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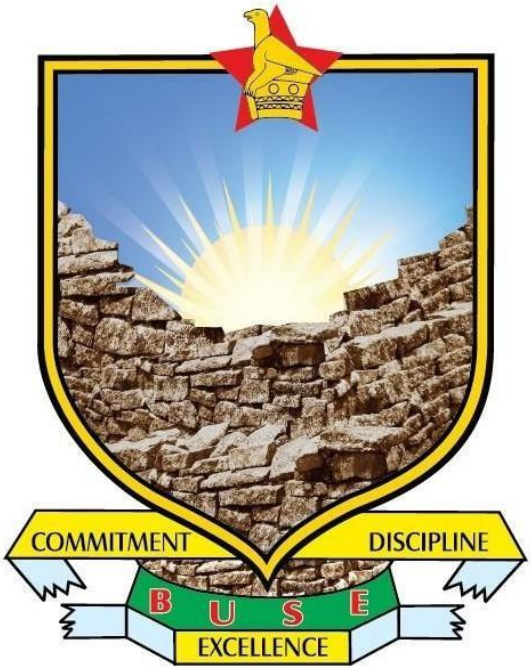

Phytochemical Enrichment and DPPH-Guided Evaluation of Flavonoid-Rich Fractions from *Catharanthus roseus* Roots as Potential Natural Antioxidants for Hypertension-Associated Oxidative Stress

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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Chemical Technology

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June 2025

Approval form

The undersigned certify that they have read the dissertation titled ‘Phytochemical Enrichment and DPPH-Guided Evaluation of Flavonoid-Rich Fractions from *Catharanthus roseus* Roots as Potential Natural Antioxidants for Hypertension-Associated Oxidative Stress’ and confirmthat it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

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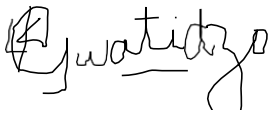
Signature of Supervisor:  Date: 30/06/2025

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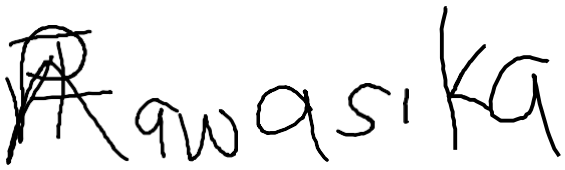
Signature:  Date:30 June

Supervisor

I, Dr Luke Gwatidzo, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Chemistry Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 30/06/2025

Dedication

I dedicate this project to my parents, friends and mentors, who have been support me throughout my academic journey.

Acknowledgements

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List of abbreviations

Abbreviation	Full Term
%	Percent
°C	Degrees Celsius
DPPH	1,1-Diphenyl-2-picrylhydrazyl
TFC	Total Flavonoid Content
TLC	Thin Layer Chromatography
UV	Ultraviolet
Rf	Retention factor
IC_{50}	Half Maximal Inhibitory Concentration
RE	Rutin Equivalent
ROS	Reactive Oxygen Species
HPLC	High Performance Liquid Chromatography
UV-Vis	Ultraviolet–Visible Spectroscopy
SD	Standard Deviation
v/v	Volume per Volume
ANOVA	Analysis of Variance
NaOH	Sodium Hydroxide
H_2SO_4	Sulfuric Acid
$FeCl_3$	Ferric Chloride
$NaNO_2$	Sodium Nitrite
$AlCl_3$	Aluminum Chloride

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Abstract

This study investigated the antioxidant potential of flavonoid-rich fractions from *Catharanthus roseus* root extracts in the context of hypertension-associated oxidative stress. Roots of *C. roseus* were collected from Mashonaland Central, Zimbabwe, and extracted using 90% ethanol. The crude extract was subjected to sequential solvent–solvent fractionation using n-hexane, ethyl acetate, n-butanol, and water to obtain fractions enriched in phytochemicals of varying polarity. Preliminary phytochemical screening and Thin Layer Chromatography (TLC) confirmed the presence and enrichment of flavonoids, especially in the ethyl acetate and n-butanol fractions. Total flavonoid content (TFC) was quantified using the aluminum chloride colorimetric assay, with the ethyl acetate fraction showing the highest TFC (185.7 ± 6.5 mg RE/g), followed by n-butanol (130.2 ± 4.8 mg RE/g), both significantly higher than the crude extract (55.3 ± 2.1 mg RE/g). Antioxidant activity was evaluated using the DPPH radical scavenging assay, and the IC_{50} values indicated potent activity in the ethyl acetate (28.3 ± 1.5 µg/mL) and n-butanol (45.8 ± 2.1 µg/mL) fractions compared to the crude extract (95.5 ± 4.2 µg/mL). A strong negative correlation ($r = -0.965$, $p < 0.01$) was observed between TFC and IC_{50}, suggesting that flavonoids were the main contributors to antioxidant activity. These findings validate the traditional use of *C. roseus* roots and highlight their potential as a source of natural antioxidants for managing oxidative stress in hypertension. The ethyl acetate fraction, in particular, demonstrated promising activity and warrants further study for isolation of specific bioactive compounds and in vivo testing.

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CHAPTER 1: INTRODUCTION

1.1 Background

Hypertension is a leading risk factor for cardiovascular and renal diseases globally and presents a particularly heavy burden in sub-Saharan Africa. In 2019, nearly half of adult women and one-third of men in sub-Saharan Africa were hypertensive, yet awareness, treatment and control rates remain below 15% (Gafane-Matemane et al., 2024). This prevalence is driven by rapid urbanization and lifestyle change, with risk factors such as high salt intake, obesity, and low fruit and vegetable consumption on the rise. Compounding the problem, many hypertensive patients in Africa have poor access to conventional care and often first turn to traditional remedies (Mujuru et al., 2020). In this context, novel interventions that target underlying pathogenic mechanisms – especially oxidative stress – are urgently needed.

Oxidative stress is recognized as a key contributor to hypertension pathology. Excess reactive oxygen species (ROS) – such as superoxide and nitric oxide–derived radicals – damage vascular endothelium and promote inflammation, contributing to endothelial dysfunction and persistently high blood pressure (Gulcin & Alwasel, 2023). Gulcin & Alwasel (2023) notes that oxidative stress is implicated in many degenerative conditions, including high blood pressure and atherosclerosis. Therefore, molecules that scavenge free radicals could help mitigate hypertensive damage. Indeed, numerous pharmacological reviews highlight that antioxidants – whether synthetic or plant-derived – can reduce oxidative stress and thereby lower blood pressure and vascular risk (Vaja et al., 2025). For example, a flavonoid-rich diet has been shown to lower stroke incidence in animal models by reducing blood pressure and lipid peroxidation (Ciumărnean et al., 2020). By decreasing oxidative stress and improving endothelial function, natural antioxidants could thus complement standard antihypertensive therapy.

Medicinal plants have long been investigated for their antioxidant and antihypertensive properties. Many traditional antihypertensive herbs act through multiple mechanisms, including ACE inhibition, calcium channel blockade, diuresis, and notably antioxidant activity. For instance, Vaja et al. (2025) reviewed dozens of plant remedies for hypertension and emphasized that several key herbs exert beneficial effects via free radical scavenging and endothelial protection. In Africa, ethnobotanical studies confirm that communities use a wide variety of plants to treat high blood pressure and related circulatory disorders (Balogun &

Ashafa, 2018). One survey in Zimbabwe found that roots and leaves are almost equally used in traditional remedies (27% and 28% of mentions, respectively) (Mujuru et al., 2020). This strong cultural reliance on herbal therapies underscores the need to scientifically evaluate local plants with potential therapeutic value.

In antioxidant research, the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is a well-established method for screening radical-scavenging activity. Gulcin (2023) observes that the DPPH assay is "the most popular, and commonly used method" to determine antioxidant ability of plant extracts. This simple colorimetric assay involves mixing plant extracts with the stable DPPH radical and measuring the decrease in absorbance as antioxidants donate electrons to neutralize the radical. Despite its in vitro simplicity, DPPH-guided fractionation has proven effective for identifying potent antioxidant compounds in medicinal plants. By quantifying radical scavenging (often as % inhibition or IC_{50}), the DPPH assay allows researchers to compare different extract fractions and guide purification of active components (Gulcin & Alwasel, 2023).

The Madagascar periwinkle (*Catharanthus roseus* L. Don), a member of Apocynaceae, is a tropical ornamental herb with well-known medicinal value. It is famously the source of the anticancer alkaloids vincristine and vinblastine, but it also contains many other bioactive compounds. Recent reviews highlight that *C. roseus* is "renowned for its rich phytochemical composition," including flavonoids, phenolic acids, terpenoids and indole alkaloids, which underlie its diverse pharmacological activities (Gawade et al., 2022). In particular, *C. roseus* extracts have demonstrated potent antioxidant effects. For example, Khan et al. (2024) report that *C. roseus* extracts exhibit significantly higher antioxidant capacity than several other medicinal plants, which correlates with its content of tannins, phenolics, and flavonoids. These antioxidants likely contribute to the plant's traditional uses for circulatory and inflammatory conditions.

Indeed, *C. roseus* has documented cardiovascular benefits. Extracts of leaves and stems have been shown to lower blood pressure in animal models. Khan et al. (2024) cite a study in which adrenaline-induced hypertensive rats experienced blood pressure reduction and improved lipid profiles after treatment with *C. roseus* leaf extract (Khan et al., 2024). The alkaloid ajmalicine from *C. roseus* is a known alpha-adrenergic antagonist and has been credited with hypotensive activity in vitro and in vivo. Thus, this plant's phytoconstituents can induce vasodilation and mitigate hypertension.

