IN-VITRO EVALUATION OF THE ANTIOXIDANT ACTIVITY OF ETHANOL-EXTRACT OF *Jatropha tanjorensis* STEM BARK

BY

OKAFOR, CHIBUIKE MISHACK

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GODFREY OKOYE UNIVERSITY

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A PROJECT RESEARCH REPORT SUBMITTED TO THE DEPARTMENT OF CHEMICAL SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.Sc.) IN BIOCHEMISTRY

SUPERVISOR: DR. O. C ENECHI

JULY, 2026

APPROVAL

This research titled "**In-Vitro Evaluation of the Antioxidant Activity of Ethanol-Extract of *Jatropha tanjorensis* Stem Bark**" has been assessed and approved by the Committees of Department of Chemical Sciences and Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, Nigeria.

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CERTIFICATION

This is to certify that this research titled "**In-Vitro Evaluation of the Antioxidant Activity of Ethanol-Extract of *Jatropha tanjorensis* Stem Bark**" was carried out by **Okafor, Chibuike Mishack** with Registration Number GOU/U22/BCH/314, under the supervision in the Department of Chemical Sciences, Godfrey Okoye University, Enugu, Nigeria.

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DEDICATION

The first and foremost purpose of this research project is to God alone, who has shown constant love and grace, wisdom and strength and divine guidance to make this work possible. I have taken refuge in his fidelity all this way, and it has been the reason for me to complete this research.

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and indirectly in the successful completion of this research. May God richly bless you all.

ABSTRACT

Growing demand for natural antioxidants is attributed to the adverse effects of synthetic antioxidants and resurgence of medicinal plants into use. The current investigation was undertaken to assess in vitro antioxidant activity of ethanol extract of stem bark of *Jatropha tanjorensis*. Phytochemical screening (in vitro), antioxidant vitamins (Vitamins A, C & E) and DPPH radical scavenging assay and Total Antioxidant Capacity (TAC) assay of the extract were performed according to standard lab procedures. In vitro Phytochemical screening of ethanol extract showed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Cardiac Glycosides and presence of no phenolic compounds. Moderate free radical scavenging activity was demonstrated in DPPH assay ranging between (25.68 ± 1.36 to 30.73 ± 0.72%) for the concentrations tested.The TAC of the extract was consistently high ranging from (74.98 ± 0.05% to 92.05 ± 0.05%) and was comparable with the ascorbic acid standard. Vitamin analysis revealed high level of Vitamin A (81.14 ± 0.80 mg/g) and moderate level of Vitamin C (4.78 ± 0.37 mg/g) and detectable level of Vitamin E (1.60 ± 0.12 mg/g) found in the extract suggested that these antioxidant vitamins contribute to the antioxidant activity observed. The presence of both bioactive phytochemical and antioxidant vitamins has led to the synergistic interaction, which could be responsible for the antioxidant activity of the extract. Hence, the ethanol extract of stem bark of *Jatropha tanjorensis* had shown good antioxidant capacity and may be considered as potential natural antioxidant resources for treatment and prevention of oxidative stress related diseases.

Keywords: *Jatropha tanjorensis*, antioxidant activity, DPPH, total antioxidant capacity, phytochemicals, Vitamins A, C and E, stem bar.

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LIST OF ABBREVIATION

AOAC	Association of Official Analytical Chemists
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CAT	Catalase
DCPIP	2,6-Dichlorophenolindophenol
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GPx	Glutathione Peroxidase
HPLC	High Performance Liquid Chromatography
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
ROS	Reactive Oxygen Species

SD	Standard Deviation
SOD	Superoxide Dismutase
SPSS	Statistical Package for the Social Sciences
TAC	Total Antioxidant Capacity
UHPLC-ESI-MS	Ultra-High Performance Liquid Chromatography–Electrospray Ionization–Mass Spectrometry
UV-Vis	Ultraviolet-Visible Spectrophotometer

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Reactive Oxygen species ROS or reactive oxygen species and definitions of oxidative stress: When ROS production levels in the body exceed the capacity of the body to deal with such levels via antioxidant defence mechanism The highly reactive chemical molecules which occur in nature as a byproduct of cellular respiration and include molecules such as Superoxide radical, hydroxyl radical, Hydrogen Peroxide, etc. ROS plays critical roles in various physiological activities as intercellular signals in biological organisms. Although in healthy state overproduction of ROS results in oxidative damage of major targets including lipids, protein and nucleic acids and disruption of cellular order leading to several age associated pathologies like cancers, cardiovascular diseases (CVDs), diabetes mellitus, neurodegenerative disorders, inflammatory diseases, and etc. (Huang *et al*., 2020).

Antioxidants are compounds that help living cell against oxidative damage. These compounds stabilize the reactivity of free radicals by stopping their action through donation of electron to the unstable free radical without becoming unstable itself and without initiation a harmful chain reaction, hence preserved cellular viability and maintains normal physiology. However, although it is now commonplace for synthesized antioxidants such as BHT (butylated hydroxytoluene), and BHA (butylated hydroxyanisole) to be used in pharmaceutical and Food applications to prolong oxidation the increased awareness of possible hazards associated with them has

sparked a recent demand for the discovery and utilization of alternative naturally occurring antioxidants (Igbinosa *et al*., 2011).

Phytochemical constituents like flavonoid, phenolic acid, tannin, alkaloids, saponins, and terpenoids in medicinal plants are responsible for exhibiting promising antioxidant activity. These naturally occurring compounds help protect cells against oxidative damage through different biological actions, including scavenging free radicals, chelating transition metal ions, limiting lipid peroxidation, and stimulating the body's natural antioxidant defence mechanisms. Because of these diverse therapeutic properties, medicinal plants continue to attract considerable scientific interest as promising sources of safe and effective natural antioxidants for disease prevention and health promotion (Igbinosa *et al*., 2011).

Jatropha tanjorensis, which is native to the tropical world and it falls under Euphorbiaceae family. It is commonly known as “Hospital too far” for the people practicing traditional healing because this plant is well recognized traditionally and used in the treatment of diseases such as anemia, diabetes mellitus, hypertension, malaria, and inflammatory conditions as well as infectious diseases. In addition, *J. tanjorensis* is one of the traditional consumed leafy vegetables in Nigeria and various other regions in West Africa for nutrition and health benefits (Omobayowa *et al*., 2021).

Many studies have shown that species in the Jatropha genus have immense medicinal significance owing to their abundant bioactive phytochemical constituents (Igbinosa *et al*., 2011). Ethanol has good dissolving power for most compounds which are polar to moderately non-polar such as the phytochemical constituents thus leading to good

recovery of the active component(s) from the plant material. The presence of large amounts of flavonoids and phenolic compounds that exhibit very good antioxidant capacity in the ethanolic extracts of medicinal plants may mean that there is a tendency to evaluate the antioxidant activity of ethanol stem bark of *Jatropha tanjorensis* in order to substantiate the efficacy in traditional medicine and possibly establish an effective and natural safe antioxidant from plant source in the pharmaceutical industry.

