

**ANTI-OXIDANT SCREENING OF METHANOL EXTRACT OF LEAVES OF *Ficus exasperata* VAHL**

**BY**

**CHUKWUEMEKE, KESTER. C.**

**PHA1505908**

**SUPERVISED BY:**

**DR. OSAYEMWENRE ERHARUYI (PhD)**



**DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY.**

**UNIVERSITY OF BENIN, BENIN-CITY,**

**EDO STATE,**

**NIGERIA**

**SEPTEMBER, 2023**

**ANTIOXIDANT SCREENING OF METHANOL EXTRACT OF LEAVES OF *Ficus exasperata* Vahl**

**BY**

**CHUKWUEMEKE, KESTER. C.**

**PHA1505908**

**SUPERVISED BY:**

**DR. OSAYEMWENRE ERHARUYI (PhD)**



**A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL  
CHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE  
AWARD OF DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY  
OF BENIN, BENIN-CITY, EDO STATE, NIGERIA**

**SEPTEMBER, 2023**

## CERTIFICATION

This is to certify that this project work was carried out by **CHUKWUEMEKE KESTER. C.** in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin-City, Edo State, Nigeria.

---

DR. OSAYEMWENRE ERHARUYI (PhD)  
PROJECT SUPERVISOR

---

DATE

---

DR. EMMANUEL E. ODION  
HEAD OF DEPARTMENT

---

DATE

## **DEDICATION**

To God and My Family.

## ACKNOWLEDGEMENTS

First and foremost, I show my deepest appreciation to Jehovah, for His unwavering guidance, blessings, and love. To my project supervisor, Dr. Osayemwenre Erharuyi, I am deeply honored to be under your tutelage and guidance without which this work would not be done.

To My mother, Mrs. J. O. Okoh, who held and made me who I am. You exemplify everything I aim to be, words cannot express my gratitude, I love you. This work and my education would not have gotten to this level without the love, support and encouragement from Mr. and Pharm (Mrs.) Iloba. Thank you would never be enough. God continue to bless your efforts. My big mummy and big daddy, Mr. and Mrs. Obi, I am who I am today because of your guidance and wisdom. Thank you.

My appreciation also goes to the staff of Pharmaceutical Chemistry especially Mr. Thompson, Mr. Charles, Mrs. Ukato, for their love and support through my sojourn in Pharmacy school and project work in the department. Never to forget Mr. Ekiomado of blessed memory. Special thanks go to my lecturers who have continued to impart immense knowledge in me and make me the Pharmacist I am becoming. Special mention to Mr. Isaac without whom this project would not have made any headway. My project group members are the best one could ask for, thank you Latifah, Jethro and Anna. An immense thank you and God bless you to the whole Natural Product Research Laboratory team, Isabella, Precious, Ifunanaya and Erica.

The efforts of Mr. and Mrs. Edosoghe-Omorogbe cannot go unmentioned as they have been a huge support system and encouraged me in my times of needs. My siblings Joel, Gozim, Chukwuyem, Madonna, Pascal, Michael, Ebube, Kelvin, Bernard, O. V. O, Ifeanyi deserve a

special mention for being who they are, I love you and appreciate you. Thank you, guys. Rhema and Light, my little nieces, I love you both.

My friends; Farrow, Darth Clinton, Storme, Ethrigan, Yoshiro, Queen, the Ebony girls (Pharm (Dr). Sophia, Madame Valerie, HRM Catherine), Chief Omono, Empress Jada, Tonia, Marvel, Franca, Osasu, Efeoma, Jacey Intl, Martins, Asher, Daniel, Joy-Good Morning, Yvonne, Ehis, Chucks, CEO Wigz My ME, Joshua, Fraulein Maria, King Setale, CEO And Staff of Neem Pharmacy, Shyne, Joshua, Ijeoma.

Lastly, and most importantly, I appreciate myself for being there for me not giving up even when things were bleak, for the strength and courage to continue. Thank you, Kester.

## TABLE OF CONTENTS

CERTIFICATION .....	iii
DEDICATION .....	iv
ACKNOWLEDGEMENTS .....	v
TABLE OF CONTENTS .....	vii
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
ABSTRACT .....	xi
CHAPTER ONE .....	1
INTRODUCTION AND LITERATURE REVIEW	
1.1 TRADITIONAL MEDICINE .....	1
1.2 MEDICINAL PLANTS .....	3
1.3 PHYTOCHEMICAL BASIS .....	4
1.4 BRIEF HISTORY OF MEDICINAL PLANTS .....	13
1.5 SAFETY OF HERBAL MEDICINES .....	18
1.6 <i>Ficus exasperata</i> .....	24
PLANT CLASSIFICATION .....	24
1.6.2 PHYTOCHEMICAL PROPERTIES OF <i>Ficus exasperata</i> Vahl .....	35
1.7 EFFECT OF FREE RADICALS AND NEED FOR ANTIOXIDANTS .....	39
1.7.1 REACTIVE OXYGEN SPECIES (ROS): .....	39
1.7.2 REACTIVE NITROGEN SPECIES (RNS) .....	40
1.8 ANTIOXIDANTS .....	43
1.9 JUSTIFICATION OF THE STUDY .....	44
1.10 AIM AND OBJECTIVES OF STUDY .....	45
CHAPTER TWO .....	46
MATERIALS AND METHODS	
2.0 MATERIALS .....	46
2.1 SOLVENTS AND REAGENTS .....	46
2.2 GLASSWARE .....	47

2.3	EQUIPMENT .....	48
2.4	COLLECTION AND PREPARATION OF PLANT MATERIALS .....	49
2.5	EXTRACTION OF CRUDE POWDERED SAMPLE .....	49
2.6	PHYTOCHEMICAL SCREENING OF <i>Ficus exasperate</i> Vahl LEAVES .....	49
2.7	DETERMINATION OF POLYPHENOLS .....	52
2.7.1	TOTAL PHENOLS .....	52
2.7.2	TOTAL FLAVONOIDS .....	53
2.8	(2,2-diphenyl-1-picrylhydrazyl) DPPH RADICAL SCAVENGING ASSAY .....	54
2.9	TOTAL ANTIOXIDANT CAPABILITY .....	55
3.0	DATA ANALYSIS .....	56
CHAPTER THREE .....		57
RESULTS		
3.1.	ORGANOLEPTIC PROPERTIES .....	57
3.2.	PERCENTAGE YEILD OF METHANOL EXTRACT OF LEAVES <i>Ficus exasperata</i> Vahl.....	57
3.3	PHYTOCHEMICAL CONSTITUENTS .....	57
3.4	TOTAL POLYPHENOLIC CONTENT.....	59
3.4.1	TOTAL PHENOLS .....	59
3.6:	DPPH FREE RADICAL SCAVENGING ACTIVITY.....	62
3.6.1	HALF-MAXIMAL INHIBITORY CONCENTRATION (IC <sub>50</sub> ).....	66
3.7:	TOTAL ANTIOXIDANT CAPACITY (TAC).....	67
CHAPTER FOUR.....		72
DISCUSSION		
4.1	PHYTOCHEMICAL CONSTITUENTS.....	72
4.2	TOTAL POLYPHENOLIC CONTENT.....	74
4.4	DPPH FREE RADICAL SCAVENGING ACTIVITY .....	74
4.5	TOTAL ANTIOXIDANT CAPABILITIES (TAC).....	76
CHAPTER FIVE .....		78
CONCLUSION		
REFERENCES .....		79
APPENDIX.....		86

## LIST OF TABLES

TABLE 3.3.1.....	59
TABLE 3.4.2.....	60
TABLE 3.4.4.....	62
TABLE 3.5.2.....	63
TABLE 3.5.3.....	63
TABLE 3.5.4.....	65
TABLE 3.6.2.....	66
TABLE 3.6.3.....	69

## LIST OF FIGURES

FIGURE 1.1.....	11
FIGURE 1.2.....	11
FIGURE 1.3.....	11
FIGURE 1.4.....	11
FIGURE 1.5.....	12
FIGURE 1.6.....	12
FIGURE 1.7.....	12
FIGURE 1.8.....	12
FIGURE 1.9.....	25
FIGURE 1.10.....	25
FIGURE 1.11.....	25

## ABSTRACT

Free radicals have been implicated in the occurrence of oxidative stress. They have also been found to be important in the pathophysiology of a number of disease conditions. This therefore, underlies the need for very effective antioxidants. *Ficus exasperata* has been used traditionally for the treatment and management of numerous disease conditions. Assessment of antioxidant properties creates opportunities for further research into the pharmacological, toxicological properties and clinical relevance of *Ficus exasperata*.

The plant sample was collected, identified, dried, and extracted. Plant sample was assessed for its phytochemical constituents, Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), using spectrophotometric methods. Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, and Total Antioxidant Capabilities (TAC), the antioxidant properties of the plant extract was assessed using specified methods.

Glycosides, alkaloids, flavonoids, phenols were among the phytochemicals observed to be present. Total Phenolic Content (TPC) was  $1.82 \pm 0.47$  mg GAE/g of extract, Total Flavonoid Content (TFC) was found to be  $47.69 \pm 23.2$  mg QE/g of extract. The  $IC_{50}$  of ascorbic acid and extract for DPPH scavenging activity was determined to be 1.31  $\mu$ g/mL and 1.91  $\mu$ g/mL, respectively. The  $IC_{50}$  for TAC of the extract was determined to be 1.23  $\mu$ g/mL, while that of the standard (ascorbic acid) was found to be  $8.82 \times 10^{-9}$   $\mu$ g/mL.

The methanol extract of the leaves of *Ficus exasperata* Vahl showed excellent antioxidant potential and thus should be examined for pharmacological, toxicological and clinical significance.

## **CHAPTER ONE**

### **INTRODUCTION AND LITERATURE REVIEW**

#### **1.1 TRADITIONAL MEDICINE**

Traditional medicine encompasses the collective wisdom, abilities, and customs rooted in the unique beliefs, theories, and accumulated experiences of diverse cultures, whether comprehensible by scientific standards or not. It is employed both for sustaining well-being and for addressing the full spectrum of physical and mental health concerns, including prevention, diagnosis, enhancement, and healing.<sup>[1]</sup> This is greatly contrasted from scientific medicine or evidence-based medicine. In line with the "Traditional Medicine Strategy 2014–2023," the World Health Organization (WHO) expressed its commitment to assisting Member States in formulating forward-looking policies and executing strategies aimed at enhancing the contribution of traditional medicine to the well-being of their populations.<sup>[2]</sup> Traditional medical practices encompass a diverse range of systems, including but not limited to traditional European, Chinese, Korean, African, Ayurvedic, Siddha, Unani, Iranian, Islamic, Muti, and Ifá medicines. The scientific fields dedicated to the examination of these traditional systems encompass herbalism, ethnomedicine, ethnobotany, and medical anthropology.

Traditional medicine and medicinal plants are interconnected in several ways. Traditional medicine often relies on the use of various plants and plant-derived substances for healing and treating ailments.

Historical Use: Traditional medicine systems, which have been practiced for centuries in different cultures worldwide, frequently incorporate the use of medicinal plants. Indigenous

peoples and local communities have relied on the knowledge of specific plants and their healing properties to address health issues.

**Plant-Based Remedies:** Traditional healers or practitioners in various regions have developed a deep understanding of the therapeutic properties of specific plants. They use these plants to create remedies, including decoctions, infusions, poultices, and salves, to treat a wide range of illnesses and conditions.

**Cultural Knowledge:** Traditional medicine is often closely tied to the cultural knowledge and practices of a particular community or ethnic group. This knowledge is passed down through generations and encompasses the use of medicinal plants, rituals, and holistic approaches to health and well-being.

**Biodiversity:** Traditional medicine systems are often closely connected to the local biodiversity of a region. The diversity of plant species in an area contributes to the rich repertoire of medicinal plants available to traditional healers. Different ecosystems may have unique plants with specific medicinal properties.

**Plant Chemistry:** Traditional medicine relies on the bioactive compounds found in medicinal plants. These compounds can have various effects on the body, such as anti-inflammatory, analgesic, antimicrobial, or antioxidant properties. Traditional healers have learned to harness these compounds for therapeutic purposes.

**Holistic Approach:** Traditional medicine often takes a holistic approach to health, considering the physical, mental, and spiritual aspects of well-being. Medicinal plants are used not only for treating physical ailments but also for addressing spiritual and emotional imbalances.

**Empirical Knowledge:** Traditional medicine practitioners have developed empirical knowledge about the effectiveness of medicinal plants over generations. This knowledge is based on observations, trial and error, and the experiences of individuals within the community.

**Integration with Modern Medicine:** In some cases, traditional medicine and modern medicine can be integrated. Medicinal plants have provided valuable leads for the development of pharmaceutical drugs. Scientists have identified and isolated active compounds from medicinal plants to create modern medicines.

**Sustainability and Conservation:** The reliance on medicinal plants in traditional medicine highlights the importance of sustainable harvesting and conservation efforts. Overharvesting of certain plant species can lead to biodiversity loss and affect the availability of traditional remedies.

**Global Interest:** Traditional medicine and the use of medicinal plants have gained global attention. Researchers and scientists are increasingly studying traditional remedies to understand their efficacy and safety. This has led to the recognition of certain traditional medicinal practices and plants in healthcare systems worldwide.

In summary, traditional medicine and medicinal plants are deeply intertwined, with plants serving as the foundation of many traditional healing practices. This connection reflects the rich cultural and ecological diversity found across different regions and communities.

