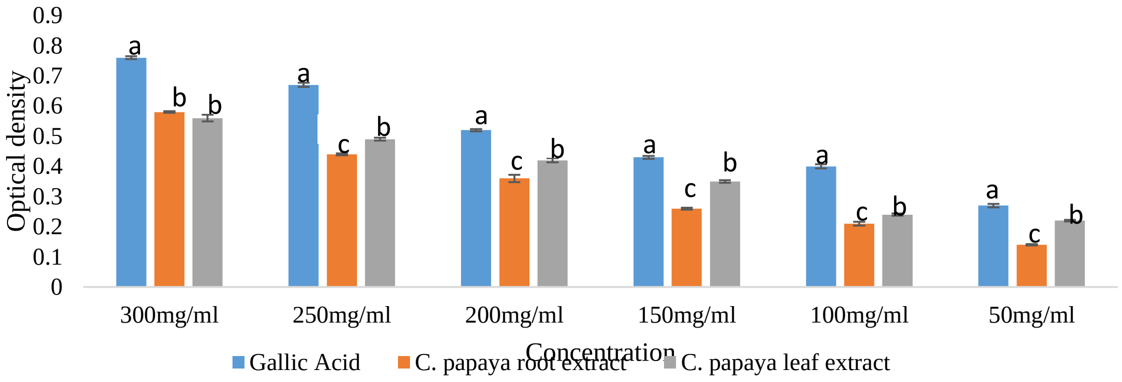

Figure 10: *In vitro* Total Phenolic Content of Methanolic Leaf and Root Extracts of *C.papaya*

4.3.6 *In vitro* Hydroxyl Radical Scavenging Activity of Methanolic Leaf and Root Extracts of *C.papaya*

The hydroxyl radical scavenging activity of *C. papaya* leaf and root extracts increased in a concentration-dependent manner (Figure 11; Appendix 6.6). Across most concentrations tested, there was no statistically significant difference between the root and leaf extracts ($p > 0.005$), except at 200 and 150 mg/ml, where a significant difference was observed ($p < 0.05$). In addition, both extracts consistently showed lower scavenging activity compared to the standard, gallic acid, which was significantly more potent across all concentrations tested ($p < 0.05$). The IC_{50} values was 71.6 µg/ml for gallic acid, 110.3 µg/ml for the root extract and 117.5 µg/ml for the leaf extract (Appendix 6.6).

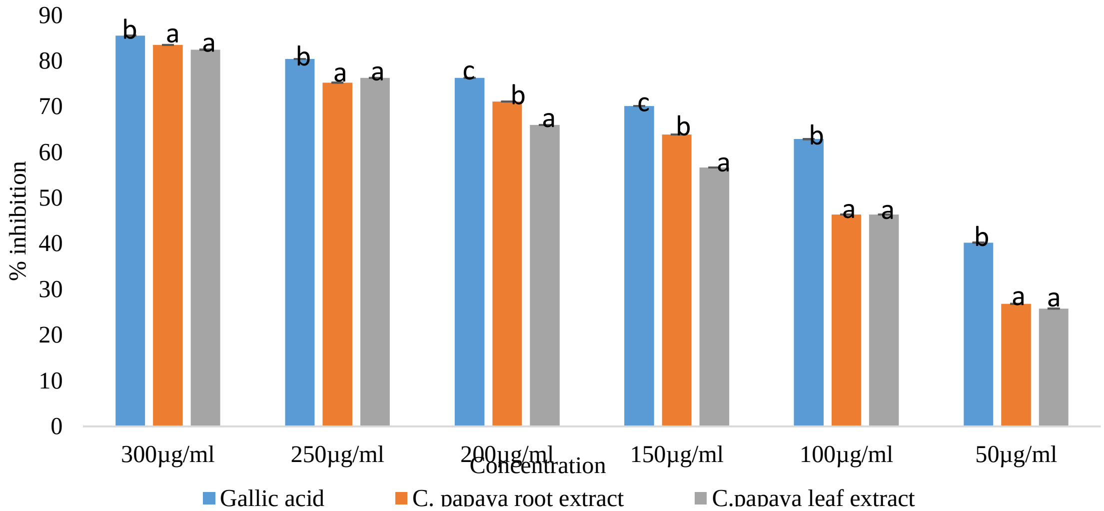

Figure 11: *In vitro* hydroxyl radical scavenging activity of methanolic leaf and root extracts of *C.papaya*

CHAPTER FIVE

DISCUSSION

The GC–MS analysis of the methanolic leaf and root extracts of *Carica papaya* Linn. revealed a diverse range of phytochemicals, including flavonoids, phenolic acids, terpenes, fatty acids, sterols, vitamins, and alkaloid derivatives. These compounds display an extensive range of pharmacological properties, particularly antioxidative and antipyretic properties. The following sections detail each compound, provides insight into its pharmacological roles and synergistic effects, and supporting the traditional use of *C. papaya* in managing oxidative stress and fever-related conditions.

Flavonoids such as quercetin ($C_{15}H_{10}O_7$) and myricetin ($C_{15}H_{10}O_8$) were prominent in the leaf extract. Quercetin is documented for its robust antioxidant properties, capable of scavenging reactive oxygen species (ROS), inhibiting lipid peroxidation, and upregulating antioxidant enzymes, including catalase and superoxide dismutase (Qi *et al*., 2022). Additionally, quercetin exerts antipyretic effects by modulating pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are key mediators of fever and systemic inflammation (Li *et al.*, 2016). Myricetin, similarly, possesses antioxidant, anti-inflammatory, and antinociceptive properties, with mechanistic actions that include inhibition of cyclooxygenase (COX) enzymes, downregulation of nuclear factor kappa B (NF-κB), and enhancement of endogenous antioxidant defenses (Yang *et al.*, 2022). Both quercetin and myricetin in the leaf extract suggest a synergistic effect that reinforces the extracts efficacy against oxidative stress related conditions.