DPPH free radical scavenging activity, nitric oxide radical scavenging activity, ferric reducing antioxidant power (FRAP) and hydrogen peroxide radical scavenging assays together with determination of total phenolic content are some standard experiments conducted in laboratory to assess the antioxidant activity of a plant extract. They are largely known to be reliable and reproducible procedures for antioxidant capacity measurement (Uroko *et al*., 2020). Following the background knowledge above, the aim of this work is to investigate the in vitro antioxidant potential of ethanol stem bark of *Jatropha tanjorensis*. It is anticipated that the findings of the study will provide sufficient scientific basis to support its ethnobotanical use as a readily available source of natural antioxidant.

1.2 Statement of the Problem

Cancer, diabetes, cardiovascular disease and neurodegenerative disease that the body suffers from because of reactive oxygen, these diseases becoming common and major public concern throughout the globe. Although synthetic antioxidants are available to help control oxidative stress, but these can cause undesirable side effects

in the long term and may even be carcinogenic. (Borquaye *et al*., 2020).

Because they are easily accessible, cheap, and less deadly, medicinal plants have continued to be the focus of scientific interest. While it is traditionally believed to have some efficacy in treating ailments, it is worth noting that not much is documented about the antioxidant activity of *Jatropha tanjorensis*, more specifically the ethanol extract of its stem bark. The majority of the studies that have been conducted have been on the leaves and crude extracts not stem bark extract (Oladele, 2020).

Hence, the antioxidant activity of ethanol extract of *Jatropha tanjorensis* stem bark should be explored to substantiate its ethnomedicinal use and establish its possible application against oxidative stress related ailments.

1.3 Aim of the Study

The aim of this study was to assess the in-vitro antioxidant activity of ethanol extract of *Jatropha tanjorensis* stem bark.

1.4 Specific Objectives

The study Objectives were:

1. Identification of phytochemical constituents in ethanol extract of *Jatropha tanjeronesis* stem bark
2. Quantification of antioxidant vitamin composition in ethanol extract of *Jatropha tanjeronesis* stem bark
3. To determine the total antioxidants capacity extract ethanol of *Jatropha tanjeronesis* stem bark.

4. Pengukuran free radical scavenging activity of ethanol extract of *Jatropha tanjeronesis* stem bark by DPPH method.
5. Pengujian antioxidant activity of the extract compared to antioxidant standard (Ascorbic acid)

CHAPTER TWO

LITERATURE REVIEW

2.1 Oxidative Stress

Oxidative stress is a state where reactive oxygen species production exceeds antioxidant defenses. Reactive oxygen species are chemically active molecules produced during life-supporting processes such as mitochondria respiration and other chemical reactions occurring in cells. An accumulation of the over the level of reactive oxygen species, cause oxidation and damage to biomolecules such as protein, DNA, and lipid, leading to the development of cellular oxidative stress (Borquaye *et al*., 2020). Oxidative stress is one of the factors involved in pathogenesis of some non-infectious chronic disease such as cancer, diabetes mellitus, hypertension, rheumatoid arthritis,

parkinson disease, Alzheimer disease and Aging. Free radicals has the ability to induce lipid peroxidation on membrane of cell causing malfunction of cells (Omoboyowa *et al*., 2020).

2.2 Free Radicals

These are atoms or molecules with one or more unbound electrons and are thus unstable and readily react with molecules in cells in an attempt to become stable. Free radicals include OH0 hydroxyl free radical, O2– superoxide anion radical, NO0 nitric oxide and Igbinosa *et al*. 2011. They include sources both in and outside the body, with in body examples such as the electron transport chain in the mitochondria, enzymatic processes and inflammation and exogenous factors like smoke from cigarettes, pesticide chemicals and pollution.

2.3 Antioxidants

Those chemicals that inhibit the oxidation process and neutralize the free radicals are called antioxidants. These antioxidants neutralize the oxidation damage of biological systems by

donating electrons to free radicals. They can either be enzymatic or non-enzymatic. Enzymes used in the system are: SOD, CAT and GPX Non enzymatic antioxidants are Vit C, Vit E, GP, polyphenols and Flavonoids. The plant-origin natural antioxidants have been of high interest due to their safety and other pharmacological properties, (Borquaye *et al*., 2020). Antioxidant bioactive phytochemicals are present in medicinal plants. For their potential to act as free radical scavengers and inhibit oxidation,

phenolic compounds and flavonoids are of particular interest. All the studies conducted so far have revealed that ethanolic extracts of medicinal plants have a higher antioxidant activity because ethanol can extract phytochemicals properly. (Borquaye *et al*., 2020). The benefits of antioxidants from plants have been linked to heart disease, inflammation, cancer and neuro-degenerative diseases.

2.4 Botanical Profile of *Jatropha tanjorensis*

Jatropha tanjorensis is a member of the Euphorbiaceae family. A perennial shrub that is widely spread in tropical Africa. Generally planted near houses, farms and used as a vegetable in southern Nigeria.

Different ethnic groups and regions in Nigeria, have names for the plant.

Yoruba: *Efo Iyana-Ipaja* (or just *Iyana-Ipaja*) and *Ewe Lapalapa*

Igbo: *Ugu-Oyibo* (meaning "foreign pumpkin")

Tiv: *Kpan gyedam*

English Common Names: Catholic vegetable, miracle leaf, tree spinach, and Reverend Father vegetable.

Nigeria Pigon: hospital too far (Osiyi *et al*. 2023)

Table 1: Taxonomic Classification

Taxonomic Rank.	Classification
Order	*Malpighiales*
Family	*Euphorbiaceae*
Genus	*Jatropha*
Species	*Jatropha tanjeronesi*

The plant has green leaves, soft stems and a characteristic latex that is produced by Euphorbiaceae plants, (Oladele *et al*., 20220)

Figure 1. *Jatropha tanjeronesis* plant

The presence of such compounds is essential to provide such plants with their medicinal and antioxidant benefits. The phenolics groups of these compounds have -OH group which are capable of donating hydrogen atoms to scavenging free radicals (Omoboyowa *et al*., 2021)

2.4.2 Medical and Health Benefits of *Jatropha tanjorensis*

Laboratory studies have yielded evidence for several biologically relevant activities of the extracts of *J. tanjorensis*. These are the most relevant to the current project and are discussed here.

