

**UNIVERSITY OF RWANDA**

**STUDY OF ANTIOXIDANT ACTIVITIES OF VERNONIA AMYGDALINA  
AIMING AT USING IT AS A HOP SUBSTITUTE IN THE PRODUCTION  
OF RWANDAN TRADITIONAL BEERS**

**2025**

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AIMING AT USING IT AS A HOP SUBSTITUTE IN THE PRODUCTION  
OF RWANDAN TRADITIONAL BEERS**

**by**

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**Dissertation submitted in fulfilment of the requirements for the degree:**

**MASTER OF SCIENCE IN BIOTECHNOLOGY**

**In the department of Biology, School of Science**

**College of Science and Technology**

**At**

**The University of Rwanda**

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**Kigali, Rwanda 2025**



## Declaration of independent work

I Kamaliza Gaudence hereby declare that this research project submitted to the University of Rwanda, for the degree Master of Science in Biotechnology is my own original work and has not been submitted before to any Institution by myself or any other person in fulfilment of the requirements to the award of any degree or any other qualification.

Kamaliza Gaudence Reg. Number: 223027558

A handwritten signature in blue ink, appearing to be 'K.G.' or similar initials, written in a cursive style.

# DEDICATION

To Jean Marie, my husband and best friend

To my beloved children

To my family and friends

## ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all those who contributed to the success of my Master's studies. To God for granting me good health, to my supervisors Assoc. Prof. Lyumugabe Francois, Dr. Joseph Nsengimana and Mr. Hubert Kageruka for their guidance and advice for the success of my thesis. Assoc. Prof Lyumugabe's office door was always open whenever I ran into trouble or had a question about my research or writing. He consistently allowed this thesis to be my own work and steered me in the right direction. On the other hand, Dr. Joseph generously accepted to be my second supervisor on a very short request and together with Assoc. Prof. Lyumugabe guided my work to this level. Mr. Hubert Kageruka, his expertise and commitment were instrumental in the successful completion of the laboratory work, the analysis of results, and the writing of this dissertation. I am especially thankful for their constructive feedback, encouragement, and availability at every stage of this study. This work would not have been possible without their dedication and mentorship.

I also thank the European Union and ENABEL, a Belgian international cooperation agency through the KWIGIRA project and BK Foundation that funded a Master of Science in Biotechnology at the University of Rwanda, as well as all the professors from the different universities who participated in teaching the Master of Science in Biotechnology program at the University of Rwanda, for their immeasurable skills, knowledge, advice, guidelines and mentorship they provided.

My sincere gratitude goes also to the Academy of Research and Higher Education (ARES) which financed my three-month stay in Belgium at the Catholic University of Louvain for my laboratory work, to Professor Sonia COLLIN for accepting me into her laboratory and for her coordination of the INBr at UCLouvain and for leading the cooperation between the INBr and the students and researchers from other universities and higher education institutions, future researchers and workers, who are doing their internship in her laboratory.

My last but not least thanks go to the management of the University of Rwanda, College of Science and Technology, Faculty of Science, Department of Biology, for their staff development initiative by assisting staff in obtaining study leave to undertake studies and training abroad in accordance with their daily duties and responsibilities. I thank you all.

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## List of abbreviation and acronyms

AAPH: Azodiisobutyramidine dihydrochloride)

AUC: Area Under Curve

g : grams

INBr: Unité de brasserie et des industries alimentaires

LIBST: Louvain Institute of Biomolecular Science and Technology

mL: milliliter

NIRDA: National Industrial Research and Development Agency

ORAC: Oxygen Radical Absorbance Capacity

UV: UltraViolet

## Abstract

The study explores the potential of *Vernonia amygdalina* as a substitute for hops in the production of traditional Rwandan beers, with a focus on its antioxidant properties. *Vernonia amygdalina*, commonly known for its bitter taste and medicinal properties, was analyzed for its antioxidant activities using the ORAC (Oxygen Radical Absorbance Capacity) method. The study assessed extracts from different parts of the plant (leaves, flowers, and bark) and explored various extraction methods, including acetone/water/acetic acid, water, and ethanol-acetic acid solvents. The antioxidant capacities of the plant parts were evaluated, with the bark exhibiting the highest antioxidant activity (1514  $\mu\text{mol Trolox eq/g}$ ) which surpassed those of industrial Citra T90 hops (614  $\mu\text{mol Trolox eq/g}$ ). Further, the total polyphenol and flavonoid contents were determined, indicating the plant's significant antioxidant potential, particularly in the leaves and bark. The results suggest that *V. amygdalina* could serve as an effective hop substitute, offering both antioxidant and bittering properties that could improve the quality, stability, and shelf life of traditional beers. These findings contribute to the growing body of knowledge on functional ingredients in brewing and offer a promising way for the development of healthier, locally sourced beer alternatives.

**Keywords:** *Vernonia amygdalina*, antioxidant activities, traditional Rwandan beers.

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# CHAPTER 1: Introduction

## 1. 1 Background and context of the study

Besides its food use in the form of grain or fruit, sorghum and banana are used as raw materials for centuries in the production of traditional alcoholic beverages commonly known as Ikigage and Urwagwa in Rwanda (Lyumugabe and Bajyana, 2019). Apart their socio-cultural importances, Rwandan beers are very rich in calories, vitamin B and essential amino-acids and constitute an income source for the local brewers who produce them at household scale by using the traditional technologies (Maoura et al., 2009; Lyumugabe et al., 2012<sup>a</sup>). The production of these local beers depends largely on socio-economic imperatives such as the wages of agricultural workers, payment of school fees and purchase of school materials for children, payment of the mutual health insurance, wedding gifts, etc. In a way, they guarantee the stability of the family economy and punctuate the social life of the average peasant. They are shared in exchange for kindness and good neighbors and are present in all traditional and non-traditional festivals such as dowry, marriage, mourning, religious and school holidays, etc. (Musabanganji et al., 2023).

Like many other African beers, Rwandan traditional beers are prepared without the addition of hops (*Humulus lupulus*). In addition to its obvious impact on the bitterness of beer, hops inhibit the growth of pathogenic bacteria in Western beers through their alpha and beta acids ( Shaokang Sun et al., 2022). These same molecules are also responsible for the stability of the foam. Hops also increase the antioxidant activity of the finished product through their richness in polyphenols, and generate varied aromas through the presence of polyfunctional thiols (Gros et al., 2012). Unfortunately, it is a plant of temperate regions, which cannot adapt to the climate of tropical countries (Krofta et al., 2008).

Surprisingly, very little research has been conducted on the possibilities of substituting hops with tropical plants. However, recent research conducted at the INBr laboratory at UCLouvain has revealed the presence of odorous hop-type compounds, as well as their precursors, in beers brewed traditionally with the addition of *V. amygdalina* leaves (Lyumugabe et al, 2012<sup>b</sup>). Intense

antimicrobial activity (effective on both gram-positive and gram-negative bacteria) has also been demonstrated in ethanolic extracts of *V. amygdalina* leaves (Lyumugabe et al, 2017).

It therefore seems appropriate to capitalize on these initial observations by developing research on the optimization of innovative processes using local plants (*V. amygdalina*) with proven antimicrobial, bittering, and odorous properties, and therefore likely to improve the stability and organoleptic quality of unhoped beers.

As the global brewing industry evolves toward more sustainable, health-conscious, and locally sourced ingredients, the integration of functional plants such as *V. amygdalina* presents a promising innovation. However, scientific validation is needed to characterize its biochemical potential and determine its suitability in brewing applications, especially in terms of antioxidant contribution and organoleptic compatibility. This study was conducted to determine the antioxidant activity of *V. amygdalina* aimed at using it as a hop substitute to improve the quality of Rwandan traditional beers.

## 1.2 Problem Statement

The production of traditional beers faces significant challenges related to flavor quality, bitterness, and shelf life, primarily due to the lack of suitable agents such as hops. The importation of hops is economically unsustainable for many local brewers, and the absence of effective local substitutes limits the potential for innovation and quality improvement in traditional beer production. There is a critical need to identify and characterize locally available plant materials that can impart desirable antioxidant activity to local beers, thereby enhancing their sensory attributes and stability.

## 1.3 Justification of the Study

The exploration of *Vernonia amygdalina* as a hop substitute is justified by its traditional use, known characteristics such as bitterness and bioactive properties. Utilizing *V. amygdalina* in local beer production could contribute to the stability, shelf life and contamination prevention of local beer especially Ikigage and promote the use of indigenous resources. Furthermore, the study on antioxidant activities of *V. amygdalina* will provide scientific evidence for its application in brewing, potentially leading to the development of higher-quality, health-promoting traditional beers.

This study will promote the Rwandan government policy called "Made in Rwanda" which one of the objectives is to develop the agro-food industries and to enhance the local resources of Rwanda in order to contribute to Economic Development and Poverty Reduction Strategy reduction of poverty in Rwanda (EDPRS 3). It part of broader goals of food security, local economic development, and the valorization of underutilized plant species.

## 1.4 Scope of the Study

This study focuses on the extraction and evaluation of antioxidant activities of *Vernonia amygdalina*. It further investigates the application of *V. amygdalina* as a hop substitute in the brewing of traditional beer especially Ikigage. The study is limited to laboratory-scale experiments, with perspectives for further research and potential industrial application.

## 1.5 Significance of the Study

The findings of this study will contribute to the scientific understanding of *Vernonia amygdalina* as a functional ingredient in traditional beer production. By providing evidence for its use as a hop substitute, the study supports the development of improved, locally sourced local beers with enhanced quality and health benefits.

This has implications for local brewers, consumers, and policymakers interested in promoting indigenous food resources and sustainable brewing practices.

## 1.6 Objectives of the Study

### 1.6.1 General Objective

To characterize the antioxidant activities of *Vernonia amygdalina* with the aim of evaluating its potential as a hop substitute to improve the quality of Rwandan traditional beers.

### 1.6.2 Specific Objectives

1. To evaluate the antioxidant activities of *Vernonia amygdalina*.
2. To characterize the phytochemical compounds related to the antioxidant power of *Vernonia amygdalina*
3. To assess the effect of brewing on *Vernonia amygdalina* antioxidant power

## 1.7 Research Hypotheses

Hypothesis 1: *Vernonia amygdalina* exhibits significant antioxidant activity.

Hypothesis 2: The phytochemical profile of *Vernonia amygdalina* includes compounds (such as polyphenols, flavonoids, and flavanols) that contribute to its antioxidant potential.

Hypothesis 3: The brewing process alters the antioxidant capacity of *Vernonia amygdalina*.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction

Beer brewing relies on hops (*Humulus lupulus*) for bitterness, aroma, antimicrobial activity and antioxidant preservation (BarthHaas Report, 2023). However, the limited availability and high cost of hops, especially in tropical countries like Rwanda, has encouraged interest in identifying alternative, locally available botanical ingredients that can fulfill similar roles in beer production. One such candidate is *Vernonia amygdalina*, a bitter plant that grows abundantly in various regions of Sub-Saharan Africa.

In Rwanda, *Vernonia amygdalina* is not widely cultivated but grows naturally in fields, forests, and abandoned areas. It is known locally as *Umubirizi* and is primarily used in traditional medicine and as fodder for livestock, particularly cows. Unlike in countries like Nigeria and Cameroon, it is rarely consumed as a vegetable in households. Nevertheless, the plant is rich in bioactive compounds with known antioxidants, antimicrobial, and anti-inflammatory properties (Farombi & Owoeye, 2023). These qualities make *Vernonia amygdalina* a promising candidate for use as a hop substitute in beer brewing, especially to improve product stability, health benefits, and affordability.

