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Phytochemical analysis and antioxidant activity of the stem bark extract of Vernonia amygdelina in management of malaria.

By

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A research report submitted to the Department of Chemistry in partial fulfilment of the requirement for the award of a degree of Bachelors of Science Education of Busitema University.

May, 2024

DECLARATION

I AKELLO NORAH declare that this work has been done by myself. In addition, the work has not been submitted for any other degree or professional qualification. I confirm that the work is my own. My contribution and those of other authors to this work have been explicitly indicated below. Appreciate credit has been granted to the origin of some work in the thesis where reference has been made to the creation of others.

DATE 13th/05/2024.....SIGN.....

AKELLO NORAH

BU/UP/2021/1525

APPROVED AND SUPERVISED BY

DR. OWOR RICHARD ORIKO

DATE ...13/5/2024.....SIGN..

DEDICATION

With a Godly heart and much love, I do dedicate my work to my beloved mother, Omugu Betty for her endless words of encouragement and financial support towards my career.

ACKNOWLEGEMENT

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ABSTRACT

Redox imbalance between reactive oxygen species (ROS) generation and their neutralization by antioxidants in the system leads to accumulation of ROS in the system resulting into oxidative stress. Antioxidants guard against reactive oxygen free radical. Antioxidants neutralize free radicals by adding electrons to them, preventing bodily harm. Earlier studies have shown that artemisinins reduce the levels of reduced glutathione (GSH) and other antioxidants in the parasite thereby inducing oxidative stress (OS) in the parasite leading to their destruction. In traditional medicine, use of plants or their crude products as antioxidants have been reported. In this study the stem bark extract of *V. amygdelina* was phytochemically investigated and its antioxidant potential evaluated. The extract was prepared using methanol and screened for presence of saponins, flavonoids, alkaloids, tannins, phenols and glycosides. Quantitative determination of phytochemicals (phenols, alkaloids, flavonoids and tannins) was done using UV spectrophometer. Total phenol content was 33.45mg/g, flavonoids was 81.95mg/l, tannins was 66.42mg/g and alkaloids was 413.76mg/g. Antioxidant activity was determined using DPPH free radical scavenging that gave a percentage of 46.04%.The findings showed that the stem bark extract of *V. amygdelina* has antioxidant activity. Thus, phytochemicals (alkaloid, flavonoids, phenols, tannins, saponins, glycosides) observed in the stem bark extract of *V. amygdelina* could be responsible its antioxidant activity, however, there is need to formulate the syrup to determine its efficacy of antioxidant activity.

ACRONYMS

RBC: Red Blood Cells

SOD: Superoxide dismutase

ROS: Reactive oxygen species

DPPH: 2, 2 -diphenyl-1-picrylhydrazyl

FRAP: Ferric reducing ability of plasma

CUPRAC: Cupric reducing antioxidant capacity

ABTS: 2, 2-azino-bis (3- ethylbenzothiazolin-6-sulphonic acid)

MDA: Malondialdehyde

Chapter:1 INTRODUCTION

1.1 BACKGROUND

The word “malaria” comes from the Italian word “mal’aria” for “bad airs.” It was not until the 1880s and 1890s that Alphonse Laveran, Ronald Ross, Battista Grassi, were able to identify the plasmodium parasite and link the transmission of malaria to mosquitoes (Mougneau et al., 2011). Plasmodium is a parasite that feeds on blood during which it invades the red blood cells (RBC) in humans and destroy them causing shock and fever characterized with high temperatures, headache, chill, joint pain and vomiting (Studies et al., 2014). Plasmodium parasite also generates ROS via hemoglobin degradation resulting in the release of redox active byproducts, free haeme and H_2O_2, conferring oxidative insult on the host cell (Müller, 2004)

Malaria is caused by principally five *Plasmodium* species namely; *P. malariae*, *P. ovale*, *P. vivax, P.Knowles* and *P.falciparum* that is transmitted to humans through the bites of the female mosquitoes of the genus *Anopheles* (Rawal, 2020). But *P. falciparum* and *P vivax* are responsible for most malaria deaths, in humans. However, in Africa, *P.falciparum* is the most common cause of malaria than *P. vivax*(Janouskovec et al., 2019).

Malaria is one of the most dangerous diseases in the world which is amongst the most prevalent diseases worldwide. The disease especially in Africa and Asia continue to be a major threat to mankind. Malaria threatens the lives of 40% of the world’s population and over 2200 million people are affected by the disease(Lines, 1994). Each year, there are an estimated 300-500 million clinical cases and an estimated 660 000 deaths where it kills a child below five years every 30 seconds (Masu & Muloiwa, 2019). Malaria is estimated to kill between 1-3 million people annually, the majority of who are young children. Ninety per cent of malaria cases in the world occur in Sub-Sahara Africa (Patouillard et al., 2017).

Children under 5 years of age and pregnant women are the most affected by malaria and it is one of the leading causes of death among young children. Malaria during pregnancy causes severe maternal illness and anemia, and is also associated with low birth weight among newborn infants; a leading risk factor for infant mortality (Guyatt & Snow, 2004). Apart from being a major public health problem with high morbidity and mortality, it has also culminated other major socioeconomic problems causing global instability as well as poverty. (Luxemburger et al., 2001),

Immune response to malaria infection results in increased generation of reactive oxygen species (ROS) from phagocytic cells recruited in the process of combating the infection resulting into erythrocyte damage and anaemia(Pawłowska et al., 2023). Reactive oxygen species are double aged sword, being necessary for vital physiologic functions at low concentrations, while leading to lipid peroxidation, oxidative DNA and tissue damage at higher levels(Bouayed & Bohn, 2010).

Redox imbalance between ROS generation and their neutralization by antioxidants in the system leads to accumulation of ROS in the system resulting into oxidative stress (OS) (Tripathy & Kumar Mohanty, 2017). Parasitemia induced OS has been implicated in the physiopathology of malaria and the development of various complications associated with severe malaria(Pawłowska et al., 2023).