2.4.2.1 Anti Anaemic and Haematopoietic Activity:

The haematinic activity of *J. tanjorensis* has the most comprehensive documentation from its biological activities. The experiment resulted in an increase in the levels of packed cell volume, haemoglobin content and red blood cell counts when the aqueous leaf extract was orally administrated to experimental rat models having anaemia (Ikewuchi *et al*., 2021). The report of presence of iron, folate and flavonoids in the extract that protects newly formed red blood cells from oxidative damage and facilitate erythropoiesis. The haematopoietic activities found by Afolabi *et al*. (2021) have also been reported while the extract has been shown to significantly possess antioxidant activity, giving an overall pharmacological profile of the plant.

2.4.2.2 Antidiabetic Activity

Salau *et al*. (2022) have also demonstrated that in the streptozotocin induced diabetic Wistar rats, ethanol leaf extract of *J. tanjorensis* dose-dependently decreases blood glucose level to the same extent as Glibenclamide. The suggested mechanism was the inhibition of alpha-glucosidase activity, and perhaps stimulation of the remaining insulin secretion, by the pancreas. Bidirectional mechanistic links exist between type 2 diabetes and chronic inflammation, whereby each

intensifies the other; this is especially pertinent for the current study, as any plant that exhibited activity in both areas has compounded therapeutic value (Rani *et al*., 2023).

2.4.2.3 Antimicrobial Activity

The observed antibacterial effect against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was found to be very strong even in case of fungus *Candida albicans* (which is a pathogen of human) using the extracts of *J. tanjorensis* even when compared to that used as antibiotics(Afolabi *et al*., 2021; Oduola *et al*., 2020). Antimicrobial activity is pertinent to use in inflammation as infection is a common cause or a common side effect in inflammation process.

2.4.2.4 Antioxidant Activity

The interlinkage between oxidative stress and chronic inflammation is very close: oxidative stress activates pro-inflammatory transcription factors that leads to additional oxidative damage in a vicious cycle (Zhu *et al*., 2022; Rani *et al*., 2023). *J. tanjorensis* extracts have displayed good activity against free radicals using the DPPH

and ABTS method largely attributed to their high phenolic and flavonoid compounds (Afolabi *et al*., 2021). Therefore, the antioxidant and anti-inflammatory activities of *J. tanjorensis* are predicted to be mechanistically synergistic and will result in augmentation of beneficial outcomes.

2.4.2.5 Hepatoprotective Activity

There has been a substantial decrease in the activities of liver injury serum marker enzymes, ALT, AST and ALP in the carbon tetrachloride (CCl4)-induced hepatotoxic rats administered leaf extracts of *J. tanjorensis* by Oduola *et al*. (2020) implying that leaf extracts of *J. tanjorensis* possesses hepatoprotective effects. The clinical significance of the study is that liver inflammation is among the most recurrent inflammatory disease cases in clinical practice in Nigeria and the ability of any plant to afford liver tissue protection from inflammatory destruction goes a long way in enriching its medicinal value.

The observed antibacterial effect against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was found to be very strong even in case of fungus Candida albicans (which is a pathogen of human) using the extracts of *J. tanjorensis* even when compared to that used as antibiotics (Afolabi *et al*., 2021; Oduola *et al*., 2020). That study focused on the leaf fraction, but as the leaf and the stem bark of *J. tanjorensis* are said to be phytochemically similar, containing flavonoids, tannins, alkaloids and phenolics, it is scientifically logical to assume the same activity for the stem bark as well (Afolabi *et al*., 2021; Ikewuchi *et al*., 2021).

We have also reported the wound healing activity of the extracts of *J. tanjorensis* in

which experimental excision wound healing were promoted in animal model, and suggested that this activity could be the synergistic effect of the constituents possessing anti-inflammatory, antioxidant and anti-microbial activity of the plant (Eze *et al.,* 2020; Afolabi *et al.,* 2021). The stem bark is the most important part of *Pandanus odoratissimus* L. used for traditional healing and the extraction of the bark only from this part is clinically relevant and logical.

2.5 In-vitro Antioxidant Assays

2.5.1 Qualitative phytochemical analysis of ethanol extract of *Jatropha tanjorensis* stem *bark*

Qualitative phytochemical analysis is an assay performed as a preliminary test to ascertain the types of secondary metabolites found in the extract of any given plant part. The identified compounds or Phytochemicals possess various biological potentials including, but not limited to; antidiabetic, antioxidant, anti-inflammatory, anticancer and antimicrobial potentials of these compounds. Commonly examined phytochemical classes include alkaloids, tannins, saponins, flavonoids, steriods, phenolics, terpenoids, cardiac glycosides and anthraquinones. Researches that have so far focused on the phytochemical characterization of *Jatropha tanjorensis* have invariably revealed a number of compounds possess antioxidants properties. For example, Anhwanage and co-researchers have identified flavonoids, alkaloids, saponins, steroids, cardiac glycosides and reducing sugars to be present in the methanolic extracts of leaves of *J. tanjorensis*, some of which were also reported to exhibit antioxidant potentials. In a recent phytopharmacological review of *J. tanjorensis*

leaf extract, it was found that the plant has high concentration of polyphenols, flavonoids, alkaloids, tannins and saponins. It possesses significant antioxidant, anti-inflammatory, hepatoprotective and antidiabetic properties, mainly due to these phytoconstituents, (Ikalo 2025 *et al*. 2025). Likewise, Dangani *et al*. reported the phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids in root extracts of *J. tanjorensis* extracted using various solvents and they correlated with the antioxidant activity. (Dangani *et al*., 2025)

It has been recognized that the majority of antioxidant constituents of medicinal plants consist of phenolic compound like Flavonoids because these molecules are able to donate a hydrogen atom or electron, which helps in ROS neutralization and thus preventing the oxidative damage, therefore the qualitative phytochemical screening is an essential part in identifying the antioxidant potential of ethanol extract of *Jatropha tanjorensis* stem bark (Igbinosa *et al*., 2011).

2.6.2 Antioxidant vitamin composition

The antioxidant vitamins are naturally present vitamins which help to prevent cells from oxidative damage due to free radicals. The primary antioxidants are the Vitamin C (ascorbic acid), Vitamin E (tocopherols), and Vitamin A (retinol/carotenoids).

2.6.2.1 Vitamin C (ascorbic Acid)

Vitamin C is a water soluble antioxidant which removes ROS and also regenerates Vitamin E. It is involved in collagen biosynthesis, immune regulation and defense of cell components against oxidative stress.

2.6.2.2 Vitamin E (tocopherol)

Vitamin E is lipid-soluble antioxidant which prevents lipid peroxidation of cell membranes. It stops free radical chain reactions by giving hydrogen atoms to lipid radicals.