## 2.2 *Vernonia amygdalina*

### 2.2.1 Botanical Description

*Vernonia amygdalina* (Figure 1), known as ‘*Umubirizi*’ in Kinyarwanda, is a green shrub found all over Africa and belonging to the Asteraceace family (Lyumugabe et al., 2012). The leaves have a dark green colour with a characteristic odour and a bitter taste (Bosch et al, 2005). Flower heads thistle like, small, creamy white, 10 mm long, grouped in dense heads, axillary and terminal, forming large flat clusters, 15 cm in diameter, sweetly scented (Ofori et al., 2013). *Vernonia amygdalina* grows in many parts of Africa. *Vernonia amygdalina* grows naturally on riversides and lakes, in forest margins, woodland and grassland, where the average rainfall is 750-2,000mm and up to 2,800m altitudes (Jordan et al.,2024)



a)



b)

Figure 1: *Vernonia amygdalina* (a) whole plant (b) flowers

### **2.2.2 Health benefits of Bitter Leaf (*Vernonia amygdalina*)**

*Vernonia amygdalina* has been traditionally used as medicine to cure different diseases such as cancer (Edeoga et al., 2005), diabetes, diarrhea, hepatitis B and C, and jaundice (Gurmesa, 2017). Polyherbal preparations with bitter leaf as the active ingredients strengthen the immune system through many cytokines and chemokines regulations (Gurmesa, 2017). The herb aids in pancreatic healing in addition to effectively lowering blood sugar levels.

It relieves piles and cleanses the body. This wonderful herb can cure almost every human health problem. It soothes inflamed joints and eases discomfort associated with rheumatism or arthritis. Chimpanzees have been observed to eat bitter leaves in the wild when they suffer from parasite infections, and they are also utilized in zoo pharmacies.

According to ethnobotany, bitter leaf leaves can be eaten as vegetables (e.g. Ndole in Cameroon, Nigeria and Benin). Analytical data on the nutritional value of African *V. amygdalina* leaves have shown that they are rich in carbohydrates, carotenoids, vitamin C, dietary fiber and essential amino acids such as lysine, glucine, alanine and leucine, which make them a food (Igile et al., 1995; Bosch et al., 2005). For emesis, ambrosia brought on by loss of appetite, diarrhea, and other gastrointestinal tract issues, several herbalists and naturopathic physicians advise their patients to take aqueous preparations of bitter leaf.

### **2.2.3. Bitterness Components in *Vernonia amygdalina***

Bitterness in plants is often attributed to secondary metabolites such as alkaloids, terpenoids, and phenolic compounds. In *Vernonia amygdalina*, the primary bitterness components are sesquiterpene lactones, particularly vernodalin, vernomygdin, and vernolepin (Adesomoju et al., 1985; Jisaka et al., 1992). These compounds not only contribute to the plant's bitter taste but also exhibit various biological activities, including anti-inflammatory, anti-malarial, and anti-cancer properties (Toyang & Verpoorte, 2013).

### **2.2.4 Antioxidant Activities of *Vernonia amygdalina***

Antioxidant activity is a crucial attribute for improving the quality and shelf-life of beers.

*Vernonia amygdalina* has been extensively studied for its antioxidant properties for other purposes not for beer production. The plant's extracts have demonstrated significant free radical scavenging activities, reducing power, and inhibition of lipid peroxidation (Farombi et al., 2005; Adejuwon et al., 2015). The antioxidant activities of *V. amygdalina* are primarily due to its high content of phenolic compounds and flavonoids. Farombi et al. (2005) reported that the methanolic extract of *V. amygdalina* leaves exhibited considerable antioxidant activity, comparable to standard antioxidants like ascorbic acid. Adejuwon et al. (2015) further characterised the antioxidant components using HPLC and found that the flavonoids and phenolic acids present in the extracts were responsible for the observed activities.

### **2.2.5 Phytochemicals properties related to antioxidant activities**

Plants produce a wide array of phytochemicals to protect themselves against oxidative stress, and many of these compounds offer similar benefits when consumed by humans. Among the most studied are flavonoids, flavanones, and phenolic compounds. These bioactives are integral to the antioxidant potential of many herbs, including *Vernonia amygdalina*, and are also found in hops (*Humulus lupulus*), which are traditionally used in beer brewing.

#### ***Flavonoids***

Flavonoids are a diverse group of polyphenolic compounds with a characteristic C6-C3-C6 backbone structure. They are subdivided into several classes including flavonols, flavones, isoflavones, flavanones, and anthocyanins (Panche et al., 2016). These compounds exert strong

antioxidant, anti-inflammatory, and antimicrobial properties due to their ability to scavenge free radicals, chelate metal ions, and modulate cellular signaling pathways.

In *V. amygdalina*, flavonoids such as luteolin, quercetin, apigenin, and their glycosides have been identified in both ethanol and aqueous leaf extracts (Alara et al., 2017). These compounds contribute significantly to the plant's total antioxidant capacity and have demonstrated free radical scavenging activity in assays such as DPPH and ABTS. For instance, Igile et al. (1994) reported that *V. amygdalina* flavonoids inhibited lipid peroxidation and protected against oxidative damage in vitro.

Flavonoids from hops, such as xanthohumol and isoxanthohumol, also enhance the antioxidant stability of beer (Stevens & Page, 2004). The inclusion of *V. amygdalina* as a hop substitute can therefore maintain, or even enhance, these beneficial properties in beer.

### **Flavanones**

Flavanones are a subclass of flavonoids predominantly found in citrus fruits but also present in *V. amygdalina*. These include naringenin, hesperidin, and eriodictyol, which have shown potent antioxidant and anti-inflammatory activity (Panche et al., 2016). In *V. amygdalina*, metabolomic profiling has identified dihydroflavones and glycosylated flavanones, which contribute to both bitterness and health-promoting activity (PeerJ, 2021).

These compounds have been studied for their protective effects against oxidative stress-related diseases and are considered promising ingredients for functional foods. Their relative thermal stability during brewing processes also supports their inclusion in beer formulations (Alara et al., 2017).

### **Phenolic Compounds**

Phenolics are a broader group of antioxidant compounds that include flavonoids as a subclass. They consist of simple molecules such as gallic acid and caffeic acid, and complex polymers like tannins. These compounds are capable of neutralizing free radicals by donating electrons or hydrogen atoms, and they often work synergistically with flavonoids (Shahidi & Ambigaipalan, 2015).

In *V. amygdalina*, total phenolic content (TPC) has been reported as high as 3211.6 mg gallic acid equivalents (GAE) per 100 g of dry leaf material (Ugwuowo et al., 2021). Bedie Mbow et al. (2022) also found significant TPC and total flavonoid content in aqueous extracts, highlighting their strong antioxidant potential.

In the brewing context, phenolics from hops and other botanicals contribute not only to antioxidant activity but also to astringency, mouthfeel, and colloidal stability in beer. The substitution of hops with *V. amygdalina* can therefore retain or even improve these functional properties (Stevens & Page, 2004).

### 2.3 Beer Production and Potential of *Vernonia amygdalina* as a Hop Substitute

Hops (*Humulus lupulus*) are a fundamental ingredient in beer production, it is added at different times during the beer brewing process to achieve different effects, primarily bitterness, flavor, and aroma and preservative qualities. The primary bitterness compounds in hops are alpha acids, which isomerise during boiling to form iso-alpha acids. Hops also contribute to the beer's antioxidant properties through prenylflavonoids such as xanthohumol (Stevens et al., 1997; Gerhäuser, 2005). However, the cultivation of hops is geographically limited and can be affected by climate change, leading to supply chain vulnerabilities (Hagen et al., 2005). This has prompted research into alternative bitterness and antioxidant sources for beer production.

*Vernonia amygdalina* presents a promising alternative to hops in beer production. A few studies have explored the use of botanicals as hop substitutes. For example, Nkouam et al. (2018) investigated the use of *Aframomum melegueta* (alligator pepper) as a hop substitute and found it could impart desirable bitterness and aroma to beer.

However, there is limited research specifically on *Vernonia amygdalina* as a hop substitute. A study by Onyemaobi et al. (2020) explored the use of *V. amygdalina* in traditional Nigerian beers and found that it could enhance the beer's bitterness and shelf-life due to its antioxidant properties.

Nevertheless, comprehensive studies characterising the impact of *V. amygdalina* on the sensory and chemical properties of beer, particularly Ikigage beers are still needed.

## 2.4 Role of antioxidants in beer

Antioxidants in beer is critical for both beer quality and potential health benefits. Antioxidants help protect the beer during production and storage and may also contribute to biological effects when consumed. Beer is a homogeneous liquid because its constituents are evenly distributed throughout the medium. Thus, the effectiveness of putative antioxidants depends on how well these compounds compete with other beer constituents as reaction partners with dangerous reactive species (radicals and reactive oxygen species). Because polyphenols are present in such small amounts, they are unable to effectively absorb highly reactive radicals and act as antioxidants before the radicals react with other, much more common compounds in beer, especially ethanol. (Zhao et al., 2010).

## CHAPTER 3: METHODOLOGY

### 3.1 Sampling and Preparation of Plant Material

Samples of *Vernonia amygdalina* leaves, flowers and bark was collected from the botanical garden of the NIRDA/Huye research center located in Huye district in the Southern province of Rwanda. On the day of collection, the samples were washed with pure water, placed in an oven at 40 °C for 48 hours (in the NIRDA/Huye research center laboratory), then ground as finely as possible and vacuum-packed in plastic bags for their subsequent analyses in the “Unité de brasserie et des industries alimentaires (INBr) de l'institut LIBST de l'Université Catholique de Louvain” in Belgique.

### 3.2 Extractions with various solvent systems

#### 3.2.1 Extraction with acetone/water/acetic acid

Extraction step was performed before other analyses. One gram of each dry sample (leaves, flowers, bark) underwent one or three successive solid/liquid extractions with 10 ml of acetone/water/acetic acid (70:29.5:0.5, v/v/v) for 10 minutes of ultrasonic agitation.

After 10 minutes of centrifugation at 3000 g, the supernatant was filtered with a glass fiber filter before antioxidant or polyphenol analyses. All experiments were performed in duplicate.

#### 3.2.2 Water Extraction at room temperature

Fifty mL of water were added to 5 g of *V. amygdalina*, and the extraction at room temperature was carried out for 10 minutes in an ultrasonic bath. After 10 minutes of centrifugation at 3000 g, the supernatant was filtered through a glass fiber filter before analyzing the antioxidant power and polyphenols.

#### 3.2.3 Extraction with boiling water

Five g of leaves were extracted with 50 mL of water. The mixture was boiled for one hour. After one hour, the lost volume was compensated with boiling water. The mixture was then centrifuged for 10 minutes at 3000 g before filtration through a glass fiber filter. The filtrate was retained for further analysis.

### 3.2.4 Extraction using water, ethanol and acetic acid solvent system

A 10-minute extraction or a three-day infusion was performed to mimic dry hopping. First, 50 mL of the water/ethanol (90/10) solvent system acidified with 0.05% acetic acid was added to 5 g of Vernonia leaves, and the extraction was carried out at room temperature for 10 minutes in an ultrasonic bath. After 10 minutes of centrifugation at 3000 g, the supernatant was filtered through a glass fiber filter before antioxidant and polyphenol analyses. On the other hand, 5 g Vernonia leaves were infused in 50 mL of solvent system and the mixture was kept for 3 days at 4 degrees Celsius before centrifugation for 10 minutes at 3000 g. The supernatant was filtered with a glass fiber filter and the filtrate was kept for further analysis.