Kaempferol derivatives, including kaempferol-3-O-glucoside and other glycosides ($C_{21}H_{20}O_{11}$), play a role in suppressing inflammatory mediators and antioxidant protection (Li *et al.*, 2019). The leaf extract also contained phenolic acids such as gallic acid derivatives ($C_7H_6O_5$), caffeic acid ($C_9H_8O_4$), catechol ($C_6H_6O_2$), chlorogenic acid derivatives ($C_{16}H_{18}O_9$), ferulic acid ($C_{10}H_{10}O_4$), rosmarinic acid ($C_{18}H_{16}O_8$), and cinnamic acid ($C_9H_8O_2$). These compounds are known for their strong antioxidant potential, primarily through ROS scavenging, lipid peroxidation inhibition, and protection against DNA damage (Gao *et al.*, 2022; D'Archivio *et al.*, 2012). Phenolics such as caffeic acid and chlorogenic acid also demonstrate anti-inflammatory and hepatoprotective effects, reinforcing the therapeutic relevance of the leaf extract in mitigating oxidative stress-related damage (Liu *et al.*, 2022). Eugenol, carvacrol, and thymol, phenolic terpenoids in the leaf extract, provide additional anti-inflammatory and antimicrobial activity, indirectly contributing to fever reduction (Olayanju *et al.*, 2024).

Terpenes such as limonene ($C_{10}H_{16}$), linalool ($C_{10}H_{18}O$), α-pinene ($C_{10}H_{16}$), β-pinene ($C_{10}H_{16}$), camphene ($C_{10}H_{16}$), myrcene ($C_{10}H_{16}$), terpinolene ($C_{10}H_{16}$), and longifolene ($C_{15}H_{24}$) were also detected in the leaf extract. These terpenes contribute to antioxidative and anti-inflammatory profile by modulating pro-inflammatory mediators and supporting cellular defense mechanisms (Kim *et al.*, 2020). Vitamins identified in the leaf extract, such as ascorbic acid derivatives ($C_6H_8O_6$) and α-tocopherol ($C_{29}H_{50}O_2$), are potent antioxidants that stabilize cell membranes, scavenge free radicals, and synergize with flavonoids to mitigate oxidative damage.

The root extract of *C. papaya* showed a different phytochemical profile, with notable flavonoids including quercetin, 3-Rhamnosyl-Glucosyl Quercetin ($C_{27}H_{30}O_{16}$), kaempferol derivatives, isoquercitrin ($C_{21}H_{20}O_{12}$), and myricetin derivatives. Rutin (3-Rhamnosyl-Glucosyl Quercetin) was most abundant. These flavonoids exhibit comparable antioxidative and anti-inflammatory actions to those in the leaves, with mechanisms involving ROS scavenging, downregulation of NF-κB signaling, inhibition of COX and inducible nitric oxide synthase (iNOS), and modulation of cytokine production (Bhatt *et al.*,2021; Azeem *et al.*, 2023). The glycosylated forms, such as isoquercitrin and kaempferol glycosides, enhance water solubility and bioavailability, potentially improving systemic absorption and efficacy (Mohammed *et al.*, 2025).

Phenolic compounds in the root extract, including caffeic acid, cis-2, 5-Dimethoxy-4-ethoxy-β-methylstyrene, and 6-Hydroxy-4, 4, 7a-trimethyl-5, 6, 7, 7a-tetrahydrobenzofuran-2(4H)-one, contribute to antioxidative defense and anti-inflammatory activity. Their mechanisms include inhibiting lipid peroxidation, scavenging free radicals, and diminishing pro-inflammatory enzyme expression (Gao *et al.*, 2022; D'Archivio *et al.*, 2012). Fatty acids and esters such as n-hexadecanoic acid, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid (Z, Z), and 4 Heptafluorobutyryloxyhexadecane provide membrane-stabilizing properties, anti-inflammatory effects, and additional antioxidant support, enhancing the pharmacological potential of the root extract (Kotlyarov & Kotlyarova *et al.*, 2021; Fratianni *et al.*, 2021).

Terpenes and terpenoid alcohols in the root extract including phytol ($C_{20}H_{40}O$), trans-farnesol ($C_{15}H_{26}O$), and squalene ($C_{30}H_{50}$), act as potent antioxidants and anti-inflammatory agents. These compounds stabilize cell membranes, reduce free radicals, and modulate inflammatory signaling pathways (Luo *et al.*, 2022; Apiri *et al.*, 2025). Compounds such as resperpine ($C_{33}H_{40}N_2O_9$) contribute alkaloid-mediated immunomodulatory, anti-inflammatory, and antipyretic activities (Olayanju *et al.*, 2024). Lactones and terpenoid esters identified in roots, including 4, 8, 12, 16-Tetramethylheptadecan-4-olide and (2E, 6E)-3, 7, 11-Trimethyldodeca-2, 6, 10-trienyl propionate, exhibit additional anti-inflammatory and cytoprotective effects by modulating oxidative and inflammatory mediators.

The combined presence of flavonoids, phenolics, terpenes, fatty acids, sterols, vitamins, and alkaloids in both leaf and root extracts in this study highlights a complementary pharmacological profile. Flavonoids and phenolics in the leaf extract provide strong antioxidative and antipyretic effects by scavenging ROS, protecting cellular components, and suppressing inflammatory mediators. Sterols, fatty acids, and alkaloids in the root extract mainly mediate immune modulation, anti-inflammatory responses, and fever reduction (Bakrim *et al.*, 2022; Nattagh-Eshtivani *et al.*, 2022). The synergistic interaction of these compounds strengthens the therapeutic efficacy, validating traditional practices that utilize multiple plant parts for the holistic management of fever and oxidative stress.