2.6.2.3 Vitamin A and carotenoids

Vitamin A and carotenoids, the precursors of vitamin A, have antioxidant properties through singlet oxygen quenching and peroxyl radicals scavenging. They also are involved in immune function and cellular differentiation. Often, plant extracts containing high levels of antioxidant vitamins have greater free radical scavenging properties. A number of studies have shown positive correlation between antioxidant vitamin content and total antioxidant activity of medicinal plants. (Alam *et al*., 2012).

Consequently, determination of antioxidant vitamins in the ethanol extract of *Jatropha tanjorensis* stem bark is important since these vitamins can play a significant role in the anti-oxidant activity. (Alam *et al*., 2012).

2.6.3 Total antioxidant capacity (TAC)

This represents the synergistic action of all antioxidants in the biological fluid or the plant extract. This provides a holistic parameter rather than mere the sum total of quantified antioxidants and the capacity of the sample to detoxify the free radical.

Phosphomolybdenum is one of the widely accepted methods for evaluating TAC. Molybdenum (VI) in acid media is reduced to molybdenum (V) by antioxidants to give a molybdenum (V)-phosphate green colored complex which may be quantified spectrophoretically at 765 nm (Alam, 2012)

This assay is beneficial since it evaluates water soluble and fat soluble antioxidants and offers an overall evaluation of antioxidants. The higher the TAC the more antioxidant activity.

Total antioxidant activity is greatly due to phenolic compounds, flavonoids, vitamins, tannins and other phytochemicals. The total phenolic content has been shown to have strong positive correlations with TAC values of medicinal plants. (Igbinosa *et al*., 2011). Hence, the total antioxidant activity of the ethanol extract of *Jatropha tanjorensis* stem bark is imperative to assess its antioxidant activity.

2.6.4 DPPH radical scavenging assay

The DPPH assay is one commonly used assay for determination of antioxidant activity and it measures the capacity of antioxidant to donate a hydrogen atom for the DPPH radicals stabilization, (Igbinosa *et al*., 2011).

2.7 Previous Studies on *Jatropha tanjeronesis*

In this section, related studies on the phytochemical constituents of *Jatropha tanjorensis*, antioxidant vitamins and its overall antioxidant activity of *Jatropha tanjorensis* as demonstrated by DPPH radical scavenging activity and total antioxidant activity. From the phytochemical study of leaves of *Jatropha tanjorensis* conducted by

Anhwange *et al*. (2019) revealed that there exist Flavonoids, Alkaloids, Steroids, Saponins, cardiac glycosides and reducing sugars in leaves. In conclusion the plant are very rich in biologically active substances that exhibit both antioxidant and antimicrobial effects. The authors stated that the plants are good sources of bio-active compounds that possess the ability to antioxidant and antimicrobial actions (Anhwange *et al*., 2019).

In this research work, antioxidant activity of *Jatropha tanjorensis* leaf methanolic extract was carried out in vitro and in vivo and it showed that extract contains a potent antioxidant in scavenging activity on the DPPH radical which depends on dose. The DPPH radical scavengimg capacity significantly increased the superoxide dismutase and catalase activity and supports the folk claim of plant use for various diseases associated with the oxidation stress (Madubuike, 2019). LC-MS/MS analysis for the presence of phenolics and alkaline elements in *Jatropha tanjorensis* leaf by Arun and Brindha, (2015) indicates the presence of number of phenolic and some flavonoids and derivatives that has been known for antioxidant properties.

They found that the phytochemical constituents profile had strong relationship with the plant biological activities (Arun and Brinda 2015). The flavonoid fractions of *Jatropha tanjorensis* were assayed for their antioxidant activities which was strong in the both DPPH and lipid peroxidation activities and they were analyzed by UHPLC-ESI-MS for flavonoid identification which indicates the present of different flavone glycosides and flavonoids as responsible to act as antioxidants (Arun *et al*., 2014).

Similarly phytochemical constitution and antioxidant activities of *Jatropha*

tanjorensis root extracts were carried out using various solvents by Dangani *et al*., 2025.

They concluded that there were significantly high contents of phenolics, flavonoids, tannins, and alkaloids in the extracts and they found that antioxidant activities was positively correlated to high phytochemical constituents (Dangani *et al*., 2025).

The ethnomedicinal and phytopharmacological properties of *Jatropha tanjorensis* leaf have been summarized by Ikalo *et al*. (2025). The review emphasized that polyphenolics, flavonoids, tannins and other phytochemicals responsible for free radical scavenging and antioxidant properties contribute to the antioxidant action of plant. (Ikalo *et al* 2025). Antioxidant activity of the ethanol, methanol and aqueous extracts of Jatropha were determined by Igbinosa *et al*. And were found to have a rich amount of phenols and exhibit substantial free radical scavenging DPPH ability in the ethanol extract. (Igbinosa *et al*., 2011).

The antioxidant activity of the methanol leaf extract of *Jatropha tanjorensis* was evaluated by Aiwonegbe *et al*. And found to have significant antioxidant and anti-inflammatory activities which is associated to the presence of phytochemicals in the plant. (Aiwonegbe *et al*.,). Sterols were isolated from the leaves of *Jatropha tanjorensis* and the anti-inflammatory potential was determined by in vitro assays and molecular docking simulation studies (Omoboyowa *et al*., 2021).

A study by Aiwonegbe *et al*. reported significant antioxidant activity of methanol leaf extract of *Jatropha tanjorensis* against DPPH radicals, (Aiwonegbe *et al*., 2021).

The leaves also yielded sterol compounds that also exhibited anti-inflammatory activities in vitro. (Omoboyowa *et al*., 2021).

Based on these studies, it has been found that some bioactive compounds present in *Jatropha tanjorensis* leaf and root exhibited a high level of antioxidant activity which is less investigated in the ethanol extract of *Jatropha tanjorensis* stem bark, hence the present study was justifiable because it focuses on the ethanol extract of *Jatropha tanjeronesis* stem bark.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

An experimental laboratory design was used to assess the in-vitro antioxidant activity of ethanol extracts of stem bark of *Jatropha tanjorensis*. The study comprised of extraction of plant material, phytochemical screening, antioxidants vitamin content determination, DPPH radical scavenging assay and total antioxidant capacity determination.

3.2 The collection and authentication of plant material.

Jatropha tanjorensis was harvested from Nsukka. Stem bark was fresh collected and was authenticated by Late Mr. Alfred Ozioko a taxonomist in Bioresources Development and Conservation program (BDCP) Research centre Nsukka Enugu State. Stem bark was then air-dried at room temperature and puverized.