Complex host-parasite interactions modulate the balance between antioxidants and ROS since both the host and parasite have the capacity of generating them(Becker et al., 2004). This is compounded by the observation that some antimalarial drugs such as chloroquine, primaquine and artemisinin and their derivatives induce ROS production and therefore constitute source of oxidation. Earlier studies have shown that artemisinins reduce the levels of reduced glutathione (GSH) and other antioxidants in the parasite thereby inducing OS in the parasite leading to their destruction (Chakraborti et al., 2019).

However, due to the complexity of the parasite life cycle and transmission of the disease, combating it has been a very difficult task to overcome. So many, antimalarial drugs such as quinine (derived from the *cinchona bark*) and artemisinin (from *qinghao*) chloroquine, amodiaquine, used in the nineties are no longer effective in treating malaria(Gregson & Plowe, 2005). Rather, malaria parasite has become resistant against these treatments and continues to develop new mechanisms for survivals(Lines, 1994).

Thus, this calls for need for an alternative against malaria and medicinal plants continue to be an attractive source of antimalarial drugs. Therefore, in this study, *V. amygdelina* in the genus Vernonia will be investigated to identify bioactive components found in it, determine its antioxidant activity and to formulate a herbal syrup from its stem bark extract for management of malaria.

1.2 STATEMENT OF THE PROBLEM

A large number of people especially children below the age of 5 years and pregnant women die as a result of malaria. Malaria is estimated to kill between 1-3 million people annually, the majority of who are young children (Patouillard et al., 2017). Malaria is caused by plasmodium falciparum. Plasmodium falciparum generates ROS via hemoglobin degradation resulting in the release of redox active byproducts, free haeme and H_2O_2, conferring oxidative insult on the host cell, erythrocyte. Redox imbalance between ROS generation and their neutralization by antioxidants in the system leads to accumulation of ROS in the system resulting into oxidative stress (Müller, 2004).

The ministry of health together with the natural drug authority has recommended so many antimalarial, antioxidant drugs such as chloroquine, quinine, amodiaquine, artemisinin (Nguta et al., 2010). However, due to the complexity of the parasite life cycle and transmission of the disease, plasmodium falciparum has become resistant against these treatments and continues to develop new mechanisms for survivals making the treatment of malaria still challenging (Mita & Tanabe, 2012).

Therefore, there is a crucial need for an alternative and traditional medications (local herbs) have been the option to replace these drugs. *V. amygdelina* in the genus Vernonia is known to have antimalarial activity but its antioxidant activity is not yet known. Thus, this study aims at identifying bioactive components present in *V amygdelina*, determine the efficacy of its antioxidant activity and to formulating herbal syrup from its stem bark extract for management of malaria.

1.3 **RESEARCH OBJECTIVES**.

1.3.1 GENERAL OBJECTIVE.

To investigate the phytochemical composition and antioxidant activity of the stem bark extract of *V amygdelina* for management of malaria.

1.3.2 SPECIFIC OBJECTIVES.

1. To determine the phytochemical composition of the stem bark extract of *V amygdelina*.

2. To analyze the efficacy of antioxidant activity of the stem bark extract of *V amygdelina* for management of malaria.

1.4 JUSTIFICATION

Most of the antimalarial drugs have been derived from medicinal plants including quinine, chloroquine, amodiaquine are derived from the *cinchona bark* and artemisinin is derived from *qinghao* (Nguta et al., 2010). Therefore, it is also possible to identify interesting antimalarial compounds from the stem bark extract of *V. amygdelina*.

1.5 SCOPE.

This research will focus on explorative studies on the stem bark extract of *V. amygdelina*. It will majorly aim at the comparative analysis of the quantities of bioactive components found in both aqueous and organic extracts of the stem bark of *V. amygdelina* and the research will be purely experimental

Chapter:2

Chapter:3 LITERATURE REVIEW.

3.1 Description of *V. amygdelina*

In recent years, the use of herbal products for preventive and therapeutic purposes has been increasing among the population. This is mostly due to the belief that 'natural' implies safety(Loundou, 2008). The Vernonia genus has about one thousand species, and members are widely used as food and medicine. Vernonia species can easily adapt to their habitat according to different environments(Babu et al., 2023). In forests, they can be found next to water sources but also in forest margins, woodlands, grasslands up to 2800 m in altitude, and a mean annual rainfall of 750-2000 mm. Vernonia is the largest genus with approximately 1000 species of shrubs, out of which *V. amygdelina* is the most prominent(Toyang & Verpoorte, 2013).

V amygdelina is scientifically classified into the kingdom Plantae that is an angiosperm of the order Asterales. *V. amygdelina* belongs to the Asteraceae family and genus Vernonia(Danladi et al., 2018). The Asteraceae family consists of herbs, shrubs, or, less commonly, trees. They are considered the largest family of flowering plants with approximately 1620 genera and about 23,600 species(Wulchafo, 2019). *V amygdelina* is usually cultivated by stem planting, and it doesn't produce seeds. *V. amygdelina* is a perennial soft wooded shrub with a height of about 2 to 10 m with stem diameter up to 40 cm (Jayaweera et al., 2022).

Leaves of plant are petiolate in shape arranged alternatively to each other usually 10-15 x 4-5 cm in size and color is medium to dark green, with or without sparse hairs above, with fine, soft, pale hairs below and conspicuous red-veining; apex and base tapering, base always almost symmetric, margin entire or very finely toothed. Flower heads are 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 regular and bisexual, and then they develop into fruits (Jayaweera et al., 2022).