## **1.2 MEDICINAL PLANTS**

A medicinal plant is a botanical species that possesses substances within its various parts, capable of being employed for therapeutic uses, or that serve as essential elements in the

synthesis of beneficial pharmaceuticals.<sup>[3]</sup> Whether employed in traditional medicine or modern medicine, medicinal plants are utilized with the goal of promoting health, being administered for a specific illness, or both. Health advantages to those who use medicinal plants as medicines, financial benefits to those who harvest, prepare, and distribute them for sale, and societal benefits including job possibilities, tax revenue, and a more productive labor force are the three fundamental forms of benefits that medicinal plants may offer.<sup>[4]</sup> Due to their affordability and accessibility compared to contemporary pharmaceuticals, medicinal plants are frequently employed as folk medicine in non-industrialized societies. However, a lack of strong scientific backing, subpar drug development procedures, and inadequate funding stifle the development of plants or extracts with potential medical use.<sup>[5]</sup> Numerous chemical compounds are produced by plants for a variety of purposes, including defense and protection against insects, fungus, illnesses, and herbivorous mammals.<sup>[6]</sup>

### **1.3 PHYTOCHEMICAL BASIS**

Each botanical specimen engages in the synthesis of a repertoire of chemical compounds, endowing them with a discernible evolutionary advantage, a strategic adaptation that enhances their ecological fitness and competitiveness within their respective ecosystems.<sup>[7]</sup>

These chemical compounds can be referred to as metabolites, which can be grouped into two (2) major categories;

- Primary metabolites
- Secondary metabolites

Primary metabolites: Primary metabolite stands as a category of metabolites intricately entangled in the essential processes of ordinary growth, development, and reproductive activities within an organism. These metabolites serve indispensable physiological roles intrinsic to the organism's well-being, and they exhibit a ubiquitous presence across various organisms or cellular systems. Additionally, they are often denoted as central metabolites, emblematic of their pivotal status in sustaining fundamental life functions.<sup>[8]</sup>

These compounds, comprise a comprehensive spectrum of chemical entities, including:

- Carbohydrates,
- Proteins,
- Lipids,
- Hormones, and
- Nucleic acids.

These collectively contribute to the intricate web of biochemical reactions that underpin the growth, development, and overall physiological well-being of plants.

Secondary metabolites: Secondary metabolites, distinct from primary metabolites, do not play a direct role in the vital functions of growth, development, and reproduction within organisms. These organic compounds are not immediate prerequisites for a plant's survival but rather contribute to various aspects of their normal growth and development. Secondary metabolites emerge through biosynthetic processes rooted in primary metabolites, yet they exhibit a more confined distribution within the plant kingdom, typically being restricted to specific taxonomic

groups such as species, genera, families, or closely related clusters of families.<sup>[9]</sup> While secondary compounds lack an overt function in a plant's primary metabolic activities, they often serve ecological purposes. They can function as attractants for pollinators, serve as chemical adaptations to environmental stresses, or act as defensive agents against microorganisms, insects, higher-level predators, and even other plants (known as allelochemicals). Compared to primary metabolites, secondary metabolites are frequently found in smaller quantities. Furthermore, the synthesis of secondary metabolites is intricate, often taking place in specialized cell types and at distinct developmental stages, which can make their extraction and purification challenging. Consequently, secondary metabolites, when employed commercially as biologically active compounds (including pharmaceuticals, flavors, fragrances, and pesticides), tend to be higher in value but lower in volume compared to primary metabolites, which are bulk chemicals. <sup>[9]</sup>

In summary, secondary metabolites are typically substantial organic molecules that necessitate a multitude of specific enzymatic steps for their production, contrasting with primary metabolites. They are often considered specialty materials or fine chemicals due to their specialized nature and are intricately linked to various ecological and defensive functions within the plant world.

These include; Alkaloids, Phenolics, Tannins, Saponins, Terpenes, Polyphenols, Flavonoids, Carotenoids, Glycosides, and so on.

**Alkaloids:** Alkaloids constitute a category of naturally occurring organic compounds characterized by their fundamental chemical structure, which includes at least one nitrogen atom. This group encompasses not only compounds with basic properties but also includes related compounds that can be neutral or even slightly acidic. Occasionally, synthetic compounds that share a similar structural framework may also be referred to as alkaloids. Apart from the core

elements of carbon, hydrogen, and nitrogen, alkaloids may also incorporate oxygen or sulfur into their chemical composition. In rarer cases, they may contain elements like phosphorus, chlorine, or bromine. <sup>[10][11][12]</sup> Alkaloids demonstrate a diverse array of pharmacological actions including analgesia, local anesthesia, cardiac stimulation, respiratory stimulation and relaxation, vasoconstriction, muscle relaxation and toxicity, as well as antineoplastic, hypertensive and hypotensive properties. The activity of alkaloids against herbivores, toxicity in vertebrates, cytotoxic activity, the molecular targets of alkaloids, mutagenic or carcinogenic activity, antibacterial, antifungal, antiviral and allelopathic properties have been reported in literature. Many alkaloids are sufficiently toxic to animals to cause death if eaten. Several (e.g., nicotine and anabasine) are used as insecticides. The following are basic types of alkaloids: acridones, aromatics, carbolines, ephedras, ergots, imidazoles, indoles, bisindoles, indolizidines, manzamines, oxindoles, quinolines, quinozolines, phenylisoquinolines, phenylethylamines, piperidines, purines, pyrrolidines, pyrrolizidines, pyrroloindoles, pyridines and simple tetrahydroisoquinolines.

**Flavonoids:** The largest class of naturally occurring phenolic chemicals is represented by flavonoids. About 500 of these compounds are known to exist in their unbound form, and researchers currently know about more than 2,000 of them. A chroman ring that is embellished with an aromatic ring at positions 2, 3, or 4 forms the basis of the structure of flavonoids. Anthocyanins, flavones, and flavonols are the most common subtypes of flavonoids. Flavonoids can be divided into distinct groups based on the level of oxidation in the middle ring (also known as ring C). Recent studies have shown that drugs containing flavonoids, such as *Glycyrrhiza glabra* (licorice root), *Chamaemelum nobile* (Roman chamomile), and *Ginkgo biloba* (gingko), have therapeutic effects.

**Tannins:** Tannins are polyphenols which have the ability to precipitate protein. There are two major types of tannins:

- Hydrolyzable tannins
- Condensed tannins.

**Hydrolyzable tannins:** Gallic and hexahydroxydiphenic acids are two phenolic acid molecules that are joined by ester bonds to a central glucose molecule to generate hydrolyzable tannins. Gallotannins and ellagitannins, which are made up of units of gallic acid and ellagic acid respectively, are the two main forms of hydrolysable tannins.

**Condensed tannins:** Also known as proanthocyanidins, are substances with structures based on oligomeric flavonoid precursors. These compounds differ in the type of connections between flavonoid units, hydroxylation patterns, stereochemistry of the pyran ring's carbons 2, 3, and 4, and the inclusion of extra substituents. Some medications contain both hydrolyzable and condensed tannins, such as those found in *Camellia sinensis* (tea), *Hamamelis virginiana* leaves, and bark.

Tannin-containing drugs act as antidiarrhoeals and have been employed as antidotes in poisoning by heavy metals and alkaloids. For example, epigallocatechin-3-gallate, the active principal in tea, has been shown to be antiangiogenic in mice.

**Saponins:** Saponins are molecules containing a polycyclic aglycone moiety connected to a carbohydrate unit (a monosaccharide or oligosaccharide chain), either a steroid (steroidal saponins) or a triterpenoid (triterpenoidal saponins). The saponin molecule's aglycone segment is referred to as the genin or sapogenin. Saponins are pervasive in the plant kingdom, having been

identified in over 500 plant species across at least 90 distinct plant families. These compounds have been extracted from various parts of plants, including leaves, stems, roots, bulbs, flowers, and fruits. However, they tend to be most concentrated in the roots of several species, such as *Digitalis purpurea* (foxglove), *Dioscorea villosa* (wild yam), *Eleutherococcus senticosus* (Siberian ginseng), *Gentiana lutea* (gentian), *Glycyrrhiza spp.* (licorice), and *Panax ginseng* (Korean ginseng).

**Phenolics:** The majority of secondary metabolites within the plant kingdom are presumed to belong to the phenolic category. These compounds exhibit a broad spectrum of structures, ranging from uncomplicated structures comprising a single aromatic ring to intricate polymeric substances. A common feature among them is the presence of one or more phenol groups. These phenolic compounds are prolific in plant sources and exert significant influence over the sensory attributes, including flavor, color, and taste, in numerous food products, beverages, and herbal preparations. From a pharmacological perspective, certain phenolics are highly esteemed for their anti-inflammatory properties, exemplified by compounds like quercetin, or their hepatoprotective capabilities, as observed in the case of silybin. Additionally, some phenolic compounds, such as genistein and daidzein, exhibit phytoestrogenic attributes, while others, like naringenin, demonstrate insecticidal properties. A multitude of phenolic substances, notably within the flavonoid category, are recognized for their robust capacity to scavenge free radicals and act as antioxidants.

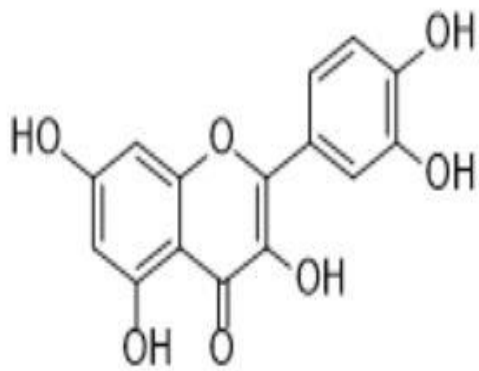
**Terpenes:** Terpenes represent the most extensive and exceptionally varied assortment of secondary compounds encountered in plant organisms. The term "terpene" is etymologically rooted in "turpentine," which, in turn, traces its origins to the ancient French word "ter(e)bintb," signifying "resin." From a chemical standpoint, terpenes all emerge from the intricate assembly

of 5-carbon isoprene units with diverse structural arrangements. The classification of a terpene is contingent upon the specific count of isoprene units residing within its molecular framework.

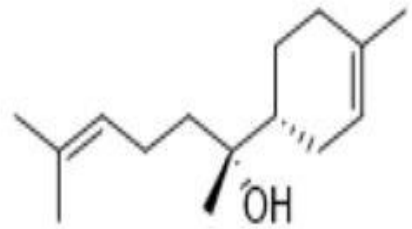
They can be classified into;

- Hemiterpenes,
- Monoterpenes,
- Sesquiterpenes,
- Diterpenes,
- Triterpenes,
- Sesterterpenes. <sup>[9]</sup>

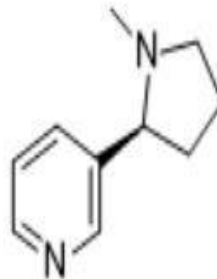
## EXAMPLE OF SOME COMMON PHYTOCHEMICALS



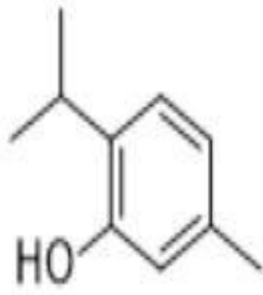
Quercetin



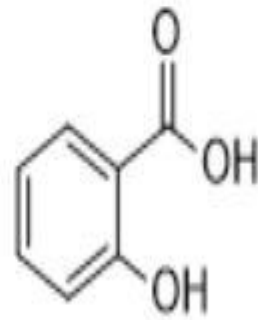
Bisabolol



nicotine

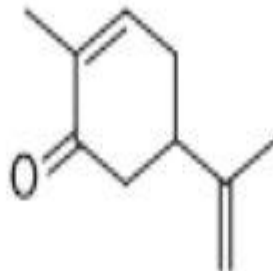


thymol



salicylic acid

Rectangular Snip



carvone

## 1.4 BRIEF HISTORY OF MEDICINAL PLANTS

Since prehistoric times, therapeutic plants, often known as medicinal herbs, have been identified and employed in traditional medical procedures. Humans have employed plants as remedies, but not always successfully. This includes many plants that are currently used as culinary herbs and spices. On clay tablets from ancient Sumeria, dating back to about 3000 BC, hundreds of medicinal herbs, including myrrh and opium, are listed. Over 800 plant remedies, including aloe, juniper, mandrake, castor bean, cannabis, and garlic, are listed in the ancient Egyptian Ebers Papyrus.

Emperor Shen Nung's "Pen T'Sao," a Chinese treatise on roots and grasses, was written in 2500 BC and covers 365 medications (dried sections of medicinal plants). Many of these remedies are still used today, including the following: Podophyllum, ginseng, jimson weed, cinnamon bark, ephedra, camphor, *Theae folium*, the great yellow gentian, and *Rhei rhisoma*.

There are references to 63 plant species from the Minoan, Mycenaean, Egyptian, and Assyrian cultures in Homer's epics The Iliad and The Odysseys, written around 800 BC. Some of them were given names in honor of mythological figures from these epics; for example, Elecampane (*Inula helenium* L. Asteraceae) was given the name Elena in honor of the goddess who served as the focal point of the Trojan War. The name of the Artemisia genus of plants, which were thought to protect and restore health, was taken from the Greek word artemis, which means "healthy".

The castor oil plant was mentioned by Herodotus in 500 BC, the aromatic hellebore and garlic by Orpheus, and the sea onion (*Scilla maritima*), mustard, and cabbage by Pythagoras.

Hippocrates' writings (c. 459–370 BC) list 300 therapeutic plants, categorized by physiological action: Opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as adstringents; and wormwood and common centaury (*Centaureum umbellatum* Gilib) as remedies for fever.