### 3.3 Evaluation of the antioxidant activities of Vernonia amygdalina by the ORAC method

The ORAC (Oxygen Radical Absorbance Capacity) method is a universal method used to assess the antioxidant power of many food matrices. It consists of measuring the capacity of antioxidants present in the sample to protect the fluorescent probe (fluorescein) against the presence of free radicals (“Radical Oxygen Species”) generated by AAPH (2,2'-Azodiisobutyramidine dihydrochloride).

The protocol to follow is as follows:

- Prepare a 75 mM phosphate buffer solution, pH 7.4 (this is used to dilute all solutions);
- Fill a 96-well microplate with the following: for blanks: 25  $\mu$ L of buffer solution; for the standard antioxidant: 25  $\mu$ L of Trolox® solution in increasing concentrations (8, 16, 24, 32, and 40  $\mu$ M); for samples: 25  $\mu$ L of the sample in increasing dilutions; *Vernonia* : 0X, 20X, 100X, 1000X, 5000X, 8000X, 10000X, 12500X and 20000X. (Figure 2)



Figure 2: Tubes containing samples

- Add 250  $\mu\text{L}$  of 55.5 nM fluorescein to all wells;

- Place the microplate (Figure 3) in a fluorescence and luminescence reader (Figure 4): Heat the plate at  $37^\circ\text{C}$  for 10 min; Dispense 25  $\mu\text{L}$  of 153  $\mu\text{M}$  AAPH into each well; Measure the fluorescence of each well every minute for 50 min;

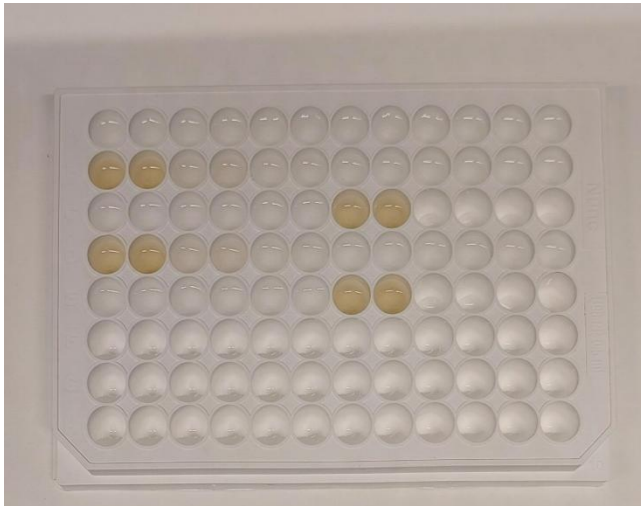


Figure 3: Microplate containing samples and fluorescein

- Collect, using BioTek Gen 5 Version 1.10 software (Figure 4), a visual representation of the fluorescence curves of each well as a function of time and the calculated value of the area under each curve (AUC);



Figure 4: Fluorescence and luminescence reader

- Create a linear regression line by calculating the antioxidant power provided by one  $\mu\text{M}$  of Trolox® (determined from the difference between the AUC of the standard samples and the AUC of the blank). Collect the slope (a) and intercept (b) values needed to calculate the ORAC value (see equation below);
- choose an appropriate concentration for each sample (i.e., the highest concentration at which the sample was unable to protect fluorescein for 50 min; the curve should reach the bottom of the graph,
- calculate ORAC value as follow (Figure 5):

$$\text{ORAC value } (\mu\text{mole Trolox eq/g}) = \frac{AUC_{\text{sample}} - AUC_{\text{blank}} - b}{a} \times \text{dilution}$$

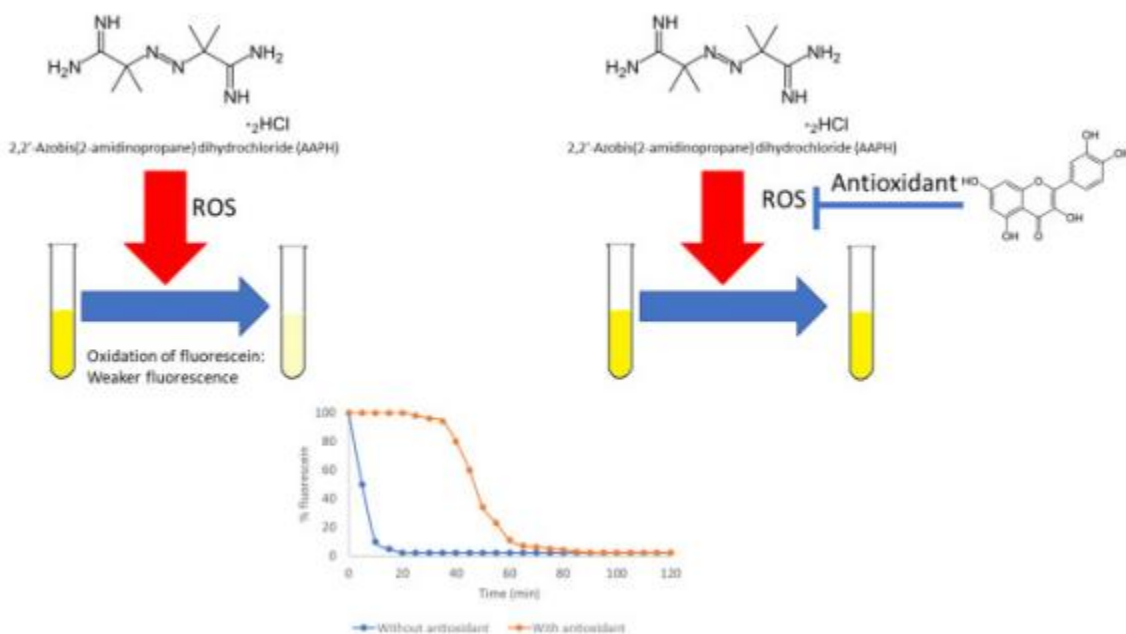


Figure 5: ORAC dosage principle

## 3.4 Phytochemical Analysis

### 3.4.1 Total Polyphenol Content

The total polyphenol content was assessed by using the Folin-ciocalteu method (Figure 6). The Folin-Ciocalteu reagent is a mixture of phosphotungstic and phosphomolybdic acids that can be reduced by phenols to blue-colored tungsten and molybdenum oxide. This test is used to quantify polyphenols and proteins, but can also respond to the presence of other easily oxidizable compounds.

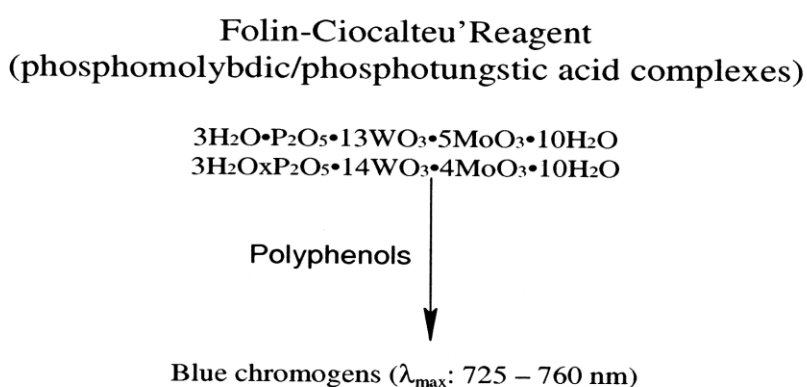


Figure 6: Principle of dosage of total polyphenols by the Folin- Ciocalteu method

To a volume of 100  $\mu\text{L}$  of extract from each sample obtained by solid/liquid extraction, 500  $\mu\text{L}$  of Folin-Ciocalteu reagent diluted 1/10 was added. The resulting mixture was kept for 1 min at room temperature, then 1.5 mL of 75 g/L sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was added and then top up with 10 mL of pure water. The resulting solution was kept for 30 min at room temperature. Absorbance readings were performed using a UV-visible spectrophotometer at a wavelength of 760 nm against a blank (100% pure water and consisting of the same proportions of the reagents introduced into the extracts). Gallic acid was used as a reference standard for establishing the calibration curve and for quantifying total polyphenol contents expressed in mg of gallic acid equivalent per gram of extract (mg eqGA/g of extract).

### 3.4.2 Flavonols and flavones Content determination by UV Spectrophotometer

The flavonols and flavones content was evaluated by colorimetric method with aluminum chloride (Figure 7), described by Aiyegoro and Okoh (2010), using UV Spectrophotometer

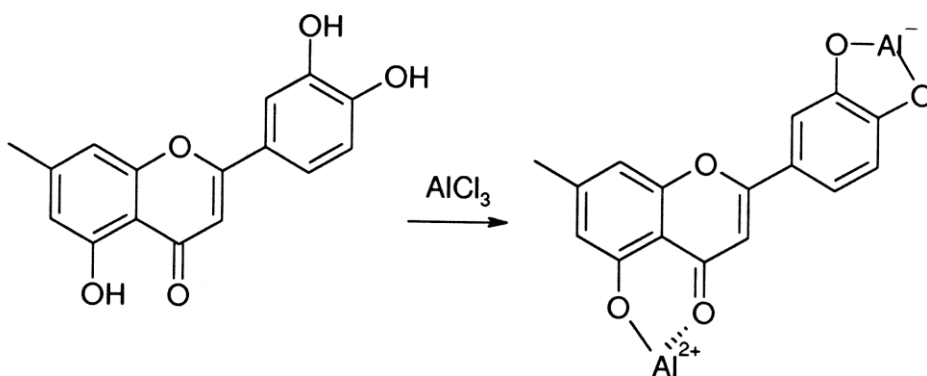


Figure 7: Principle of dosage of flavonols and flavones

To 0.1 ml of aluminum chloride ( $\text{AlCl}_3$ , 10%), 0.2 ml aliquot of an extract was added, followed by 0.1 ml of potassium acetate ( $\text{CH}_3\text{COOK}$ , 1 M); 1.5 ml of ethanol (95%) and 2.8 ml of distilled water followed. The whole mixture was well homogenized and incubated at room temperature, and the absorbance was read at 410 nm against the reagent blank 30 minutes later. Quercetin (0–1000  $\mu\text{g/ml}$ ) served as a standard: a stock solution (550 ppm) of quercetin was prepared by dissolving 2.75 mg of quercetin in 5 mL of methanol/ethanol. This standard solution was serially diluted to obtain different concentrations of 27.5 ppm, 55 ppm, 110 ppm, 165 ppm, and 275 ppm, 0 ppm (blank). The following sequence of steps was followed using a 15 mL glass tube: 2.0 mL of ethanol followed by 0.20 mL of clear sample extract or quercetin, 0.20 mL  $\text{AlCl}_3$  (10% w/v), vortex mixing and equilibration time of 5.0 min, 0.20 mL of  $\text{CH}_3\text{COONa}$  (1.80 g/mL), and the final volume was brought to 5.0 mL using ethanol.

### 3.4.3 Total flavanoids assay

Total flavanoids primarily refer to flavan-3-ols and flavan-3,4-diols, as well as their adducts (proanthocyanidins). In this case, however, only the higher unit will interact with the reagent used in the assay method (Figure 8).

It should be noted, however, that the yield of the coloring compound varies depending on the type of flavanoid involved. The degree of polymerization and the configuration of the heterocycle appear to modify the response of the flavanoids to the chromogen.

The principle of the assay is simple. As shown in the following figure, it involves a condensation between the flavanoid compound and p-dimethylaminocinnamaldehyde.