3.2.1 Equipment

The equipment used were obtained from the Department of Biochemistry and other Scientific shops within Enugu town. And they are as follows

Equipment	Manufacturer
Analytical balance	
Beaker	Payrex
Rotary evaporator	

Electric blender

Conical Flask. Payrex

Glass funnel

Filter paper. Whatman

Measuring Cylinder. Pyrex

Seperatory funnel

Micropipette. Perfect

Spatula. Pyrex

752N

UV-VIS Spectrophotometer. Searchtech

Syringe. Lifespan

Test tube. Pyrex

Weighing balance

Water bath

3.2.2 Chemical and Reagent

The chemical used were bought from scientific shops in Enugu and were of analytical grade, while some others were obtained from the laboratory of the Department of Biochemistry Godfrey Okoye University Enugu. The chemicals include:

70% ethanol

Distilled water

Hager's reagents

Wagner's reagents

Mayer's reagents

Glacial acetic

Ferric chloride

Conc H_2SO_4

Baljet's reagent

NaOH solution

Dilut HCL

Dilut iodine solution

Ferric chloride

Ascorbic acid

Sulphuric acid

Sodium phosphate

Ammonium molybdate

DPPH reagents

Methanol

Acetone

n-hexane

retinol acetate

DCPIP dye

Metaphosphoric acid

Xylene

a-Tocopherol

3.3 Preparation of Plant Material

Stem bark of *Jatropha tanjeronesis* was washed and grounded and cleaned out all adhered contaminants. Dried air in a clear well ventilated location at 25 -30 degrees C to obtain constant weight. Air dried material obtained, and then ground into very fine powder in electric grinder. Powdered material was dried for some time under the ambient temperature in desiccator, prior to extraction.

3.3.1 Stem bark was powdered and macerated in 70% ethanol for 72 h with intermediate shaking. The extract was filtered through a muslin cloth, subsequently

through Whatman no.1 filter paper. The filtrate was concentrated under vacuum at 40C in a rotary evaporator.

3.4 Qualitative Phytochemical Analysis

Qualitative phytochemical screening of ethanol extracts was done using the methods of Junaid R shaikh and MK patil (2020)

3.4.1 Test for Alkaloids

Extraction procedure

To 5 mL of the 1% hydrochloric acid solution, about 0.5 g of the extract was added and warmed slightly. The filtrate was obtained after filtering the solution and was treated for alkaloids.

(a) Mayer's Test

Two drops of Mayer’s reagents was dropped in 1mL of filtrate.

A creamy white precipitate formed was evidence for the presence of alkaloids.

(b) Wagner's Test

A few drops of Wagner's reagent were added to the filtrate.

Absent of Alkaloids were detected by the lack of formation of brown or reddish-brown precipitate.

(C) Hager’s Test

2 ml of Hagers reagents were added to few ml of the filtrate.

A creamy white precipitate formed indicate the present of Alkaloids.

3.4.2 Test for Flavonoids

(a) Conc. H_2SO_4 Test

Conc. H_2SO_4 were added to few ml of plant extract

The flavonoids were indicated by the formation of Orange colour.

(b) Alkaline Reagent Test

Two millilitres of sodium hydroxide solution were added to the extract followed by dilute hydrochloric acid.

Flavonoids were confirmed by a yellow colour which disappeared after adding acid.

3.4.3 Test for Tannins

(a) Braymer's test:

This is the Ferric Chloride Test.

A mile filtrate were mixed with 3ml distilled water and 3 drops of ferric chloride solution

The presence of tannins was indicated by blue-green colour.

(b) 10%NaOH test

Quantity of 4ml 10%NaOH was added in 0.4ml plant extract and was shaked very well.

Absence of formation of emulsion indicate the absence of tannins.

3.4.4 Test for Saponins

(a) Frothing Test

Distilled water 10ml was shaken vigorously with 1 g of extract.

Saponins were indicated by the formation of forming.

3.4.5 Test for Phenols

Iodine test

Few drops of dilute iodine solution were added to the 1ml of extract.

Absent of transient red colour indicate the lack of phenols in the extracts

3.4.6 Test for Cardiac Glycosides

(a) Keller-Killiani Test

To the extract, 2 ml of glacial acetic acid with ferric chloride was added and slowly overlaid with concentrated sulfuric acid.

A brown ring at the border was a sign of cardiac glycosides.

(b) Baljet's test

A drop of baljet's reagents was added in two ml of the extract

A yellow- orange colour indicate the present of Cardiac Glycosides

3.5 Determination of Antioxidant Vitamin Composition

The antioxidant vitamins A, C and E were measured by standard AOAC methods.

3.5.1 UV-VIS Spectrophotometeric Vitamin A determination in plants extract of

Jatropha tanjeronesis using Sharmas., *et al* (2021)

One gram of the exact were weighed into a conical Flask and 20ml of was added and shaked for 30 minutes and filtered. The filtrate was transferred into a separatory funnel containing 20ml n-hexane and distilled water were added slowly to facilitate phase separation. The upper organic layer containing carotenoids and vitamin A compound were collected. Which is 25ml a know volume.

Preparation of standard curve

A stock solution of vitamin A standard where prepared in n-hexane and working standards of 2ug/ml, 4ug/ml, 6ug/ml, 8ug/ml, 10ug/ml were also prepared. Absorbance was measured at 325 against n-hexane blank. Plot of absorbance versus concentration was ploted to obtain a calibration curve.

3.5.2 The titration method of determining vitamin C composition is determined using AOAC official method 967.21(2023).

Preparation of DCPIP Dye Solution

Distilled water was used to dissolve o.1g of DCPIP to make up 1 liter and stored in amber bottle and refrigerated because it is light sensitive.

Standardization of Dye

10ml Ascorbic acid was pippetted into the flask, add 10ml metaphosphoric acid then titrate with 10ml dcpip solution, record end point when light pink remained permanent

for 15secs (dye factor).

Procedure for sample

Exactly one gram sample was ground into metaphosphoric acid solution and filtered to get clear solution. Ten mile of filtr ate solution was pipette in a cone flask and was taken to titration with standardise DCPIP solui 0 10 3N solution. 1. 0 of dye added continuously keep swirling till faint pink color sustained for 15 s and volume of dye was noted .

3.5.3 Determination of Vitamin E using a method used by AL. Iman., *et al* (2023)

One gram of dried sample was extracted using 20ml n-hexane and filtered. The exact was collected and it absorbance was measured at 292nm using the n-hexane as blank. Quantified using a calibration curve prepared from standard a-Tocopherol solutions.

3.6 Determination of DPPH Radical Scavenging Activity

The antioxidant activity of the extracts in ethanol was evaluated using a stable DPPH radical, which has an absorption maximum at 515nm, according to method described above, with a minor adaptation. 2.4 mg of the radical were dissolved in 100 ml methanol, in order to prepare a stock solution of the radical. Extracts, Ascorbic acid (standard) were individually prepared as 100 mg/ml and subsequently 5 concentrations (50, 25, 12.5, 6.25, and 3.125) of each and the standard was made through serial dilution.