Dioscorides, known as "the father of Pharmacognosy," was the most well-known author on plant medicines in ancient times. As a military doctor and pharmacognosist for Nero's Army, he researched therapeutic plants wherever the Roman Army went. He published "De Materia Medica" around 77 AD. Until the late Middle Ages and the Renaissance, this canonical source of ancient history, which has been translated numerous times, provides a wealth of information about the medicinal herbs that made up the basic materia medica. 657 of the 944 medications that are discussed are plant-based, with details on their external appearance, location, method of collection, method of production, and therapeutic outcome. The names of the plants in various languages are also included, along with information about the regions where they might be found or farmed. The majority of the plants are those with a minor effect, but there are also mentions of some that contain alkaloids or other substances that have a stronger impact (fragrant hellebore, false hellebore, poppy, buttercup, jimson weed, henbane, deadly nightshade). Willow, camomile, garlic, onion, marshmallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, and false hellebore are among Dioscorides' favorite domestic plants. The herb known as chamaemelon, or *Matricaria recucita* L., is used as an antiphlogistic to treat wounds, stings, burns, and ulcers as well as to wash and rinse the eyes, ears, nose, and mouth. Because of its mild carminative effect, it is especially suitable for use with kids. Dioscorides believed it to have an emasculating effect, stating that "The flower, root, and entire plant accelerate menstruation,

the release of the embryo, and the discharge of urine and stone, provided that they are used in the form of an infusion and baths". Dioscorides distinguished between many *Mentha* species that were grown and used to treat headache and stomach ache. White willow was employed as an antipyretic, while sea onion and parsley bulbs were used as diuretics, gynecological uses for oak bark, and more. According to Dioscorides, *scillae bulbus* was also used as an antihydrotic, expectorant, and heart stimulant. It is important to note that Dioscorides alluded to the possibility of drug forgery when discussing both domestic substances like opium made from milk sap and a yellow poppy (*Glaucium flavum*) as well as more expensive foreign substances brought in by Arab traders from the Far East, such as iris, calamus, caradmom, incense, etc.

The earliest list of interchangeable pharmaceuticals having a similar or identical mechanism of action (parallel drugs) was created by Galen, the most eminent Roman physician and pharmacist (131 AD–200 AD), in his work "De succedanus." From today's perspective, several of the alternatives that have been suggested do not make sense in a pharmaceutical setting and are wholly unsuitable. He also contributed a number of novel plant medicines that Dioscorides had not mentioned, such as *Uvae ursi folium*, which is still used today as a mild diuretic and uroantiseptic.

Herbal medicine in the medieval ages and antiquity bears witness to a captivating chronicle of botanical wisdom and therapeutic endeavors that reverberate through the annals of history. During these epochs, when medical science was in its nascent stages, herbalism emerged as the cornerstone of healthcare practices.

In the medieval ages, monasteries evolved into bastions of herbal knowledge and experimentation. Monastic orders, often fortified with the veneration of learning and meticulous

record-keeping, cultivated a treasure trove of medicinal plants within their cloistered gardens. These sacred institutions became epicenters of herbal cultivation and medication production, driven by the doctor-monks who diligently tended to these botanical pharmacies. Notable among these botanicals were sage, anise, mint, Greek seed, savory, and tansy, among others. The illustrious medical institution in Salerno, attributed to the legacy of Charles the Great (742 AD–814), was instrumental in advancing the field of medicine during this era. Under his guidance, detailed in his "Capitularies," provisions were made for the cultivation of medicinal plants on public estates. This decree encompassed an extensive list of nearly 100 distinct plants, many of which continue to find applications in contemporary medicine. Among these botanical treasures were sage, sea onions, iris, mint, common centaury, poppies, and marsh mallow, exemplifying the enduring relevance of these ancient herbal remedies.

These historical epochs underscore the enduring significance of herbal remedies. Herbalists and physician-monks of yore, armed with an intricate understanding of botanicals, navigated the complexities of plant-based medicines. Their pioneering efforts not only contributed to the preservation of knowledge but also laid the foundation for the evolution of modern pharmacology, as many of the plants they studied continue to shape contemporary medicine. The annals of herbal medicine in the medieval and ancient world remain a testament to humanity's enduring quest for healing and the profound interplay between nature's bounty and the advancement of medical science.

In antiquity, the utilization of medicinal plants predominantly centered around uncomplicated pharmaceutical preparations, encompassing methods such as infusions, decoctions, and macerations. However, during the Middle Ages, and notably within the span extending from the 16th to the 18th centuries, a discernible shift occurred in healthcare practices.

This transformation was marked by an escalating demand for compound drugs, intricate formulations that incorporated not only medicinal plants but also components derived from the animal and mineral realms. The pinnacle of these compound concoctions was exemplified by theriac, a prized elixir produced through the amalgamation of an extensive array of medicinal plants, rare animal substances, and minerals. This extraordinary therapeutic compound garnered considerable esteem and commanded a premium price in the market.

The early 19th century represents a pivotal juncture in the understanding and utilization of medicinal plants, marking the genesis of scientific pharmacy. This era witnessed a cascade of breakthroughs, commencing with the momentous discovery, substantiation, and subsequent isolation of alkaloids from a variety of plants. Notable instances include the extraction of alkaloids from poppy in 1806, ipecacuanha in 1817, strychnos in 1817, quinine in 1820, and pomegranate in 1878, among others. These pioneering efforts heralded a scientific revolution, propelling the study of pharmacologically active compounds found within botanical sources.

As chemical techniques and methodologies underwent refinement, the exploration of medicinal plants yielded an array of additional active substances. This comprehensive inquiry led to the isolation of various compounds, including glycosides, tannins, saponosides, essential oils, vitamins, hormones, and more. These discoveries ushered in an era of scientific rigor and precise understanding of the diverse constituents inherent in medicinal flora.

However, by the late 19th and early 20th centuries, a concern emerged regarding the potential relegation of medicinal plants from therapeutic practice. Several scholars argued that pharmaceutical preparations derived from these plants exhibited certain drawbacks owing to the action of enzymes, which induced substantial alterations during the drying process of medicinal

plants. Notably, it was discerned that the efficacy of medicinal plants was intrinsically linked to the manner in which they were dried.

During the 19th century, there emerged a shift in favor of therapeutics, alkaloids, and glycosides isolated in their purest form, thereby superseding the utilization of the original plant-derived drugs. However, subsequent investigations illuminated that while pure alkaloids demonstrated swifter action, the holistic effects of alkaloid-based drugs proved to be more comprehensive and enduring.

Advancements in the early 20th century brought forth stabilization methods for freshly harvested medicinal plants, particularly those containing labile active components. Additionally, extensive research was dedicated to understanding the optimal conditions for the cultivation and manufacturing of medicinal plants. These multifaceted endeavors aimed to preserve the efficacy and therapeutic potential of medicinal flora, ensuring their enduring significance in the realm of healthcare and pharmacology. <sup>[13]</sup>

## **1.5 SAFETY OF HERBAL MEDICINES**

In light of the substantial surge in global herbal medicine consumption, concerns about the safety of these remedies have come to the forefront. Currently, there exist misconceptions and preconceived notions that cast doubt on the safety of herbal medicine. Consequently, there is a pressing need for a comprehensive, unbiased, and equitable approach to understanding, interpreting, and disseminating information about the safety of herbal treatments. Such an approach should prioritize objectivity, neutrality, and widespread public awareness.

In accordance with a proverbial wisdom in Chinese culture, it is recognized that "all medicines have their own side effects." In essence, this adage underscores the dual nature of medicinal

interventions, akin to a double-edged sword. Medications possess the inherent capability to either alleviate ailments and uphold well-being, or conversely, to induce harm to the human body. It is imperative to acknowledge that virtually all efficacious pharmaceuticals, without exception, have the potential to generate adverse drug reactions. Herbal medicines, although rooted in nature, are not exempt from this principle.

Hence, the judicious use of herbal medicine necessitates a meticulous approach, one that involves the precise determination of dosage, treatment duration, and applicability to the specific syndrome in question. The indiscriminate and excessive use of herbal remedies should be unequivocally discouraged. Instead, the focus must be on the prudent and tailored utilization of herbal treatments, a practice that optimizes their therapeutic benefits while mitigating the associated risks.

There are a number of causes of adverse events to herbal medicines, which can be divided into “direct” and “indirect” reasons.

**Direct or innate toxicity:** The underlying cause of this phenomenon lies in the inherent toxicity exhibited by certain herbs, either when administered within the bounds of standard therapeutic dosages or when consumed in excess. Observable instances of adverse reactions linked to the usage of herbs like *Ephedra*, *Aristolochia*, and *Aconitum* have served as concrete illustrations of the capacity of herbs to elicit toxic responses within the human body.

**Contamination, adulteration, misidentification or substitution and Inadequate processing and preparation:** The occurrence of detrimental consequences linked to the utilization of herbal remedies can often be traced back to a multitude of factors, including the potential contamination of herbal products with hazardous metals, the surreptitious inclusion of unauthorized substances,

the misidentification or replacement of herbal constituents, or the inappropriate processing and preparation of herbal formulations. These multifaceted issues have, on occasion, yielded distressing outcomes. To illustrate, instances where *Caulis Akebiae* was substituted with *Caulis Aristolochiae Manshuriensis* or *Stephania tetrandra* was replaced by *Aristolochia fangchi* have given rise to a severe and concerning condition known as "aristolochic acid nephropathy." This serves as a poignant example of how erroneous identification and substitution within the herbal ingredient repertoire can precipitate profound health concerns.

Wrong indication: The inappropriate utilization of herbal medicines can lead to negative or even potentially dangerous effects on health. A case in point is the herb known as "Ma Huang," which traditionally holds a place in Chinese medicine for its application in alleviating respiratory congestion. However, a stark contrast emerges in the United States, where Ma Huang was marketed and sold as a dietary supplement, specifically formulated for weight reduction.

The critical issue arose when individuals in the United States, seeking weight loss, began using Ma Huang in large quantities, often exceeding recommended dosages. This excessive consumption had dire consequences, including the tragic loss of at least twelve lives. Moreover, it led to numerous instances of heart attacks and strokes, which underscored the serious health risks associated with the misuse and overuse of herbal remedies. This example serves as a poignant reminder of the imperative need for a nuanced understanding of herbal medicines, proper dosing, and informed usage to prevent unintended and detrimental health outcomes.

Herb-drug interaction: The realm of herbal medicines is characterized by the inherent complexity of these remedies, each comprising a diverse array of active constituents. This intricate amalgamation of active ingredients heightens the likelihood of interactions between herbal

medicines and conventional pharmaceutical drugs. Furthermore, individuals who resort to medicinal herbs typically grapple with chronic health conditions that necessitate concurrent use of prescribed medications. This confluence of factors significantly amplifies the potential for herb-drug interactions, a domain fraught with clinical implications. A retrospective cross-sectional study conducted on these subject unearthed compelling insights. It revealed that a substantial 36.4% (ranging from 34.2% to 38.6%) of individuals were concurrently using herbal medicinal products alongside antipsychotic treatments.

Within this context, specific herbal regimens, such as those featuring *Radix Bupleuri*, *Fructus Gardenia*, *Fructus Schisandrae Chinensis*, *Radix Rehmanniae*, *Akebia Caulis*, and *Semen Plantaginis*, when used in conjunction with medications like quetiapine, clozapine, and olanzapine, were found to be associated with a heightened risk—nearly 60%—of adverse clinical outcomes. These findings underscore the intricate interplay between herbal remedies and conventional drugs, underscoring the importance of vigilance and informed decision-making in managing the potential risks of herb-drug interactions in individuals with chronic health conditions. St. John's Wort, an herbal remedy used for mild to moderate depression, can interact with various antidepressant medications, including selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs). This interaction can lead to a potentially dangerous condition known as serotonin syndrome, characterized by symptoms like agitation, confusion, rapid heart rate, and high blood pressure. *Ginkgo biloba*, a popular herbal supplement for cognitive function, can increase the risk of bleeding when taken with blood-thinning medications like warfarin or aspirin. This can result in excessive bleeding and bruising. Garlic supplements, often used for their potential cardiovascular benefits, may enhance the effects of anticoagulant medications like heparin and warfarin, increasing the risk of bleeding complications.

These examples highlight the diverse range of herb-drug interactions that can occur when herbal remedies and pharmaceutical drugs are used concurrently. It underscores the importance of healthcare providers being aware of a patient's use of herbal supplements to ensure safe and effective treatment.

Insufficient basic research on herbal medicine safety: Sheng Nong's Herbal, which stands as the inaugural classic within the realm of Chinese materia medica, meticulously documented a compendium of 365 herbs. These botanical specimens were judiciously categorized into three tiers—high grade, moderate, and inferior—predicated on the degree of toxicity inherent in each herb. Fast forward to the Chinese Pharmacopoeia of 2010, and we find 83 distinct varieties of Chinese materia medica that have officially been acknowledged and designated as possessing toxic attributes. These substances have been further stratified into three overarching categories, denoting high, medium, and low levels of toxicity. It is pertinent to note that much of the information concerning the efficacy and toxicity of these herbal entities remains rooted in traditional knowledge and clinical observations. Regrettably, the current classification system for toxicity lacks a concrete foundation in scientific standards and empirical, objective experimental data. Gaps persist in our comprehension of toxic herbs, the specific organs they may target with their adverse effects, the permissible range of safe dosages, the margin of safety within effective dosages, and the minimum threshold of toxicity. <sup>[14]</sup>

Consequently, establishing a comprehensive understanding of the toxicological profiles and adverse effects associated with each herbal medicine assumes paramount importance. This knowledge serves as the cornerstone for ensuring the judicious and secure utilization of herbal remedies within the realm of healthcare.