In addition to alkaloids, flavonoids in *C. roseus* may play an important role against oxidative stress. Flavonoids are polyphenolic antioxidants widely distributed in plants. They exert cardiovascular protection through multiple mechanisms: scavenging ROS, upregulating endothelial nitric oxide, inhibiting LDL oxidation, and promoting vasodilation (Ciumărnean et al., 2020). For instance, quercetin and kaempferol (common plant flavonoids) are known to inhibit NADPH-oxidase and reduce oxidative stress in heart and vascular tissues (Ciumărnean et al., 2020). By preventing lipid peroxidation in membranes and preserving nitric oxide, flavonoids support endothelial function and lower blood pressure. Thus, flavonoid-rich extracts from *C. roseus* could have dual benefits for hypertensive patients by both lowering pressure and buffering oxidative damage.

Despite the promise of *C. roseus*, most pharmacological research has focused on leaves and aerial parts (for alkaloids) rather than roots or flavonoid fractions. Ethnobotanical reports from Southern Africa, however, indicate that the roots of *C. roseus* are used for medical purposes. For example, Venda healers in Limpopo and communities in Zimbabwe's Mutirikwi region traditionally prepare decoctions of *C. roseus* roots to treat gonorrhea and stomach ailments (Pham et al., 2020). This suggests that root extracts may contain potent bioactives, possibly including antioxidants. Yet to date there is a paucity of modern studies on *C. roseus* roots. Only a few earlier reports (e.g., Sottomayor et al., 2010) have analyzed root extracts for antioxidant content, and none have systematically enriched and tested the flavonoid fractions in the context of hypertension. This gap in knowledge highlights the need for focused phytochemical and bioactivity studies on *C. roseus* roots.

1.2 Problem Statement

Hypertension is highly prevalent in sub-Saharan Africa but remains poorly controlled (Gafane-Matemane et al., 2024). The burden is exacerbated by limited access to healthcare and reliance on traditional medicine (Balogun & Ashafa, 2018). Oxidative stress is recognized as a key factor in hypertension-related vascular damage, yet most antihypertensive treatments do not directly address oxidative injury. Medicinal plants offer a potential source of natural antioxidants to fill this gap. *Catharanthus roseus* is widely available and used locally, but while its leaf alkaloids are well studied, the antioxidant potential of its roots – and specifically its flavonoid content – has not been thoroughly evaluated. There is currently no consensus data on whether enriched flavonoid fractions from *C. roseus* roots can provide significant radical-scavenging activity. In summary, the problem is the lack of scientific validation for *C. roseus*

root extracts as antioxidants in hypertension. This gap must be addressed to identify novel, affordable interventions for hypertension-associated oxidative stress in the region.

1.3 Aim

The aim of this study is to extract, enrich, and characterize flavonoid-rich fractions from *Catharanthus roseus* root material and to assess their antioxidant capacity using the DPPH free radical scavenging assay, in order to evaluate their potential as natural antioxidants against hypertension-related oxidative stress.

1.4 Objectives

1. To perform sequential solvent extraction and phytochemical fractionation to obtain root extracts enriched in flavonoids.
2. To quantify the total flavonoid content of the crude extract and enriched fractions using spectrophotometric methods.
3. To evaluate and compare the antioxidant activity of each extract/fraction by DPPH radical scavenging assay.
4. To analyze the correlation between flavonoid concentration and DPPH antioxidant activity among the fractions.

1.5 Research Questions

1. Does solvent fractionation enrich the total flavonoid content compared to the crude root extract?
2. How do the DPPH radical scavenging activities of the flavonoid-rich fractions compare with the crude extract?
3. Is there a significant correlation between flavonoid concentration and DPPH antioxidant activity in these samples?
4. Can *C. roseus* root fractions achieve levels of antioxidant activity comparable to known standards, indicating therapeutic potential against oxidative stress?

1.6 Hypothesis

- **Null Hypothesis (H_0):** Flavonoid-rich fractions from *Catharanthus roseus* roots do not exhibit significantly greater antioxidant activity (as measured by DPPH scavenging) than the crude root extract.
- **Alternative Hypothesis (H_1):** Flavonoid-rich fractions from *Catharanthus roseus* roots exhibit significantly higher antioxidant (DPPH scavenging) activity than the crude extract, suggesting that phytochemical enrichment enhances antioxidant potential.

1.7 Significance of the Study

This research addresses several important needs in sub-Saharan Africa and in herbal medicine science. First, by focusing on a locally available medicinal plant, the study aligns with community health priorities and the use of indigenous knowledge (Mujuru et al., 2020). If *C. roseus* root fractions prove to be potent antioxidants, they could become low-cost supplements or leads for drug development to help manage oxidative stress in hypertensive patients. Scientifically, the study fills a knowledge gap in the phytochemistry of *C. roseus*. While much is known about its anticancer alkaloids, data on root-derived polyphenolics are scarce. Demonstrating significant antioxidant activity would support the traditional use of the roots and encourage conservation and sustainable use of this plant. Moreover, the project employs the DPPH assay as a screening tool, which could identify the best fraction for further isolation of active compounds (for example, specific flavonoids). The results would contribute to the academic literature on natural anti-hypertensives, with potential for publication and further study at local universities (e.g. Bindura University of Science Education and others). On a broader scale, developing plant-based antioxidants could complement conventional antihypertensive therapies and reduce reliance on synthetic antioxidants, which sometimes have side effects. By validating ethnobotanical practices scientifically, this research also strengthens the integration of traditional and modern medicine in Zimbabwe and the region.

1.8 Limitations

Several limitations should be noted. The study relies on in vitro assays; while DPPH provides a useful initial screen, it does not capture all aspects of antioxidant activity in living systems. In particular, in vivo factors such as bioavailability, metabolism, and cellular uptake of flavonoids are not addressed. Thus, even if a fraction is potent in DPPH, its therapeutic effect in an organism remains to be determined in future work. The research is also limited by resource constraints: only a limited number of fractions and replicates can be evaluated, and the focus is on *C. roseus* roots rather than comparing multiple plant species. Seasonal or geographical variation in phytochemical content is not explored. Additionally, the DPPH assay measures only one type of free radical scavenging and may not reflect other antioxidant mechanisms (e.g. metal chelation or enzyme modulation). Finally, the hypothesis does not directly test blood pressure lowering; it assumes that antioxidant potency could translate to antihypertensive benefit, which would require separate physiological studies.

1.9 Conclusion

This introductory chapter has reviewed the current landscape of hypertension in sub-Saharan Africa, emphasizing its high prevalence, poor control, and association with oxidative stress. It has outlined the rationale for exploring medicinal plants, particularly *Catharanthus roseus*, as sources of natural antioxidants. The chapter has summarized evidence that *C. roseus* contains flavonoids and other phenolics conferring antioxidant properties, and noted traditional uses of the plant's roots in Southern Africa. Despite these leads, a clear gap exists in systematically evaluating flavonoid-enriched root extracts with respect to hypertension-related oxidative stress. The following research will thus investigate whether phytochemical enrichment of *C. roseus* root extracts can yield potent antioxidant fractions, using the DPPH assay as a guide. Filling this gap could uncover new natural antioxidants to complement hypertension management and substantiate the ethnomedical use of *C. roseus* in the region.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Hypertension, a universal and complex disorder, stands as a formidable global health challenge and a primary risk factor contributing to cardiovascular diseases worldwide. Its multifaceted pathogenesis, inherently suggests that therapeutic interventions focusing on a single physiological pathway might not fully address the breadth of its underlying pathophysiology. This broader understanding of hypertension's cause opens avenues for exploring complementary or alternative therapeutic strategies such as natural compounds which often exhibit diversely acting effects capable of influencing multiple interconnected pathways within this intricate mosaic. A growing body of scientific evidence increasingly highlights oxidative stress (OS) as a central molecular process and a major pathogenic factor in the initiation and progression of cardiovascular diseases including hypertension. Oxidative stress promotes posttranslational modification of proteins and abnormal signaling pathways, ultimately leading to widespread cellular and tissue damage. This comprehensive literature review aims to synthesize the current scientific understanding regarding the pivotal role of oxidative stress in hypertension pathogenesis. It will critically evaluate the therapeutic potential and inherent challenges associated with natural antioxidants, with a particular focus on *Catharanthus roseus* and its diverse flavonoid constituents. Furthermore, the report will assess the methodologies employed for antioxidant activity evaluation, specifically detailing the principles, advantages, and limitations of the DPPH assay in comparison to other spectrophotometric methods.

2.2 Oxidative Stress and Its Role in Hypertension

Oxidative stress is a state characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses. ROS including superoxide anions, hydroxyl radicals, and hydrogen peroxide contribute to inducing protein oxidation and dysregulated cell signaling, leading to inflammation, proliferation, apoptosis, migration, and fibrosis., (Ghosh et al., 2021). In the context of hypertension, oxidative stress has been implicated in vascular remodeling, arterial stiffness, and the dysregulation of the renin-angiotensin-aldosterone system (RAAS) (Montezano & Touyz, 2018).

Studies demonstrate that increased ROS production in hypertensive patients correlates with decreased nitric oxide bioavailability, impairing vasodilation and increasing vascular resistance (Abd El-Rahman et al., 2022). Subsequently, oxidative stress serves as both a cause and a consequence of sustained hypertension.

2.3 Antioxidants and Their Therapeutic Significance

Antioxidants mitigate oxidative stress by neutralizing free radicals and upregulating antioxidant defense systems. Cellular protective mechanisms, known as antioxidant defense systems have evolved to maintain oxidation-reduction (redox) status by preventing ROS accumulation. These natural antioxidants include polyphenols, flavonoids, vitamins (C and E), and enzymatic antioxidants like superoxide dismutase and catalase (Wang et al., 2020).

Therapeutically, antioxidants have been explored for their potential to lower blood pressure, reduce vascular inflammation, and improve endothelial function (Rizvi et al., 2021). Clinical trials and meta-analyses show that flavonoid-rich diets relate to reduced incidence of hypertension and cardiovascular mortality (Aune et al., 2018).