The shrub's bark is densely pubescent at the early stage; when the plant gets matured, the bark turns from grey to brown(Mark S. Ashton et al., 1997). The fruits are shaped into 10-ribbed achene lengths up to 1.5 – 3.5 mm. The most frequently used member of the Vernonia genus is Vernonia amygdelina (VA). VA is commonly known as a bitter leaf due to the bitter taste of the leaves(Egharevba, 2014). The shrub is commonly found in tropical countries like Kenya, Uganda,

and Tanzania, where it is domesticated. It can also be found in some regions of Asia, including Malaysia. VA is consumed as a green leafy vegetable throughout West and Central Africa as a source of due for its nutritional and medicinal properties (Cowen, 1952).

V amygdelina is used as a medicinal herb from the time when zoo pharmacologists observed that the chimpanzees sucked juice of *V. amygdelina* to enhance their body fitness and increase strength. Their appetite was also found to be enhanced with reduction in problems like constipation and diarrhea(Yeap et al., 2010). The Bitter taste of plant was ideal identification to choose the appropriate plant and plant part for intake. The native of Africa especially the illiterate and economically weak patient used to use this plant for the medicinal purpose, due to cultural and economic reasons(Egharevba, 2014).

V. amygdelina was used traditionally for various diseased conditions such as leaves and roots of plant were used for Stomach disorder, skin wound, diarrhea, scabies, ascariasis, tonsillitis, and fever and worms' infection(Sofowora et al., 2013). It is also useful in Malaria, fever, constipation, diabetes, anti-helminth(Fawwaz et al., 2023). *V. amygdelina* has been reported to be safe and considered as a lead herbal drug candidate with good efficacy, fewer side effects, and reduced toxicity for clinical trials(Bahekar & Kale, 2013). Both written and oral literature indicates that *V. amygdelina* has been used as a medicinal herb for a long period of time(Arekemase et al., 2013).

Figure 1 Shows stem bark of *V.amygdelina*

3.2 **The phytochemical analysis of *V. amygdelina***

The qualitative phytochemical analysis of *V amygdelina* revealed that both phlobatannins and anthraquinones are absent in all the extracts. It also confirms the presence of saponin, tannin, flavonoid, terpenoids and cardiac glycosides in all the extracts(Yadav et al., 2014). Quantitative

phytochemical constituents and it reveals saponin as the most abundant phytochemical in all the extracts (methanol, ethyl acetate, hot water, and cold water). African stem bark was found to contain steroids/triterpenoids, glycosides, saponins, tannins, and flavonoids(Akinyemi et al., 2023). The extracts were prepared with the reflux method using methanol, butanol, isopropanol, ethyl acetate, chloroform, and n-hexane(Farid et al., n.d.).

3.3 The antimalarial activity of *V. amygdelina*

V. amygdelina was traditionally used for the treatment of malaria in southern region of Nigeria, Kenya, Uganda, and Asia(Suleman et al., 2018). There are also some reported studies which show the activity of ethanolic extract of plant against Plasmodium berghei in the dose dependent manner, Omoregie. The aqueous extract of *V amygdelina* possesses anti-malarial activity on Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae

3.4 The anti-oxidant activity of *V amygdelina*

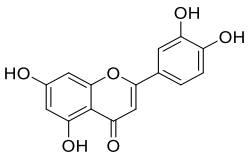
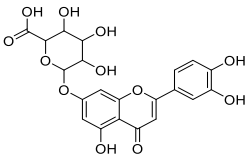
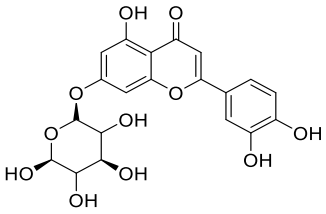
Antioxidant activity measurement methods have improved greatly in recent decades. Thus, early antioxidant efficiency assessments focused on lipid oxidation (Yilmaz et al., 2003). Antioxidant activity has been measured by scavenging multiple forms of free radicals or ROS, reducing power, and metal chelation using various chemical tests and susceptible and automated detection systems(Mbow et al., 2022). Oxidizing substrates have expanded from food model systems to chemical substances, biological materials, cellular pathways, and even living tissues(Floegel et al., 2011).

Numerous tests, including the DPPH, FRAP, CUPRAC, ABTS, Hydroxyl radical scavenging, and phenanthroline techniques, are available for the direct assessment of hydrogen atom transfer or electron transfer from antioxidants to free radicals(Shah & Modi, 2015). The findings show that *V. amygdelina*. extract exhibit antioxidant activity(Farombi & Owoeye, 2011). According to the findings, *V amygdelina* stem bark extract with methanol solvent provided the maximum antioxidant activity(Rafael et al., 2023).

With respect to *V. amygdelina,* in order to combat common antioxidants like ascorbic acid, gallic acid, and quercetin that are polar and represent the structure of all antioxidants in general that leaves are utilized, propagated for consumption as a vegetable due to its medicinal properties(Rafael et al., 2023).

The spray dried water extract of the stem bark of *V. amygdelina* was tested with DPPH radical scavenging activity and the results of this study showed significant increase in IC_{50} in comparison to vitamin C and E treated groups(Shalaby & Shanab, 2013). In this study, the activity of antioxidant enzymes such as SOD, MDA level and total antioxidant capacity was evaluated for 14 days. The SOD activity was significantly increased in in both *V. amygdelina* extract and vitamin c treated groups. This effect was associated with decreased MDA level, which is a major metabolite of lipid peroxidation (Malik Al-Rubaei et al., 2014). The anti-oxidant ability of *V. amygdelina* extract that can enter in blood plasma is far more in comparison to vitamin C. The reason for the antioxidant activity of *V amygdelina* should be the presence of flavonoids such as (Luteolin, luteolin 7-O-β-glucuronide and luteolin 7-O-β- glucoside) which are reported to have antioxidant properties(Mbow et al., 2022).