Patients who are concurrently using both herbal medicines and conventional medications, which is a frequent scenario, frequently omit disclosing their herbal medicine use to their healthcare providers. Similarly, patients often neglect to inform their herbal medicine practitioners about their ongoing use of conventional medications. This communication gap poses a considerable challenge in ensuring the safety and efficacy of healthcare interventions. It is imperative that healthcare professionals and herbal medicine practitioners adopt a proactive and empathetic approach by directly and persistently inquiring about the full spectrum of substances their patients are consuming. This includes a comprehensive assessment of not only prescription medications but also herbal remedies and other self-care health products. Effective communication and transparency are paramount. To mitigate the potentially severe risks associated with the misuse or unmonitored combination of herbal medicines and conventional drugs, it is indispensable to prioritize the education of healthcare professionals, herbal medicine practitioners, and patients alike. Equipping these stakeholders with comprehensive knowledge and awareness is a pivotal step in safeguarding the well-being of individuals who seek diverse avenues of care, encompassing both traditional and modern approaches to healthcare. <sup>[15]</sup>

## 1.6 *Ficus exasperata*

### PLANT CLASSIFICATION

Kingdom	<i>Plantae</i>
subkingdom	<i>Viridaeplantae</i>
Phylum	<i>Tracheophyta</i>
Subphylum	<i>Euphyllophytina</i>
Infraphylum	<i>Radiatopses</i>
Class	<i>Magnoliopsida</i>
Subclass	<i>Dilleniidae</i>
Superorder	<i>Urticanae</i>
Order	<i>Urticales</i>
Family	<i>Moraceae</i>
Tribe	Ficeae
Genus	<i>Ficus</i>
Specific epithet	<i>exasperata</i> – Vahl.
Botanical name	<i>Ficus exasperata</i> Vahl

#### Common Names

English: Sandpaper fig tree, white fig tree,

French: Papier de verre,

Swahili: Msasa, Banyun <sup>[16]</sup>

#### Local Names in Nigeria

Igbo: Nkpu

Urhobo: Ebiememe

Yoruba: Afomo, Ewe epin

Anioma: Akwukwo anwilinwa

Hausa: Kulle, Yakule



**FIG 1.9 LEAVES, RIPPENED AND YOUNG FRUITS OF *Ficus exasperata***

Plant Description: The genus *Ficus*, usually known as fig, belongs to the Mulberry family (Moraceae), which includes about 1,100 species of trees, shrubs, climbers, and creepers. Chiefly found in tropical and sub-tropical regions, the genus is identified with a number of features, although their key characteristics being that they produce fruits with many seeds and an inflorescence inside them known as syconium.<sup>[17]</sup>

The genus is very important due to its high ecological, nutritional, medicinal and economic values. Some species of the *Ficus* genus are used as food, others have medicinal values, while most are used for food and medicine.<sup>[16]</sup>

According to *Devi et al*, different plant part of different species of *Ficus*, are used to manage and treat a variety of ailments as well as for food.<sup>[16]</sup> The bark, leaves, fruits and latex are considered to be very effective in diabetes, skin diseases, ulcers, dysentery, diarrhea, stomachache, hemorrhoids and as carminative, astringent, anti-inflammatory, anti-oxidant and anti-cancer agents.<sup>[17]</sup>

Due to its scabrous surface, the leaves are used for polishing wooden slates and furniture. They are also used for scrubbing utensils among the rural populations. They are a good source of feed as animal keepers use the leaves to feed sheep, goats, etc. The leaves are also used for the stabilization of palm oil to enhance the qualities by elimination of saponins and the foaming tendency as well as improving the carotenoid levels in the oil this results in reduced free fatty acids, acid level and peroxide value.<sup>[16]</sup>

Koshimizu *et al.*, opined that, medicinal plants that are used by nonhuman primates are valuable targets in the search for naturally occurring compounds of biological as well as physiological

importance. This arises after observing the peculiar manner at which chimpanzees feed on the leaves of *Ficus exasperata*.<sup>[18]</sup>

### **1.6.1 TRADITIONAL AND MEDICINAL USES OF *Ficus exasperata* Vahl.**

In the domain of traditional medicine, various components of *Ficus exasperata* Vahl. from the Moraceae family find application as multifaceted remedies. These plant constituents serve a spectrum of therapeutic purposes, including their use as analgesics, antiarthritics, diuretics, agents for wound healing, antiparasitics, vermifuges, abortifacients, ecboolics, and treatments for conditions such as hemorrhoids and venereal diseases. Additionally, these botanical elements also serve a utilitarian role as fodder for animals, underscoring their multifaceted significance in both traditional medicine and agricultural practices.<sup>[19]</sup>

The leaves of *Ficus exasperata* hold a position of great esteem in the realm of traditional medicine, where they are harnessed for an array of therapeutic applications. In the context of French Guinea, the leaves are employed to prepare a decoction that serves as a remedy for various gastrointestinal maladies. These versatile leaves find usage in the treatment of a wide spectrum of ailments, including hemostatic ophthalmia, respiratory conditions such as coughs, hemorrhoids, anxiety disorders, epilepsy, hypertension, rheumatism, arthritis, cancer, intestinal discomforts, colic episodes, as well as for managing bleeding and wounds. Across regions such as Nigeria, the Republic of Congo, and the Central African Republic, the leaves of *Ficus exasperata* assume the role of an antipyretic agent, effectively combatting feverish conditions. This diverse range of therapeutic applications underscores the multifaceted and invaluable role that these leaves play in addressing various health concerns within traditional healing practices.

In Nigeria, the young leaves are prescribed as a common anti-ulcer remedy.

In Ivory Coast, the leaves of *Ficus exasperata* are harnessed in a multitude of therapeutic contexts, demonstrating their versatile medicinal value. When combined with palm oil, these leaves play a pivotal role in managing cardiac arrhythmias, offering a potential remedy for this cardiac irregularity. Furthermore, a concoction of these leaves blended with the zesty infusion of lemon juice is effectively employed to combat a spectrum of respiratory tract infections, encompassing conditions such as asthma, bronchitis, tuberculosis, and emphysema.

The viscid, non-milky sap extracted from this plant holds medicinal significance, being employed in the treatment of hemostatic ophthalmia and stomachaches. Moreover, fresh leaves are applied externally to staunch bleeding, and within the domain of herbal therapeutics, these leaves are incorporated into polyherbal remedies, addressing concerns such as insomnia.

Across the terrain of Sierra Leone, dried leaf powder assumes a distinctive role in the management of vaginal rash, while the leaf's infusion is ingested orally as an abortifacient. The water extract derived from dried leaves is administered both orally and as an abdominal rub to induce contractions during childbirth. Additionally, dried leaf powder is enlisted for external application within a medicinal blend devised to address eruptive skin diseases.

In Gambia, the leaves undergo boiling, and the resulting steam is inhaled as a remedy for chest pain. In Ghana, the sap sourced from *Ficus exasperata* is employed to staunch bleeding. Moreover, intriguingly, the plant has been observed to hasten placental expulsion in cows following calf delivery and is utilized by traditional birth attendants to expedite human childbirth.

The roots of *Ficus exasperata* contribute to medicinal traditions as well, being utilized to manage conditions such as asthma, dyspnea, and venereal diseases. In Tanzania, a decoction crafted from the dried root bark is administered orally as a treatment for asthma. Fresh leaves serve a dual role,

both as a topical remedy for throat inflammation and tonsillitis, as well as an anthelmintic agent for combating parasitic infections. Dried flowers are ingested to alleviate throat pain and also hold value as an ascaricide, targeting intestinal parasites.

The application of *Ficus exasperata* extends to addressing skin ailments, where a paste derived from its roots, either used in isolation or combined with the crushed root of *Croton roxburghii* within a coconut milk emulsion, finds utility in treating eczema. These myriad applications across diverse cultural contexts underscore the profound botanical significance and rich medicinal heritage of *Ficus exasperata*.<sup>[16]</sup>

While numerous pharmacological activities have been ascribed to diverse extracts from various components of *Ficus exasperata*, comprehensive information regarding the active constituents remains elusive. Nevertheless, it has been reported that the leaves, stem bark, and roots of this plant species harbor a medley of chemical compounds, including steroids, flavonoids, phlobatannins, tannins, and saponins.

In a separate investigation conducted by Ogunleye *et al.* (2003), an aqueous leaf extract was found to contain an array of phytochemicals, encompassing saponins, alkaloids, tannins, flavonoids, and cardiac glycosides. Furthermore, Dongfack *et al.* (2008) conducted research leading to the isolation of a novel, yet unnamed acylglucosylsterol and an unconventional fatty acid from the leaves of *Ficus exasperata*.<sup>[20][21]</sup>

In the broader context of the *Ficus* genus, which comprises numerous species with extensive traditional applications, *Ficus exasperata* remains relatively uncharted in terms of its phytochemical composition. Given this knowledge gap, there exists a pressing need for comprehensive investigations aimed at characterizing the diverse extracts of *Ficus exasperata*.

Such endeavors are imperative to unlock the full therapeutic potential of this plant and to illuminate the intricacies of its phytochemical profile, offering promising avenues for in-depth research and therapeutic applications.

#### PHARMACOLOGICAL ACTIVITIES OF *Ficus exasperata* Vahl

Within the realm of *Ficus exasperata*, the leaves have emerged as a focal point of extensive research endeavors conducted by scholars worldwide. These botanical constituents have been subjected to thorough scrutiny, resulting in a wealth of knowledge concerning their diverse pharmacological attributes.

The leaves of *F. exasperata* have garnered widespread attention due to their multifaceted pharmacological properties, rendering them a subject of immense scientific interest. Researchers from across the globe have undertaken rigorous investigations into the therapeutic potential encapsulated within these leaves, uncovering a spectrum of compelling activities.

One notable facet of their pharmacological profile is their antidiabetic effect. Studies have illuminated the leaves' capacity to modulate glucose levels, offering promise in the management of diabetes mellitus. Their hypotensive properties, on the other hand, entail the ability to exert a blood pressure-lowering influence, holding potential significance in the management of hypertension.

Moreover, the leaves have been celebrated for their robust antioxidant activity, effectively neutralizing harmful free radicals within the body and conferring protective effects against oxidative stress. This antioxidant prowess underscores their potential utility in preventing or mitigating various chronic diseases linked to oxidative damage.

The anti-inflammatory attributes of *F. exasperata* leaves are equally noteworthy. They exhibit the capacity to alleviate inflammation, a hallmark of numerous pathological conditions, thereby opening avenues for therapeutic interventions in various inflammatory disorders. Furthermore, their antiarthritic properties make them a candidate for managing joint-related ailments, offering relief to individuals grappling with arthritic conditions.<sup>[16]</sup>

In summation, the leaves of *F. exasperata* stand as a testament to the rich pharmacological diversity embedded within the plant kingdom. Their multifaceted activities, ranging from antidiabetic and hypotensive effects to antioxidant, anti-inflammatory, and antiarthritic properties, underscore their significance in contemporary medicine and the potential they hold for the development of novel therapeutic interventions. The thorough exploration of these leaves by researchers worldwide underscores the ongoing quest to harness nature's botanical treasures for the betterment of human health and well-being.

In a study conducted by Taiwo *et al.* (2010), the impact of an aqueous extract derived from the leaves of *Ficus exasperata* was scrutinized with regard to its influence on glucose metabolism in rats exhibiting glucose intolerance induced by fructose consumption. The findings of this investigation yielded noteworthy insights into the potential therapeutic attributes of *F. exasperata* leaf extract.

The researchers deduced that the administration of *F. exasperata* leaf extract demonstrated a positive effect by mitigating glucose intolerance induced by fructose intake in the rat model. Importantly, they posited that these effects could potentially be attributed to the extract's ability to stimulate insulin secretion by the pancreas. Moreover, it was postulated that the extract might enhance insulin sensitivity within various organs, particularly the muscles and the liver. These

effects bear semblance to the actions of sulfonylurea drugs, a class of pharmaceutical agents commonly employed to manage diabetes.

In essence, this study underscores the potential of *Ficus exasperata* leaves as a valuable natural resource with promising implications for the management of glucose intolerance and diabetes. The observed effects, which encompass enhanced insulin secretion and heightened insulin sensitivity in critical organs, hold the prospect of contributing to the development of novel therapeutic strategies, akin to the mechanisms of action seen in established pharmaceutical interventions like sulfonylureas.<sup>[22]</sup>

Adewole *et al.* (2011) conducted an investigation aimed at assessing the hypoglycemic capabilities of *Ficus exasperata* leaf extract in a population of diabetic rats induced by streptozotocin. These rats encompassed both spontaneously hypertensive specimens and obese Zucker rats, collectively serving as subjects within the study. The findings of this study led Adewole *et al.* to draw a significant conclusion regarding the potential therapeutic benefits of *Ficus exasperata* leaf extract. Their research outcomes provided compelling evidence that the leaf extract indeed exhibits hypoglycemic properties, meaning it has the ability to lower blood glucose levels. Additionally, the extract displayed hypolipidemic properties, which imply a capacity to reduce elevated lipid levels in the bloodstream.

These observations align with the traditional and ethnomedical practices of the people in Western Nigeria, where *F. exasperata* has long been utilized as a remedy for managing diabetes. The study's results essentially validate the indigenous knowledge and folklore surrounding the plant, substantiating its historical use as a therapeutic agent for individuals grappling with diabetes. In essence, the scientific investigation affirms the efficacy of *Ficus exasperata* leaf

extract as a promising natural resource in the management of diabetes, bridging the gap between traditional wisdom and contemporary medical understanding.<sup>[23]</sup>

In a study by Abotsi *et al.* (2010) the antiarthritic potential of a hydroethanol extract derived from *Ficus exasperata* leaves was assessed in rats afflicted with Freund's adjuvant-induced arthritis. The extract was orally administered over a period of 29 days at varying doses ranging from 30 to 300 grams per kilogram of body weight. To mimic this debilitating condition for experimental purposes, researchers often turn to animal models, such as the adjuvant-induced arthritis model in rats. This model replicates certain key aspects of human rheumatoid arthritis, including chronicity, polyarticular involvement, and erosive joint damage. Remarkably, the results revealed a dose-dependent reduction in arthritic edema (swelling) within the rats' affected paws, with an impressive 34% reduction noted. Furthermore, the extract demonstrated the ability to prevent the spread of edema from the affected paw to the opposite, unaffected paw, thereby indicating its capacity to inhibit the systemic progression of the disease. Beyond these symptomatic improvements, the *Ficus exasperata* leaf extract exhibited its antiarthritic potential by suppressing pathological changes in bone structure. Notably, it achieved a remarkable 95% reduction in the radiological index, underscoring its ability to mitigate structural damage associated with arthritis.<sup>[24]</sup>

In essence, this study highlights the promising antiarthritic properties of the *Ficus exasperata* leaf extract, shedding light on its potential as a therapeutic agent for rheumatoid arthritis, a condition characterized by significant joint inflammation and degradation.