Comparatively, the leaf extract is richer in flavonoids and phenolic compounds, conferring higher antioxidative activity and ROS scavenging potential. Conversely, the root extract is abundant in sterols, fatty acids, terpenes, and alkaloids, enhancing antipyretic, immunomodulatory, and anti-inflammatory properties. This distinction supports the notion that different plant parts can be deliberately used for specific therapeutic purposes, while their combined application may yield enhanced overall medicinal effects (Olayanju *et al.*, 2024). The GC-MS profiles of papaya leaf and root extracts reveal a complex phytochemical activity that supports their complementary pharmacological roles and reinforces their traditional usage in managing oxidative stress and fever-related conditions.

This study evaluated the antipyretic and antioxidative properties of the methanolic root and leaf extracts of *C. papaya.* The assessment of antipyretic properties of the extracts was done using turpentine induced pyrexia rats. Turpentine is a well-characterized exogenous pyrogen which is widely used in experimental models because of its predictable mechanism and substantial fever response. Moreover, Estella *et al.* (2022) showed in their research study that it is less easy for experimental animals to acquire tolerance to turpentine than to other pyrogens. Thus, turpentine was selected as a pyrogen in this study. Turpentine induces fever by stimulating immune cells such as macrophages and monocytes to release pro-inflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), which in turn act as endogenous pyrogens (Estella *et al.*, 2022). These cytokines promote the synthesis of prostaglandins, particularly prostaglandin E2, in the hypothalamus, leading to an elevated set-point for body temperature and resultant fever (Patel *et al.*, 2022).

In this study, both methanolic root and leaf extracts of *C. papaya* demonstrated appreciable antipyretic activity, reflected by significant reductions in rectal temperature over the four-hour test period. The extracts exhibited a clear dose-dependent response: higher doses (100 mg/kg and 150 mg/kg) consistently produced greater reductions in rectal temperature compared to the lower dose (50 mg/kg). At the second, third, and fourth hours, the antipyretic effects of the extracts at 100 mg/kg and 150 mg/kg were statistically comparable to those of the standard drug, paracetamol. These findings are similar to recent work by Nagaveni *et al.* (2011), who reported significant antipyretic effects of methanolic fruit extracts of *Mangifera indica* in pyretic animal models. Similarly, Soumya Santra *et al.* (2024) demonstrated comparable dose-dependent antipyretic effects of methanolic extracts of *Azadirachta indica*, with higher doses producing efficacy equivalent to standard antipyretic drugs.

These findings are consistent with previous research on other medicinal plants. Essien *et al*. (2015) reported significant antipyretic activity of the methanolic leaf extract of *Pseudocedrela kotschyi* in brewer's yeast- and amphetamine-induced pyrexia in rats. Similarly, Abdur *et al*. (2014) demonstrated that methanolic extracts and different fractions of *Diospyros lotus* markedly reduced yeast-induced hyperthermia in mice. Ullah *et al.* (2020) highlighted the role of flavonoid-rich extracts in effectively reducing pyrexia by modulating proinflammatory cytokines and prostaglandin pathways. These findings support the concept that plant-derived extracts rich in bioactive compounds can provide potent antipyretic effects comparable to conventional drugs, especially at higher doses.

NSAIDs and paracetamol are widely used to manage fever by blocking the cyclooxygenase (COX) enzyme pathway, thereby preventing the biosynthesis of prostaglandins that elevate the hypothalamic set-point for temperature regulation (Charde *et al*., 2025). Over time after a thorough investigation, it is believed that mechanism underlying temperature reduction is the inhibition of prostaglandin synthesis within the hypothalamus, a pathway shared by standard antipyretic drugs such as paracetamol and nonsteroidal anti-inflammatory drugs. The methanolic leaf and root extracts showed a slightly slower onset of antipyretic effect compared to paracetamol during the first hour of the test this may reflect the pharmacokinetic delay required for absorption and metabolic activation of active phyto-constituents in the methanolic root and leaf extracts of *C. papaya* (Sun *et al.*, 2019). This pattern is consistent with findings from recent studies reporting similar lag phases for plant extracts rich in flavonoids and alkaloids before maximal antipyretic effects are observed (Ullah *et al.*, 2020; Soumya Santra, *et al.*, 2014).

The methanolic root and leaf extracts of *C.papaya* demonstrated a dose dependent response on rectal temperature lowering effect in turpentine-induced pyretic rats, this was consistent with Soumya Santra, *et al.* (2014) who observed that methanolic leaf extracts of *Azadirachta indica* produced dose-dependent antipyretic effects in yeast-induced pyrexia models, using paracetamol as the standard reference drug; at higher doses, the extracts efficacy became statistically comparable to paracetamol, despite a slower onset during the first hour. Another study by Arika *et al.* (2019) demonstrated a dose-dependent antipyretic effect of DCM: Methanolic leaf and root bark extracts of *C. edulis* explaining the dose dependent difference may be as a result of passive diffusion of the active principles across the cell membrane in the peritoneal cavity. Thus the credibility that the antipyretic action of *C. papaya* methanolic extracts observed in this study is similarly mediated by inhibition of prostaglandin synthesis in the hypothalamus, with the timing and magnitude of effect shaped by the extracts pharmacokinetics and phytochemical composition.

The dose ranges employed in this study were comparable to those reported by Arika *et al*. (2019), who investigated the antipyretic effects of dichloromethane–methanolic leaf and root bark extracts of *Carissa edulis* using doses of 50, 100, and 150 mg/kg body weight in rats. Similarly, Abdur *et al*. (2014) evaluated the antipyretic activity of methanolic extracts and various solvent fractions of *Diospyros lotus* at doses of 50 and 100 mg/kg body weight. In the present study, the methanolic leaf and root extracts of *Carica papaya* at lower doses (50 and 100 mg/kg body weight) were less effective compared to the higher dose of 150 mg/kg body weight, possibly due to faster metabolism and inactivation at lower concentrations. At lower doses (50 mg/kg), the root extract produced significantly greater reductions in rectal temperature from the second hour onwards compared to the leaf extract. This may reflect faster-acting bioactive compounds in root extracts (Ogundele *et al.*, 2017). In contrast, at higher doses, 100 mg/kg and 150 mg/kg extracts, achieved comparable efficacy over time suggesting saturation of pharmacological targets.