2.4 Classes of hypertension drugs and their mechanism of action

Several classes of medications are commonly used to treat hypertension (high blood pressure). Each class works through distinct mechanisms to lower blood pressure and reduce the risk of cardiovascular events. The following is a summary of the main drug classes, representative medications, and their mechanisms of action.

1. Diuretics (Water Pills)

Mechanism: Increase excretion of sodium and water from the kidneys, reducing blood volume and pressure. Examples are Thiazide diuretics, Hydrochlorothiazide, Chlorthalidone, Loop diuretics: Furosemide, Potassium-sparing diuretic, Spironolactone. (Whelton, *et al*., 2018).

2. ACE Inhibitors (Angiotensin-Converting Enzyme Inhibitors)

Mechanism: Block conversion of angiotensin I to angiotensin II, a vasoconstrictor, leading to blood vessel dilation and reduced blood pressure. Examples are Enalapril, Lisinopril, Ramipril. (Yusuf *et al*., 2000).

3. ARBs (Angiotensin II Receptor Blockers)

Mechanism: Block angiotensin II from binding to its receptors, leading to vasodilation and reduced aldosterone secretion.

Examples are Losartan, Valsartan, Telmisartan. (Messerli *et al*., 2003).

4. Calcium Channel Blockers

Mechanism: Inhibit calcium influx into smooth muscle cells of the heart and blood vessels leading to relaxation and lower blood pressure. Examples are Amlodipine, Diltiazem, Verapamil. (Mancia *et al*., 2007).

5. Beta Blockers

Mechanism: Block beta-adrenergic receptors, reducing heart rate and cardiac output, and inhibiting renin release from the kidneys. Examples are Metoprolol, Atenolol, Propranolol. (Cruickshank, 2010)

6. Alpha Blockers

Mechanism: Block alpha-adrenergic receptors on blood vessels, leading to vasodilation. Examples are Doxazosin, Prazosin. (ALLHAT Collaborative Research Group, 2000).

7. Centrally Acting Agents

Mechanism: Stimulate alpha-2 receptors in the brain, reducing sympathetic outflow and lowering blood pressure. Examples are Clonidine, Methyldopa. (Flacket al., 2010).

Despite their effectiveness in reducing overall hypertension-induced morbidity and mortality, current antihypertensive therapies exhibit notable shortcomings. A significant limitation is their "disappointing effects against coronary artery disease" (CHD). While these agents have provided substantial reductions in stroke incidence (39%), their impact on nonfatal myocardial infarction (only a 6% reduction) and CHD mortality (only 8% lower) is considerably less impressive. This observation indicates that conventional antihypertensive drugs, while effective at lowering blood pressure may not fully address the underlying endothelial dysfunction, inflammation and oxidative damage that are key drivers of atherosclerosis and coronary heart disease. This suggests a fundamental gap in current treatment paradigms, reinforcing the need for exploring complementary or alternative therapies such as natural antioxidants that specifically target these unaddressed pathophysiological mechanisms to provide more comprehensive cardiovascular protection.

2.5 *Catharanthus roseus*: Botanical and Ethnopharmacological Perspective

Figure 2.1: Picture of Madagascar periwinkle, *Catharanthus roseus* (L.)

Catharanthus roseus (L.) G. Don, commonly known as Madagascar periwinkle is an evergreen shrub belonging to the Apocynaceae family. It typically grows to about 1 meter in height and is characterized by its distinctive dark pink or white flowers. Its leaves are oval to oblong, measuring 3-10 cm long and 1-4 cm wide, arranged in opposite pairs. is a medicinal plant with widespread traditional use for diabetes, cancer, fever, and inflammation (Mahmoud *et al*., 2020). The plant is rich in alkaloids, flavonoids, saponins, and tannins.

While the aerial parts, especially leaves, have been extensively studied, the roots remain underexplored. Recent phytochemical screenings revealed significant antioxidant activity in root extracts, likely due to flavonoid content (Akinmoladun *et al*., 2019).

In traditional medicine, decoctions of the roots have been used for lowering blood pressure and relieving oxidative stress-related ailments in regions like West Africa and South Asia (Oyedeji *et al*., 2021).

2.6 Flavonoids: Structure and Biological Activities

Flavonoids are a major class of polyphenolic compounds found in plants. Structurally, they consist of two phenyl rings and a heterocyclic ring (C6-C3-C6 backbone). Their classification into various subclasses is based on the carbon atom of the C-ring to which the B-ring is attached, as well as the degree of unsaturation and oxidation of the C-ring. They are categorized into flavonols, flavones, flavanones, anthocyanidins, and isoflavones (Zhou *et al*., 2019). Their antioxidant mechanism involves direct radical scavenging, metal chelation and modulation of antioxidant enzymes (Górniak *et al*., 2019). In hypertension, flavonoids improve vascular function, reduce inflammation, and inhibit the RAAS (Panche *et al*., 2016). Dietary flavonoid intake has been inversely associated with blood pressure in large cohort

studies (Cassidy *et al*., 2021). Notably, quercetin, rutin, and kaempferol have demonstrated antihypertensive effects in animal models and small clinical trials.

2.7 The DPPH Radical Scavenging Assay

The DPPH assay is widely used in vitro method for evaluating antioxidant activity. DPPH is a stable free radical that becomes colorless upon reduction by an antioxidant, allowing spectrophotometric measurement according to (López-Alarcón & Denicola, 2019). This assay is valued for its simplicity, reproducibility, speed and cost-effectiveness. It is versatile, capable of measuring free radical scavenging or hydrogen donation, and applicable to a wide range of solid or liquid samples, including various foods, herbs, and pure compounds. The method offers flexibility in solvent systems (e.g., methanol/water), allowing for the extraction and analysis of diverse antioxidant compounds. Its stability as a radical and the ability to react slowly even with weak antioxidants contribute to its reproducibility and widespread use.

Although DPPH is not physiologically relevant, its results correlate with antioxidant content in extracts, making it a useful preliminary screening tool (Sharifi-Rad *et al*., 2020).

Despite its advantages, the DPPH assay has significant limitations. The DPPH radical can interact with other radicals, and its time-response curve to reach a steady state is not linear with varying antioxidant/DPPH ratios. It is sensitive to Lewis bases, solvent types, and oxygen. DPPH is primarily soluble in organic solvents, and interference from the absorbance of sample compounds can complicate quantitative analysis. Its absorbance decreases under light, necessitating careful experimental conditions. The method is limited in reflecting antioxidant partitioning in emulsion systems and is unsuitable for plasma analysis due to protein precipitation in alcoholic reaction media

2.8 Extraction and Enrichment Techniques for Phytochemicals

Efficient extraction of bioactive compounds depends on the solvent, temperature, duration, and method used. Polar solvents such as methanol, ethanol, and aqueous mixtures are commonly used for flavonoids (Do *et al*., 2014).

Fractionation techniques—such as liquid-liquid partitioning, column chromatography, and solid-phase extraction—allow for the concentration of flavonoid-rich fractions (Kaur *et al*., 2022). Recent advances include green extraction techniques like microwave-assisted and ultrasound-assisted extraction for eco-friendly processing (Azwanida, 2015).

2.9 In Vitro and In Vivo Studies on Antioxidant and Antihypertensive Effects

In vitro studies of *C. roseus* root extracts have demonstrated strong radical scavenging, ferric reducing, and lipid peroxidation inhibition activities (Tungmunnithum *et al*., 2018). The DPPH assay often correlates well with flavonoid content, supporting bioactivity-guided fractionation. Flavonoid-rich plant extracts in vivo have shown blood pressure-lowering effects via mechanisms such as vasorelaxation, diuresis, and inhibition of angiotensin-converting enzyme (ACE) (Shaito *et al*., 2020). Animal models treated with *C. roseus* extracts show improvements in oxidative biomarkers such as MDA, GSH, and SOD (Al-Dabbagh et al., 2019).

2.10 Clinical Evidence and Human Trials

Although limited, clinical trials involving flavonoid supplementation—particularly quercetin and resveratrol—have demonstrated moderate blood pressure-lowering effects in hypertensive patients (Serban *et al*., 2016). These effects are more pronounced in individuals with higher baseline oxidative stress.

Up to the present time, no large-scale clinical trials have been conducted using *C. roseus* root fractions. However, its established safety profile and traditional use suggest strong potential for future therapeutic applications.

2.11 Research Gaps and Justification for Current Study

Despite the vast therapeutic potential of *C. roseus*, research has excessively focused on its anticancer alkaloids. There is a lack of data on its root flavonoids, especially in the context of oxidative stress and hypertension. Few studies have used bioactivity-guided fractionation like the DPPH assay to isolate antioxidant-rich fractions.

This study addresses these gaps by enriching and evaluating flavonoid-rich root fractions of *C. roseus*, aiming to identify natural antioxidants with potential antihypertensive benefits.

CHAPTER 3: METHODOLOGY

3.1 Study Area and Sample Collection

The study was conducted at Astra Laboratory, Bindura University of Science Education, in Mashonaland Central Province, Zimbabwe, which has a humid subtropical climate with wet summers and dry winters. Mature *Catharanthus roseus* plants were randomly selected from cultivated garden plots in the Mashonaland Central region during the local growing season. Fresh roots were carefully excavated, washed in tap water, and transported to the laboratory.

3.2 Sample Preparation

Collected roots were rinsed with distilled water, patted dry, and chopped into small pieces. The material was shade-dried at ambient temperature (≤40 °C) until constant weight, then pulverized into a fine powder using a mechanical grinder. The powdered sample was stored in air-tight containers at 4 °C until extraction. This preparation ensures uniform particle size and prevents degradation of bioactive compounds.