These various concentrations of extract/standard was mixed with methanolic solution of the radical. After vigorous shaking of the mixture, the samples were kept for 30 min at room temperature in darkness. Absorbance of the reaction mixture was read at 515nm Spectrophotometrically. DPPH radical with the absence of antioxidant was also recorded and called blank. Also DPPH with methanol as mixture was prepared and measured at the same wavelength, as the standard. All the determination were performed in triplicate. The capability to scavenge the DPPH radical was calculated. Brand Williams., *et al* (1995).

The percentage inhibition was obtained by dividing the following:

% Inhibition = [(A0 − A1)/A0] × 100

Where:

A0 = absorbance of the blank at t=0

A1 = absorbance of the Antioxidant at t= 30min

The calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant (Vitamin C).

J Agric Food chem (1994)

3.7 The Total Antioxidant Capacity is determined as described below

The Total Antioxidant Capacity was determined as follows:

The total antioxidant capacity was measured by the phosphomolybdenum method as described by Prieto *et al*., (1999).

Preparation of Reagent

Preparation Reagent Solution (5mL) - - 0.6 M sulfuric acid (5mL), 28 mM sodium phosphate (5mL), 4 mM ammonium molybdate (5mL).

Procedure

Add the three milliliters of phosphomolybdenum reagent to the extract solution with various concentrations and the volumes were 0.3 mL of different concentrations respectively. Incubate at water bath at 95C for 90 min. Measure the absorbance at 765 nm at room temperature after cooled down. The total antioxidant activity was expressed as AAE with standard calibration curve by using ascorbic acid, the antioxidant capacity were estimated by using following formular : antioxidant capacity (AAE) = ((control absorbance)-(sample absorbance))/(control absorbance) * 100%.

3.8 Statistical Analysis

The data expressed were Mean SD (standard deviation), and conducted 3 times. Statistical analyses performed using SPSS version 25.0. Data were compared to analyze the differences between means, if necessary one-way ANOVA conducted, and $p < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Analysis of Ethanol Extract of *Jatropha tanjeronesis* Stem Bark

As shown in table 1, phytochemical analysis of the ethanol extract of *Jatropha tanjeronesis* Stem Bark shows the presence of Alkaloid, Flavonoid, Cardiac Glycosides, Tannins, Saponins and phenolic was not detected in iodine test.

Table 1: Photochemical analysis of ethanol extract of *Jatropha tanjeronesis* stem bark

Phytochemicals	Method	Relative Presence
Alkaloid	Hager test	++
	Mayer test	++
	Wagners test	ND
Flavonoids	Alkaline reagent test	++
	Conc. H_2SO_4	++
Cardiac Glycosides	Kellar-Kilani test	++
	Baljet test	++
Phenolic	Iodine	ND
Tannins	Braymer's test	++
	10% NaOH test	ND
Saponins	Form test	++

Key: + Slightly present
++ Highly present
ND Not Detected

4.2 % DPPH inhibition of the ethanol extract of *Jatropha tanjeronesis* stem bark

As shown in Table 2, % DPPH inhibition of ethanol extract of *Jatropha tanjeronesis* stem Bark decreased as concentration was reduced. When compared with Ascorbic acid, 3.125mg/ml of *Jatropha tanjeronesis* had relative %DPPH inhibitory activity as to 6.25mg/ml of ascorbic acid.

Table 2 - %DPPH inhibition of ethanol extract of *Jatropha tanjeronesis* Stem Bark

Sample	**Concentration (mg/ml)**	**% DPPH Inhibition (Mean ± SD)**
Jatropha tanjeronesis	50	30.73 ± 0.72
Jatropha tanjeronesis	25	30.09 ± 0.04
Jatropha tanjeronesis	12.5	29.13 ± 0.69
Jatropha tanjeronesis	6.25	30.14 ± 0.63
Jatropha tanjeronesis	3.125	25.68 ± 1.36
	50	83.78 ± 0.86
Ascorbic Acid (Standard)		
Ascorbic Acid	25	82.75 ± 0.55
Ascorbic Acid	12.5	26.88 ± 1.37
Ascorbic Acid	6.25	21.66 ± 0.10
Ascorbic Acid	3.125	19.39 ± 1.60

Values expressed as Mean ± SD, n=3

4.3 %TAC of ethanol extract of *Jatropha tanjeronesis* stem bark

As shown in table 3, in % TAC of Ethanol Extract of *Jatropha tanjeronesis* Stem Bark 25mg/ml of *Jatropha tanjeronesis* showed highest activity of 90.84% when compared with other concentrations and this was comparable with 6.25mg/ml of ascorbic acid.

Table 3. %TAC of ethanol extract of *Jatropha tanjeronesis* Stem Bark

Sample	**Concentration (mg/ml)**	**% TAC Activity (Mean ± SD)**
Jatropha tanjeronesis	50	92.05 ± 0.05
Jatropha tanjeronesis	25	90.84 ± 0.02
Jatropha tanjeronesis	12.5	91.25 ± 0.03
Jatropha tanjeronesis	6.25	89.91 ± 0.03
Jatropha tanjeronesis	3.125	74.98 ± 0.05
Ascorbic Acid (Standard)	50	92.49 ± 0.01
Ascorbic Acid	25	92.45 ± 0.02
Ascorbic Acid	12.5	92.36 ± 0.08

Ascorbic Acid	6.25	90.72 ± 0.51
Ascorbic Acid	3.125	89.04 ± 0.02

Values expressed as Mean ± SD, n=3

4.4 Vitamin Content of ethanol extract of *Jatropha tanjeronesis* stem bark

Vitamins

In table 4, *Jatropha tanjeronesis* contains 81.14mg/g of Vitamin A, 4.78mg/g of Vitamin C and 1.60mg/g of Vitamin E.

Table 4. Vitamin content of ethanol extract of *Jatropha tanjeronesis* Stem Bark

Sample	**Vitamin A (mg/g)**	**Vitamin C (mg/g)**	**Vitamin E (mg/g)**
Jatropha tanjeronesis	81.14 ± 0.80	4.78 ± 0.37	1.60 ± 0.12

Values expressed as Mean ± SD, n=3

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The qualitative phytochemical analysis of ethanol extract of stem bark of *Jatropha tanjorensis* showed presence of alkaloids, flavonoids, cardiac glycosides, tannins and saponins but had no phenolic compound in it. The presence of these types of secondary metabolites suggested that stem bark contained other bioactive components which can contribute to its antioxidant and medicinal properties. Lack of detectable phenolics could be due to the type of extraction solvent used, the geographical origin of the plant, environmental conditions, the time of the year when it is harvested, plant maturity, or analytical detection limits. Alkaloids found in this study corroborated the report of Madubuike *et al*. (2015)

Likewise, flavonoids have been detected, which supports the findings of Arun *et al*. (2014) Flavonoids are one of the strongest natural antioxidants as they donate hydrogen atoms or electrons to neutralize the ROS and prevent lipid peroxidation. The presence of these compounds was found to explain the amount of DPPH radical scavenging activity and the high total antioxidant capacity found in this investigation.