That the dose level of 100mg/kg body weight of the methanolic root extracts of *C. papaya* at the fourth hour, was more effective than higher dose of 150 mg/kg. This observation might be due to the non-linear or bell-shaped dose–response patterns often reported in studies of plant extracts and natural compounds (Soumya Santra, *et al.*, 2014). Such patterns may arise when an intermediate dose achieves optimal receptor binding and pharmacological activity, whereas higher doses can trigger faster metabolism and clearance of active constituents, reducing their effective concentration at the target site over time (Jia *et al.*, 2022). Additionally, very high doses may activate physiological feedback mechanisms such as increased production of pyrogenic cytokines or upregulation of prostaglandin synthesis that partially offset the antipyretic effect. These complex interactions are characteristic of multi-component phytochemical extracts, where the balance between absorption, metabolism, and receptor interactions determines the net effect at different time points (Sun *et al.*, 2019). Thus, the slightly stronger effect of the 100 mg/kg root extract at the fourth hour likely reflects an optimal balance between pharmacokinetic and pharmacodynamics processes, highlighting that higher doses do not always guarantee proportionally greater efficacy.

A comparative evaluation of the methanolic leaf and root extracts of *Carica papaya* revealed that both extracts possess appreciable antipyretic activity, yet with subtle differences in magnitude and timing of effect. At lower doses (50 mg/kg), the root extract consistently produced significantly greater reductions in rectal temperature compared to the leaf extract, particularly from the second hour onwards. This suggests the root extract may contain higher concentrations of more readily absorbable or potent bioactive compounds, such as alkaloids and low-molecular-weight flavonoids, which can diffuse more rapidly across biological membranes (Ullah *et al.,* 2020).These differences may also arise from pharmacokinetic factors: alkaloids and low molecular weight flavonoids abundant in roots may cross cell membranes more readily, leading to faster onset (Zhong *et al.*, 2022); whereas larger polyphenols common in leaf extracts may require metabolic activation, delaying their effect (Nagaveni *et al.*, 2011).

The negative control group, which received turpentine to induce pyrexia followed by vehicle only (10% DMSO), maintained consistently elevated rectal temperatures throughout the four-hour observation period, showing only minimal fluctuations that were not statistically significant. This pattern is expected and supports the validity of the experimental design. Turpentine acts as an exogenous pyrogen by stimulating immune cells to release endogenous pyrogens, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α), which in turn promote the synthesis of prostaglandin E2 in the hypothalamus, elevating the thermoregulatory set-point (Estella *et al*., 2022). In the absence of an active antipyretic agent, there is no inhibition of this prostaglandin-mediated pathway, and thus the fever response persists. Furthermore, the vehicle used (10% DMSO) lacks known antipyretic activity at the administered dose, serving purely as a solvent for extract administration (Sun *et al.*, 2019). Consequently, the persistent high temperature in the negative control group confirms that the observed reductions in rectal temperature was due to the plant extract and paracetamol pharmacological effects.

Quantitative phytochemical screening revealed that the methanolic leaf and root extracts of *C.papaya* contains phenolic, alkaloids, flavonoids, fatty acid, vitamins and terpenoids. Recent pharmacological studies increasingly support the role of phytochemicals such as flavonoids, alkaloids, and saponins in mediating antipyretic activity through inhibition of cyclooxygenase (COX) enzymes and modulation of proinflammatory cytokines (Chen *et al.*, 2021b; Zhong *et al.*, 2022). Flavonoids, for instance, have been shown to reduce fever by suppressing TNF-α and inhibiting prostaglandin E2 synthesis in the hypothalamus, thereby lowering the thermoregulatory set-point (Ullah *et al.*, 2020). Saponins can act synergistically with other bioactive compounds to enhance antipyretic effects, by inhibiting prostaglandin biosynthesis and modulating cytokine release (Zhong *et al.*, 2022). In addition, pharmacological evaluations have confirmed that alkaloids exert significant antipyretic activity, likely through combined effects on COX inhibition and suppression of pyrogenic cytokines (Chen *et al.*, 2021b).

The presence of these phytochemicals in the methanolic root and leaf extracts of *Carica papaya*, as identified in earlier phytochemical screening studies provides a plausible mechanistic basis for the dose-dependent antipyretic activity (Ogundele *et al.*, 2017). These findings suggest that the antipyretic effect of *C. papaya* extracts may arise from a synergistic interaction of flavonoids, alkaloids, and saponins, each targeting different steps in the fever pathway, and together leading to significant reductions in rectal temperature comparable to standard drugs.

On the other hand, antioxidative effect was evaluated using DPPH radical scavenging assay, ferric reducing power assay, hydroxyl Radical (-OH) scavenging activity, lipid peroxidation activity, total phenolic and total flavonoids content**.** A cellular redox imbalance occurs when the pro-oxidants overwhelm the natural defense system which leads to excessive reactive oxygen species (ROS) production. These ROS, including superoxide, hydroxyl radicals, and hydrogen peroxide, are potent initiators of oxidative damage to lipids, proteins, and DNA, contributing to oxidative stress (Jomova *et al.*, 2023). Antioxidants act by donating electrons or hydrogen atoms, interrupting radical chain reactions and restoring redox equilibrium (Jakubek *et al.*, 2024). Given the toxicity and potential carcinogenicity associated with certain synthetic antioxidants research interest has increasingly shifted toward natural antioxidants derived from plant sources (Shubina *et al.*, 2023). Plant-derived compounds are considered safer, effective alternatives for mitigating oxidative damage and managing degenerative diseases (Chaudhary *et al.*, 2023; Jomova *et al.*, 2023). This study demonstrated an *In vitro* antioxidant and free radical scavenging properties of methanolic leaf and root extracts of *Carica papaya*.