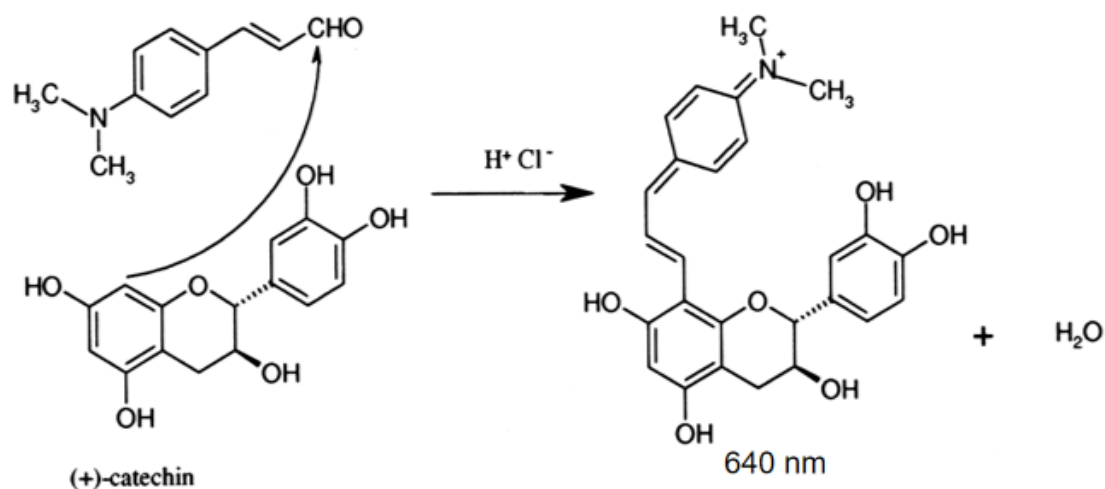


Figure 8: Principle of flavanoid dosage

### ***Apparatus***

Spectrophotometer and cuvette with a 10 mm path length, water bath: 20°C, vortex, volumetric pipettes: 2 x 1 mL, 1 x 5 mL, and 1 x 10 mL, Erlenmeyer flask: 750 mL. Per sample: 1 x 750 mL Erlenmeyer flask, 2 x 100 mL volumetric flasks with stoppers, 3 test tubes.

### ***Preparation of reagents***

Concentrated hydrochloric acid, methanol, p-Dimethylaminocinnamaldehyde: 1 g/L chromogenic solution. Dissolve 500 mg of p-dimethylaminocinnamaldehyde in a pre-cooled mixture of 125 mL of concentrated hydrochloric acid and 350 mL of methanol. Make up to 500 mL with methanol. Prepare the reagent weekly and store it in the dark. Deionized water is also required.

### ***Preparation of the 'Test' and 'Blank' Solutions***

To 1 mL of the extract add 5 mL of chromogenic solution and mix. This will give you the 'Test' solution. The 'Blank' solution will consist of 1 mL of water and 5 mL of chromogenic solution.

### ***Determination of the Constituents***

Using a pipette, transfer a portion of the 'Test' solution into a cuvette after rinsing/conditioning it. Repeat the procedure for the blank. Read the absorbance results at a wavelength of 640 nm.

The following formula is used to determine the total flavanoid content of the sample:

$$F = [(A_t - A_b) \times 33.5]$$

where F = total flavanoid content of the sample, in [ppm] of catechin equivalents;

A<sub>t</sub> = absorbance value of the 'test' solution at 640 nm;

A<sub>b</sub> = absorbance value of the 'blank' solution at 640 nm;

### **3.5 Data analysis**

Data were analyzed using BioTek Gen 5 Version 1.10 software for ORAC results and other results were analysed using descriptive statistics and excel.

# CHAPTER 4: RESULTS PRESENTATION

## 4.1 Antioxidant activities of Vernonia amygdalina

### 4.1.1 Antioxidant activities of Vernonia amygdalina in different parts of the plant by the ORAC method

The ORAC values reported in (figure9) represent the results antioxidant activities of Vernonia amygdalina in different parts of the plant (Leaf, bark and flower) by the ORAC method from BioTek Gen 5 Version 1.10 software (area under curve (AUC) formula and curves. Results shown in table (Table1) represent the calculations of ORAC concentration using excel.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	16.323	12.283	19.061	18.873	21.696	21.201	25.36	24.816	28.732	28.245	30.186	29.508	AUC: Formula Result [485/20,528/2 0]
B	52.607	52.738	49.936	50.002	50.878	50.79	29.787	28.982	15.63	15.621	13.753	11.838	AUC: Formula Result [485/20,528/2 0]
C	13.82	14.853	14.239	15.158	13.991	13.552	51.413	52.673					AUC: Formula Result [485/20,528/2 0]
D	52.867	52.986	49.932	49.903	50.397	50.284	46.473	30.46	17.137	17.828	14.095	12.02	AUC: Formula Result [485/20,528/2 0]
E	15.567	15.406	15.221	14.941	14.492	13.81	52.821	54.505	19.214	19.651			AUC: Formula Result [485/20,528/2 0]
F	50.023	49.984	49.577	49.504	50.112	50.301	35.808	33.13	18.834	17.629	15.289	13.694	AUC: Formula Result [485/20,528/2 0]
G	15.443	16.994	16.786	15.928	20.194	19.725		49.626	49.493	49.414	49.549		AUC: Formula Result [485/20,528/2 0]
H	50.788	50.333	35.237	35.767	34.829	19.375	17.997	27.139	14.32	14.203	17.425	11.852	AUC: Formula Result [485/20,528/2 0]

	1	2	3	4	5	6	7	8	9	10	11	12	
A	12.16	12.701	15.969	15.131	17.389	19.142	20.786	24.491	23.05	24.595	25.114	26.092	AUC: Formula Result [485/20,528/2 0]
B	46.849	47.021	48.501	48.478	50.098	50.074	29.103	29.302	17.221	17.131	12.938	11.495	AUC: Formula Result [485/20,528/2 0]
C	12.642	13.404	13.594	13.889	14.695	15.526	46.836	46.669					AUC: Formula Result [485/20,528/2 0]
D	47.384	47.038	48.396	48.409	49.99	49.623	26.405	26.421	14.124	13.9	14.226	16.184	AUC: Formula Result [485/20,528/2 0]
E	15.146	13.196	19.404	12.821	11.887	11.549	46.752	46.334					AUC: Formula Result [485/20,528/2 0]
F													AUC: Formula Result [485/20,528/2 0]
G													AUC: Formula Result [485/20,528/2 0]
H													AUC: Formula Result [485/20,528/2 0]

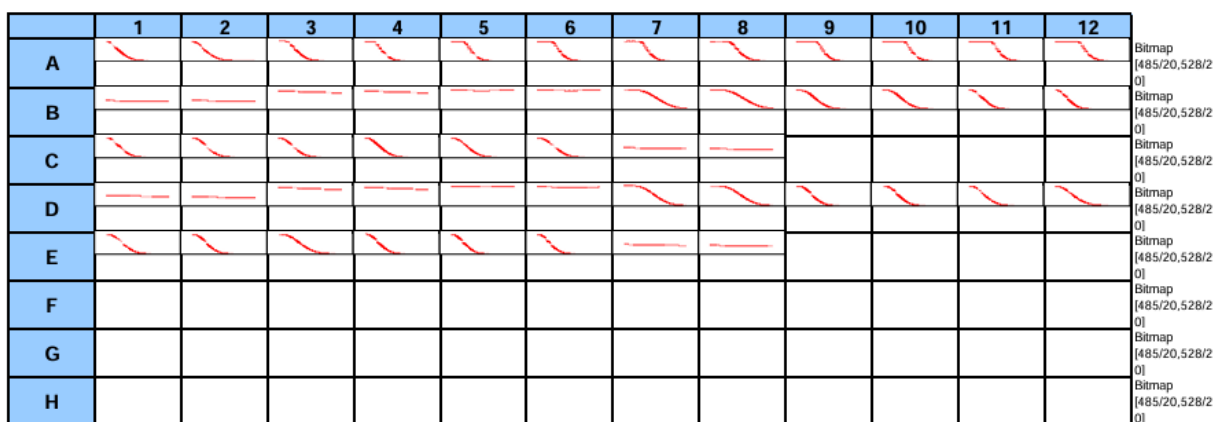
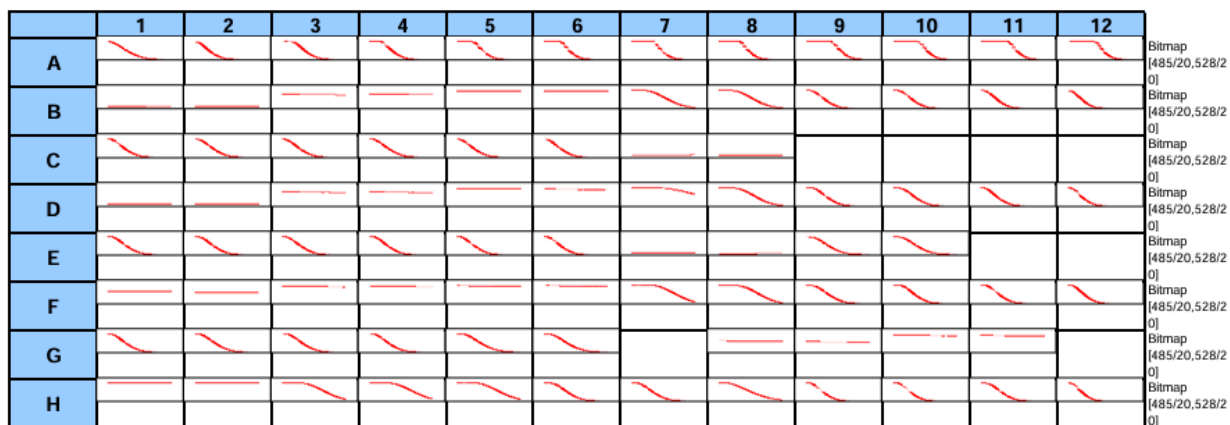


Figure 9: Results representation of the antioxidant activities of *Vernonia amygdalina* in different parts of the plant (Leaf, bark and flower) by the ORAC method.

Table 1: Calculations of ORAC concentration using excel

auc Trolox	14.303	18.967	21.4485	25.088	28.4885	29.847		
auc net Trolox	0	4.664	7.1455	10.785	14.1855	15.544		
	0	8	16	24	32	40		
Leaves								
auc	52.6725	49.969	50.834	29.3845	15.6255	12.7955	14.3365	14.6985
auc net	38.3695	35.666	36.531	15.0815	1.3225	-1.5075	0.0335	0.3955
orac	95.51834	88.6322	90.83546	36.20097	1.15512	-6.05323	-2.12812	-1.20606
fd	1	20	100	1000	5000	8000	10000	12500
orac value	95.51834	1772.644	9083.546	36200.97	5775.599	-48425.9	-21281.2	-15075.8
auc	52.9265	49.9175	50.3405	38.4665	17.4825	13.0575	15.4865	15.081
auc net	38.6235	35.6145	36.0375	24.1635	3.1795	-1.2455	1.1835	0.778
orac	96.16531	88.50102	89.57845	59.33393	5.885125	-5.38589	0.80107	-0.23179
fd	1	20	100	1000	5000	8000	10000	12500
orac value	96.16531	1770.02	8957.845	59333.93	29425.62	-43087.1	8010.698	-2897.35
Bark								
auc	50.0035	49.5405	50.2065	34.469	18.2315	14.4915	16.2185	16.357
auc net	35.7005	35.2375	35.9035	20.166	3.9285	0.1885	1.9155	2.054
orac	88.72007	87.54075	89.23714	49.15181	7.792919	-1.73332	2.665563	3.018339
fd	1	20	100	1000	5000	8000	10000	12500
orac value	88.72007	1750.815	8923.714	49151.81	38964.6	-13866.5	26655.63	37729.24
auc	49.5595	49.4815	50.5605	35.502	27.102	22.568	14.2615	14.6385
auc net	35.2565	35.1785	36.2575	21.199	12.799	8.265	-0.0415	0.3355
orac	87.58915	87.39047	90.13882	51.78299	30.38716	18.83851	-2.31915	-1.35889
fd	1	20	100	1000	5000	8000	10000	12500
orac value	87.58915	1747.809	9013.882	51782.99	151935.8	150708.1	-23191.5	-16986.1
Flower								
auc	46.935	48.4895	50.086	29.2025	17.176	12.2165	13.023	13.7415
auc net	34.5045	36.059	37.6555	16.772	4.7455	-0.214	0.5925	1.311
orac	100.1655	104.7443	109.4468	47.93432	12.51016	-2.09809	0.277467	2.393814
fd	1	20	100	1000	5000	8000	10000	12500
orac value	100.1655	2094.887	10944.68	47934.32	62550.81	-16784.7	2774.669	29922.68
auc	47.211	48.4025	49.8065	26.413	14.012	15.205	14.171	16.1125
auc net	34.7805	35.972	37.376	13.9825	1.5815	2.7745	1.7405	3.682
orac	100.9785	104.4881	108.6236	39.71782	3.190574	6.704566	3.65891	9.377614
fd	1	20	100	1000	5000	8000	10000	12500
orac value	100.9785	2089.761	10862.36	39717.82	15952.87	53636.52	36589.1	117220.2