In light of the traditional medicinal use of young leaves from *Ficus exasperata* as a prevalent anti-ulcer remedy in Nigeria, researchers embarked on an investigation to assess the anti-ulcer

properties of an aqueous leaf extract of this plant. The study, which involved Wistar rats, aimed to evaluate the extract's efficacy against ulceration induced by aspirin. The results of this investigation revealed a compelling and dose-dependent reduction in the ulcer index, denoting a marked mitigation of ulcer formation with increasing extract dosage. Furthermore, the extract exhibited the capacity to delay small intestinal transit in mice, an effect that carries notable therapeutic significance for individuals suffering from ulcers. This delay in intestinal transit can be particularly beneficial in ulcer patients, as it allows for more effective healing and recovery. Notably, the study demonstrated that the *Ficus exasperata* leaf extract had a dose-dependent inhibitory effect on charcoal meal transit, with the highest inhibition, reaching an impressive 81%, observed at a dosage of 400 milligrams per kilogram. This inhibition significantly delayed gastric emptying, which is beneficial for ulcer management. Moreover, the extract induced alterations in gastric secretion parameters, including an increase in pH levels and a reduction in both the volume and total acidity of gastric secretions. These changes further underscore the extract's potential in mitigating the harmful effects of excess stomach acid, which is often implicated in ulcer formation and exacerbation.<sup>[25]</sup>

In sum, the investigation illuminated the remarkable anti-ulcer properties of the *Ficus exasperata* leaf extract, showcasing its ability to reduce ulcer formation, delay intestinal transit, and modulate gastric secretion parameters. These findings offer promising insights into the therapeutic potential of this natural remedy for individuals grappling with ulcers, reaffirming the value of traditional medicinal knowledge in contemporary medical research.

### 1.6.2 PHYTOCHEMICAL PROPERTIES OF *Ficus exasperata* Vahl

The investigation encompassed the isolation of various constituents from *Ficus exasperata* leaves and subjected them, alongside their respective crude extracts, to rigorous phytochemical screening. Within the leaves, four distinct constituents were isolated and subsequently analyzed. The results of the phytochemical screening unveiled the presence of cardiac glycosides and saponins. Notably, flavonoids, phenols, and tannins were notably absent in the isolated fractions but were present in the crude extract. Similarly, from the petroleum ether extract of the stem bark, three constituents were isolated and subjected to phytochemical screening. This scrutiny revealed the presence of alkaloids, saponins, cardiac glycosides, terpenoides, and sterols within these isolates.

It is noteworthy that both the leaves and stem bark of *Ficus exasperata* contain bioactive constituents belonging to significant phytochemical categories. These phytochemical groups are likely contributors to the plant constituents' bioactivity, underscoring their potential therapeutic significance and paving the way for further research into the plant's medicinal properties.<sup>[26]</sup>

The leaf of *Ficus exasperata* underwent a comprehensive analysis, encompassing proximate composition, mineral content, antinutrient assessment, phytochemical profiling, and antimicrobial activity. The results from the study by Ajayi *et al.*(2012) of these analyses provide valuable insights into the nutritional and bioactive properties of this botanical specimen.

Proximate composition analysis revealed that the leaf exhibited a rich nutritional profile. It contained a substantial amount of carbohydrates (38.73%), crude protein (16.85%), crude fat (13.75%), ash (11.76%), moisture (10.65%), and crude fiber (8.26%). These values fell within the expected range for dry leafy vegetables, highlighting its potential as a dietary resource.

Antinutrient screening unveiled the presence of bioactive compounds within the leaf. These compounds included phytate (112.82%), oxalate (4.502 mg/100g), flavonoids (3.21%), cyanide (0.543 mg/kg), and tannins (0.207 mg/ml). While some of these compounds can have negative effects on nutrient absorption and utilization, their levels are within acceptable limits. The leaf of *Ficus exasperata* was also found to be a source of essential minerals, including calcium (500.00 ppm), phosphorus (217.65 ppm), magnesium (187.20 ppm), and iron (168.20 ppm), underscoring its potential contribution to dietary mineral intake.

Phytochemical analysis revealed the presence of various bioactive compounds, including flavonoids, saponins, cardiac glycosides, and alkaloids. These phytochemicals are known for their potential health benefits and may contribute to the plant's medicinal properties.<sup>[27]</sup>

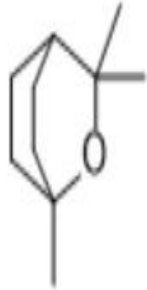
Overall, this comprehensive analysis sheds light on the multifaceted properties of *Ficus exasperata* leaf, encompassing its nutritional value, bioactive constituents, and potential health benefits.

A study conducted by Adebayo *et al.* in 2009 entailed an extensive phytochemical screening, which yielded intriguing findings regarding the constituents of interest. The results of this screening unveiled the presence of a range of bioactive compounds within the examined specimen. Notably, the detected compounds included flavonoids, saponins, tannins, steroids, and phlobatannins, signifying the botanical specimen's richness in diverse phytochemicals. Of particular note, the analysis revealed an absence of alkaloids and anthraquinones, further enhancing our understanding of the plant's chemical composition. These findings provide valuable insights into the phytochemical profile of the specimen, elucidating its potential applications and medicinal properties.<sup>[28]</sup>

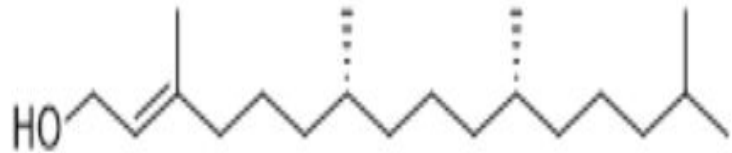
The composition of the volatile leaf oil from *Ficus exasperata* Vahl (Moraceae) was explored in a study conducted by Mubo *et al.* in 2009, with the findings duly reported. The oil extraction process involved hydrodistillation, and the subsequent analysis of its constituents was facilitated through gas chromatography-mass spectrometry (GC-MS), a powerful analytical technique renowned for its ability to identify and quantify complex compound mixtures.

The analysis illuminated the composition of the leaf oil, revealing a predominant presence of two major compound classes: terpenoids and aliphatic compounds. Within this diverse array of compounds, several emerged as quantitatively significant. Among these prominent constituents, 1,8-cineole stood out, constituting approximately 13.8% of the oil's composition. Additionally, (E)-phytol was identified as a noteworthy component, making up roughly 13.7% of the oil. Further contributing to the oil's complexity, p-cymene was found at a level of approximately 11.4%. Furthermore, the analysis elucidated that all other compounds, while not as abundant as the key constituents mentioned above, were nevertheless present at levels exceeding 2%. This comprehensive investigation into the volatile leaf oil's composition, as documented by Mubo *et al.* in 2009, deepens our understanding of the chemical profile of *Ficus exasperata* Vahl, shedding light on the diverse array of compounds that contribute to its aromatic and potentially therapeutic properties.<sup>[29]</sup>

**EXAMPLES OF SOME PHYTOCHEMICAL CONSTITUENTS IDENTIFIED FROM LEAVES *Ficus exasperata* Vahl.**

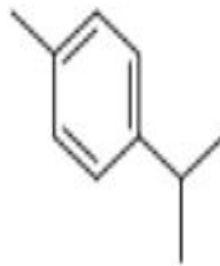


1,8-cineole



(E)-phytol

Rectangular Snip



p-cymene

## **1.7 EFFECT OF FREE RADICALS AND NEED FOR ANTIOXIDANTS**

Free radicals are chemical entities characterized by the presence of unpaired electrons in their outermost electron shell, rendering them highly reactive and unstable.<sup>[34]</sup> These unpaired electrons instill in free radicals a propensity to form bonds with electrons from other molecules, thereby instigating a diverse array of biological and chemical reactions. Consequently, free radicals possess the potential to trigger chain reactions and inflict damage upon cellular constituents, thereby contributing to a spectrum of health-related issues, including but not limited to the processes of aging, the development of cancer, and the onset of neurodegenerative disorders. Within the body, there exist intrinsic defense mechanisms, such as antioxidants, which serve the crucial function of counteracting and regulating the levels of free radicals. These antioxidants play a pivotal role in maintaining equilibrium in the intricate interplay between free radicals and protective agents. However, when this delicate balance between free radicals and antioxidants becomes disrupted, it can precipitate a state of oxidative or nitrosative stress, culminating in consequential harm to cells and tissues.

It is noteworthy that among the various free radicals, the most prevalent and detrimental species include reactive oxygen species (ROS) and reactive nitrogen species (RNS). These entities are particularly notorious for their potential to exert deleterious effects on biological systems.<sup>[43]</sup>

### **1.7.1 REACTIVE OXYGEN SPECIES (ROS):**

Reactive Oxygen Species (ROS) encompass a category of chemically reactive molecules infused with oxygen, which arise as inherent byproducts of cellular metabolic activities. These ROS production mechanisms predominantly unfold within cellular organelles like mitochondria and peroxisomes, as well as through the involvement of enzymes such as NADPH oxidases. Within

the realm of physiological processes, ROS serve pivotal roles, orchestrating functions such as cell signaling, immune responses, and the intricate regulation of gene expression.

It is crucial to acknowledge that while ROS are indispensable for these vital cellular functions, their excessive generation can precipitate a condition known as oxidative stress. This oxidative stress is characterized by an imbalance between the production of ROS and the body's antioxidant defense mechanisms. In such instances, the heightened presence of ROS can wreak havoc by inflicting damage upon essential biomolecules, including DNA, proteins, and lipids. This damage, in turn, contributes to the development and progression of a myriad of diseases and disorders.<sup>[32][35][44]</sup>

### **1.7.2 REACTIVE NITROGEN SPECIES (RNS)**

Reactive nitrogen species (RNS) constitute a group of free radicals that originate from nitrogen compounds. Similar to reactive oxygen species (ROS), RNS exhibit a multifaceted nature, and their influence on living organisms hinges on their concentration levels. These species can yield both favorable and detrimental effects within biological systems, depending on the specific context and the precise quantities in which they are present.<sup>[34]</sup> To elaborate further, RNS, like ROS, are molecules with unpaired electrons that make them highly reactive. In moderation, they play crucial roles in various physiological processes, such as immune responses and cell signaling. However, when their production surpasses the body's ability to manage and neutralize them, a state of imbalance, known as nitrosative stress, can ensue. This excessive presence of RNS can lead to damage in the form of oxidative modifications to DNA, proteins, and lipids, contributing to the development of numerous diseases and disorders.

In essence, the intricate interplay of RNS in biological systems underscores the importance of maintaining a delicate equilibrium to harness their beneficial aspects while mitigating their potential for harm.

Some categories of RNS include; Nitric oxide, Nitrogen dioxide, Nitrosamine, S-nitrosothiols, etc.

Oxidative stress represents a complex physiological condition that arises from a delicate equilibrium disrupted within the body. At its core, this condition is marked by a notable disparity between the generation of free radicals, encompassing both reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the body's inherent capacity to counteract their potentially harmful effects through a defense mechanism mediated by antioxidants. This intricate equilibrium ordinarily maintains a state of harmony within the body's biological systems. Reactive oxygen species and reactive nitrogen species, while inherently reactive due to their unpaired electrons, serve pivotal roles in various physiological processes. They partake in essential functions such as cell signaling, immune responses, and the intricate orchestration of gene expression.<sup>[35]</sup> However, when the delicate balance between the production of these free radicals and the body's antioxidant defenses is disrupted, oxidative stress emerges as a consequence. This disruption often results in the overproduction of ROS and RNS or a reduction in the body's antioxidant capacity. As a result, these highly reactive species can run amok within the body, inflicting damage on vital biomolecules like DNA, proteins, and lipids.<sup>[32]</sup> The repercussions of oxidative stress extend beyond this molecular-level damage, as it is intricately linked to the development and progression of a wide spectrum of diseases and disorders. These may include chronic conditions such as neurodegenerative diseases, cardiovascular diseases, cancer, and aging-related ailments.<sup>[34]</sup>

In essence, oxidative stress serves as a critical concept in understanding the intricate interplay between free radicals and antioxidants in the context of human health. Striking the right balance is vital to maintain optimal physiological functioning and minimize the risk of oxidative stress-related pathologies. An excess production of free radicals, combined with insufficient antioxidant defenses, can result in a condition known as oxidative stress. This phenomenon is closely linked to the development and progression of a multitude of diseases, spanning across a range of health domains, including cardiovascular health, skin conditions, neurodegenerative disorders, and cancer. To combat the potentially harmful effects of free radicals, the human body employs natural defense mechanisms.

Among these defense mechanisms are antioxidants, which include well-known compounds like vitamins C and E, as well as glutathione. Additionally, enzymes such as superoxide dismutase (SOD) and catalase play crucial roles in neutralizing free radicals and maintaining the delicate balance of redox processes within the body. <sup>[32]</sup>

It's worth noting that free radicals aren't solely adversaries; they also serve as tools within the immune system and inflammatory responses to combat pathogens. However, when inflammation becomes chronic, it can lead to a sustained production of free radicals, exacerbating oxidative stress.<sup>[35]</sup> The cumulative damage inflicted by free radicals over time has also been closely associated with the aging process. Understanding the pivotal role of free radicals in oxidative stress is instrumental in both the prevention and management of various diseases that result from oxidative damage. Striking a harmonious equilibrium between the production of free radicals and the body's antioxidant defenses is paramount for maintaining the health of individual cells and, by extension, overall well-being. Balancing these factors is vital for preserving cellular health and promoting a state of optimal wellness.<sup>[36]</sup>

## 1.8 ANTIOXIDANTS

Antioxidants are molecules that play a crucial role in safeguarding our body's cells against oxidative damage inflicted by free radicals. Their primary function is to shield cells from harm by neutralizing free radicals through a process that involves donating electrons, and in doing so, they themselves undergo oxidation.<sup>[37]</sup> These antioxidants can be broadly classified into two main categories: endogenous, which are naturally produced within the body, and exogenous, which are acquired from external sources such as our diet or through supplements. Examples of well-known antioxidants encompass vitamins C and E, beta-carotene, as well as the valuable compound glutathione.<sup>[38]</sup> The significance of antioxidants extends to a range of health benefits, which encompass a reduced risk of chronic ailments like heart disease, cancer, and neurodegenerative disorders.<sup>[39]</sup> Their role in mitigating oxidative stress and preserving cellular integrity underscores their vital contribution to overall health and well-being.