The *In vitro* DPPH radical inhibitory assay showed that methanolic root and leaf extracts of *Carica papaya* have concentration-dependent DPPH radical scavenging activity, with the root extract exhibiting slightly greater potency than the leaf, although both remained less effective than the standard, ascorbic acid. This pattern reflects the extracts capacity to donate hydrogen atoms or electrons to neutralize free radicals, which helps mitigate oxidative stress. The DPPH assay relies on the principle of measuring the reduction of the stable DPPH radical to its non-radical form, which is indicated by color change from deep purple to pale yellow (Baliyan *et al*., 2022). These findings align with previous research showing that natural plant extracts commonly exhibit similar antioxidant behaviors. For instance, the methanolic whole plant extract of *Biophytum sensitivum* showed a concentration-dependent DPPH scavenging effect with a maximum inhibition of about 43.96% (Pallab *et al.*, 2013). Similarly, leaf, flower, and stem extracts of *Thymelaea hirsuta* also demonstrated significant, dose-dependent free radical scavenging activity (Amari *et al.*, 2014). Such observations reinforce the reliability of the concentration-dependent trend observed in *C. papaya* extracts.

The FRAP assay showed that methanolic root and leaf extracts of *Carica papaya* have dose-dependent ferric reducing antioxidant power, with the root extract displaying higher activity. This reflects the extracts capacity to act as electron donors, neutralizing oxidized intermediates before they propagate damage. Increased absorbance in the FRAP assay directly indicates an electron transfer, thus signifying greater reductive potential (Platzer *et al*., 2021). The findings align with those of Nisa *et al.* (2019), who reported significant antioxidant activity and high total flavonoid content in *C. papaya* leaf extracts, although values varied by variety, maturity, and solvent type. Their results emphasize that solvent choice and plant part strongly influence the measured antioxidant capacity, consistent with our observation that the root extract had higher reducing power than the leaf. Recent studies have also shown that phenolic-rich extracts tend to exhibit higher FRAP activity (Kumar *et al.*, 2020; Walasek-Janusz *et al.*, 2025). Additionally, methanolic extract of *Hypericum salsolifolium* exhibited the highest FRAP values among tested solvents, this was attributed to its high phenolic content (Seyrekoglu *et al.*, 2022). The stronger performance of the root extract suggests that it may contain higher levels or more effective types of phenolic compounds, flavonoids, or other reductive phytochemicals compared to the leaf. Such compounds play a key biological role by interrupting radical chain reactions and stabilizing reactive oxygen species (Shubina *et al.*, 2023). This study showed that ferric reducing power of *C. papaya*, especially its root, as a promising natural source of antioxidants for managing oxidative stress.

The methanolic root and leaf extracts of *Carica papaya* demonstrated clear concentration-dependent inhibition of lipid peroxidation, with the root extract showing a greater potency than the leaf. This suggests that the root contains higher levels or more effective types of bioactive compounds capable of halting oxidative chain reactions. Lipid peroxidation, which involves free radical attack on cellular lipids, generates malondialdehyde (MDA) as one of its primary byproducts a widely recognized biomarker of oxidative damage (Chandimali *et al.*, 2025). Elevated MDA levels have been linked to the pathogenesis of various disorders, including inflammatory conditions, certain cancers, atherosclerosis, diabetes mellitus, and neurodegenerative diseases such as Alzheimer’s (Chandimali *et al.*, 2025). MDA accumulation also contributes to lysosomal degradation and mitochondrial dysfunction, further amplifying cellular injury (Dash *et al.*, 2024). Limiting lipid peroxidation is therefore crucial for preserving membrane integrity and preventing progression of oxidative stress–related diseases (Chandimali *et al.*, 2025).

The findings of this study align with other studies reporting similar anti-lipid peroxidation effects from plant-derived bioactives. Essential oils from *Eryngium creticum* have shown comparable inhibition of lipid peroxidation, demonstrating the broader capacity of natural plant extracts to disrupt radical-induced membrane damage (Nusair *et al*., 2022). The findings support the potential of *C. papaya* root extract as an effective natural agent for reducing oxidative stress by inhibiting lipid peroxidation pathways, as confirmed its high antioxidant and flavonoid content (Nisa *et al.*, 2019).

In this study, both root and leaf extracts of *Carica papaya* demonstrated a concentration-dependent increase in total flavonoid content, with the leaf extract consistently showing higher flavonoid levels than the root. Flavonoids are polyphenolic compounds known for their potent antioxidant properties, primarily through their ability to donate hydrogen atoms and electrons, scavenge reactive oxygen species and chelate transition metal ions (Panche *et al.*, 2016). The higher flavonoid content in the leaf extract likely contributes to its antioxidant capacity, although the root extract exhibited stronger activity in some radical scavenging assays, suggesting that other non-flavonoid compounds may also play a significant role.

The results are aligning with findings by Nisa *et al.* (2019), who reported substantial flavonoid accumulation in *C. papaya* leaves across different varieties and solvent extractions. Recent studies have also highlighted that the antioxidant potential of plant extracts often correlates with total flavonoid and phenolic content, as observed in *Annona muricata* leaves (Orak *et al.*, 2019). These bioactive compounds interrupt oxidative chain reactions and stabilize free radicals, thereby protecting cellular components from oxidative damage. In addition to antioxidant defense, flavonoids have been associated with additional health benefits, including anti-inflammatory, anti-cancer, and cardioprotective effects (Zahra *et al.*, 2024). Given rising concerns over synthetic antioxidants and their potential adverse effects (Shubina *et al.*, 2023). The high flavonoid content observed in *C. papaya*, especially in its leaf extract, reinforces its value as a natural and potentially safer alternative for managing oxidative stress–related conditions.