Table 1 shows the structures of Luteolin, luteolin 7-O-β-glucuronide and luteolin 7-O-β- glucoside

Luteolin		(Mbow et al., 2022)
Luteolin 7-β-glucuronide		(Mbow et al., 2022)
Luteolin 7-β-glucoside		(Mbow et al., 2022)

Chapter:4 METHODS AND MATERIALS

4.1 Collection of plant material

The fresh stem bark of *V. amygdelina* was collected from its natural habitat from Namwaya village, West Mudama County, Tororo district. Dust materials were removed from the plant materials by washing with distilled water dried at room temperature under shade. The shade dried plant samples were ground into powder using an electric motor and the powder of the sample was stored in an air tight bag (container).

4.2 Extraction

4.2.1 Organic extraction (OE).

A 10g portion of the shade dried powdered sample of the stem bark of *V. amygdelina* was dissolved in 100ml of 80% methanol in a conical flask. The mixture was shaken for 5minutes using a vortex machine after which the mixture was heated at 45^0c for 15minutes using a boiler stirring machine .The mixture was cooled and then filtered using Whatsman No 1 filter paper to get rid of the suspended particles. The filtrate was concentrated to dryness using a rotary evaporator to obtain a paste. The extract was preserved in airtight container at 4°c for further use.

4.3 Phytochemical tests

Organic extracts of the stem bark of *V amygdelina* was subjected to various phytochemical tests to identify the various bioactive components in *V amygdelina* as stated in the literature(DA COSTA et al., 2020). The tests were as follows:

4.3.1 Test for flavonoids.

Ferric chloride test:

3 drops of neutral ferric chloride solution were added to 1 mL of an alcoholic solution of a crude extract (dissolve a little part of crude extract in ethyl alcohol and filter) using a test tube. Formation of blackish red colour indicated the presence of flavonoids

Lead-acetate test:

2 drops of aqueous basic lead acetate solution were added to 1 mL of alcoholic extract using a test tube. The appearance of a reddish-brown bulky precipitate indicated the presence of flavonoids.

4.3.2 Test for glycosides.

Sulphuric acid test:

1 mL of aqueous solution of crude extract was added to 1 mL of concentrated Sulphuric acid and allow to stand for 2 min. The formation of reddish colour indicated the presence of glycosides.

4.3.3 Test for saponins.

A little part of the crude extract was mixed with 20 mL of distilled water and then agitated in a graduated cylinder (test tube also can be used) for 10 min. Foam formation indicated the presence of saponins.

4.3.4 Test for Alkaloids

Wagner's test:

1 mL of Wagner's reagent (iodine in potassium iodide) was added to the 1 mL of an acidic solution of crude extract. The formation of reddish-brown precipitate indicated the presence of alkaloids.

Dragendorff's reagent test:

2 mL of Dragendorff's reagent (a solution of potassium bismuth iodide prepared from basic bismuth nitrate, tartaric acid, and potassium iodide) and 2 mL of diluted hydrochloric acid were added to the acidic solution of crude extract. An orange-red colour precipitate indicated the presence of alkaloids.

4.3.5 Test for phenols compounds:

Ferric chloride test:

1 mL of ferric chloride solution was added to 5 mL of an aqueous/ alcoholic solutions of crude extract (dissolve little part of crude extract in 5 mL of water or ethyl alcohol and filter) using a test tube. Formation of an intense colour indicated the presence of phenols.

4.3.6 Test for steroids.

Salkowski test:

1 mL of concentrated Sulphuric acid was added to 5 mL of a chloroform solution of crude extract (dissolve a little part of crude extract in chloroform and filter) using a test tube. After shaking, the

test tube allows standing for 5 min. Lower layer turning into red in colour indicated the presence of steroids.

Liebermann-Burchard test:

2 drops of acetic anhydride was added to 5 mL of a chloroform solution of crude extract followed by adding 1 mL of concentrated Sulphuric acid through the wall of the test tube and allowed to stand for 5 min. Formation of a reddish-brown ring at the junction of the two layers and a green colour in upper layer indicated the presence of steroids.

4.3.7 Test for tannins

Ferric chloride test:

2 drops of ferric chloride solution were added to 1 mL of aqueous solution of crude extract (dissolve a little part of the crude extract in 1 mL of distilled water and filter) using a test tube. A blackish precipitate indicated the presence of tannins.

Lead acetate test:

3 drops of aqueous basic lead acetate solution were added to 1 mL of aqueous solution of crude extract using a test tube. Reddish-brown bulky precipitate indicated the presence of tannins.

4.3.8 Test for terpenoids

Fresh plant material was treated with 5 mL of 1% aqueous hydrochloric acid for about 4 h. The extract thus obtained is treated with 1 mL of Trim-Hill reagent (10 mL of acetic acid, 1 mL of 0.2% copper sulphate in water and 0.5 mL of concentrated hydrochloric acid) in a test tube followed by heating in a water bath. There was no observable change which indicated the absence of terpenoids.

4.4 QUANTITATIVE ANALYSIS

4.4.1 Total Phenol content

Folin-Ciocalteu method is widely used to estimate the total phenol content in a plant extract. In this method, a methanolic solution of the extract (1 mg/ml) is added to 2.5 ml of 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml 7.5% Na_2CO_3 or $NaHCO_3$. Blank is similarly prepared which contains 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml of 7.5% of Na_2CO_3 or NaHCO3. Thereafter, the samples are incubated in a thermostat at 45 °C for 45 min. The absorbance is determined using a spectrophotometer at λmax 765 nm. The samples should be construed. Based on the measured absorbance, the concentration of phenolics is read (mg/ml) from the calibration line. Thereafter, the total phenol content in a plant

extracts is expressed in terms of gallic acid prepared in triplicate for each analysis so that a mean value of absorbance can be obtained. The same procedure should be repeated for the standard solution of gallic acid and the calibration line is equivalent (mg of GA/g of extract