Numerous fruits, including berries, a variety of vegetables such as spinach, and certain nuts like walnuts, as well as seeds, represent abundant reservoirs of antioxidants.<sup>[40]</sup> These remarkable compounds assume a pivotal role in the preservation of vibrant and healthy skin by acting as formidable shields against the deleterious repercussions of UV radiation and environmental pollutants.<sup>[36]</sup> Furthermore, current scientific investigations are delving into the promising realm of antioxidants with respect to their prospective anti-aging properties. This exploration is driven by their remarkable capacity to counteract oxidative stress, a process intricately linked to the aging phenomenon. Antioxidants are thus emerging as potential allies in the quest for sustaining youthful vitality and extending the overall quality of life.

## 1.9 JUSTIFICATION OF THE STUDY

One significant focus of this study is to investigate the antioxidant potential of *F. exasperata* leaves. Oxidative stress is implicated in the development of various diseases, making the exploration of natural antioxidant sources a subject of great importance in both research and healthcare. By examining the phytochemical composition of *F. exasperata* leaves, the study aims to shed light on the potential mechanisms underlying its antioxidant effects.

Additionally, the research seeks to bridge the gap between traditional knowledge and scientific evidence. Traditional healers have employed *F. exasperata* for its medicinal benefits for generations, and this study could provide scientific validation for these traditional uses. Understanding the antioxidant potential of *F. exasperata* may have implications for human health. If the plant extract proves to be a potent antioxidant, it could pave the way for the development of natural antioxidants or nutraceuticals that could aid in preventing or mitigating diseases associated with oxidative stress. Moreover, this study addresses a scientific gap by providing empirical data on the antioxidant properties of *Ficus exasperata* leaves. Despite its extensive traditional use, there may be limited scientific documentation on this aspect of the plant's properties.

In conclusion, the research study on the antioxidant screening of *Ficus exasperata* leaves is driven by the need to explore and validate the antioxidant potential of this plant. It aligns with the broader objectives of discovering natural sources of antioxidants and advancing our understanding of the pharmacological applications of traditional medicinal plants in contemporary healthcare.

### **1.10 AIM AND OBJECTIVES OF STUDY**

The aim of the study was to investigate the antioxidant properties of the methanol extract of the leaves of *Ficus exasperata* Vahl.

The objectives were to:

1. Determine the phytochemical constituents of the methanol extract of the leaves of *Ficus exasperata* Vahl.
2. Quantify the concentration of overall phenolic and flavonoid contents.
3. Evaluating the antioxidant activity of *Ficus exasperata* Vahl.

## CHAPTER TWO

### MATERIALS AND METHODS

#### 2.0 MATERIALS

##### 2.1 SOLVENTS AND REAGENTS

2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck)

Acetic anhydride (Sigma-Aldrich, Germany)

Alcoholic  $\alpha$ -naphthol (Sigma-Aldrich, Germany)

Alkaloidal reagents (Dragendoff's, Wagner's, Hager's, Mayer's reagents)

Aluminum chloride

Ammonia

Ammonium phosphomolybdate

Ascorbic acid (Sigma-Aldrich, Germany)

Chloroform (JHD, China)

Distilled water

Fehling solution A & B (Sigma-Aldrich, Germany)

Ferric chloride (Merck)

Folin Ciocalteu's reagent (Merck)

Gelatin solution

Gallic acid (Sigma-Aldrich, Germany)

Hydrochloric acid (JHD, China)

Lead acetate

Methanol (JHD, China)

Petroleum ether (JHD, China)

Potassium acetate

Quercetin (Sigma-Aldrich, Germany)

Sodium carbonate (Sigma-Aldrich, Germany)

Sodium chloride (Merck)

Sodium phosphate

Sodium hydroxide (Merck)

Sulphuric acid (JHD, China)

## **2.2 GLASSWARE**

Beakers

Conical flasks

Funnels

Glass jars

Measuring cylinders

Pipettes

Reagent bottles

Stirrer

Test tubes

All glass wares used were products of Pyrex (England), Fiolax and Chengdu (China).

### **2.3 EQUIPMENT**

Analytical balance

Drying oven (Gallenkamp, England)

Mechanical grinder (Viking Exclusive, Joncod)

Refrigerator (LG, China)

Rotary evaporator (Stuart, England)

Ultraviolet-Visible spectrophotometer (Spectrumlab, China)

Vacuum pump (Stuart, England)

Water bath

Weighing scale

## **2.4 COLLECTION AND PREPARATION OF PLANT MATERIALS**

Fresh leaves of *Ficus exasperata* Vahl were collected around Ugbowo campus (6°23'41.5" N 5°37'16.6" E), in April 2023. The leaves were spread out under shade to air dry for a few days, after being sorted and adulterants and contaminants have been removed. The drying process was completed with the use of an oven dryer. The dried leaves were then ground into powder with the aid of a mechanical grinder and stored in an air-tight container until ready for use.

## **2.5 EXTRACTION OF CRUDE POWDERED SAMPLE**

The powdered plant material (1.5 kg) was extracted with 6.6 L of methanol by maceration at room temperature for 7 days. The extract was filtered and concentrated *in vacuo* in a rotary evaporator at 45°C and at reduced pressure. The concentrated extract was weighed and the percentage yield calculated based on the initial weight of the crude powdered sample.

## **2.6 PHYTOCHEMICAL SCREENING OF *Ficus exasperate* Vahl LEAVES**

A comprehensive series of chemical examinations were meticulously conducted on the unprocessed powdered sample. These analyses were performed in strict adherence to universally accepted and rigorously defined protocols to discern the existence of an array of phytochemical components. An approximate quantity of 5 grams of the unprocessed powdered sample was subjected to a boiling for 30 minutes in 75 mL of distilled water. Subsequently, the resulting solution was subjected to a hot filtration process and left undisturbed to attain a state of cooling. The resultant filtrate, obtained, was subsequently employed in the execution of the subsequent series of tests.

### **General Tests for Alkaloids**

- Two drops of Dragendorff's reagent were added to 2 mL of the filtrate.
- Two drops of Wagner's reagent were added to 2 mL of the filtrate.
- Two drops of Hager's reagent were added to 2 mL of the filtrate.
- Two drops of Mayer's reagent were added to 2 mL of the filtrate.

### **Tests for Carbohydrates**

- Molisch's Test

To 2 mL of filtrate was added 2 drops of 1% alcoholic naphthol followed by 2 mL of concentrated sulphuric acid at a slanting position.

### **Tests for Reducing Sugars**

- Fehling's Test

To 2 mL of filtrate was added 2 drops of Benedict's reagent (a mixture of equal volumes of Fehling's solution A and B). The resulting solution was heated over a boiling water bath for 3 minutes.

### **Test for Saponins**

- Frothing Test

The filtrate (1 mL) was diluted with 10 mL distilled water and shaken vigorously for one minute.

- Fehling's Test

To 10 mL of the filtrate was added 5 mL of dilute  $\text{H}_2\text{SO}_4$ . The mixture was boiled for 15 min, filtered and cooled. 2.5 mL of the filtrate was made alkaline with 20% NaOH solution and boiled with 0.1 mL each of Fehling's solutions A and B for 2 minutes.

➤ Lieberman Burchard's Test for Steroidal saponins or Phytosterols

A mixture of 1 mL chloroform and few drops of acetic anhydride was added to 2 mL of the filtrate. To the final mixture was added 2 drops of concentrated sulphuric acid.

### **Test for Tannins**

➤ Gelatin Test

To 2 mL of the filtrate was added 2 mL of 1% gelatin solution in 10% NaCl.

### **Test for Phenolic compounds**

➤ Ferric chloride Test

To 2 mL of filtrate was added 5 mL of distilled water followed by 2 drops of 5% ferric chloride solution. A blank test was done by adding 2 drops of 5% ferric chloride solution to 5 mL of distilled water.

➤ Folin Ciocalteu's Test

To 5 mL of filtrate was added 0.5 mL 10 % Folin Ciocalteu's phenol reagent followed by 5 mL of 7%  $\text{Na}_2\text{CO}_3$ .

### **Test for Flavonoids**

➤ Alkaline reagent Test

To 2 mL of filtrate was added few drops of 20% sodium hydroxide solution followed by few drops of dilute hydrochloric acid solution.

➤ Lead acetate Test

Few drops of lead acetate solution were added to 2 mL of the filtrate.

### **Test for Anthraquinone Derivatives**

➤ Bontreger's Test

The filtrate (2 mL) was shaken with 2 mL of petroleum ether. The ether layer was washed with 2 mL distilled water and then shaken with dilute ammonia solution.

## **2.7 DETERMINATION OF POLYPHENOLS**

### **2.7.1 TOTAL PHENOLS**

The assessment of the total phenolic content in the extracts was performed using the methodology outlined by Kim *et al.*, 2003.

A 0.5 mL portion of the extract solution, having a concentration of 1 mg/mL, which was prepared by dissolving 0.01 g in 10 mL of methanol, was mixed with 4.5 mL of distilled water. Subsequently, 0.5 mL of Folin Ciocalteu's reagent (previously diluted with water at a ratio of 1:10 v/v) was added to this mixture. After thorough mixing, the tubes were left at room temperature for 5 minutes. Following this, 5 mL of a 7% sodium carbonate solution and 2 mL of deionized distilled water were introduced to the samples. After another round of thorough mixing, the samples were incubated at room temperature for a duration of 90 minutes. The absorbance of the samples was measured using a UV spectrophotometer set at 750 nm. The total phenolic content was quantified and expressed in milligrams of gallic acid equivalents (GAE) per gram of

extract (mg GAE/g extract). To establish a standard curve, gallic acid was used as standard at six different concentrations, specifically 12.5, 25, 50, 75, 100, and 150 mg/L. <sup>[45]</sup> All preparations were made in triplicates.

The graph equation was used to calculate the total phenol content. Results were analyzed and interpreted.

### **2.7.2 TOTAL FLAVONOIDS**

The determination of total flavonoid contents was conducted following the procedure outlined by Ebrahimzadeh *et al.* in 2008.

To determine the total flavonoid content, a 0.5 mL sample of the extract (with a concentration of 1 mg/mL) was meticulously combined with 1.5 mL of methanol. Following this, 0.1 mL of a 10% aluminum chloride solution was introduced into the mixture, along with 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. This amalgamation was left to incubate at room temperature for a duration of 30 minutes. Subsequently, the absorbance of the solution was quantified using a spectrophotometer at a wavelength of 415 nm. The outcomes of this analysis were then expressed in terms of milligrams of quercetin equivalents (mg QE) per gram of the extract (mg QE/g extract). To facilitate this assessment, a standard curve was established using quercetin at six different concentrations in triplicates (12.5, 25, 50, 75, 100, and 150 mg/L). <sup>[46]</sup>

The graph equation was used to calculate the total flavonoids content. Results were analyzed and interpreted.

**2.8 (2,2-diphenyl-1-picrylhydrazyl) DPPH RADICAL SCAVENGING ASSAY** The assessment of the crude methanol extract's ability to scavenge DPPH radicals was conducted following the procedure outlined by Jain *et al.*, in 2008. This method involves measuring the extract's capacity to neutralize DPPH radicals, which are commonly used to evaluate antioxidant activity. The DPPH assay quantifies how effectively the extract can counteract these radicals, providing valuable insights into its antioxidant potential.

A methanol solution containing 0.1 mM DPPH was prepared. Subsequently, 1.0 mL of this DPPH solution was combined with 3.0 mL of the extract dissolved in methanol, which had varying concentrations of (1, 2, 5, 10, 20, 50, 100 and 200 mcg/mL) of the extract. The resulting mixture was vigorously mixed and then placed in a dark environment at room temperature for a duration of 30 minutes. Following this incubation period, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. Ascorbic acid was employed as a reference standard for comparison in this assay, and same range of concentrations as that of sample was prepared. [47]

The evaluation of DPPH radical scavenging activity involved the utilization of the following formula:

$$\left[ \frac{(A_0 - A_1)}{(A_0)} \right] \times 100$$

Where:

A<sub>0</sub> is absorbance of DPPH radical + methanol (blank),

A<sub>1</sub> is absorbance of DPPH radical + sample extract/standard.

The IC<sub>50</sub> value, which indicates the concentration required for the sample to effectively scavenge 50% of the DPPH free radical, was determined through this process.

All experiments were done in triplicates.

## 2.9 TOTAL ANTIOXIDANT CAPABILITY

The assessment of total antioxidant capability (TAC) in the methanol extract derived from *Ficus exasperata* leaves was conducted, using ascorbic acid as the standard reference. The methodology closely aligned with the protocol delineated by Moonmun *et al.*, 2017, with slight modifications tailored to the unique parameters of this research.

Phosphomolybdate reagent was meticulously prepared by mixing 30 mL of 28 mM sodium phosphate, 30 mL of 0.6 M sulfuric acid, and 30 mL of 4 mM ammonium molybdate solutions. Subsequently, 0.3 mL of different concentrations (ranging from 20, 50, 100, 150, 200 and 250 mcg/mL) of the extract solution were introduced to 3 mL of the phosphomolybdate reagent. This amalgam was then incubated in a dark environment for a duration of 90 minutes. The subsequent evaluation involved the measurement of absorbance at 765 nm. Using ascorbic acid as standard is same varying concentrations, for comparison.<sup>[48]</sup>

The evaluation of percentage (%) TAC radical scavenging activity involved the utilization of the following formula:

$$\left[ \frac{(A_0 - A_1)}{(A_0)} \right] \times 100$$

Where:

A<sub>0</sub> is absorbance of the phosphomolybdate reagent (blank),

$A_1$  is absorbance of phosphomolybdate reagent + sample extract/standard.