In this study, both root and leaf extracts of *Carica papaya* exhibited a concentration-dependent increase in total phenolic content, with no statistically significant difference observed between the leaf and the root. Phenolic compounds are widely recognized for their strong antioxidant activity, primarily due to their ability to donate hydrogen atoms or electrons, neutralize reactive oxygen species, and chelate pro-oxidant metal (Yousif *et al*., 2021). The similar phenolic profiles in the root and leaf extracts suggest that both plant parts contribute comparably to the antioxidant defense. The findings were consistent with those of Nisa *et al.* (2019), who reported high total phenolic content in *C. papaya* leaves across various maturity stages and extraction solvents, highlighting the influence of plant part and processing on phenolic yield. Recent studies have further established strong correlations between higher phenolic content and enhanced antioxidant potential in diverse plant extracts, including *Punica granatum* peels and *Moringa oleifera* leaves (Sweidan *et al.*, 2023; Arshad *et al.*, 2025). These bioactive compounds play a crucial role in mitigating oxidative stress by interrupting lipid peroxidation chain reactions and protecting cellular components.

Phenolics have been linked to a range of health-promoting effects beyond antioxidant defense, such as anti-inflammatory, cardioprotective, and anti-carcinogenic properties (Shahidi & Ambigaipalan, 2015). Given safety concerns over synthetic antioxidants (Shubina *et al.*, 2023), the comparable and substantial phenolic content in both root and leaf extracts of *C. papaya* reinforces its potential as a natural and safer source of antioxidants for managing oxidative stress–related diseases.

Both the root and leaf extracts of *Carica papaya* displayed significant hydroxyl radical scavenging activity, suggesting their effectiveness in neutralizing one of the most reactive species of reactive oxygen species. Hydroxyl radicals (•OH), generated via Fenton chemistry, can rapidly oxidize lipids, proteins, and DNA, contributing to aging, neurodegeneration, and carcinogenesis (Pham-Huy *et al.*, 2008). Therefore, compounds that scavenge •OH are critical in retaining cellular homeostasis. Recent studies using electron paramagnetic resonance (EPR) have confirmed the value of plant extracts in hydroxyl radical scavenging. Sanna & Fadda (2022) used an EPR-based method on ginger and blueberry extracts, showing efficient neutralization of •OH generated by different Fenton systems. This highlights the precision and reliability of EPR in validating antioxidant mechanisms. Furthermore, advanced fractionation of *Moringa oleifera* extracts has also revealed potent •OH scavenging that correlates with high concentrations of flavonoids and phenolics (El-Sherbiny *et al.*, 2024). These findings suggest that polyphenolic compounds play a major role in protecting against oxidative stress by directly trapping hydroxyl radicals.

The comparable activity observed in *C. papaya* root and leaf extracts likely reflects the presence of similar phytochemical profiles, consistent with high phenolic and flavonoid content (Nisa *et al.*, 2019). Given increasing concerns about synthetic antioxidant safety (Shubina *et al.*, 2023), these naturally derived extracts offer compelling potential as safer, biologically effective antioxidants capable of targeting highly reactive radical species. Overall, the findings demonstrate that while both *C. papaya* leaf and root extracts have significant antioxidant potential, the root extract was consistently more potent in radical scavenging and reducing power assays, while the leaf extract contained more flavonoids. These differences support their complementary therapeutic potential.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The first objective of the study aimed at determining the phytochemical profile of methanolic leaf and root extracts of *Carica papaya* Linn the variety of Sunrise Solo. The plant was used traditionally to manage fever and oxidative stress. However, there is limited scientific evidence on its pharmacological use especially on the leaves and roots. The GC-MS was used to analyze the methanol extracts from leaves and roots collected in Karingani Ward, Tharaka Nithi County, Kenya. The analysis revealed several phytochemical constituents, including flavonoids (quercetin and kaempferol derivatives), phenolics (caffeic and ferulic acids), terpenoids (squalene and myrcene), alkaloids (reserpinine), and fatty acids (n-hexadecanoic acid). These compounds are known for their antioxidant and antipyretic properties. The results confirmed that both parts of the plant contain significant bioactive constituents, supporting the traditional use of *C. papaya* in treating fever and oxidative-related conditions.

The second objective focused on evaluating the antipyretic effects of methanolic leaf and root extracts of *C. papaya in vivo* using male albino Wistar rats. Fever was induced by intramuscular injection of turpentine oil, and the extracts were administered intraperitoneally at doses of 50, 100, and 150 mg/kg, with paracetamol serving as a positive control. The problem addressed was the dependence on synthetic antipyretics such as NSAIDs and paracetamol, which are associated with adverse cardiovascular, hepatic, and gastrointestinal effects. The results showed that both extracts significantly reduced elevated rectal temperatures in a dose- and time-dependent manner, with the root extract demonstrating stronger activity at lower doses. Temperature reduction patterns were comparable to those observed with paracetamol, particularly after the second hour of treatment. The discussion highlighted that the antipyretic effect was due to the presence of phytochemicals such as flavonoids and phenolic compounds that inhibit prostaglandin E2 synthesis via cyclooxygenase (COX) suppression. These findings provide scientific validation for the plant traditional use in managing fever.

The third objective assessed the *in vitro* antioxidant activities of the methanolic leaf and root extracts using multiple non-enzymatic assays. The rationale was to explore *C.*

papaya as a natural, affordable alternative to synthetic antioxidants, which are often relatively expensive and less effective. Results demonstrated that both extracts exhibited strong antioxidant activities in a concentration-dependent manner, with high total phenolic and flavonoid contents correlating positively with radical scavenging efficacy. The leaf extract showed slightly higher antioxidant potential, possibly due to higher levels of flavonoids and phenolic acids. The discussion concluded that the antioxidative capacity of *C. papaya* supports its potential therapeutic use in mitigating oxidative stress-related diseases such as cancer, cardiovascular disorders, and neurodegeneration, thereby the plant should be used a potential natural antioxidant supplement to manage oxidative stress–related disorders.