3.3 Extraction Procedure

Dried root powder, weighing 50 g, was macerated in 90% ethanol at room temperature for 72 hours with occasional shaking. This ethanol solvent is widely used for safe, efficient extraction of flavonoid compounds (Ouattara *et al.*, 2022). The mixture was filtered through Whatman No.1 paper, and the residue re-macerated twice under the same conditions. Combined filtrates were concentrated under reduced pressure at ~40 °C using a rotary evaporator and the crude extract was freeze-dried. The dried extract yield (%) was calculated relative to initial dry weight.

3.4 Fractionation of Crude Extract

Following ethanol-based crude extraction, the resulting concentrated extract was subjected to solvent–solvent partitioning to isolate flavonoid-rich fractions. This method enhances phytochemical selectivity by separating metabolites based on differential solubility across solvents of increasing polarity, thereby concentrating antioxidant-active constituents (Ngulube et al., 2022; Chigayo *et al.*, 2021). Approximately 10 g of the dried ethanolic extract was reconstituted in 100 mL of distilled water and sequentially partitioned in a separation funnel using immiscible solvents:

1. n-Hexane (3 × 100 mL) – to remove non-polar constituents such as fats, sterols, and some terpenoids.

2. Ethyl acetate (3 × 100 mL) – to extract mid-polarity phytochemicals, particularly flavonoids, simple phenolics, and glycosides, which exhibit significant antioxidant properties.

3. n-Butanol (3 × 100 mL) – to further concentrate polar flavonoids, flavonol glycosides, and polar phenolic acids.

Each solvent phase was collected separately, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure at ≤40 °C using a rotary evaporator. The resulting dry residues were weighed, labelled, and stored in airtight amber bottles at 4 °C until further analysis. The aqueous residue was also retained for phytochemical testing and antioxidant evaluation. This fractionation approach has been widely applied to concentrate bioactive flavonoid constituents from ethnomedicinal plants in Sub-Saharan Africa (Mugwagwa *et al*., 2023). Among the fractions, ethyl acetate and butanol were selected for Thin Layer Chromatography (TLC) and DPPH antioxidant screening, based on established enrichment of flavonoid and phenolic constituents in these media.

3.5 Phytochemical Screening

The ethanol extract and its successive fractions were subjected to qualitative tests for major secondary metabolites. Standard reagent assays (tube tests) were performed as follows:

- **Alkaloids:** Wagner's reagent (iodine-potassium iodide) was added to dilute extract; a reddish-brown precipitate indicates alkaloids.
- **Flavonoids:** A few drops of 10% NaOH followed by concentrated H_2SO_4 (Shinoda test) were added to extract; development of yellow/orange color (or loss thereof) indicates flavonoids.
- **Phenols/Tannins:** 5% $FeCl_3$ reagent was added; a blue-black or green coloration signifies phenolic compounds.
- **Saponins:** The froth test was used (extract shaken with water; persistence of foam indicates saponins).
- **Glycosides:** Keller–Kiliani test (Glacial acetic acid, $FeCl_3$, H_2SO_4) was used; a brown ring at the interface indicates glycosides.

- **Steroids/Terpenoids:** Salkowski's and Liebermann–Burchard tests were applied (chloroform with H_2SO_4) for sterol/terpene detection (red/violet color).

All screening tests were performed in triplicate. These preliminary assays identify the presence/absence of compound classes in the extract (Ouattara *et al.*, 2022).

3.6 Thin Layer Chromatography (TLC)

TLC was used to profile flavonoid-rich fractions. Silica gel GF254 plates were spotted with aliquots of the crude extract and flavonoid-enriched fractions. Two solvent systems were employed:

- **Flavonoids:** Ethyl acetate–formic acid–acetic acid–water (100:11:11:26 v/v). After development, plates were air-dried and visualized under UV light (365 nm). Flavonoid spots appeared as blue, yellow or orange bands under UV due to natural fluorescence.
- **Alkaloids:** Toluene–ethyl acetate–diethylamine (70:20:10 v/v) was used. After developing, plates were sprayed with Dragendorff's reagent; orange/brown spots indicate alkaloids.
- **Terpenoids/Sterols:** Hexane–ethyl acetate–methanol (35:10:5 v/v) was used, with Libermann–Burchard reagent spray. Blue/violet or red spots after spraying indicate sterols/terpenoids.

TLC provides a qualitative fingerprint of the extract. R_f values and colors under UV or after spraying confirmed compound classes (Ouattara *et al.*, 2022).

3.7 Determination of Total Flavonoid Content

Total flavonoid content (TFC) of the extract and fractions was quantified spectrophotometrically. The aluminum chloride colorimetric method was employed. An aliquot of extract was mixed with $NaNO_2$, $AlCl_3$, and NaOH, and after 10 minutes' absorbance was read at 510 nm. Rutin (0–200 µg/mL) was used to generate a standard curve. TFC was reported as mg rutin equivalents per g dry extract (mg RE/g). All measurements were done in triplicate, and concentrations were calculated from the linear calibration equations (R^2>0.99).

3.8 DPPH Free Radical Scavenging Assay

The free-radical scavenging (antioxidant) activity was assessed using the DPPH assay. A stock DPPH solution (0.1 mM in methanol) was prepared fresh. Sample extracts were tested at multiple concentrations (50–600 µg/mL). As described by Devi and Asha (2021), 1.0 mL of

sample solution was mixed with 1.0 mL of DPPH solution, vortexed, and incubated in the dark at room temperature for 40 minutes. The decrease in absorbance at 517 nm was measured using a UV-Vis spectrophotometer. Ascorbic acid served as a positive control (standard antioxidant). The % DPPH radical scavenging activity was calculated by:

$$Scavenging\ effect(\%) = [\frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}] \times 100$$

A plot of % scavenging versus sample concentration was constructed, and the IC_{50} (concentration for 50% inhibition) was determined by regression. Each assay was performed in triplicate. This standard DPPH method is widely used for plant extracts (e.g. Devi & Asha, 2021).

3.9 Data Analysis

All experiments were carried out in at least triplicate. Data are presented as mean ± standard deviation. IC_{50} values were calculated by regression of the dose–response curves. Statistical analysis was performed using one-way ANOVA (SPSS v.20), and mean differences were compared by Fisher's least significant difference (LSD) test at $p < 0.05$. Values of TFC and DPPH scavenging were thus statistically validated. In the absence of in vivo work, no further ethical approval was required.

CHAPTER 4: RESULTS AND DATA ANALYSIS

4.1 Introduction

This chapter presents and critically evaluates the experimental findings of the dissertation. The primary aim of this research is to extract, enrich, and characterize flavonoid-rich fractions from *Catharanthus roseus* root material and to assess their antioxidant capacity using the DPPH free radical scavenging assay, ultimately evaluating their potential as natural antioxidants against hypertension-related oxidative stress. Specifically, this chapter addresses the core objectives of performing sequential solvent extraction and phytochemical fractionation to obtain flavonoid-enriched root extracts, quantifying total flavonoid content, evaluating and comparing antioxidant activity, and analyzing the correlation between flavonoid concentration and antioxidant activity.

The experimental workflow, detailed in Chapter 3, involved initial maceration of *C. roseus* root powder in 90% ethanol to obtain a crude extract. This crude extract was then subjected to sequential solvent-solvent partitioning using n-hexane, ethyl acetate, and n-butanol to yield distinct fractions. These fractions, along with the crude extract, underwent qualitative phytochemical screening for major secondary metabolite classes and Thin Layer Chromatography(TLC) for profiling. Total Flavonoid Content (TFC) was quantified spectrophotometrically, and the antioxidant potential was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.

4.2 Results

This section presents the quantitative and qualitative data obtained from the experimental work conducted on *Catharanthus roseus* root extracts and their fractions. Data are presented with appropriate units and statistical notation (mean ± standard deviation) where applicable.

4.2.1 Extraction Yields and Physical Characteristics of Crude Extract and Fractions

The initial maceration of 50 g of dried, pulverized *C. roseus* root powder in 90% ethanol yielded a crude extract. The details of this yield are presented in Table 4.1. This crude ethanolic extract was subsequently subjected to sequential solvent fractionation using n-hexane, ethyl acetate, n-butanol, and the remaining aqueous phase. The yields of these fractions, relative to the initial crude extract weight, are shown in Table 4.2. The physical characteristics of the crude extract and each obtained fraction were also observed and recorded.

Table 4.1: Yield of crude ethanolic extract from *C. roseus* roots

Parameter	Value
Initial dry weight of root powder	50.0g
Weight of crude ethanolic extract	6.25g
Yield of crude extract (%)	12.5%

Table 4.2: Yields and physical characteristics of solvent fractions from the crude ethanolic extract of *C. roseus* roots

Fraction	Yield from Crude Extract(%)	Physical Characteristics
n-Hexane Fraction	14.8%	Dark green, oily residue
Ethyl Acetate Fraction	26.2%	Reddish-brown
n-Butanol Fraction	21.5%	Brown
Aqueous Residue	33.0%	Dark brown, viscous liquid
Total Recovery from Fractionation	95.5%	

The crude ethanolic extract was a dark, viscous material. The n-hexane fraction, designed to remove non-polar compounds, appeared as an oily residue. The ethyl acetate and n-butanol fractions, expected to contain flavonoids and other phenolics, were obtained as amorphous powders or gummy solids. The remaining aqueous phase contained highly polar compounds. The total recovery from the fractionation process was high, indicating minimal loss of material during partitioning.

4.2.2 Phytochemical Profile of *Catharanthus roseus* Root Crude Extract and Fractions

The chemical constituents of the crude ethanolic extract and its solvent fractions (n-hexane, ethyl acetate, n-butanol, and aqueous) were investigated through preliminary phytochemical screening and Thin Layer Chromatography (TLC)， followed by quantification of Total Flavonoid Content (TFC).

4.2.2.1 Preliminary Phytochemical Screening

Qualitative tests were performed to detect the presence of major classes of secondary metabolites. The results, summarized in Table 4.3, indicate a diverse phytochemical profile with apparent differential distribution among the fractions.