The concentration of extract at which 50% inhibition is observed ( $IC_{50}$ ) is calculated in mcg/ml and compared with the standard.

### **3.0 DATA ANALYSIS**

All the results acquired were recorded and analyzed using Microsoft Excel Spreadsheet. The Mean, Standard Deviation, Co-efficient of Determination ( $R^2$ ) and the Standard Error of Mean (SEM) were evaluated.

## CHAPTER THREE

### RESULTS

#### 3.1. ORGANOLEPTIC PROPERTIES

Colour: Dark green

Odour: Pungent

Texture: Rough

Taste: Bitter

#### 3.2. PERCENTAGE YIELD OF METHANOL EXTRACT OF LEAVES *Ficus exasperata* Vahl

Weight of powdered leaves = 1500 g

Weight of extract = 65.29 g

Percentage (%) yield of crude extract =  $\frac{65.29}{1500} \times 100 = 4.35\%$

#### 3.3 PHYTOCHEMICAL CONSTITUENTS

The aqueous extract of powdered leaves of *Ficus exasperata* Vahl, was found to contain;

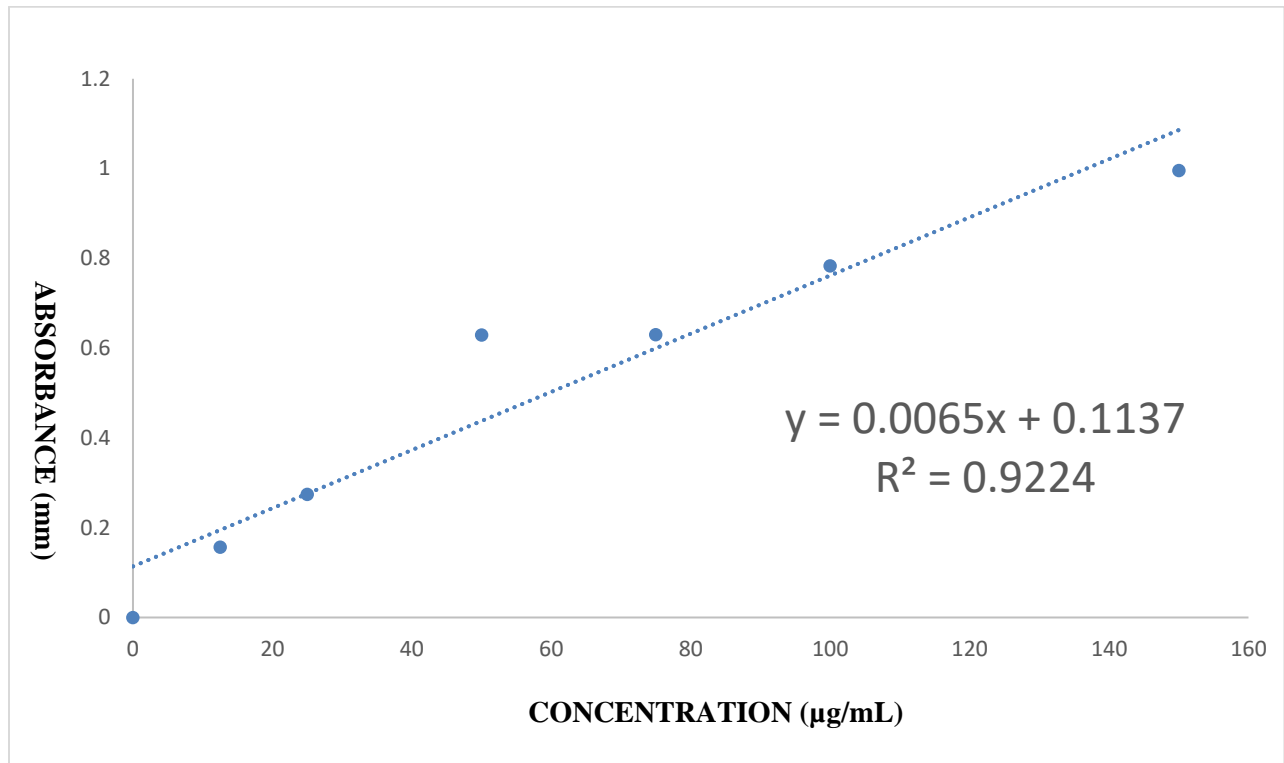
Glycosides, Alkaloids, Flavonoids, Phenolics and Reducing sugars.

**TABLE 3.1 RESULT OF PHYTOCHEMICAL SCREENING**

<b>PHYTOCHEMICAL</b>	<b>RESULT</b>
<b>Glycosides</b>	+
<b>Alkaloids</b>	+
<b>Saponins</b>	-
<b>Tannins</b>	+
<b>Flavonoids</b>	+
<b>Phenolics</b>	+
<b>Anthraquinones</b>	-
<b>Proteins</b>	-
<b>Phytosterols</b>	-
<b>Reducing sugars</b>	+

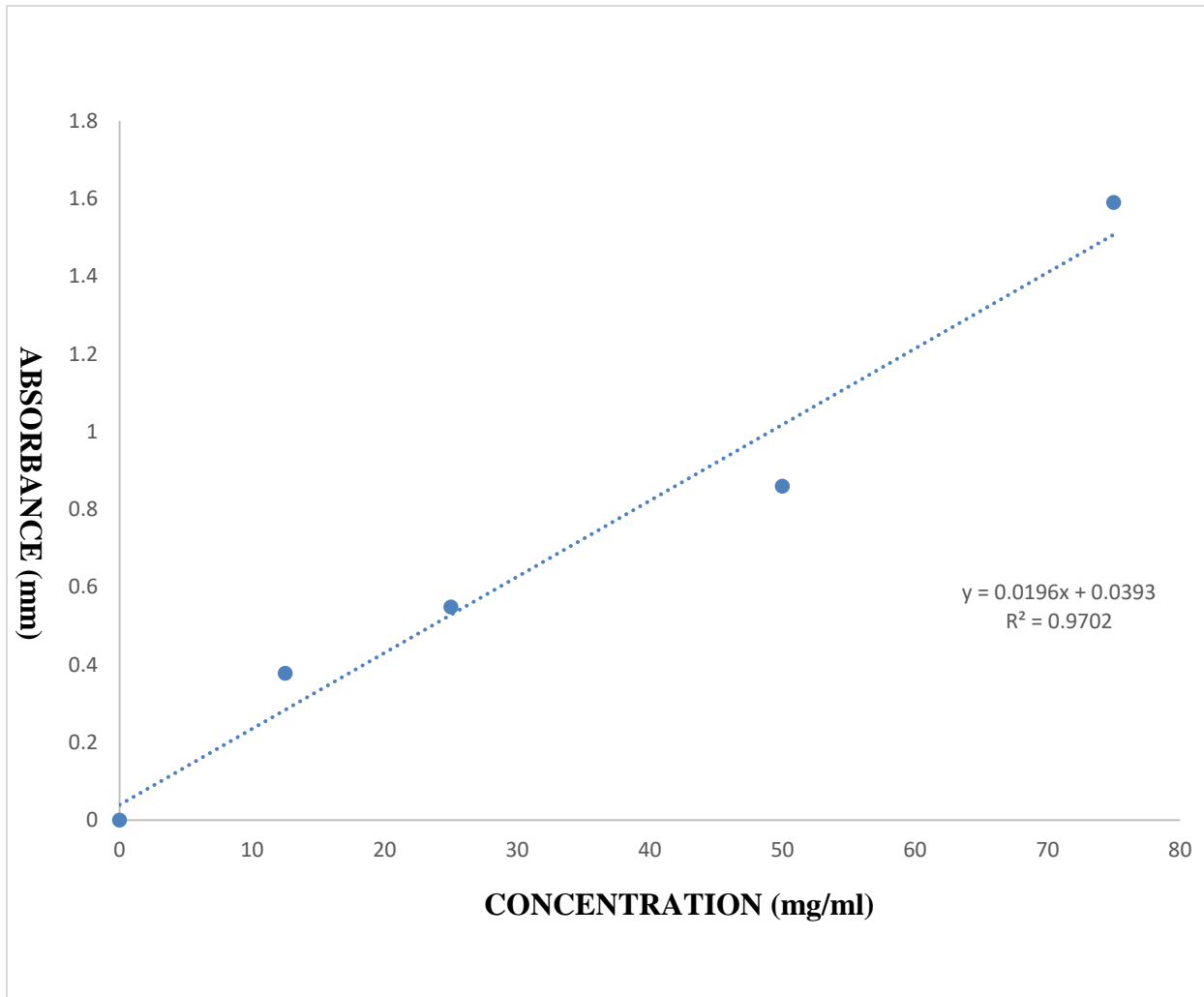
### 3.4 TOTAL POLYPHENOLIC CONTENT

#### 3.4.1 TOTAL PHENOLS



**FIGURE 3.1: Gallic acid calibration curve for total phenols**

### 3.5: TOTAL FLAVONOIDS



**FIGURE 3.2: Quercetin calibration curve**

**TABLE 3.2 TOTAL POLYPHENOLIC CONTENT OF METHANOL EXTRACT OF LEAVES OF *Ficus exasperata* Vahl.**

<b>EXTRACT</b>	<b>TOTAL POLYPHENOLIC CONTENT</b>			<b>MEAN</b>	<b>STANDARD DEVIATION</b>	<b>STANDARD ERROR OF MEAN</b>
	<b>TOTAL PHENOLIC CONTENT</b>					
	<b>(mgGAE/g Extract)</b>					
	<b>-0.2615</b>	<b>-3.1846</b>	<b>-2.0000</b>	<b>-1.8154</b>	<b>1.4703</b>	<b>0.8489</b>
	<b>TOTAL FLAVONOID CONTENT (mgQE/g Extract)</b>					
	<b>-</b>	<b>31.2755</b>	<b>64.1173</b>	<b>47.6964</b>	<b>23.2227</b>	<b>16.4209</b>

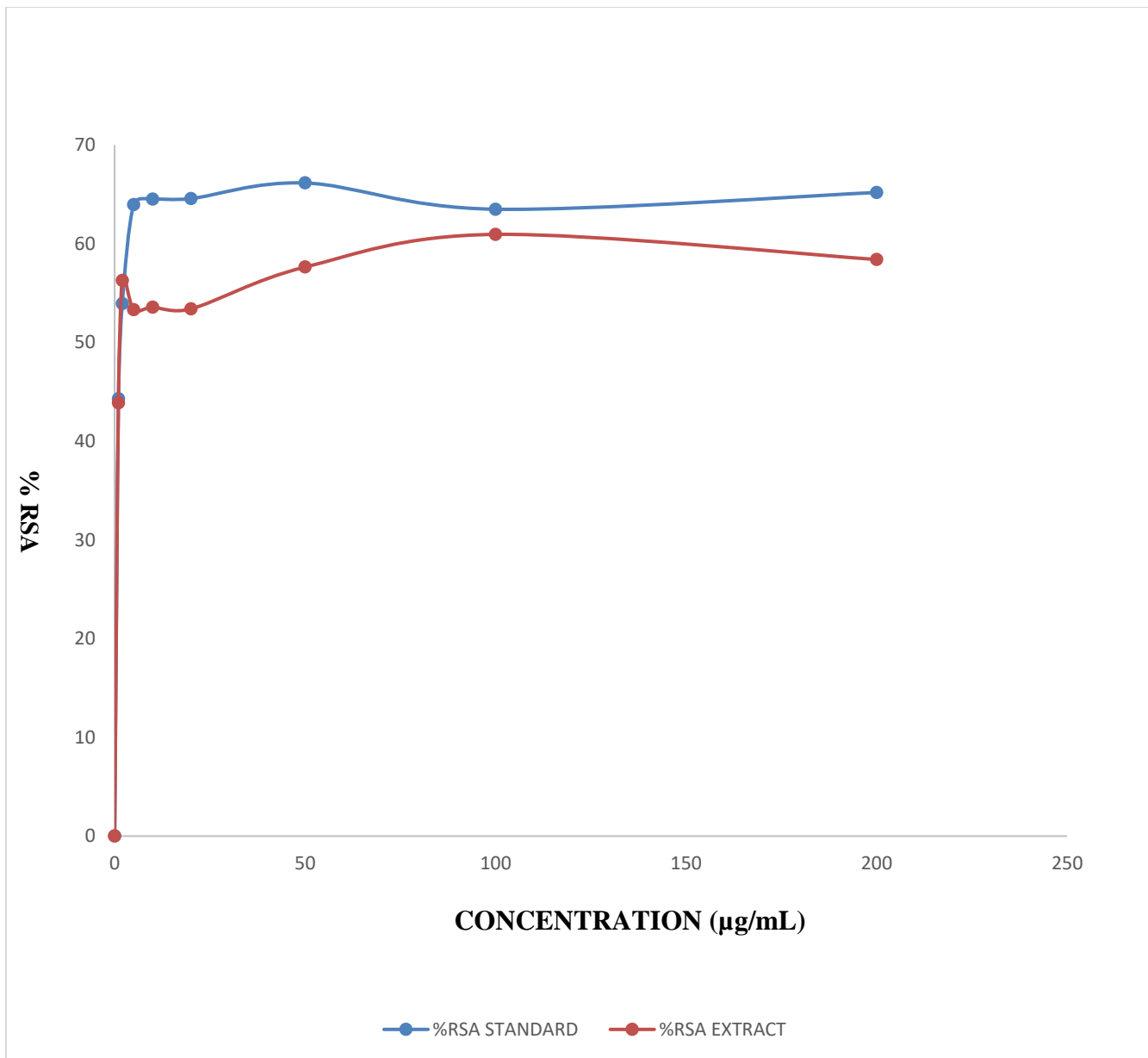
### 3.6: DPPH FREE RADICAL SCAVENGING ACTIVITY.