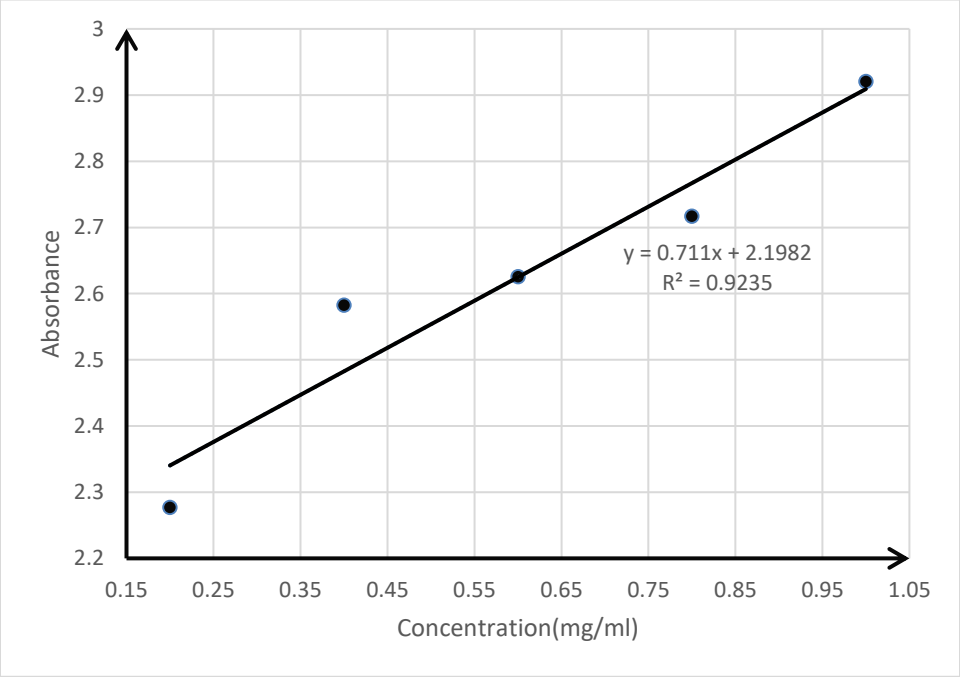


Figure 2: Standard calibration curve for phenols

The absorbance of phenols in the extract of *V. amygdelina* at 292nm was obtained using the UV spectrometer and recorded in table 8 below.

From figure 6, the regression equation is $y = 0.711x + 2.1982$

Where y is absorbance and x is concentration.

Total quantity of phenols $= \frac{GAE \times 1 \times 100}{weight\ of\ sample}$

GAE is gallic acid equivalent, 1 is dilution factor and 100 is the volume of 80% methanol used to dissolve the sample.

4.4.2 Total Flavonoid contents

The aluminium chloride spectrophotometric assay was used to estimate total flavonoid content in an extract. In this method, the sample contained 1 ml of a methanol solution of the extract in the

concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution was dissolved in methanol. The samples were incubated for 1 h at 25 °C. The absorbance was determined using spectrophotometer at λ_{max} 415 nm. The samples were be prepared in triplicate for each analysis so that a mean value of absorbance can be obtained. The same procedure was repeated for the standard solution of any flavonoid such as quercetin and the calibration line is construed. Based on the measured absorbance, the concentration of flavonoids is read (mg/ml) on the calibration line. Thereafter, total flavonoid content in a plant extracts was expressed in terms of quercetin equivalent (mg of QU/g of extract). Rutin can also be used as a standard in place of quercetin and the results can be expressed in terms of rutin equivalent (mg of RU/g of extract).

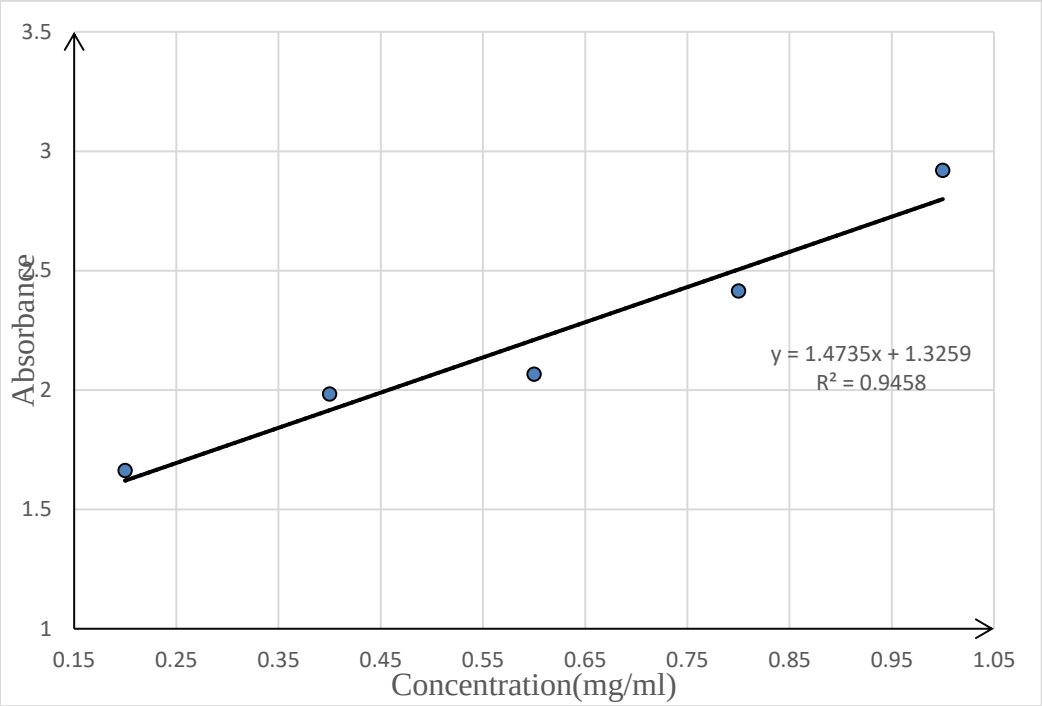


Figure 3: Standard calibration curve for flavonoids

The absorbance of flavonoids in the extract of V. amygdelina at 420nm was using the UV spectrometer as shown in table 4 below

The regression equation is $y = 1.473x + 1.3259$

Where y is absorbance and x is the concentration.

$$\text{Total quantity of flavonoids} = \frac{QE \times 1 \times 100}{weight\ of\ the\ sample}$$

Where QE is quercetin equivalent, 1 is dilution factor and 100 is the volume of 80% methanol used to dissolve the sample.