6.2 Conclusion

The quantitative phytochemical analysis of *Carica papaya* leaf extracts revealed phytochemicals such as quercetin, limonene, myricetin, rosmarinic acid, ferulic acid and kaempferol derivatives, which exhibit potent antioxidative and antipyretic activities through ROS scavenging, inhibition of lipid peroxidation, and suppression of pro-inflammatory mediators. The root extract, contained sterols, fatty acids, quercetin, terpenes such as squalene, reserpine, and kaempferol glycosides which exhibits immunomodulatory, antioxidative, and antipyretic effects.

The antipyretic activity of methanolic root and leaf extracts of *Carica papaya* in turpentine-induced pyrexia in male Albino Wistar rats demonstrated dose-dependent antipyretic effects. The higher doses of (100 mg/kg and 150 mg/kg) produced reductions in rectal temperature comparable to the standard drug, paracetamol. The root extract demonstrated a faster onset and greater efficacy at lower doses, while both extracts achieved antipyretic effects comparable to paracetamol at higher concentrations. These was attributed to presence of phytochemicals such as kaempferol, quercetin, terpenoids and rosmarinic acid thus underscoring their potential as phytotherapeutic agents for fever management.

The antioxidant effects were concentration-dependent, with the root extract consistently exhibiting stronger activity in radical scavenging (DPPH), reducing power (FRAP), and lipid peroxidation inhibition. In contrast, the leaf extract contained significantly higher flavonoid content, which supports its traditional use in herbal medicine. While both extracts showed comparable phenolic content and moderate hydroxyl radical scavenging ability, the root extract emerged as a more potent antioxidant. These was attributed to presence of phytochemicals such as sterols, squalene, kaempferol, quercetin, caffeic acid and reserpinine.

6.3 Recommendations

From this study, the following recommendation can be made:

i. The quantitative analysis of methanolic leaf and root extracts of *C. papaya* contained various phytochemicals associated with antipyretic and antioxidative effect.
ii. The methanolic leaf and root extracts of *C. papaya* demonstrated significant antipyretic activity hence the plant should be considered as alternative source for developing novel, plant-based fever management therapies.
iii. The methanolic leaf and root extracts of *C. papaya* showed a strong antioxidant properties thus should be used a potential natural antioxidant supplement to manage oxidative stress–related disorders.

6.4 Areas for Further Studies

i. It is recommended to use solvent of lower polarity or higher polarity than methanol for extraction in order to compare results among the fractions.
ii. Further research should examine the precise molecular targets, such as cyclooxygenase isoforms and cytokine pathways, to clarify how *Carica papaya* extract reduce fever.
iii. Further research should be conducted using in vivo models to confirm the antioxidant efficacy and bioavailability of *Carica papaya* extracts under physiological conditions

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APPENDICES

Appendix 1: Dried *Carica papaya*

(i) Roots

(ii) Leaves

Appendix 2: Heavy duty blender that was used for grinding the leaves and root of *C.papaya*

Appendix 3: The powdered leaf and root of *Carica papaya* were left to soak for 24 hours

Appendix 4: Decantation and Filtration of the root and leaf extracts

Appendix 5: The rectal temperature being taken using thermometer model YB-009

Appendix 6: Antioxidant Assay Results

Appendix 6.1: *In vitro* DPPH Radical Scavenging Activity of Methanolic Leaf and Root Extract of *C. papaya*

Concentration	**1000mg/ml**	**750mg/ml**	**500mg/ml**	**250mg/ml**	**125mg/ml**	**62.5mg/ml**	**IC50**
Ascorbic Acid	0.18±0.004[c]	0.24±0.003[c]	0.35±0.005[b]	0.45±0.004[b]	0.58±0.007[b]	0.62±0.029[b]	312.7mg/ml
C. papaya root extract	0.26±0.006[a]	0.39±0.001[a]	0.47±0.008[a]	0.57±0.01[a]	00.68±0.004[a]	0.77±0[a]	640.6mg/ml
C.papaya leaf extract	0.23±0.004[b]	0.34±0.006[b]	0.46±0.01[a]	0.55±0.011[a]	0.65±0.011[a]	0.72±0.023[a]	572.8mg/ml

Appendix 6.2: *In vitro* FRAP Radical Scavenging Activity of Methanolic Leaf and Root Extract of *C. papaya*

Concentration	**250µg/ml**	**200µg/ml**	**150µg/ml**	**100µg/ml**	**50µg/ml**	**25µg/ml**	**IC 50**
Ascorbic Acid	0.85±0.012[a]	0.67±0.006[a]	0.58±0.006[a]	0.45±0.006[a]	0.37±0.011[a]	0.28±0.001[a]	147.5µg/mL
C. papaya root extract	0.67±0.016[b]	0.51±0.005[b]	0.42±0.015[b]	0.34±0.005[b]	0.23±0.004[b]	0.13±0.005[b]	219.1µg/mL
C.papaya leaf extract	0.56±0.005[c]	0.39±0.006[c]	0.31±0.006[c]	0.26±0.005[c]	0.23±0.004[b]	0.14±0.006[b]	251.4µg/mL

Appendix 6.3: *In vitro* Lipid Peroxidation Radical Scavenging Activity of Methanolic Leaf and Root Extract of *C. papaya*