Results presented in table (Table 2) show that *Vernonia amygdalina* has exceptional antioxidant activity in different parts of the plant (leaves, bark and flowers) and the part that shows the highest antioxidant activity was the bark of *V. amygdalina* (1514) and the lowest have been the flowers (1315)

Table 2: ORAC values for extracts of different parts of *V. amygdalina* by acetone/water/acetic acid

	Vernonia leaves	Vernonia flower	Vernonia bark
ORAC values ( $\mu\text{mol Trolox eq/g}$ ) Three successive extractions	1513	1315	1514

#### 4.1.2 Antioxidant activities of *Vernonia amygdalina* leaves extracted from different solvent systems

The ORAC values reported in (Figure 10) represent the results of antioxidant activities of *Vernonia amygdalina* leaves extracted from different solvent systems by the ORAC method from BioTek Gen 5 Version 1.10 software (area under curve (AUC) formula and curves. Results shown in (Table 3) represent the calculations of ORAC concentration in excel using the equation represented in (Figure 11).

	1	2	3	4	5	6	7	8	9	10	11	12	
<b>A</b>	11.488	12.551	20.257	20.069	26.697	26.267	26.934	27.714	30.139	30.035	32.037	29.995	AUC: Formula Result [485/20,528/20]
<b>B</b>	49.797	49.642	49.514	49.484	50.047	50.029	38.386	38.524	25.562	25.75	20.013	20.209	AUC: Formula Result [485/20,528/20]
<b>C</b>	18.308	19.136	20.295	18.684	17.151	16.622	49.149	49.303	49.278	49.433	50.187	50.158	AUC: Formula Result [485/20,528/20]
<b>D</b>	40.485	42.128	23.187	23.616	26.496	26.307	22.804	22.657	20.981	21.139	18.088	15.882	AUC: Formula Result [485/20,528/20]
<b>E</b>	48.87	48.412	49.506	49.472	51.28	51.167	30.743	30.51	22.481	20.956	20.307	18.076	AUC: Formula Result [485/20,528/20]
<b>F</b>	14.675	14.939	15.342	15.35	20.422	15.632	49.474	49.326	49.477	49.405	51.181	51.464	AUC: Formula Result [485/20,528/20]
<b>G</b>	30.117	32.155	19.929	20.038	17.119	16.685	17.881	16.663	17.789	15.435	14.975	14.124	AUC: Formula Result [485/20,528/20]
<b>H</b>													AUC: Formula Result [485/20,528/20]

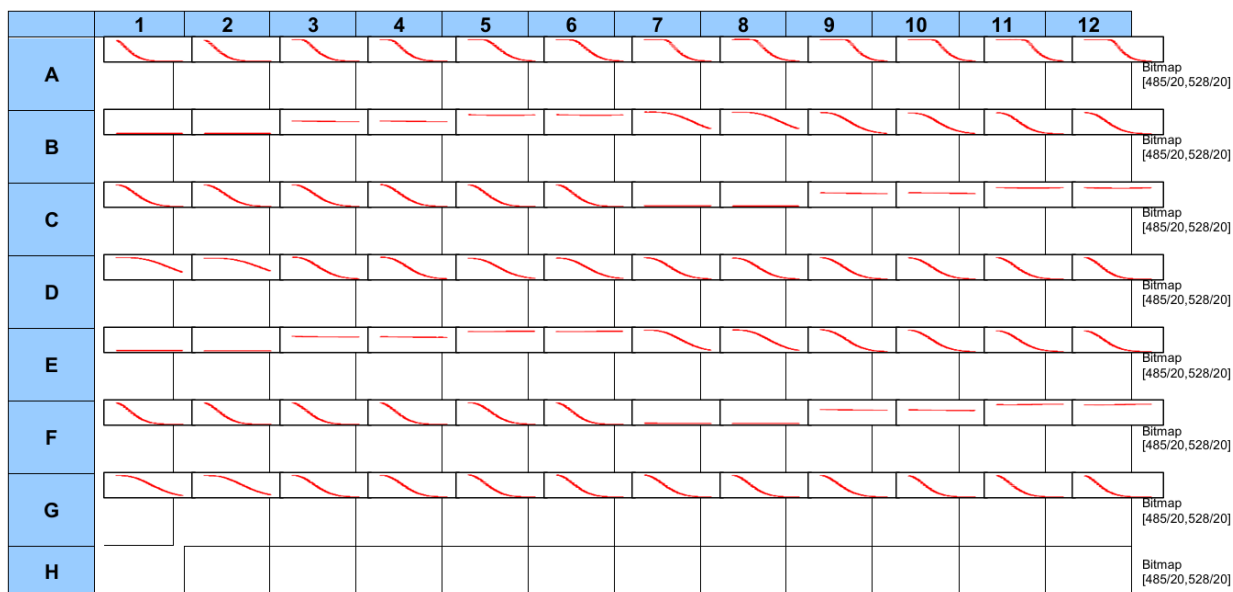


Figure 10: Representation of results from BioTek Gen 5 Version 1.10 software

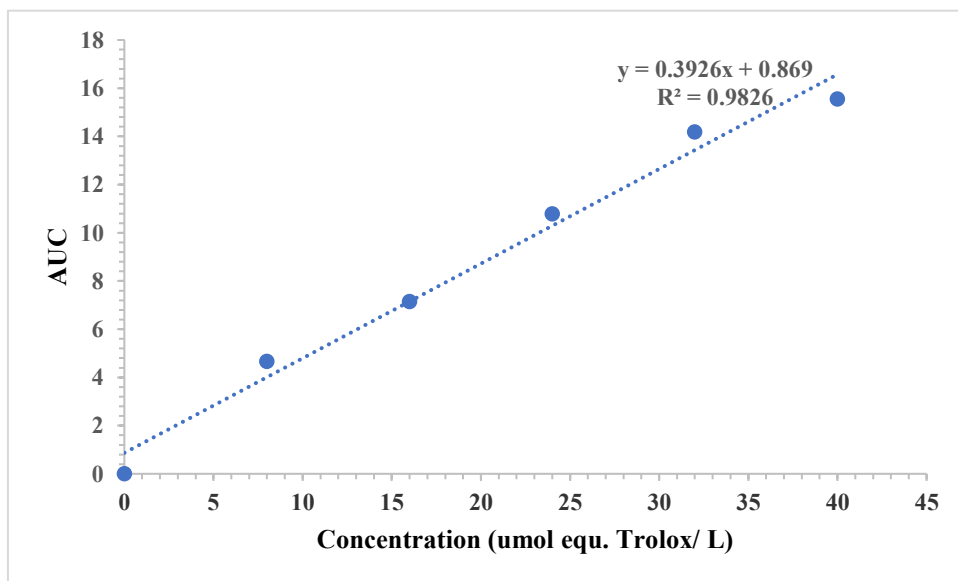


Figure 11: Trolox calibration curve used as a reference standard

Table 3: ORAC results of *Vernonia amygdalina* leaves extracted from different solvents system.

solvent cold water									
auc	49.494	49.467	50.99	27.9845	19.4905	18.8815	20.4735	17.439	17.158
auc net	35.476	35.449	36.972	13.9665	5.4725	4.8635	6.4555	3.421	3.14
orac	77.88587	77.82361	81.33525	28.29052	8.705557	7.30136	10.9721	3.975329	3.327415
fd	1	20	100	1000	5000	8000	10000	12500	20000
orac conc	77.88587	1556.472	8133.525	28290.52	43527.78	58410.88	109721	49691.61	66548.31
auc	49.7825	49.6245	50.366	44.8365	24.4105	21.909	17.993	21.23	18.036
auc net	35.7645	35.6065	36.348	30.8185	10.3925	7.891	3.975	7.212	4.018
orac	78.55107	78.18677	79.89647	67.14688	20.0498	14.28199	5.252709	12.71639	5.351856
fd	1	20	100	1000	5000	8000	10000	12500	20000
orac conc	78.55107	1563.735	7989.647	67146.88	100249	114255.9	52527.09	158954.9	107037.1

Boiling water									
auc	49.7195	49.499	50.038	38.455	25.656	20.111	18.722	19.4895	16.8865
auc net	37.7	37.4795	38.0185	26.4355	13.6365	8.0915	6.7025	7.47	4.867
orac	76.18235	75.69082	76.89233	51.072	22.54102	10.18034	7.084039	8.794918	2.992421
fd	1	20	100	1000	5000	8000	10000	12500	40000
orac conc	76.18235	1513.816	7689.233	51072	112705.1	81442.71	70840.39	109936.5	119696.8
auc	49.226	49.3555	50.1725	41.3065	23.4015	26.4015	22.7305	21.06	16.985
auc net	37.2065	37.336	38.153	29.287	11.382	14.382	10.711	9.0405	4.9655
orac	75.08226	75.37093	77.19215	57.42844	17.51538	24.20285	16.01962	12.29581	3.211993
fd	1	20	100	1000	5000	8000	10000	12500	20000
orac conc	75.08226	1507.419	7719.215	57428.44	87576.91	193622.8	160196.2	153697.6	64239.86

water, acetic acid and ethanol									
auc	48.552	49.587	50.498	34.64	18.1025	27.0105	28.2065	18.506	15.746
auc net	34.534	35.569	36.48	20.622	4.0845	12.9925	14.1885	4.488	1.728
orac	75.71386	78.1003	80.20083	43.63638	5.505188	26.04473	28.8024	6.435555	0.071709
fd	1	20	100	1000	5000	8000	10000	12500	20000
orac conc	75.71386	1562.006	8020.083	43636.38	27525.94	208357.9	288024	80444.43	1434.171
auc	49.2165	49.007	50.9125	23.6905	16.364	19.8025	23.6105	23.2665	14.2475
auc net	35.1985	34.989	36.8945	9.6725	2.346	5.7845	9.5925	9.2485	0.2295
orac	77.24602	76.76297	81.15656	18.38967	1.496657	9.424948	18.20521	17.41204	-3.38344
fd	1	20	100	1000	5000	8000	10000	12500	20000
orac conc	77.24602	1535.259	8115.656	18389.67	7483.283	75399.58	182052.1	217650.4	-67668.9

water, acetic acid and ethanol (3days)									
auc	48.641	49.489	51.2235	30.6265	21.7185	19.1915	14.807	15.346	18.027
auc net	36.6215	37.4695	39.204	18.607	9.699	7.172	2.7875	3.3265	6.0075
orac	73.7782	75.66852	79.535	33.62104	13.76371	8.130629	-1.64311	-0.4416	5.534775
fd	1	20	100	1000	5000	8000	10000	12500	20000
orac conc	73.7782	1513.37	7953.5	33621.04	68818.55	65045.03	-16431.1	-5519.95	110695.5
auc	49.4	49.441	51.3225	31.136	19.9835	16.902	17.272	16.612	14.5495
auc net	37.3805	37.4215	39.303	19.1165	7.964	4.8825	5.2525	4.5925	2.53
orac	75.47013	75.56152	79.75568	34.7568	9.896121	3.026973	3.851761	2.380517	-2.21712
fd	1	20	100	1000	5000	8000	10000	12500	20000
orac conc	75.47013	1511.23	7975.568	34756.8	49480.61	24215.78	38517.61	29756.46	-44342.4

It is also worth noting a higher antioxidant activity (Table 4) for the extraction by boiling water for one hour (1001  $\mu\text{mol Trolox eq/g}$ ).