4.4.3 Total alkaloid content:

A solution of 1 mg/mL of plant extract was prepared using dimethyl sulfoxide (DMSO). 1 mL of 2 M HCl was added to 1 mL of DMSO dissolved extracts and the resulting mixture is filtered using filter paper. The filtrate was transferred to a 250 mL separating funnel and to this solution, 5 mL of 0.1% Bromocresol green (dissolved in methanol) was added followed by 5 mL of phosphate buffer (pH 6.6). Chloroform (1 mL) was added into the separating funnel and the mixture was vigorously shaken, after which the funnel was allowed to stand to allow the mixture to separate into different layers. The lower layer was collected in a 10 mL volumetric flask. The process was repeated with 2, 3, and 4 mL of chloroform. Atropine was used to construct a standard curve using a concentration range of 1.0–0.0625 mg/mL. The absorbance of the sample and standard solutions was recorded at a wavelength of 470 nm against a reagent blank. The total alkaloid content was expressed as milligram atropine equivalent/ gram of extract (mg AE/g). All the measurements are evaluated in triplicate.

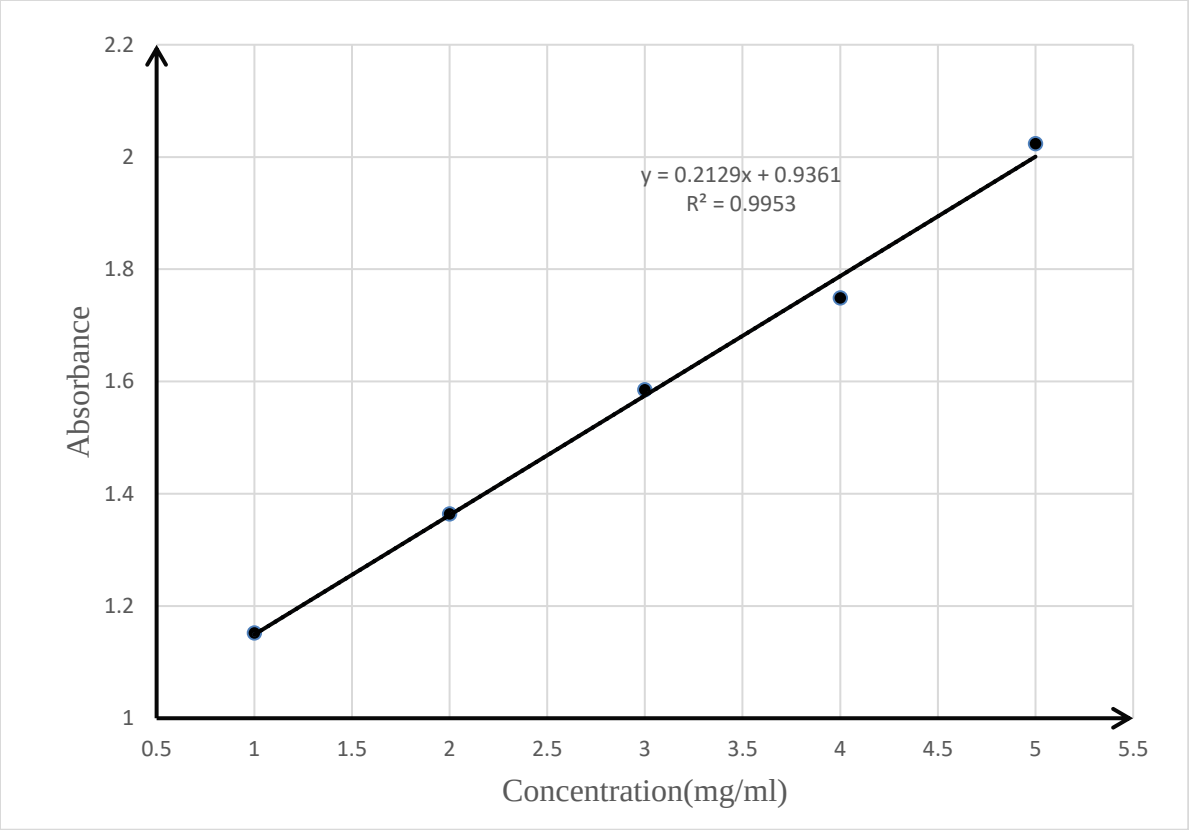


Figure 4: Standard calibration curve for alkaloids

The absorbance of alkaloids in the extract of V. amygdelina at 292nm was obtained using UV spectrophotometer and recorded in table 10 below.

From figure 7, the regression equation is, $y = 0.2129x + 0.9361$

Where y is absorbance and x is the concentration

Total quantity of alkaloids $= \frac{AE \times Dilution\ factor \times 100}{weight\ of\ the\ sample}$

Where AE is Atropine equivalent, 100 is the volume of 80% methanol used to dissolve the sample.