Concentration	**250µg/ml**	**200µg/ml**	**150µg/ml**	**100µg/ml**	**50µg/ml**	**25µg/ml**	**IC 50**
Ascorbic Acid	0.04±0.003[c]	0.09±0.006[c]	0.12±0.004[c]	0.16±0.005[c]	0.25±0.002[c]	0.27±0.003[c]	56.3µg/mL
C. papaya root extract	0.07±0.006[b]	0.13±0.005[b]	0.17±0.004[b]	0.22±0.007[b]	0.28±0.005[b]	0.33±0.01[b]	125.0µg/mL
C.papaya leaf extract	0.12±0.004[a]	0.15±0.005[a]	0.21±0.008[a]	0.28±0.01[a]	0.34±0.005[a]	0.39±0.012[a]	162.5µg/mL

Appendix 6.4: *In vitro* Total Flavonoids Content of Methanolic Leaf and Root Extract of *C. papaya*

Concentration	**300µg/ml**	**250µg/ml**	**200µg/ml**	**150µg/ml**	**100µg/ml**	**50µg/ml**	**IC50**
Quercetin	0.79±0.007[a]	0.56±0.005[a]	0.48±0.004[a]	0.32±0.005[a]	0.28±0.005[a]	0.17±0.005[a]	160.1mg/ml
C. papaya root extract	0.58±0.043[b]	0.41±0.005[c]	0.32±0.004[c]	0.24±0.004[c]	0.17±0.006[c]	0.12±0.004[b]	270.0mg/ml
C.papaya leaf extract	0.63±0.004[b]	0.45±0.004[b]	0.36±0.005[b]	0.28±0.008[b]	0.24±0.003[b]	0.13±0.004[b]	250.0mg/ml

Appendix 6.5: *In vitro* Total Phenolic Content of Methanolic Leaf and Root Extract of *C. papaya*

Concentration	**300µg/ml**	**250µg/ml**	**200µg/ml**	**150µg/ml**	**100µg/ml**	**50µg/ml**	**IC50**
Gallic Acid	0.76±0.005[a]	0.67±0.007[a]	0.52±0.004[a]	0.43±0.005[a]	0.4±0.007[a]	0.27±0.006[a]	80.55mg/ml

C. papaya root extract	0.58±0.003[b]	0.44±0.003[c]	0.36±0.012[b]	0.26±0.003[c]	0.21±0.006[b]	0.14±0.002[c]	125.0mg/ml
C.papaya leaf extract	0.56±0.011[b]	0.49±0.005[b]	0.42±0.007[c]	0.35±0.004[b]	0.24±0.004[c]	0.22±0.003[b]	162.5mg/ml

Appendix 6.*6: In vitro* Hydroxyl Radical Scavenging Activity of Methanolic Leaf and Root Extracts of *C.papaya*

Concentration	**300μg/ml**	**250μg/ml**	**200μg/ml**	**150μg/ml**	**100μg/ml**	**50μg/ml**	**IC50**
Gallic Acid	0.14±0.003[a]	0.19±0.004[a]	0.23±0.003[a]	0.29±0.003[a]	0.36±0.015[b]	0.58±0.003[b]	71.6μg/mL
C. papaya root extract	0.16±0.003[a]	0.24±0.003[a]	0.28±0.003[b]	0.35±0.006[b]	0.52±0.003[a]	0.71±0.002[a]	110.3μg/mL
C.papaya leaf extract	0.17±0.004[b]	0.23±0.004[b]	0.33±0.003[c]	0.42±0.002[c]	0.52±0.003[a]	0.72±0.003[a]	117.5μg/mL

Appendix 7: Institution Introductory Letter

CHUKA UNIVERSITY

Knowledge is Wealth *(Sapientia divitia est)* Akili ni Mali

OFFICE OF THE DIRECTOR
BOARD OF POSTGRADUATE STUDIES

Telephones: 020-2310512/18
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Website: www.chuka.ac.ke

REF: SM16/57773/23 **21st July, 2025**

Director
National Commission for Science Technology and Innovation
Off Waiyaki Way, Upper Kabete
P O Box 30623, 00100
Nairobi.

Dear Sir / Madam

RE: EVERLYNE JEBIWOTT TARUS
The above-named person is a *bona fide* student of Chuka University pursuing MSC in Biochemistry, proposal titled:– **Determination of Phytochemical Composition, Antipyretic and Antioxidative Effects of Methanolic Leaf and Root Extracts of *Carica Papaya* Linn.**

Ms. Tarus has defended at the Faculty level and is now expected to conduct research. Any assistance accorded will be highly appreciated.

Yours sincerely,

Prof. Moses Muraya, PhD

DIRECTOR
BOARD OF POSTGRADUATE STUDIES

Appendix 8: Ethics Review Letter

CHUKA UNIVERSITY

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CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18
Direct Line: 0772894438
Email: info@chuka.ac.ke.
P. O. Box 109-60400, Chuka
Website: www.chuka.ac.ke

11th July, 2025

REF: CUIERC/ NACOSTI/827
TO: **Everlyne Jebiwott Tarus**

RE: **Determination of Phytochemical Composition, Evaluation of Antipyretic And Antioxidative Effects of Methanolic Leaf and Root Extracts of *Carica papaya Linn.* (Caricaceae)**

This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812*. The approval period is 11th July, 2025 – 11th July, 2026.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC*.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC*.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr. Benjamin Kanga
SECRETARY

INSTITUTIONAL ETHICS REVIEW COMMITTEE
11 JUL 2025
P.O. Box 109-60400, CHUKA
CHUKA UNIVERSITY

Appendix 9: NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **620159**

Date of Issue: **01/August/2025**

RESEARCH LICENSE

This is to Certify that Ms.. EVERLINE JEBIWOTT TARUS of Chuka University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Tharaka-Nithi on the topic: DETERMINATION OF PHYTOCHEMICAL COMPOSITION, ANTIPYRETIC AND ANTIOXIDATIVE EFFECTS OF METHANOLIC LEAF AND ROOT EXTRACTS OF Carica papaya Linn for the period ending : 01/August/2026.

License No: NACOSTI/P/25/4177649

620159

Applicant Identification Number

Ag. Director General

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

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See overleaf for conditions