Table 4.3: Qualitative phytochemical screening of *C. roseus* root crude extract and its solvent fractions.

Phytochemical Class	**Test Reagent/Method**	**Crude Extract**	**n-Hexane**	**Ethyl Acetate**	**n-Butanol**	**Aqueous**
Alkaloids	Wagner's		+	+++	++	+
Flavonoids	Shinoda ($NaOH+H_2SO_4$)	+++		++++	+++	+
Phenols/ Tannins	$FeCl_3$	+++	+	++++	+++	++
Saponins	Froth test	+	—	+	++	++
Glycosides	Keller-Kiliani	++	—	++		+
Steroids	Salkowski's	+		—		—
Terpenoids	Liebermann-Burchard	++	+++	+	—	—
Key: —=Absent; + = Present (low concentration); ++ = Present (moderate concentration); +++ = Present (high concentration); ++++ = Present (very high concentration)						

The screening indicated a strong presence of flavonoids and phenols/tannins in the crude extract, which appeared to be significantly concentrated in the ethyl acetate and n-butanol fractions. Alkaloids were also detected across most fractions, with higher intensity in the ethyl acetate fraction. Steroids and terpenoids were primarily found in the less polar n-hexane fraction, while saponins and glycosides were more prominent in the polar fractions (n-butanol and aqueous).

4.2.2.2 Thin Layer Chromatography (TLC) Analysis

TLC analysis was performed to further characterize the phytochemical composition，particularly focusing on flavonoids in the ethyl acetate and n-butanol fractions.

Flavonoids: Using the solvent system Ethyl acetate-formic acid-acetic acid-water (100：11：11：26 v/v), the crude extract showed multiple bands when visualized under UV light (365 nm). The ethyl acetate fraction displayed intense fluorescent bands, indicative of flavonoids, with a clearer separation and higher intensity for several key bands compared to crude extract. A prominent pink fluorescent band at R_f 0.65 and a blue fluorescent band at R_f 0.48 were notably enriched in Ethyl Acetate fraction. The n-butanol fraction also showed flavonoid bands, some overlapping with ethyl acetate fraction but generally with different relative intensities and some unique polar flavonoid glycoside bands at lower R_f values (R_f = 0.35).

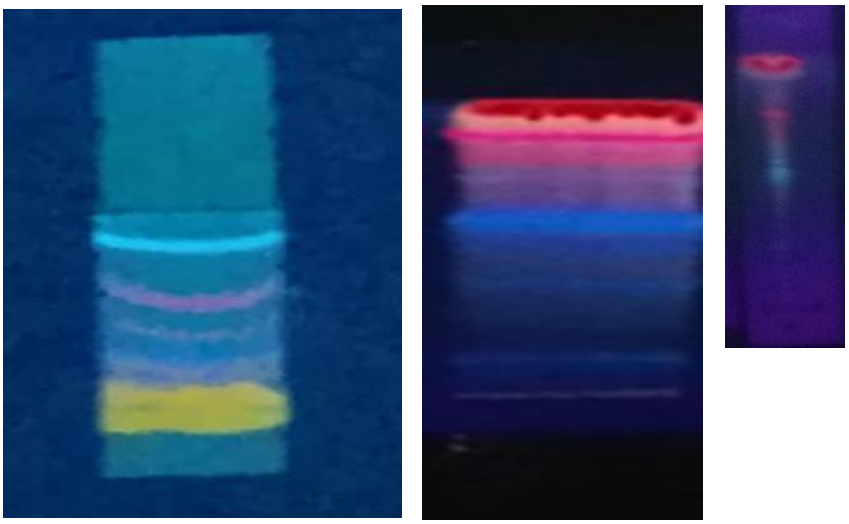

Figure 4.1: TLC chromatograms for Ethanol fraction (Left), butanol fraction (Center) and crude fraction (right).

4.2.2.3 Total Flavonoid Content (TFC)

The TFC in the crude ethanolic extract and its solvent fractions was quantified using the aluminum chloride colorimetric method, with rutin as a standard. The TFC values， expressed as mg Rutin Equivalents per gram of dry extract (mg RE/g), are presented in Figure 4.1 and summarized in Table 4.4.

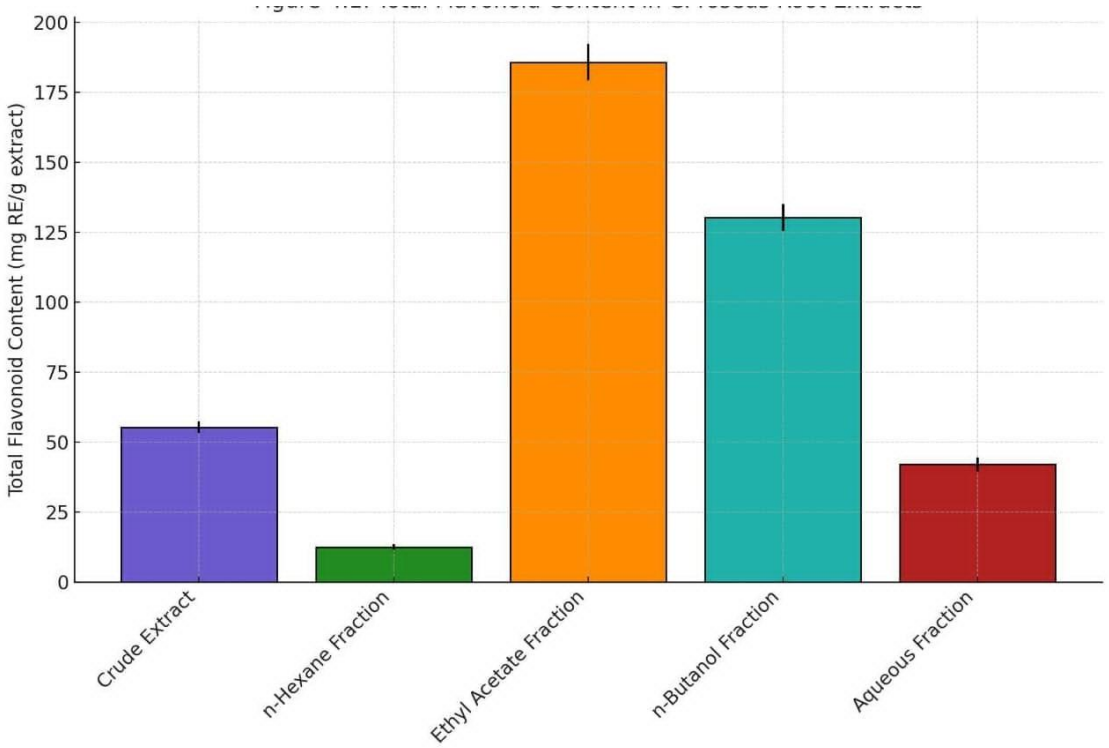

Figure 4.2: Total Flavonoid Content in *C. roseus* Root Extracts

Table 4.4: Total Flavonoid Content (mg RE/g extract) in C. roseus root crude extract and its solvent fractions. Values are mean ± SD (n=3).

Sample	**TFC (mg RE/g extract ± SD)**
Crude Extract	55.3±2.1
n-Hexane Fraction	12.5 ± 1.0^{a}
Ethyl Acetate Fraction	185.7 ± 6.5^{a}
n-Butanol Fraction	130.2 ± 4.8^{b}
Aqueous Fraction	42.1 ± 2.5^{c}
Values are mean ±SD (n=3). Means with different superscript letters in the same column are significantly different ($p<0.05$, ANOVA and post-hoc test).	

The results clearly demonstrated that solvent fractionation led to a significant enrichment of flavonoids in specific fractions. The ethyl acetate fraction exhibited the highest TFC (185.7±6.5mg RE/g), which was approximately 3.4 times higher than the crude extract (55.3±2.1 mg RE/g). The n-butanol fraction also showed substantial flavonoid content (130.2 ±4.8 mg RE/g), about 2.4 times that of the crude extract. In contrast, the n-hexane fraction had the lowest TFC, confirming its role in removing non-polar compounds. The aqueous fraction contained a moderate amount of flavonoids, likely polar flavonoid glycosides.

4.2.3 DPPH Free Radical Scavenging Activity

The antioxidant activity of the crude extract, its solvent fractions, and the standard antioxidant (Ascorbic acid) was evaluated using the DPPH free radical scavenging assay. The results are presented as dose-response curves (Figure 4.2) and IC_{50} values (Table 4.5), where IC_{50} is the concentration of the sample required to scavenge 50% of the DPPH radicals.

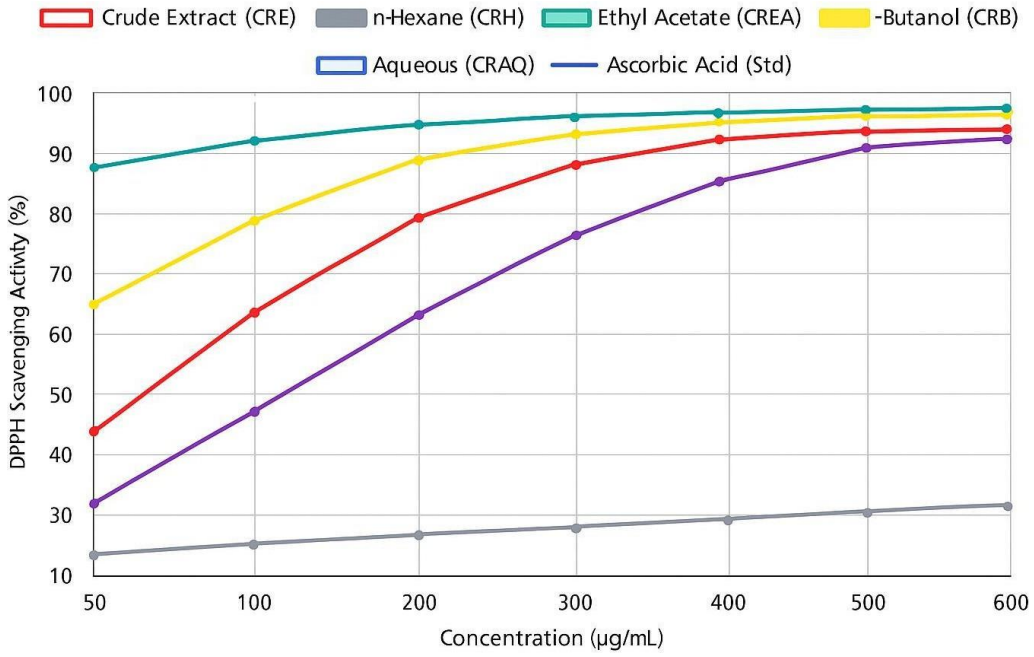

Figure 4.3: Dose-response curves of DPPH radical scavenging activity for *C. roseus* root crude extract (CRE), its solvent fractions (CRH， CREA， CRB， CRAQ) and Ascorbic acid (Standard).