4.4.4 Total tannins:

To quantify the tannin content, the Folin-Ciocalteu method was used. Briefly, 100 µL of 10 mg/mL extracts was added to a clean test tube containing 7.5 mL of distilled water. The Folin-Ciocalteu reagent (0.5 mL) was added to the mixture and vortexed thoroughly. 10 mL of a 35% solution of sodium carbonate (Na_2CO_3) was added to the mixture. The mixture in the tube was transferred to

a 10 mL volumetric flask and the volume of the mixture was made up to 10 mL with distilled water. The mixture was shaken and kept at ambient temperature for 30 min in the dark. Gallic acid was used as a standard and reference standard solutions (1.0–0.625 mg/mL) were prepared. The absorbance for the solutions was measured at 292 nm against a blank reagent blank. Tannin content was expressed as milligram gallic acid equivalence/gram of extract (mg GAE/g). All the measurements were evaluated in triplicate.

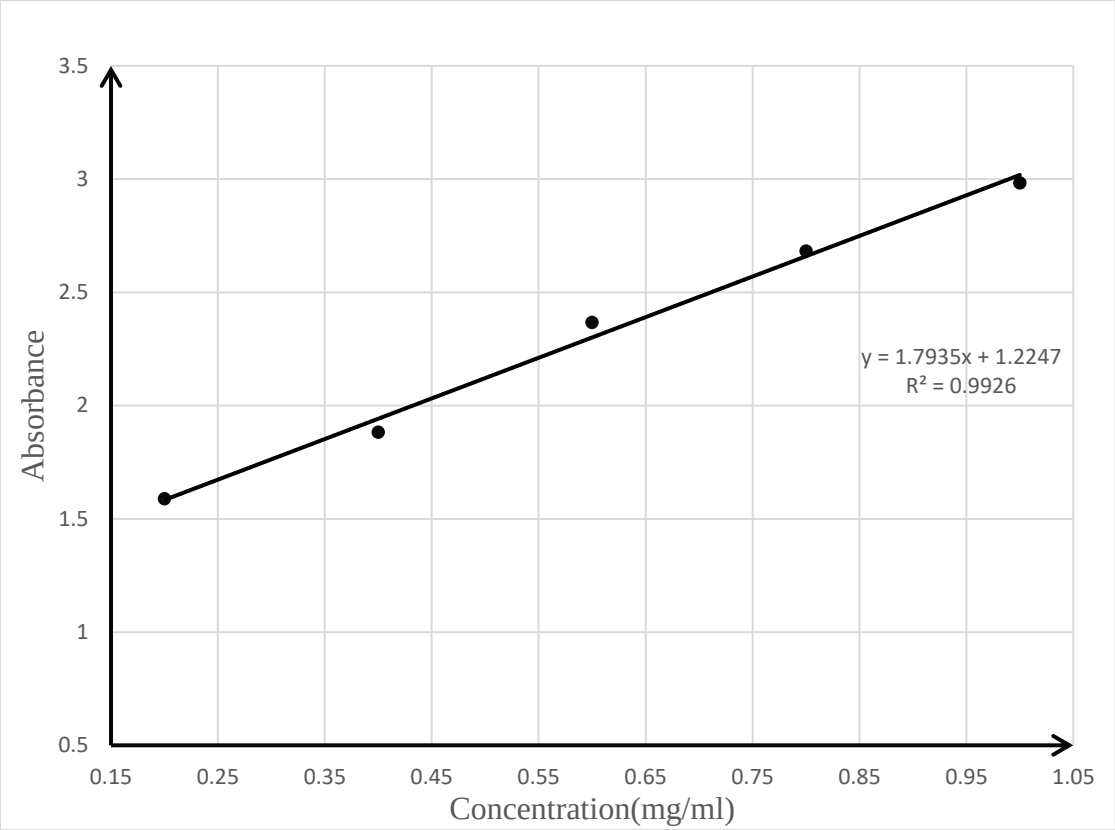


Figure 5: Standard calibration curve for tannins

The absorbance of tannins in the extract of *V. amygdelina* at 292nm was obtained using UV spectrometer and recorded in table 6 below.

From figure 5, the regression equation is $y = 1.7935x + 1.2247$

Where y is absorbance and x is concentration

Total quantity of tannins $= \frac{GAE \times Dilution\ factor \times 100}{weight\ of\ sample}$

Where GAE is gallic acid equivalent, 100 is the volume of 80% methanol used to dissolve the sample.

4.5 Testing for Free Radical Scavenging Activity

4.5.1 DPPH radical scavenging activity

This particular technique was based on the degradation of DPPH radical (2,2-diphenyl-picryhydrazyl). The DPPH radical is violet colored, the addition of antioxidant basically reduced this radical and caused the mixture to decolorize. This radical decolourization measured by spectrophotometer 446nm was proportioned to concentration of antioxidant.

Briefly, the sample stock solutions (1mg/m1) were diluted with 80% methanol to final concentrations of 60, 120, 125, 500 and 1000µm/ml. 2ml of each of the sample solutions at different concentrations was mixed with 1ml of 0.25Mm DPPH solution in methanol and allowed to react at room temperature in dark for 30 minutes. The change in color was observed in terms of absorbance using a UV- spectrometer at 446nm. And 1 ml of methanol served as a control. L-ascorbic acid was used as the standard antioxidant. The percentage of scavenging activity was calculated using the formula,

$$\% \text{ Inhibition } = \frac{(Ao - A)}{Ao} \times 100$$

Where Ao was the absorbance of the control (without extract) and A was the absorbance in the presence of the extract.