Table 4: ORAC values extracts of leaves of *V. amygdalina* by different solvents systems.

	Cold water	Boiling water	Water, Acetic acid and Ethanol	Water, Acetic acid and Ethanol (infusion of 3 days)
ORAC values ( $\mu\text{mol Trolox eq/g}$ )	671	1001	436	591

## 4.2 Characterization of the phytochemical compounds related to the antioxidant power of *Vernonia amygdalina* and assessment of effect of brewing process on *Vernonia* antioxidant power

### 4. 2.1 Total Polyphenol Content determination in different parts of the plant by Folin-Ciocalteu method

The results shown in Table 5 represent the calculation of total phenolic concentration in different parts of the plant using the equation on (Figure 12) and show that the barks have a slightly higher

polyphenol content (28,010 ppm), followed by leaves (27,480) and flowers (26,550). This corroborates the ORAC results discussed above.

The following equation was applied:

$$\text{Conc (ppm)} = \frac{(\text{abs sample} - \text{abs blank} - 0.0188)}{0.0007} \times \text{Dilution}$$

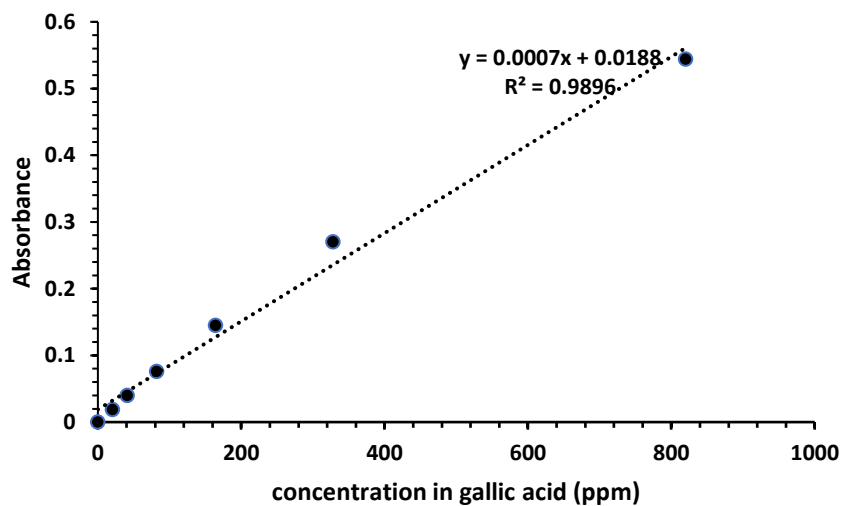


Figure 12: Calibration curve of gallic acid used as a reference standard for total polyphenols

Table 5: Polyphenol results in different parts of the plant

	Absorbance	Absorbance-net	Concentration in mg/l	concentration in mg/g	Average	Concentration in ppm	Standard Deviation
Blank	0.073						
Leaf sample 1	0.728	0.655	908.8571	27.26571	27.48	27480	303
Leaf sample 2	0.738	0.665	923.1429	27.69429			
Bark sample 1	0.746	0.673	934.5714	28.03714	28.01	28010	30
Bark sample 2	0.745	0.672	933.1429	27.99429			
Flower sample 1	0.686	0.62	858.8571	25.76571	26.55	26550	1121
Flower sample 2	0.723	0.657	911.7143	27.35143			

#### 4.2.2 Total Polyphenol Content Assessment of Vernonia leaves from different solvent system

Results presented in Table 6 represents the calculation of total phenolic content of Vernonia leaves from different solvent system by Folin-Ciocalteu method. They indicate that boiling water has a higher polyphenol content (9.7 mg/g) which verifies the ORAC results discussed above.

Table 6: polyphenol results of Vernonia leaves from different solvent systems by Folin-Ciocalteu method.

Solvent	Absorbance	Absorbance-net	concentration in mg/l	concentration in mg/g	Average	Concentration in ppm	Standard Deviation
blank	0.077						
Water 100% 1	0.578	0.501	688.8571 429	6.888571 429	6.83857 1	6839	71
Water 100% 2	0.571	0.494	678.8571 429	6.788571 429			
Boiling water 1	0.776	0.699	971.7142 857	9.717142 857	9.74757 1429	9748	43
Boiling water 2	0.776	0.699	971.778	9.778			
Ethanol acetic acid water 1	0.584	0.507	697.4285 714	6.974285 714	7.50285 7143	7503	748
Ethanol acetic acid water 2	0.658	0.581	803.1428 571	8.031428 571			
Ethanol acetic acid water 3days 1	0.737	0.66	916	9.16	9.28857 1429	9289	182
Ethanol acetic acid water 3days 2	0.755	0.678	941.7142 857	9.417142 857			

#### 4.2.3 Total Flavonoid Content Assessment in the different parts of the plant

The results presented in (Table 7) indicate the calculation of flavonoid concentration in different parts of the plant and show that the flowers have a significantly higher flavonol and flavone content than other parts of Vernonia (8686 ppm).

The following equation was applied:

$$\text{Conc (ppm)} = \frac{(\text{abs sample with chromogen} - \text{abs blank} - \text{abs without chromogen}) + 0.0119}{0.0031} \times \text{Dilution}$$

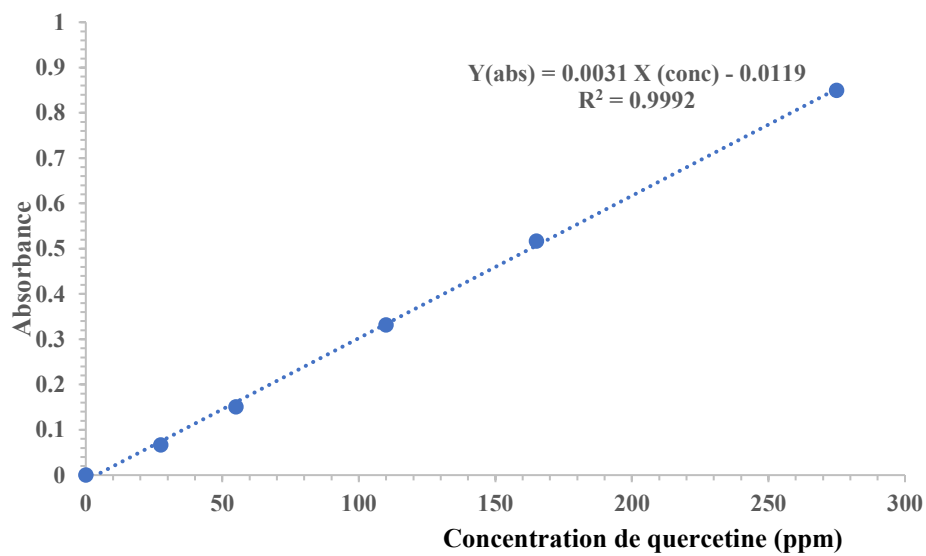


Figure 13: Calibration curve of quercetin (used as a reference standard)

Table 7: Flavonoids content in different parts of the plant

	Absorbance	Absorbance-net	Concentration en ppm	concentration en mg/g	moyenne	Concentration en ppm	Standard Deviation
<b>Blank</b>	0.072						
<b>Reference leaf</b>	0.513						
<b>Reference bark</b>	0.119						
<b>Leaf sample 1</b>	0.97	0.385	120.3548	3.610645	3.823548	3824	301
<b>Leaf sample 2</b>	1.014	0.429	134.5484	4.036452			
<b>Bark sample 1</b>	0.803	0.612	193.5806	5.807419	5.744516	5745	89
<b>Bark sample 2</b>	0.79	0.599	189.3871	5.681613			
<b>Flower sample 1</b>		0.971	309.3871	9.281613	8.686452	8686	842
<b>Flower sample 2</b>		0.848	269.7097	8.09129			

#### 4. 2.4 Total flavonoid Content Assessment of Vernonia leaves from different solvent system

The results shown in Table 8 indicate a higher extraction of flavonols and flavones with boiling water (4762 ppm). These results corroborate the total polyphenols discussed above. The extraction was even better than the acetone/water/acetic acid solvent system considered effective for polyphenol extraction.

Table 8: Results of leaf flavonoids extracted with different solvents

	Absorbance	absorbance-net	concentration en mg/l	concentration en mg/g	Moyenne	Concentration en ppm	Standard Deviation
blank	0.077						
blank solvent water	0.513						
blank solvent boiling water	1.171						
blank solvent ethanol acetic acid water	0.409						
blank solvent ethanol acetic acid water 3days	0.656						
water 1	1.004	0.414	129.7097	1.297097	1.200	1200	96
water 2	0.962	0.372	116.1613	1.161613			
Boiling water 1	2.736	1.488	476.1613	4.761613	4.762	4762	0
ethanol acetic acid water 1	0.972	0.486	152.9355	1.529355			
ethanol acetic acid water 2	1.256	0.77	244.5484	2.445484	1.987	1987	648
ethanol acetic acid water 3 days 1	1.419	0.686	217.4516	2.174516	2.350	2350	262
ethanol acetic acid water 3 days 2	1.534	0.801	254.5484	2.545484			

#### 4.2.5 Total flavanoids assay for different parts of the plant

The results shown in (Table 9) represent the concentration of flavonoids in different parts of Vernonia Amygdalina and indicate that the flavanoid content is significantly higher in the leaves than other parts of the plant.

Table 9: Flavanoids content in different parts of the plant.

	Absorbance	absorbance-net	Concentration in mg/l	concentration in mg/g	Average	Concentration in ppm	Standard Deviation
<b>Blank</b>	0.057						
<b>Reference leaf</b>	0.375						
<b>Reference bark</b>	0.084						
<b>Leaf sample 1</b>	0.746	0.314	10.519	0.31557	0.32763	328	17
<b>Leaf sample 2</b>	0.77	0.338	11.323	0.33969			
<b>Bark sample 1</b>	0.112	0.055	1.8425	0.055275	0.057285	57	3
<b>Bark sample 2</b>	0.116	0.059	1.9765	0.059295			
<b>blank</b>	0.062						
<b>Flower sample 1</b>	0.143	0.081	2.7135	0.081405	0.076883	77	6
<b>Flower sample 2</b>	0.134	0.072	2.412	0.07236			

#### 4.2.6 Total flavanoids assay for *V. amygdalina* leaves extracted using different solvent systems

The results shown in (Table 10) represent the flavanoids content of vernonia leaves extracted using different solvent systems and indicate a higher extraction of flavanoids with water at boiling temperature (272 ppm).