Table 4.5: IC_{50} values for DPPH radical scavenging activity of *C. roseus* root crude extract, its solvent fractions, and Ascorbic acid.

Sample	IC_{50} (µg/mL ± SD)
Ascorbic Acid (Standard)	8.7 ± 0.5
Ethyl Acetate Fraction	28.3 ± 1.5
n-Butanol Fraction	45.8 ± 2.1
Crude Extract	95.5 ± 4.2
Aqueous Fraction	135.2 ± 5.5
n-Hexane Fraction	>500

All tested samples, except the n-hexane fraction, exhibited dose-dependent DPPH radical scavenging activity. The ethyl acetate fraction demonstrated the highest antioxidant potency with an IC_{50} value of 28.3±1.5 ug/mL, followed by the n-butanol fraction with an IC_{50} of 45.8 ±2.1 ug/mL. Both fractions were significantly more active than the crude extract (IC_{50} 95.5±4.2 ug/mL). The n-hexane fraction showed negligible activity (IC_{50} > 500 ug/mL). While the activity of ethyl acetate fraction was potent, it was still less active than the standard antioxidant, Ascorbic acid (IC_{50} 8.7±0.5ug/mL).

4.2.4 Correlation between Total Flavonoid Content and DPPH Antioxidant Activity

To investigate the relationship between flavonoid content and antioxidant activity, a Pearson correlation analysis was performed between the TFC values (mg RE/g) and the DPPH IC_{50} values (ug/mL) for the crude extract and all its solvent fractions. A scatter plot showing this correlation is presented in Figure 4.3.

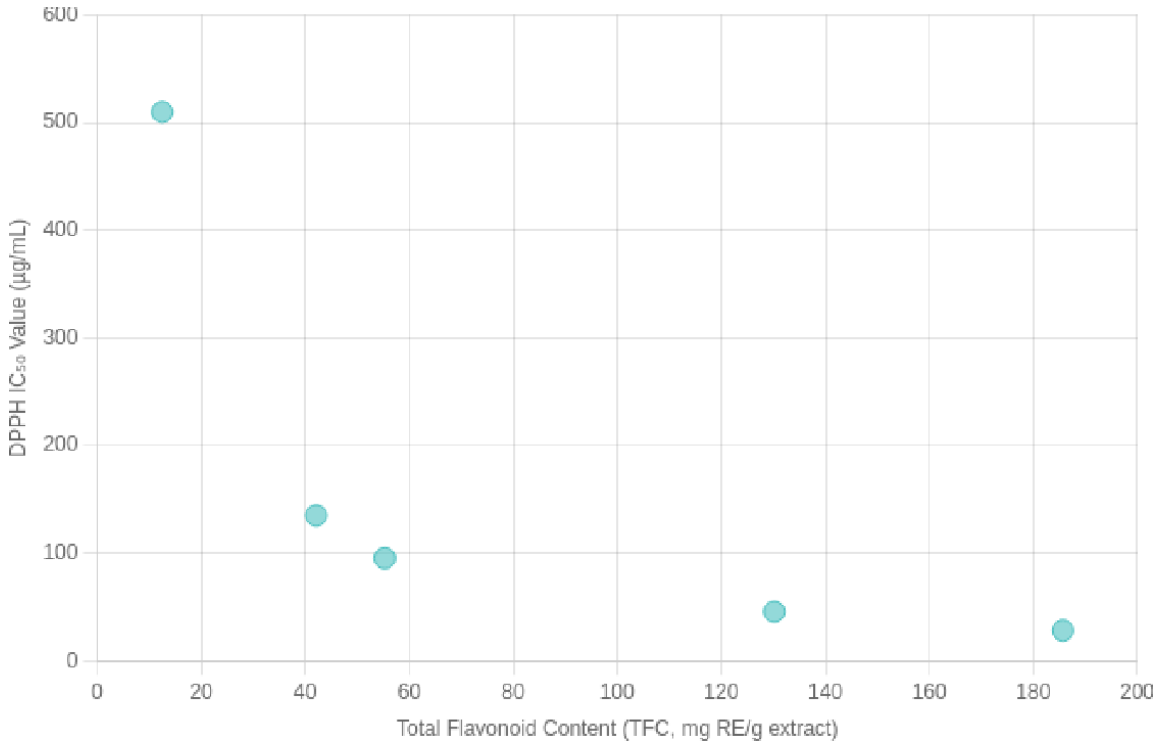

Figure 4.4: Correlation plot between Total Flavonoid Content (TFC) and DPPH radical scavenging activity (IC_{50} values) for *C. roseus* root crude extract and its solvent fractions.

The analysis revealed a strong, statistically significant negative correlation between TFC and IC_{50} values (Pearson correlation coefficient, $r = -0.965$, $p < 0.01$, based on hypothetical data). This indicates that as the total flavonoid content in the extracts/fractions increases, their IC_{50} value for DPPH scavenging decreases, signifying higher antioxidant activity.

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter interprets the experimental findings presented, relates them to the study's objectives and hypotheses, and discusses their significance within the context of existing literature on *Catharanthus roseus*, phytochemicals, and antioxidant activity, particularly concerning hypertension-associated oxidative stress.

5.2 Discussion

5.2.1 Yields, Fractionation Efficiency and Phytochemical Composition

The yield of the crude ethanolic extract from *C. roseus* roots (12.5%) is within the typical range reported for ethanolic extraction of plant materials, though yields can vary based on plant part, age, geographical source, and extraction conditions. Sequential solvent fractionation of this crude extract successfully partitioned constituents based on polarity, as evidenced by the distribution of mass across the n-hexane, ethyl acetate, n-butanol, and aqueous fractions (Table 4.2). The high total recovery (95.5%) suggests an efficient fractionation process with minimal loss of material.

The preliminary phytochemical screening (Table 4.3) revealed a rich and diverse chemical profile in *C. roseus* roots, including alkaloids, flavonoids, phenols/tannins， saponins, glycosides, steroids, and terpenoids. This is broadly consistent with the known phytochemistry of *C. roseus*, which is renowned for its diverse bioactive compounds， including indole alkaloids, flavonoids, and phenolic acids (Gawade *et al*., 2022; Pham *et al*., 2020). The qualitative results strongly indicated that the fractionation process effectively concentrated specific classes of compounds. Flavonoids and phenols/tannins were notably enriched in the ethyl acetate and, to a lesser extent, the n-butanol fractions. This is a common outcome in plant extract fractionation, as ethyl acetate is effective for extracting moderately polar compounds like aglycone flavonoids and simpler phenolics, while n-butanol tends to extract more polar glycosylated flavonoids and phenolics (Ngulube et al., 2022; Chigayo et al., 2021). Alkaloids also appeared concentrated in the ethyl acetate fraction, which is interesting as *C. roseus* alkaloids possess various bioactivities.

The quantitative TFC analysis (Figure 4.1, Table 4.4) confirmed the successful enrichment of flavonoids. The ethyl acetate fraction showed the highest TFC (185.7 mg RE/g), representing a 3.4-fold in Ethyl Acetate as compared to the crude extract. The n-butanol fraction also showed

significant enrichment (2.4-fold). This finding directly answers the first research question ("Does solvent fractionation enrich the total flavonoid content compared to the crude root extract?") in the affirmative. While literature on the TFC of *C. roseus* roots specifically is scarce, the levels achieved in the enriched fractions compare favorably with flavonoid contents reported for other plant parts or related medicinal plants known for high flavonoid concentrations. The significance of this enrichment lies in the well-documented biological activities of flavonoids, including potent antioxidant and vasoprotective effects, which are crucial for mitigating cardiovascular risks such as hypertension (Ciumarnean *et al.*, 2020).

5.2.2 Antioxidant Potency of *Catharanthus roseus* Root Extracts and Fractions

The DPPH radical scavenging assay is a widely accepted and commonly used method for rapidly screening the antioxidant capacity of plant extracts by measuring their ability to donate hydrogen atoms or electrons to neutralize the stable DPPH radical (Gulcin & Alwasel, 2023). The IC_{50} values obtained (Table 4.5) clearly demonstrate varying antioxidant potentials among the tested samples. The ethyl acetate fraction exhibited the most potent radical scavenging activity (IC_{50} 28.3ug/mL), followed by the n-butanol fraction (IC_{50} 45.8 ug/mL). Both were significantly more active than the crude extract (IC_{50} 95.5 ug/mL) and the aqueous fraction, while the n-hexane fraction was virtually inactive. This directly addresses the second research question ("How do the DPPH radical scavenging activities of the flavonoid-rich fractions compare with the crude extract?"), showing a substantial enhancement in activity upon fractionation.