The hydroxyl groups in tannins have high reducing potential; scavenges free radicals and chelate transition metals and prevent the oxidation which confirm to result from Awhwange *et al*. (2019). The tannins also posses an antibacterial, anti-inflammatory and wound healing activity that is believed to be of medicinal use to

plant. The Cardiac glycosides are present, which agree with the result of Ikalo *et al*. (2011), cardiac glycosides is believed to cause contraction of the heart, but recent researches claim some cardiac glycosides to possess some antioxidant activities as well which makes them of medicinal importance to stem bark.

The presence of saponins is consistent with the reports of Aiwonegbe *et al* (2021) which indicates that saponins help in stabilizing the biological membranes, improving immune responses and decreasing oxidative stress, which aligns with the antioxidant activities of flavonoids and tannins. Phenolic compounds were not found in the present study, but it was found in the related study done by Dangani *et al*. (2025) which may be due to difference in geographical location, climate condition, extraction solvent, extraction method, plant maturity or analytical technique. The variability in phytochemical composition in response to environmental and genetic factors has been well documented. Thus, it can be concluded that the absence of phenolics in the present investigation does not necessarily mean complete absence but only meant they were not detected with the qualitative method used.

Based on the results the phytochemical constitution obtained here provides a basis for the ethnobotanical application of stem bark of *Jatropha tanjorensis*. It may be attributed to synergistic effect of combination of phytochemicals i.e. Alkaloids, flavonoids, tannins, saponins and cardiac glycosides on scavenging activity observed in DPPH and Total Antioxidant Capacity.

DPPH Assay

The DPPH radical scavenging activity of the extract was moderate but

consistent at all tested concentrations varying from 25.68 ± 1.36% to 30.73 ± 0.72%. The stability in the inhibition at different concentrations, suggests that the antioxidant compounds in the extract are not affected by the dilution which means that there is a steady scavenging effect on the radicals rather than a dose-dependent response. The DPPH activity of the extract was lower than ascorbic acid at higher concentrations and higher than the ascorbic acid at the lowest concentration (3.125 mg/mL), indicating that the phytochemicals present in the extract might have their antioxidant activity intact in diluted physiological condition.

The moderate DPPH activity noted in the present study thus corroborates the previous reports by Madubuike *et al*. (2015) and Arun *et al*. (2014).

Total Antioxidants activity assay

The antioxidant activity of these extracts was even higher than the ascorbic acid, with values from 74.98 ± 0.05% to 92.05 ± 0.05%, respectively, when compared to the values of the ascorbic acid. This indicates that the individual free radical scavenging (DPPH) activity was moderate, but that the extract has a very high overall reducing activity which is able to scavenge multiple oxidant species. The similarity of the present study with these reports indicate that the ethanol extract was able to effectively extract many antioxidant compounds that led to the high reducing power observed.

Vitamin composition

The total vitamin A, C and E content. The extracted Vitamin A content was found

to be very high compared to others (81.14 ± 0.80 mg/g); Vitamin C and Vitamin E were also detected in the extract with a concentration of 4.78 ± 0.37 mg/g and 1.60 ± 0.12 mg/g respectively. Such vitamins are well known for their antioxidant properties and may have significantly added to the Total Antioxidant Capacity observed in this study.

The presence of high Vitamin A content indicates that the stem bark of *Jatropha tanjorensis* is rich of carotenoid precursor that could help in reducing oxidative damage to tissues, maintain the immune system and epithelial integrity. Vitamins C and E act synergically in the antioxidant defence system; vitamin C acts as a scavenger of lipid radicals and regenerates oxidized vitamin E. This synergy effect may be responsible for the high antioxidant activity of the extract although it had moderate DPPH inhibition. These findings corroborate that of Ikalo *et al*., (2011), and Madubuike *et al*., (2015). The agreement of the present study with previous work also confirms the appropriateness of ethanol as an extraction solvent for antioxidant studies.

5.2 Conclusion

The current results show that the antioxidant property of *Jatropha tanjorensis* stem bark can be attributed to the synergistic effect of phytochemicals and antioxidant vitamins present in it. It seems that despite the moderate DPPH activity, the high Total Antioxidant Capacity reported suggests that the extract has several antioxidants with diverse mechanisms. This finding confirms with previous studies by Madubuike *et al*., (2015), Arun *et al*., (2014), Awhwange *et al*., (2019), Ikalo *et al*., (2011), Aiwonegbe *et al*., (2021) and Dangani *et al*., (2025) which reported that *Jatropha tanjorensis* has

excellent antioxidant property due to its phytochemical and nutritional composition.

In conclusion this study extends the present understanding of the application of ethanol extract of stem bark of *J. tanjorensis* as a good natural source of antioxidant compounds for the prevention and control of diseases of oxidative stress

5.3 Recommendation

1. Bioactive compounds of ethanol-rich fraction should be identified by using advanced analytical techniques as HPLC, GC–MS, and LC-MS/MS.