Table 10: Results of leaf flavanoids extracted with different solvents

	Absorbance	Absorbance-net	concentration in mg/l	concentration in mg/g	Average	Concentration in ppm	Standard Deviation
blank	0.072						
Water solvent 1	0.405	0.333	11.1555	0.111555	0.10251	103	13
Water solvent 2	0.351	0.279	9.3465	0.093465			
Boiling water 1	0.885	0.813	27.2355	0.272355	0.272355	272	0
Ethanol acetic acid water 2	0.314	0.242	8.107	0.08107	0.08107	81	0
Ethanol acetic acid water 3days 1	0.395	0.323	10.8205	0.108205	0.0916225	92	23
Ethanol acetic acid water 3days 2	0.296	0.224	7.504	0.07504			

## CHAPTER 5: RESULTS DISCUSSION

### 5.1 Antioxidant Activity of Different Plant Parts by the ORAC Method

The ORAC (Oxygen Radical Absorbance Capacity) assay is widely used to evaluate antioxidant potential based on the ability of plant extracts to neutralize peroxy radicals. In this study, *Vernonia amygdalina* exhibited measurable antioxidant activity across its leaves, flowers, and bark. Among these, the bark extract (1514  $\mu\text{mol Trolox eq/g}$ ) demonstrated the highest antioxidant potential. Akinmoladun et al., 2010T reported the same findings where bark tissues often concentrate secondary metabolites with redox potential, including polyphenols and flavonoids.

Even if bark shows a higher antioxidant power, collecting samples of bark destroy the plant. Using flowers is also difficult because the flowering period of the plant happens once a year. We opted to use leaves which enough on the plant.

Although the ORAC values of the leaves were lower (1513  $\mu\text{mol Trolox eq/g}$ ), they still significantly surpassed those of industrial Citra T90 hops (614  $\mu\text{mol Trolox eq/g}$ ), Margaux et al., 2024 making *V. amygdalina* a viable natural substitute for hops in beer for antioxidant fortification.

The values obtained in this study on leaves were below those previously reported by INBr Laboratory (5234  $\mu\text{mol Trolox eq/g}$ ) Margaux et al., 2024. This discrepancy could be attributed to harvest time, or other factors known to influence antioxidant yield (Shahidi & Ambigaipalan, 2015).

### 5.2 Influence of Solvent Extraction on Antioxidant Activity

The solvent used for extraction plays a crucial role in determining the yield of antioxidant compounds. The boiling water extract yielded the highest ORAC value (1001  $\mu\text{mol Trolox eq/g}$ ) among the solvent systems tested. This suggests that heat may enhance the release of antioxidant compounds, possibly due to cell wall rupture or increased solubility of polyphenolic compounds in hot water (Zhou & Yu, 2006). The acetone-water-acetic acid mixture, though commonly regarded as efficient for polyphenol extraction, showed lower ORAC values (436  $\mu\text{mol Trolox eq/g}$ ) when compared to boiling water.

This supports the hypothesis that using *V. amygdalina* during heat-intensive brewing steps like hopping may retain or even enhance antioxidant properties. This is valuable for the development of functional beers enriched with plant-derived antioxidants.

### 5.3 Total Polyphenol Content in Different Plant Parts

The bark exhibited the highest total polyphenol content (28.01 mg/g), followed by the leaves (27.48 mg/g) and flowers (26.55 mg/g) as represented in (Figure 14) This aligns with the ORAC results and supports the direct correlation between polyphenol concentration and antioxidant capacity. Polyphenols, especially those with catechol structures, are highly effective radical scavengers and are abundant in vascular tissues like bark (Cheynier, 2012).

The variation in polyphenol content by plant part is not surprising as different tissues accumulate specific metabolites based on physiological roles. Bark, being a protective structure, tends to store higher levels of antimicrobial and antioxidative compounds.

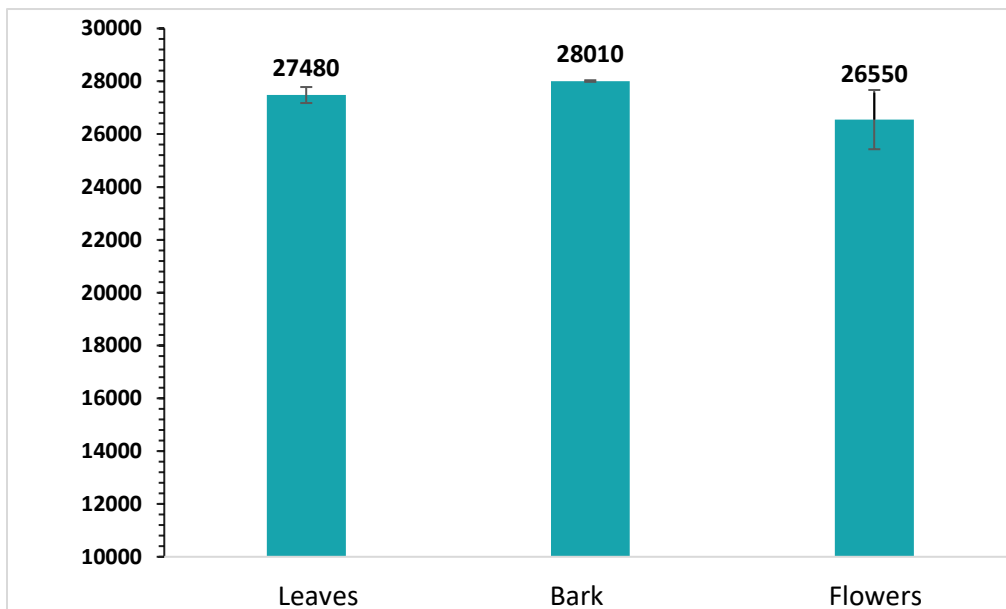


Figure 14: Total polyphenol content of extracts (acetone/water/acetic acid) of different parts of *V. amygdalina*.

## 5.4 Total Polyphenols by Solvent System

Boiling water again proved most effective, extracting 9.7 mg/g of polyphenols from *V. amygdalina* leaves, higher than cold water and the ethanol–acetic acid–water mix (Figure 15). These findings are in agreement with previous studies reporting higher polyphenol recovery using thermal methods due to improved solubilization and degradation of cell walls (Vergara-Salinas et al., 2012).

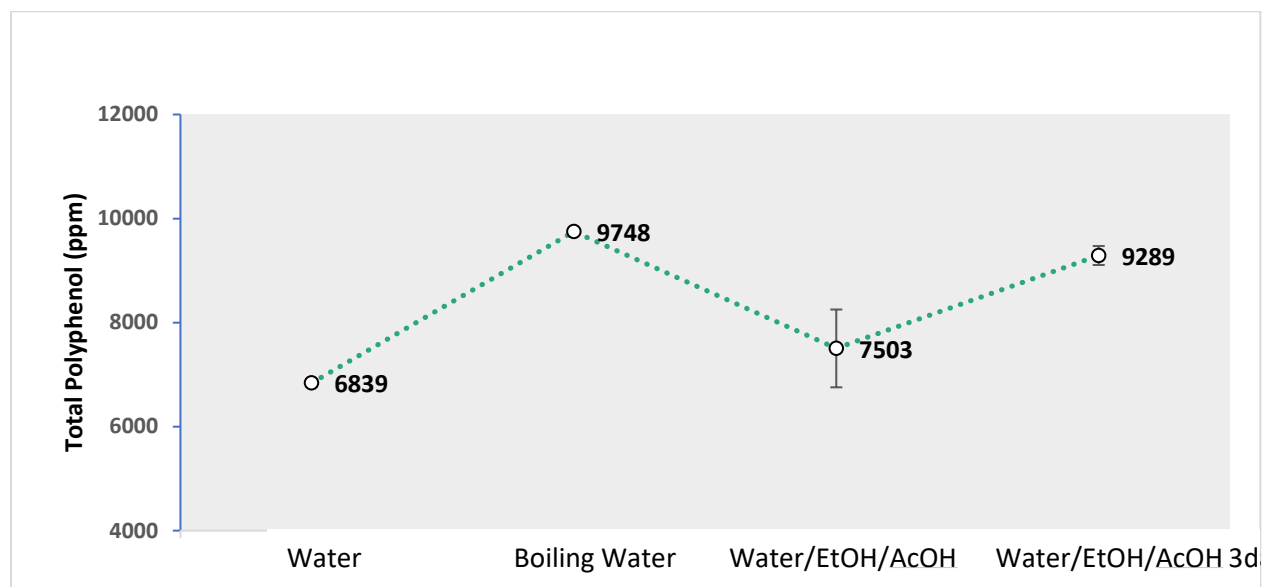


Figure 15: Total polyphenol content of *V. amygdalina* leaf extracts by different solvent systems

## 5.5 Flavonoid Content in Plant Parts

Flavonoids are a subclass of polyphenols known for their strong antioxidant and metal-chelating properties. In this study, the flowers showed the highest total flavonol/flavone content (8686 ppm), followed by bark (5744 ppm) and leaves (3823 ppm) (Figure 16). Flowers often accumulate flavonoids for UV protection and pigmentation, which may explain their elevated levels.

In contrast, when considering total flavonoid content (including other subclasses), leaves had the highest levels (328 ppm), supporting their potential as functional ingredients. These results are comparable to those reported by Okoh et al. (2014), who also observed high flavonoid contents in *V. amygdalina* leaves and flowers.

The scientific literature indicates that the total flavonoid content of hops ranges between 5 and 22,000 ppm (Margaux S et al., 2024). This indicates that the different parts of *Vernonia* could also fall within this range (here, we only measured flavonols and flavones).

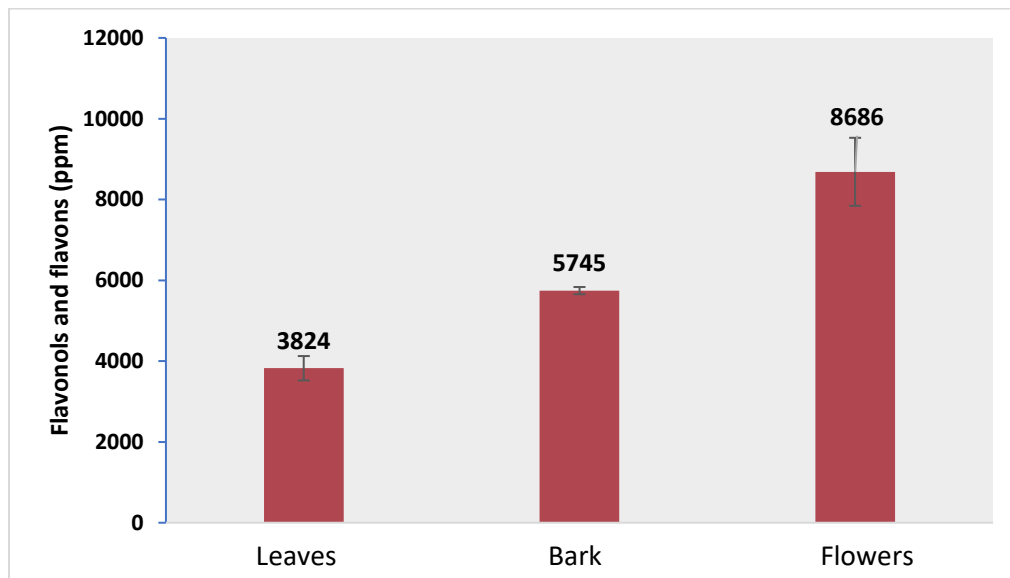


Figure 16: Flavonol and flavone content of extracts (acetone/water/acetic acid) of different parts of *V. amygdalina*

## 5.6 Effect of Solvent System on Flavonoid Extraction

Boiling water again emerged as the best solvent for extracting flavonols and flavones, yielding up to 4.76 mg/g, significantly higher than acetone-acetic acid-water systems (1.98–2.35 mg/g) (Figure 17). This confirms that heat enhances the solubility of flavonoid glycosides and aglycones, supporting its suitability for use in heated brewing processes.