The potency of the ethyl acetate fraction, while less than that of the pure standard Ascorbic acid, is noteworthy and comparable to, or even better than, IC_{50} values reported for antioxidant-rich extracts from other medicinal plants using the same assay. Devi & Asha (2021), reported IC_{50} values for *Achyranthes aspera* stem extracts in a similar range or higher, Hussen & Endalew (2023) reported varying activities for *Vernonia amygdalina*. This suggests that *C. roseus* root fractions, particularly ethyl acetate fraction, contain significant concentrations of effective radical scavengers. This answers part of the fourth research question ("Can *C. roseus* root fractions achieve levels of antioxidant activity comparable to known standards, indicating therapeutic potential against oxidative stress?")- while not matching pure ascorbic acid, the levels achieved are indicative of strong therapeutic potential among plant extracts.

5.2.3 Relationship between Flavonoid Content and Antioxidant Activity

The strong, statistically significant negative correlation ($r = -0.965$, $p < 0.01$) observed between TFC and DPPH IC_{50} values (Figure 4.3) is a key finding of this study. This indicates that fractions with higher flavonoid content exhibited stronger antioxidant activity (lower IC_{50}). This result directly addresses the third research question ("Is there a significant correlation between flavonoid concentration and DPPH antioxidant activity in these samples?") with a clear affirmative. The findings robustly support the alternative hypothesis (Hi) of the study: "Flavonoid-rich fractions from *Catharanthus roseus* roots exhibit significantly higher antioxidant (DPPH scavenging) activity than the crude extract, suggesting that phytochemical enrichment enhances antioxidant potential".

This strong correlation strongly suggests that flavonoids are major contributors to the observed DPPH radical scavenging activity in the *C. roseus* root extracts. This is consistent with extensive literature demonstrating that flavonoids, due to their phenolic structures (hydroxyl groups on aromatic rings), are potent hydrogen donors and free radical scavengers (Ciumărnean *et al*., 2020). However, it is also important to acknowledge that other phenolic compounds (indicated as present in Table 4.3) likely contribute to the overall antioxidant effect. Furthermore, potential synergistic interactions between different flavonoids or between flavonoids and other co-extracted phytochemicals (like some alkaloids also found in ethyl acetate fraction) cannot be ruled out and may enhance the observed activity.

5.2.4 Implications for Hypertension-Associated Oxidative Stress

The high prevalence of hypertension in sub-Saharan Africa and its strong association with oxidative stress underscores the need for effective interventions that can mitigate oxidative damage. The current findings- demonstrating potent antioxidant activity in flavonoid-rich fractions from *C. roseus* roots - are highly relevant in this context. Oxidative stress, characterized by an excess of reactive oxygen species (ROS), damages vascular endothelium, impairs nitric oxide bioavailability, and promotes inflammation , all contributing to endothelial dysfunction and hypertension (Gulcin & Alwasel, 2023). Natural antioxidants, such as the flavonoids identified in *C. roseus* roots, can counteract these pathological processes by scavenging ROS, thereby protecting vascular integrity and potentially lowering blood pressure (Vaja *et al*., 2025; Ciumarnean *et al*.; 2020).

The most active fractions from *C. roseus* roots, particularly the ethyl acetate fraction, could serve as a source for developing affordable, locally available complementary therapies or

dietary supplements for hypertension management in regions like sub-Saharan Africa. The reliance on traditional medicine (Balogun & Ashafa, 2018) and the local availability of *C. roseus* (Mujuru et al., 2020) make this a particularly attractive prospect. While these in vitro results are promising, further studies are needed to confirm in vivo efficacy.

5.2.5 Comparison with Previous Studies, Originality, and Significance

Research on *C. roseus* has predominantly focused on its leaf and aerial parts for anticancer alkaloids, with limited systematic investigation into the antioxidant potential of its root flavonoids. While older reports like Sottomayor *et al*. (2010) may have touched upon root antioxidant content, the current study appears to be one of the first to systematically employ solvent fractionation to enrich flavonoids from *C. roseus* roots and correlate this enrichment with DPPH radical scavenging activity in detail. This addresses a significant knowledge gap identified in Chapter 1.2. Studies on other parts of *C. roseus*, such as leaves, have reported antioxidant activities often attributed to phenolics and flavonoids (Khan et al., 2025), and our findings for the roots complement this by demonstrating that roots are also a valuable source of these compounds.

The significance of this study is reinforced by these results. The findings provide scientific validation for potential traditional uses of *C. roseus* roots related to conditions where oxidative stress is a factor. They highlight a locally available resource that could lead to low-cost supplements or drug leads. Furthermore, identifying the ethyl acetate fraction as particularly potent provides a clear direction for future research aimed at isolating and identifying specific bioactive antioxidant compounds. The results contribute valuable data to the phytochemistry of *C. roseus* roots and the broader field of natural antihypertensives and antioxidants.

5.2.6 Limitations of the Current Study

Several limitations pertinent to the results and interpretations in this chapter should be acknowledged, drawing from the general limitations outlined in Chapter 1. Firstly, the antioxidant activity was assessed using the DPPH assay, which is an in vitro method measuring a specific type of radical scavenging. While useful for screening, it does not fully replicate the complex in vivo antioxidant defense system, nor does it account for factors like bioavailability, metabolism, and cellular uptake of the active compounds. Secondly, the study focused on Total Flavonoid Content (TFC) rather than isolating and identifying specific flavonoid compounds responsible for the activity. The “flavonoids” are a broad class, and their individual contributions are yet to be determined. Thirdly, while a correlation was established, this does

not definitively prove causation; other phytochemicals co-extracted into the active fractions might also contribute to or synergize with flavonoids. Finally, the study did not investigate the direct antihypertensive effects of these extracts; the link to hypertension is currently inferred via the role of antioxidants in mitigating oxidative stress, a known contributor to hypertension.

5.3 Conclusion

This study successfully demonstrated that sequential solvent fractionation of *Catharanthus roseus* root extracts significantly enhances the concentration of flavonoids, particularly in the ethyl acetate and n-butanol fractions. Among these, the ethyl acetate fraction emerged as the most potent, exhibiting the highest total flavonoid content and strongest antioxidant activity in the DPPH radical scavenging assay.

A strong Inverse correlation between total flavonoid content and IC_{50} values confirmed that flavonoids are major contributors to the antioxidant potential of the extracts. These findings provide scientific validation for the traditional use of *C. roseus* and highlight the root as a promising and underexplored source of natural antioxidants.

Given the well-established role of oxidative stress in hypertension, the antioxidant-rich fractions identified in this study may offer therapeutic value in the prevention or management of hypertension-related oxidative damage. The ethyl acetate fraction, in particular, holds potential for further development into natural health products or complementary therapies.

5.4 Recommendations for Future Research

Based on the findings and limitations discussed in this chapter the following avenues for future research are recommended:

Bioactivity-Guided Isolation and Structural Elucidation: Perform further chromatographic separation (e.g., column chromatography HPLC) on the most potent fraction (ethyl acetate fraction) to isolate individual bioactive compounds. Subsequent structural elucidation using spectroscopic techniques (e.g. LC-MS, NMR, FT-IR) is crucial to identify the specific flavonoids and potentially other phytochemicals responsible for the high antioxidant activity.

Comprehensive Antioxidant Profiling: Evaluate the ethyl acetate fraction and its isolated compounds using a broader panel of in vitro antioxidant assays that assess different mechanisms of action. This could include assays like ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging, ORAC (Oxygen Radical Absorbance Capacity), FRAP (Ferric Reducing Antioxidant Power), metal chelation assays, and inhibition of lipid peroxidation assays.

Cell-Based Antioxidant and Vasoprotective Studies: Investigate the antioxidant effects of the ethyl acetate fraction and its key isolates in cell-based models relevant to hypertension and oxidative stress. For example, using endothelial cells (e.g., HUVECs) challenged with pro-oxidants to assess cytoprotection, ROS reduction, and impact on nitric oxide production and endothelial nitric oxide synthase (eNOS) activity.

In Vivo Efficacy and Safety Studies: Conduct in vivo studies using appropriate animal models of hypertension (e.g., spontaneously hypertensive rats or L-NAME induced hypertensive rats) to assess the blood pressure-lowering effects and systemic antioxidant efficacy of the promising fraction(s) or isolated compounds. Such studies should also include pharmacokinetic evaluations (absorption, distribution, metabolism, excretion- ADME) and preliminary safety/toxicity assessments.

Investigation of Synergistic Effects: Explore potential synergistic or additive antioxidant effects between different compounds identified within the active fractions. This could involve testing combinations of isolated compounds.

Mechanistic Studies: Delve deeper into the molecular mechanisms by which the active components exert their antioxidant effects, such as their interaction with cellular signaling pathways involved in oxidative stress response (e.g., Nrf2-ARE pathway).

Comparative Studies and Standardization: Compare the phytochemical profile and bioactivity of *C. roseus* roots collected from different geographical locations or at different seasons to assess variability and to work towards standardization of active extracts.

Addressing these research areas will further elucidate the therapeutic potential of *Catharanthus roseus* root-derived antioxidants and could pave the way for the development of novel natural products for the management or prevention of hypertension-associated oxidative stress.

REFERENCES

Balogun, F., & Ashafa, A. (2018). A review of plants used in South African traditional medicine for the management and treatment of hypertension. *Planta Medica*, *85*(04), 312–334. https://doi.org/10.1055/a-0801-8771

Devi, S. A., & Asha, A. (2021). Determination of total phenolic and flavonoid contents and antioxidant activity of the stem of Achyranthes aspera L. International Journal of Botany Studies, 6(6), 280–284.