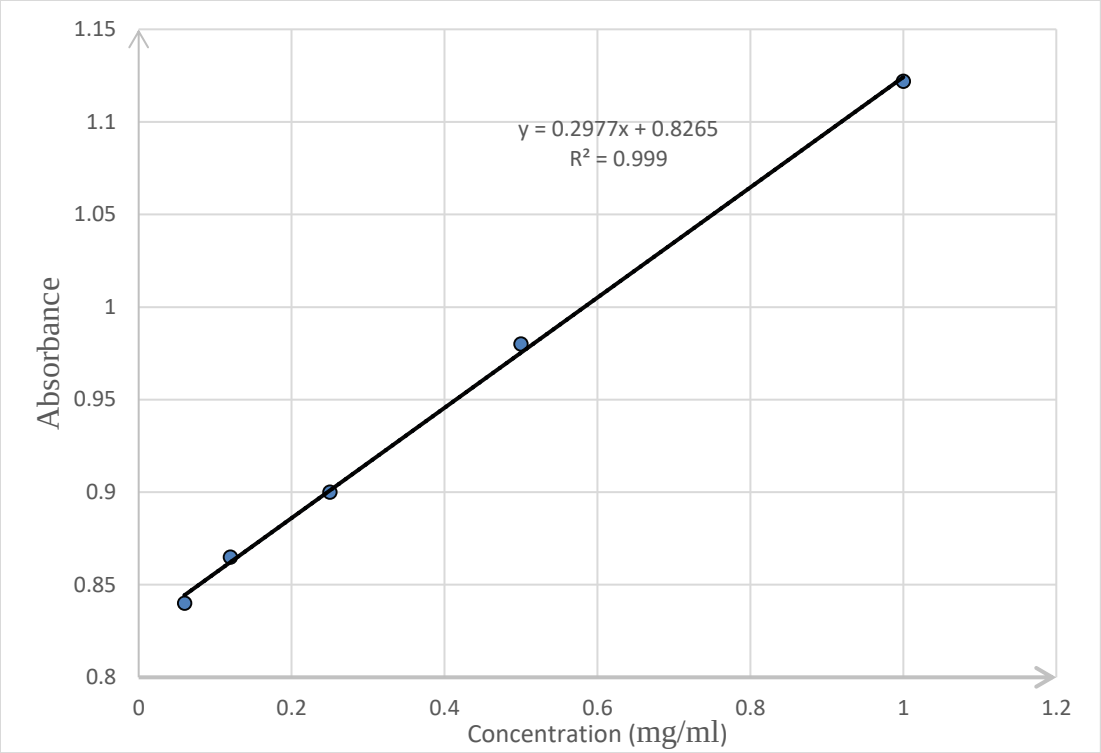


Figure 6: Standard calibration for antioxidant

% Inhibition $= \frac{(Ao-A)}{Ao} \times 100$

$$= \frac{(1.655 - 0.893)}{1.655} \times 100$$

$$= 46.04\%$$

Chapter:5 RESULTS AND DISCUSSION

5.1 Phytochemical analysis

In this study, the phytochemical screening was carried out to determine the bioactive components presence in the stem bark extract of *V. amygdelina*. The phytochemicals present were saponins, alkaloids, flavonoids, tannins, phenols and glycosides as shown in table 2. Total content of each phytochemical present was determined and expressed in mg/g of the extract. The stem bark extract of *V.amygdelina* was found to contain very high amounts of alkaloids (413.76mg/g), moderate amounts of flavonoids (81.95mg/g) and tannins (66.42mg/g) and relatively low amounts of phenols (33.45mg/g) (table 2). Very high amounts of alkaloids could be responsible for the bitterness of *V. amygdelina* (Egharevba, 2014). The findings on phytochemical components is in line with an earlier report on the plant. African stem bark was found to contain steroids/triterpenoids, glycosides, saponins, tannins, and flavonoids(Akinyemi et al., 2023). Quantitative phytochemical analysis revealed saponin as the most abundant phytochemical in all the extracts (methanol, ethyl acetate, hot water, and cold water) (Yadav et al., 2014)..

Table 2: Results of phytochemical tests of organic extract of V. amygdelina

Phytochemical	Test	Quantity Present	Quantified amount
Flavonoids	Ferric chloride test	++	
	Lead acetate test	++	81.95
Steroids	Salkowski test	+	
	Liebermann- Burchard test	+	ND
Tannins	Ferric chloride test	++	
	Lead acetate test	++	66.42
Saponins	Distilled water test	+++	ND
Phenols	Ferric chloride test	+	33.45
Glycosides	Sulphuric chloride test	++	ND
Alkaloids	Wanger's test	+++	
	Dragendorff's reagent test	+++	413.76
Terponoids	Salkowski test	-	-
Key: strongly present +++, moderately present ++, weakly present +, absent -, ND is not determined			

5.2 ANTIOXIDANT ACTIVITY

Antioxidant activity of the extract was evaluated using DPPH radical scavenging activity. The average absorbance of DPPH radical scavenging is 0.839. The relatively high percentage of inhibition of DPPH by the stem bark extract of *V. amygdelina* (46.04%) supports its use as an

antimalarial. As compared to *A. melegueta* seeds with a percentage inhibition of DPPH radical scavenging of 38.13% (Chakraborti et al., 2019), *M. chamomilla*, 20% , *M piperita*, 30% (Shalaby & Shanab, 2013), T. vulgaris, 40% (Shah & Modi, 2015). The presence of flavonoids in the stem bark extract of *V. amygdelina* could be responsible for higher free radical scavenging activity (Mbow et al., 2022).

Chapter:6 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The findings of this study showed that *V .amygdelina* contains various phytochemicals including alkaloid, flavonoids, phenols, tannins saponins and glycosides, which supports its use as a traditional medicine. These phytochemicals may be responsible for its therapeutic properties and medicinal value. *V. amygdelina* exhibits antioxidant activity as evidenced by the relatively high percentage inhibition of DPPH radical scavenging activity (46.04%).

6.2 RECOMMENDATION

It is recommended that a herbal remedy should be formulated from the stem bark extract of *V. amygdelina* to analyze the efficacy of antioxidant activity of the stem bark extract of V amygdelina for management of malaria Further should also be carried out to identify the bioactive components responsible for its antioxidant activity.

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