The flavonoid yield with boiling water also achieved over 80% efficiency compared to organic solvent systems, indicating that traditional, water-based extraction methods could be economically and environmentally preferable (Zhang et al., 2011)

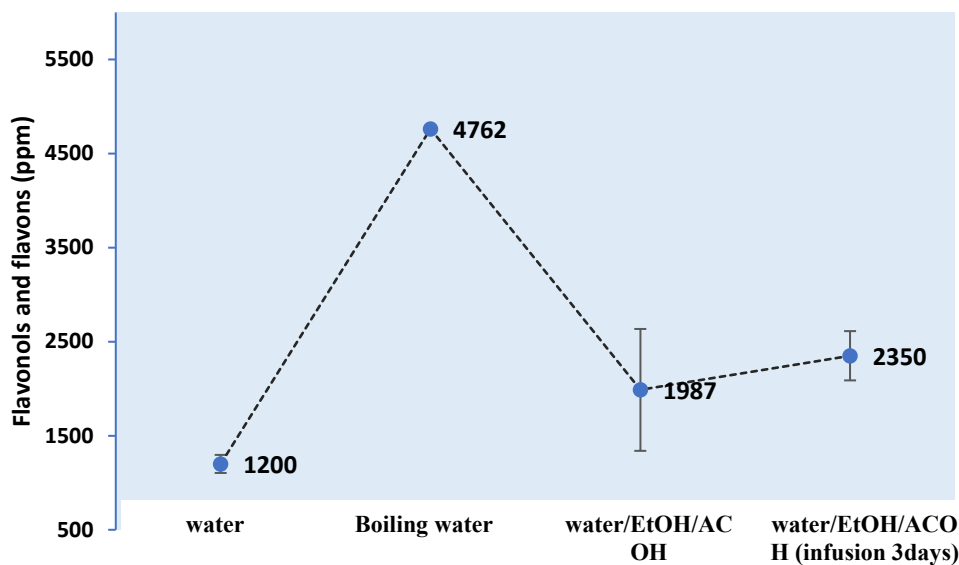


Figure 17: Flavonol and flavone content of *V. amygdalina* leaf extracts (January, 2025) by different solvent systems

### 5.7 Total Flavanoid Content in Different Parts of the Plant

In this study, the flavonoid content was significantly higher in the leaves of *Vernonia amygdalina* (328 ppm) compared to the bark and flowers, with the bark showing the lowest flavonoid concentration (1.8–1.9 ppm) (Figure 18). This distribution of flavonoid content among different plant parts is consistent with previous studies, which often report higher concentrations of flavonoids in the leaves due to their role in photoprotection and defense against oxidative stress (Nabavi et al., 2015).

Flavonoids in leaves are primarily involved in UV protection and combating oxidative stress, which might explain the higher concentrations in the leaves compared to other parts of the plant (Quideau et al., 2011). The flowers, though generally associated with high levels of anthocyanins and flavonoids for pollination, also contained significant flavonoid content (approximately 2.7 ppm). These results further highlight the diverse roles flavonoids play in various plant organs, influencing plant-pollinator interactions and protecting against environmental stressors (Oleszek et al., 2003).

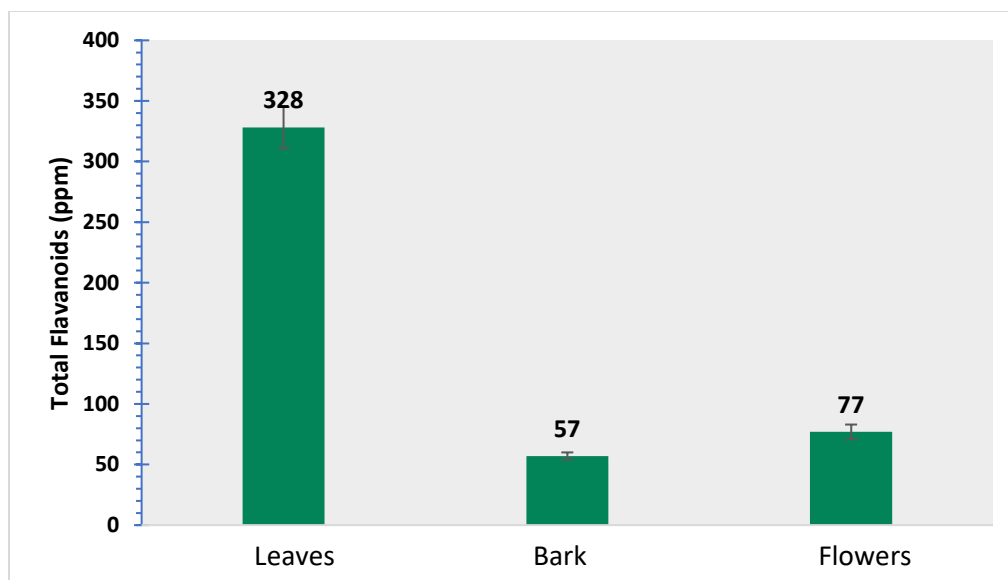
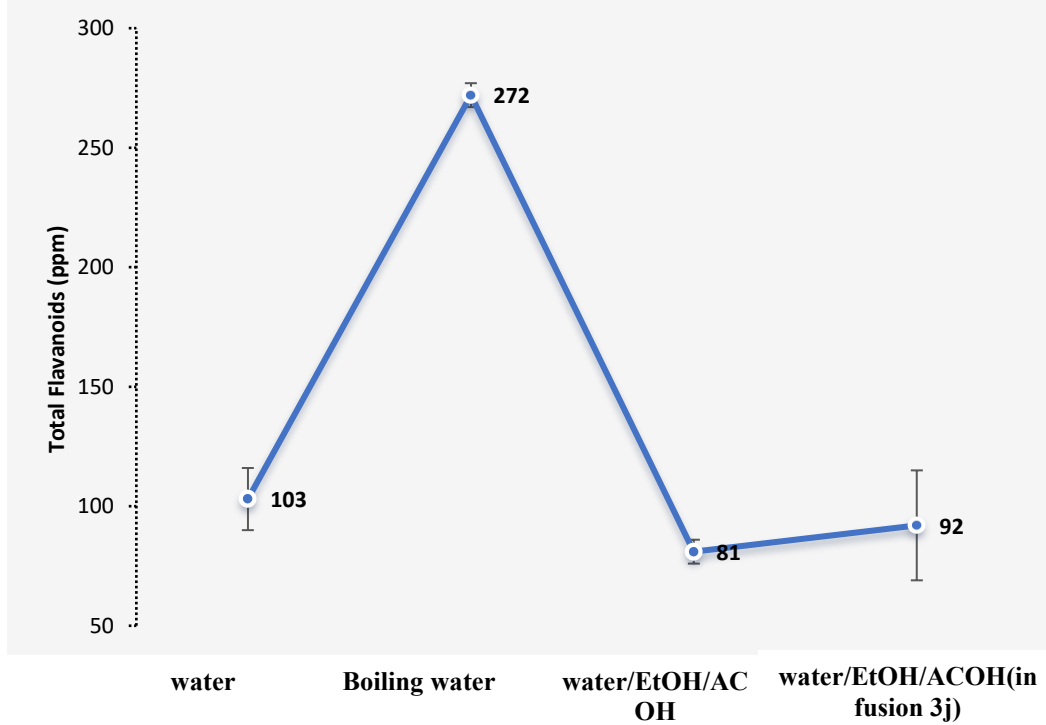


Figure 18: Total flavanoid content of extracts (acetone/water/acetic acid) of different parts of *V.amygdalina*

## 5.8 Solvent Extraction Methods for Flavanoid Yield

The solvent system plays a crucial role in the efficiency of flavonoid extraction. The boiling water extraction method proved to be the most effective, yielding 272 ppm of flavonoids (Figure 19), significantly higher than other solvents, such as the ethanol-acetic acid-water mix and the cold water extraction. The higher extraction efficiency with boiling water may be attributed to the breakdown of cellular structures, enhancing the release of flavonoids into the solution (Toniolo et al., 2012).

Figure 19: Flavanoid content of *V. amygdalina* leaf extracts by different solvent systems



## CHAPTER 6: CONCLUSION AND RECOMMENDATION

This study aimed to evaluate the antioxidant potential, polyphenol content, and flavonoid activity of different parts of *Vernonia amygdalina* (leaves, bark, and flowers), and how various extraction methods influence these bioactive components. The findings provide significant insight into the antioxidant activities of *Vernonia amygdalina* and highlight its potential as a source of natural antioxidants for use in various applications, such as in the brewing industry as a substitute for hops.

The ORAC method revealed that the bark of *Vernonia amygdalina*, exhibited the highest antioxidant activity, significantly surpassing the leaves and flowers. Although the antioxidant values for the leaves in this study (1433–1513  $\mu\text{mol Trolox eq/g}$ ) were lower than those previously reported, they still surpassed the values of hops commonly used in brewing (614  $\mu\text{mol Trolox eq/g}$ ). This suggests that *Vernonia amygdalina* could be a viable alternative for breweries seeking natural sources of antioxidants, particularly for regions where hops are less accessible.

In terms of polyphenol content, the bark exhibited the highest concentration (28,010 ppm), closely followed by the leaves, further supporting the ORAC findings. The use of boiling water as an extraction method was particularly effective, yielding higher polyphenol content compared to other solvents. This highlights the efficiency of heat in extracting bioactive compounds from *Vernonia amygdalina*, making it suitable for industrial applications where heat is typically applied.

The flavonoid content was also assessed, with the flowers showing the highest levels of flavonols and flavones (8686 ppm), followed by the bark and leaves. Boiling water proved to be the most effective extraction method for flavonoids, further corroborating the results obtained for polyphenols.

These results not only confirm the antioxidant, polyphenol, and flavonoid potential of *Vernonia amygdalina* but also emphasize the importance of extraction methods in maximizing the bioactive components. The findings suggest that using *Vernonia amygdalina* in brewing or other applications could offer significant health benefits, primarily due to its antioxidant and polyphenol properties.

## Recommendations

**Further Research on Bioactive Components:** Future studies should explore other bioactive compounds within *Vernonia amygdalina* to fully assess its potential beyond antioxidants and polyphenols. Comprehensive phytochemical analysis can reveal additional compounds with medicinal value, such as alkaloids or saponins.

**Optimization of Extraction Methods:** Since boiling water and specific solvent mixtures proved to be effective for extracting polyphenols and flavonoids, further optimization studies on these methods could enhance yield and efficiency, potentially lowering production costs for industrial use.

**Long-Term Storage and Stability Studies:** The stability of *Vernonia amygdalina* extracts should be evaluated to assess how antioxidant activity and polyphenol content change over time, particularly under different storage conditions. This is crucial for commercial applications, especially in the food and beverage industry.

**Application in Brewing Industry:** Given the high antioxidant activity of the bark and leaves, *Vernonia amygdalina* could serve as a potential substitute for hops in brewing. Further studies should investigate the sensory profile and stability of beverages made with *Vernonia amygdalina* extracts to evaluate its feasibility for large-scale production.

**Sustainability and Local Use:** As *Vernonia amygdalina* is widely available in Rwanda and other parts of Africa, it could provide an eco-friendly, locally sourced alternative to imported antioxidants in various industries, promoting local agricultural development and sustainability.

Research into the economic and environmental impacts of cultivating *Vernonia amygdalina* should be pursued to assess its potential as a cash crop for farmers in Africa.

By investigating these aspects, *Vernonia amygdalina* could become a key resource in enhancing the antioxidant content of food and beverages, with broader implications for public health and economic development.

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