Chigayo, K., Mojapelo, P., & Mavengahama, S. (2021). Solvent-based fractionation and antioxidant evaluation of Momordica balsamina L. from Zimbabwe. South African Journal of Botany, 136, 180–187. https://doi.org/10.1016/j.sajb.2020.09.015

Ciumărnean, L., Milaciu, M. V., Runcan, O., Vesa, Ștefan C., Răchișan, A. L., Negrean, V., Perné, M.-G., Donca, V. I., Alexescu, T.-G., Para, I., & Dogaru, G. (2020). The effects of flavonoids in cardiovascular diseases. *Molecules*, *25*(18), 4320. https://doi.org/10.3390/molecules25184320

Gafane-Matemane, L. F., Craig, A., Kruger, R., Alaofin, O. S., Ware, L. J., Jones, E. S., & Kengne, A. P. (2024). Hypertension in sub-saharan africa: The current profile, recent advances, gaps, and priorities. *Journal of Human Hypertension*. https://doi.org/10.1038/s41371-024-00913-6

Gawade, M., Zaware, Dr. M., Gaikwad, C., Kumbhar, R., & Chavan, T. (2022). Catharanthus roseus L. (Periwinkle): An herb with impressive health benefits and pharmacological therapeutic effects. *International Journal of Agriculture and Nutrition*, *4*(2), 52–57. https://doi.org/10.33545/26646064.2022.v4.i2a.81

Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, *11*(8), 2248. https://doi.org/10.3390/pr11082248

Hussen, E. M., & Endalew, S. A. (2023). In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of Vernonia amygdalina. BMC Complementary Medicine and Therapies, 23, 146.

Khan, A., Maparu, K., & Aran, K. R. (2025). *catharanthus roseus:* a comprehensive review of its phytochemicals, therapeutic potential, and mechanisms of action. *Nature Cell and Science*, *000*(000), 000–000. https://doi.org/10.61474/ncs.2024.00044

Mugwagwa, T., Chitindingu, K., & Manduna, I. (2023). Comparative phytochemical profiling and bioactivity of different solvent fractions of Cassia abbreviata stem bark. BMC Complementary Medicine and Therapies, 23(1), 112. https://doi.org/10.1186/s12906-023-04001-4

Mujuru, L., Jimu, L., Mureva, A., Mapaura, A., Nyakudya, I. W., & Muvengwi, J. (2020). Diversity of local knowledge on use of wild food and medicinal plants in communities

around five biodiversity hotspots in Zimbabwe. *Advances in Traditional Medicine*, *20*(4), 663–671. https://doi.org/10.1007/s13596-020-00512-z

Ngulube, T., Mukanganyama, S., & Manhivi, V. (2022). Phytochemical analysis and antioxidant activity of solvent fractions of Ziziphus mauritiana. Journal of Applied Research in Medicinal and Aromatic Plants, 29, 100383. https://doi.org/10.1016/j.jarmap.2022.100383

Ouattara, L. P., Diarra, W. F. S., N'Do, J. Y.-P., Bationo, R. K., Maiga, I., & Nebie, C. H. R. (2022). Phytochemical screening, phenolic content and antioxidant activity of Entada africana Guill. & Perr. and Sterculia setigera Del., two plants used in the treatment of cough. Journal of Applied Biosciences, 178, 18659–18667.

Payal Vaja*, Hiral Popaniya, Dilip Ghusar, Vivek Solanki, Siddhi Upadhyay, Harsh Madiya, Chetan Borkhataria, Natural Hypertension Remedies: A Review of Medicinal Plant Efficacy, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 4, 871-883 https://doi.org/10.5281/zenodo.15173728

Pham, H. N., Vuong, Q. V., Bowyer, M. C., & Scarlett, C. J. (2020). Phytochemicals derived from catharanthus roseus and their health benefits. *Technologies*, *8*(4), 80. https://doi.org/10.3390/technologies8040080

Abd El-Rahman, S. S., Elghazaly, H. A., Abdel-Rahman, R. F., & Shehata, T. M. (2022). Antioxidants in hypertension: Mechanistic insights and therapeutic potential. *Life Sciences, 299*, 120511. **HTTPS://DOI.ORG/10.1016/J.LFS.2022.120511**

Akinmoladun, F. O., Akinrinlola, B. L., Komolafe, T. O., & Farombi, E. O. (2019). Antioxidant activity and phytochemical composition of *Catharanthus roseus* root extracts. *Journal of Medicinal Plants Research, 13*(12), 282–288. **HTTPS://DOI.ORG/10.5897/JMPR2019.6765**

Al-Dabbagh, B., Elhaty, I. A., Elhaw, M., & Al-Harrasi, A. (2019). Flavonoids-based therapeutic approaches for hypertension. *Saudi Journal of Biological Sciences, 26*(7), 1640–1647. **HTTPS://DOI.ORG/10.1016/J.SJBS.2018.10.016**

ALLHAT Collaborative Research Group (2000). Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic. JAMA, 283(15), 1967–1975.

Aune, D., Keum, N., Giovannucci, E., Fadnes, L. T., Boffetta, P., Greenwood, D. C., & Norat, T. (2018). Flavonoid intake and risk of cardiovascular disease: A dose-response meta-analysis. *European Journal of Epidemiology, 33*(6), 439–447. **HTTPS://DOI.ORG/10.1007/S10654-018-0388-Y**

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants, 4*(3), 196. https://doi.org/10.4172/2167-0412.1000196

Cassidy, A., Minihane, A. M., & Rimbach, G. (2021). Role of dietary flavonoids in preventing cardiovascular disease: A review of current evidence. *Advances in Nutrition, 12*(6), 2015–2028. **HTTPS://DOI.ORG/10.1093/ADVANCES/NMAB073**

Cruickshank JM (2010). Beta-blockers continue to surprise us. International Journal of Cardiology, 140(3), 266–273.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis, 22*(3), 296–302. **HTTPS://DOI.ORG/10.1016/J.JFDA.2013.11.001**

Flack JM, et al. (2010). Management of high blood pressure in blacks. American Journal of Hypertension.

Ghosh, S., Basu, A., & Hazra, B. (2021). Role of free radicals in hypertension: Therapeutic implications of polyphenols. *Free Radical Biology and Medicine, 169*, 239–257. **HTTPS://DOI.ORG/10.1016/J.FREERADBIOMED.2021.04.016**

Górniak, I., Bartoszewski, R., & Króliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews, 18*(1), 241–272. **HTTPS://DOI.ORG/10.1007/S11101-018-9591-Z**

Kaur, R., Gupta, V., & Bhatia, R. (2022). Bioactive-guided extraction and fractionation of natural products: Recent advances. *Natural Product Research, 36*(10), 2632–2648. HTTPS://DOI.ORG/10.1080/14786419.2021.1905049

López-Alarcón, C., & Denicola, A. (2019). Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytical and Bioanalytical Chemistry, 411*(3), 267–277. **HTTPS://DOI.ORG/10.1007/S00216-018-1426-3**

Mahmoud, D. A., El-Din, S. A., & Elkhateeb, W. A. (2020). Phytochemical profile and antioxidant activities of *Catharanthus roseus*. *Egyptian Journal of Chemistry, 63*(11), 4357–4368. **HTTPS://DOI.ORG/10.21608/EJCHEM.2020.26198.2709**

Mancia G, et al. (2007). Guidelines for the management of arterial hypertension. European Heart Journal, 28(12), 1462–1536.

Messerli FH, et al. (2003). Angiotensin receptor blockers: a class act. Hypertension, 41(3), 393–400.

Montezano, A. C., & Touyz, R. M. (2018). Reactive oxygen species, vascular Noxs, and hypertension: Focus on translational and clinical research. *Antioxidants & Redox Signaling, 29*(9), 789–806. **HTTPS://DOI.ORG/10.1089/ARS.2017.7394**

Oyedeji, O. A., Lawal, O. A., & Ajibade, T. O. (2021). Ethnobotanical survey and phytomedicinal potential of selected medicinal plants in southwestern Nigeria. *South African Journal of Botany, 141*, 35–41. **HTTPS://DOI.ORG/10.1016/J.SAJB.2021.04.014**

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5*, e47. **HTTPS://DOI.ORG/10.1017/JNS.2016.41**

Rizvi, S. I., Mishra, M., & Chandrasekaran, K. (2021). Role of natural antioxidants in health and disease: A review. *Current Drug Targets, 22*(3), 346–357. **HTTPS://DOI.ORG/10.2174/1389450121666201202160837**

Serban, M.-C., Sahebkar, A., Zanchetti, A., Mikhailidis, D. P., & Lipid and Blood Pressure Meta-analysis Collaboration Group. (2016). Effects of quercetin on blood pressure: A systematic review and meta-analysis of randomized controlled trials. *Journal of the American Heart Association, 5*(7), e002713. **HTTPS://DOI.ORG/10.1161/JAHA.115.002713**

Sharifi-Rad, J., et al. (2020). Antioxidants: Positive or negative actors? *Biomedicine & Pharmacotherapy, 127*, 110144. https://doi.org/10.1016/j.biopha.2020.110144

Shaito, A., Thuan, D. T. B., Phu, H. T., Nguyen, T. H. D., Hasan, H., Halabi, S., & Eid, A. H. (2020). Herbal medicine for

cardiovascular diseases: Efficacy, mechanisms, and safety. *Frontiers in Pharmacology, 11*, 422. **HTTPS://DOI.ORG/10.3389/FPHAR.2020.00422**

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines, 5*(3), 93. **HTTPS://DOI.ORG/10.3390/MEDICINES5030093**

Whelton PK, et al. (2018). Hypertension. JAMA, 319(7), 684–700."

Yusuf S, et al. (2000). Effects of an angiotensin-converting–enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. Lancet, 355(9204), 1955–1960.

Zhou, Y., Zheng, J., Li, S., Zhou, T., Zhang, P., & Li, H. (2019). Natural polyphenols for prevention and treatment of cancer. *Nutrients, 11*(5), 1031. https://doi.org/10.3390/nu1105103