2. Biological activities should be tested by applying in vivo antioxidant models to confirm the efficacy and safety.

3. Toxicological test of the extract to confirm long term safely use of the plant.

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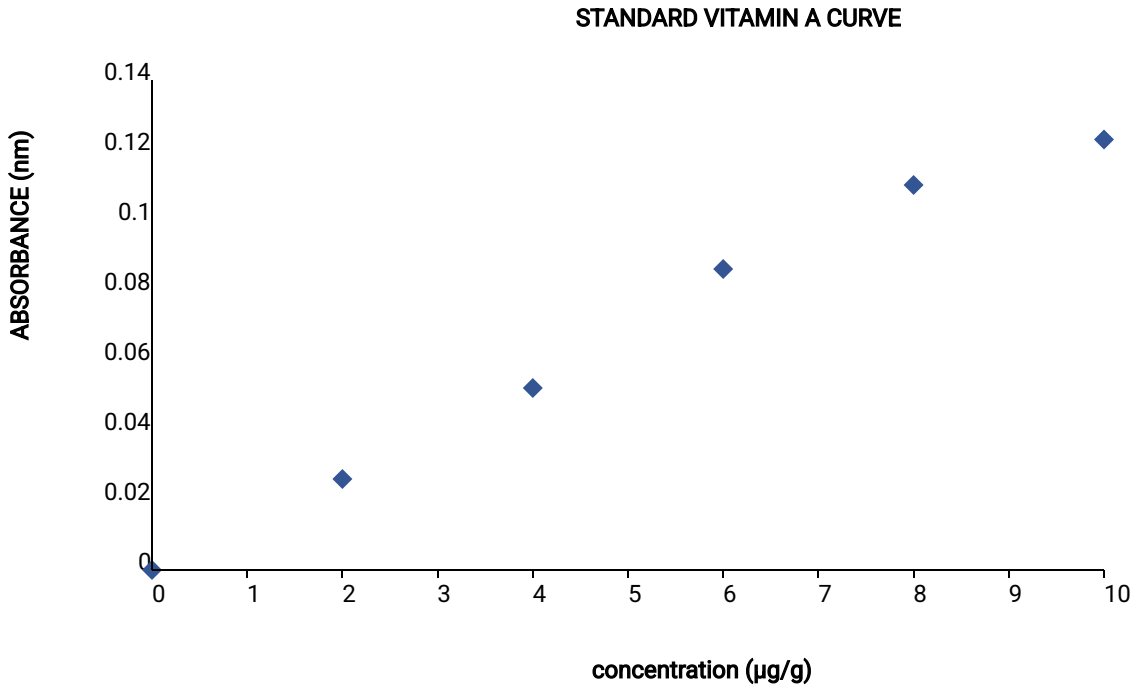
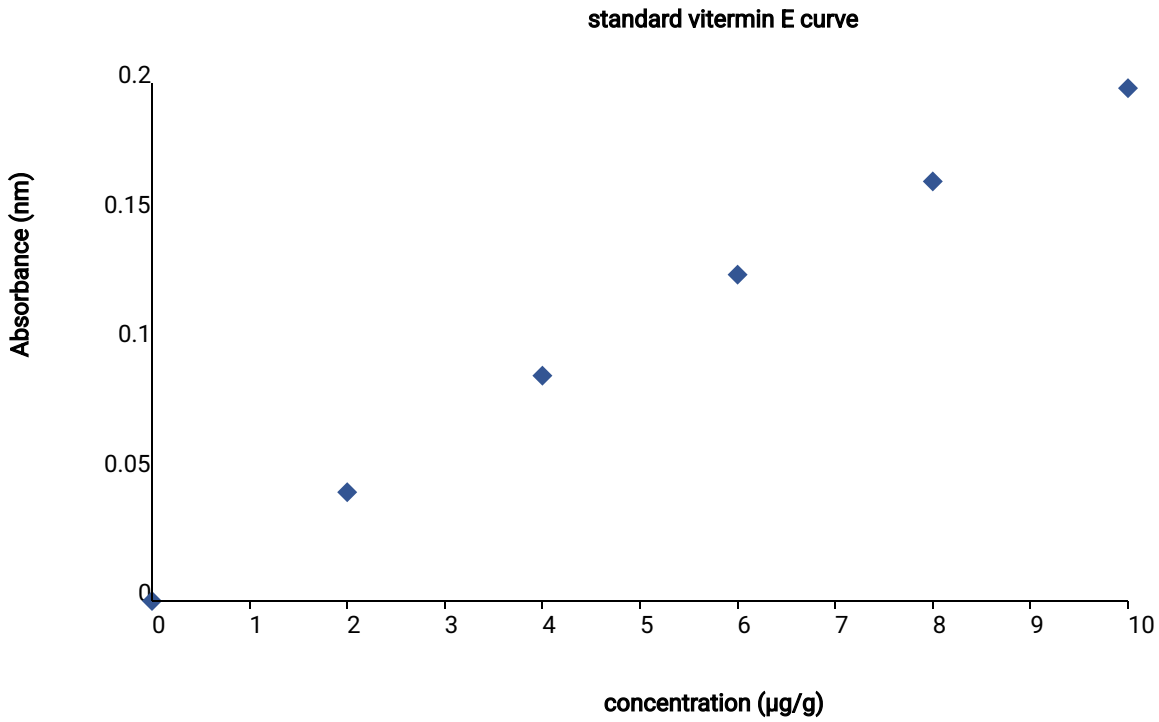
APPENDICES



Concentration (µg/g)	Absorbance (nm)
2	0.026
4	0.052
6	0.086
8	0.11
10	0.123

Appendix 1: Standard vitamin A



Concentration (µg/g)	Absorbance (nm)
2	0.042
4	0.087
6	0.126
8	0.162
10	0.198

Appendix 2: Standard vitamin E

J.T. is a biochemically rich extract with the following properties:

Property	J.T. Performance	Comparison to Ascorbic Acid
DPPH Scavenging	Moderate (~30%), consistent	Weaker at high doses; stronger at low doses
Total Antioxidant Capacity	High (89–92%)	Nearly equivalent to ascorbic acid
Vitamin A	81.14 mg/g — very high	N/A — not in ascorbic acid standard
Vitamin C	4.78 mg/g — present	Contributes to TAC activity
Vitamin E	1.60 mg/g — present	Synergistic with Vitamin C

Appendix 3: The vitamin profile of J.T. particularly its dominant Vitamin A content alongside Vitamins C and E provides a plausible biochemical basis for the strong Total Antioxidant Capacity observed. The synergistic interaction between these vitamins (the ascorbate-tocopherol antioxidant cycle) likely amplifies the net antioxidant effect beyond what any single compound could achieve alone.

S/N	SAMPLE ID	CONC (mg/ ml) of extract	% DPPH inhibition activity
	J.T	50	30.38
			31.57
			30.26

	25	30.14
		30.07
		30.07
	12.5	29.40
		29.66
		28.34
	6.25	29.49
		30.18
		30.75
	3.125	26.05
		26.82
		24.17
Ascorbic acid (standard)	50	83.28
		84.78
		83.28
	25	83.09
		82.11
		83.05
	12.5	26.11
		28.47
		26.07
	6.25	21.78
		21.65
		21.57
	3.125	17.55
		20.50
		20.13

Appendix 4: Results for antioxidant activity DPPH

S/N	SAMPLE ID	CONC (mg/ml) of extract	% TAC activity
	J.T	50	92.01
			92.11
			92.05
		25	90.82
			90.83
			90.87
		12.5	91.23
			91.24
			91.30
		6.25	89.89
			89.91
			89.95
		3.125	75.04
			74.95
			74.95
	Ascorbic acid (standard)	50	92.48
			92.50
			92.50

	25	92.44
		92.44
		92.48
	12.5	92.41
		92.41
		92.26
	6.25	90.44
		91.31
		90.41
	3.125	89.04
		89.07
		89.03

Appendix 5: Results for antioxidant activity TAC

S/N	SAMPLE ID	Vit A (mg/g)	Vit C (mg/g)	Vit E (mg/g)
1	J.T	82.061	4.44	1.48
2		80.839	5.18	1.62
3		80.534	4.73	1.72

Appendix 6: Result for vitamins