**TABLE 3.3: Concentration and percentage Radical Scavenging Activity of Ascorbic acid (Standard) and Extract**

CONCENTRATION	% RSA			MEAN % RSA	STANDARD DEVIATION	STANDARD ERROR MEAN
0	0	0	0	0	0	0
1	45.8405	46.1799	40.9168	44.3124	2.945568	1.70062
2	58.7436	44.8217	58.2343	53.9332	7.894898	4.55812
5	63.4975	63.3277	65.0255	63.95023	0.93507	0.53980
10	64.1766	65.7046	63.6672	64.51613	1.060289	0.61215
20	64.3463	65.365	64.0068	64.5727	0.706838	0.61215
50	69.1002	66.0441	63.3277	66.15733	2.887915	1.66733
100	61.4601	64.1766	64.8557	63.49747	1.796786	1.03733
200	66.0441	66.7233	62.8183	65.19523	2.086309	1.20453

**TABLE 3.4: Concentration and percentage Radical Scavenging Activity of Extract**

<b>CONCENTRATION</b>	<b>% RSA</b>		<b>MEAN % RSA</b>	<b>STANDARD DEVIATION</b>	<b>STANDARD ERROR MEAN</b>
<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>1</b>	<b>43.6333</b>	<b>44.1426</b>	<b>43.88795</b>	<b>0.360129</b>	<b>0.2546</b>
<b>2</b>	<b>58.2343</b>	<b>54.3294</b>	<b>56.28185</b>	<b>2.761181</b>	<b>1.9524</b>
<b>5</b>	<b>53.6503</b>	<b>53.82</b>	<b>53.73515</b>	<b>0.119996</b>	<b>0.0848</b>
<b>10</b>	<b>53.6503</b>	<b>53.4805</b>	<b>53.5654</b>	<b>0.120067</b>	<b>0.0849</b>
<b>20</b>	<b>53.6503</b>	<b>53.1409</b>	<b>53.3956</b>	<b>0.3602</b>	<b>0.2547</b>
<b>50</b>	<b>57.8947</b>	<b>57.3854</b>	<b>57.64005</b>	<b>0.360129</b>	<b>0.2546</b>
<b>100</b>	<b>60.7809</b>	<b>61.1205</b>	<b>60.9507</b>	<b>0.240133</b>	<b>0.1698</b>
<b>200</b>	<b>58.4041</b>	<b>58.4041</b>	<b>58.4041</b>	<b>0</b>	<b>0</b>



**FIGURE 3.3: Graph of DPPH Radical Scavenging Activity Vs Concentration**

**TABLE 3.5: TABLE FOR DETERMINATION OF HALF-MAXIMAL INHIBITORY CONCENTRATION (IC<sub>50</sub>)**

<b>SAMPLE</b>	<b>CONCENTRATION (µg/mL)</b>	<b>% RSA</b>
<b>ASCORBIC ACID</b>	<b>1</b>	<b>44.3124</b>
	<b>2</b>	<b>53.9332</b>
	<b>5</b>	<b>63.9502</b>
	<b>10</b>	<b>64.5161</b>
<b>EXTRACT</b>	<b>1</b>	<b>43.8878</b>
	<b>2</b>	<b>56.2819</b>
	<b>5</b>	<b>53.3107</b>
	<b>10</b>	<b>53.5654</b>

### 3.6.1 HALF-MAXIMAL INHIBITORY CONCENTRATION (IC<sub>50</sub>)

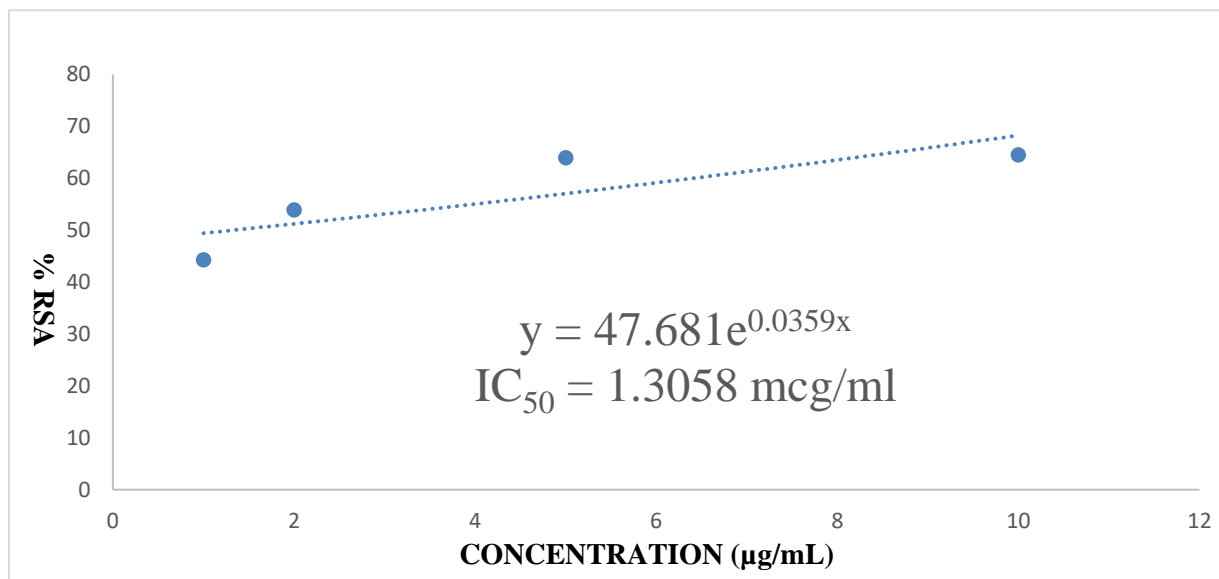


FIGURE 3.4: Regression plot for Calculating IC<sub>50</sub> Value for Ascorbic Acid

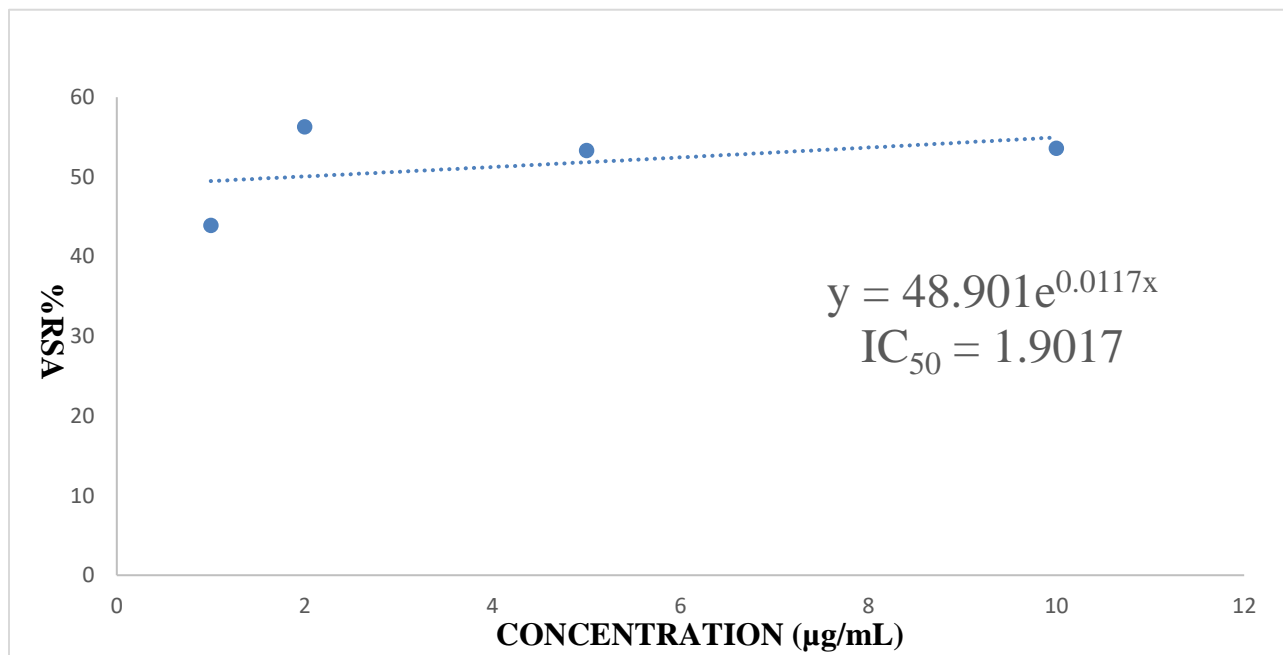


FIGURE 3.5: Regression plot for Calculating IC<sub>50</sub> Value for Methanol Extract of Leaves of *Ficus exasperata* Vahl

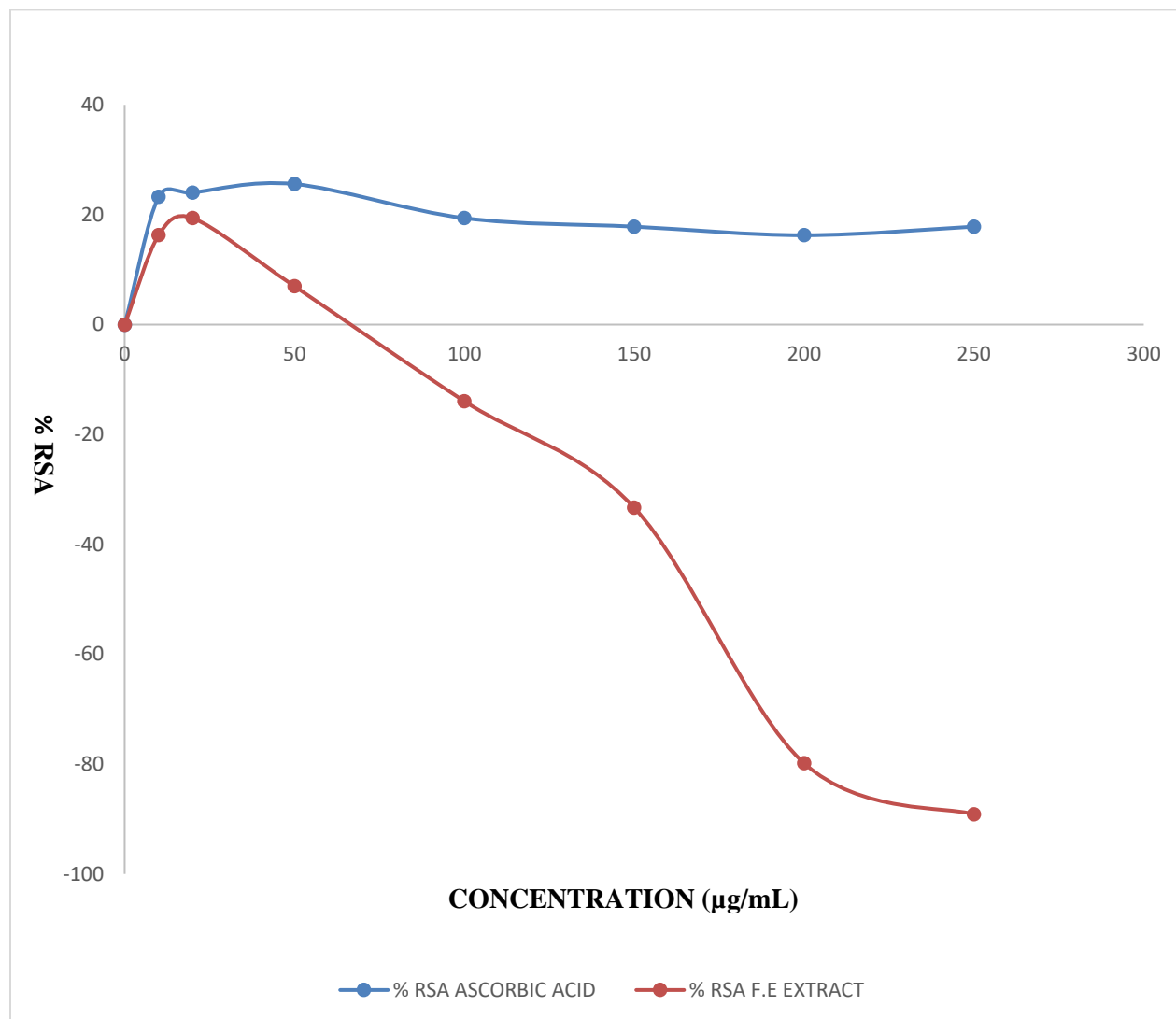
### 3.7: TOTAL ANTIOXIDANT CAPACITY (TAC)

**TABLE 3.6: CONCENTRATION AND PERCENTAGE RADICAL SCAVENGING ACTIVITY OF ASCORBIC ACID (STANDARD)**

<b>CONCENTRATION</b>	<b>% RSA</b>			<b>MEAN % RSA</b>	<b>STANDARD DEVIATION</b>	<b>STANDARD ERROR OF MEAN</b>
<b>10</b>	<b>32.5581</b>	<b>6.9767</b>	<b>30.2326</b>	<b>23.2558</b>	<b>14.14598</b>	<b>8.167187</b>
<b>20</b>	<b>23.2558</b>	<b>25.5814</b>	<b>23.2558</b>	<b>24.031</b>	<b>1.342686</b>	<b>0.7752</b>
<b>50</b>	<b>20.9302</b>	<b>30.2326</b>	<b>25.5814</b>	<b>25.5814</b>	<b>4.6512</b>	<b>2.685372</b>
<b>100</b>	<b>18.6047</b>	<b>20.9302</b>	<b>18.6047</b>	<b>19.37987</b>	<b>1.342628</b>	<b>0.775167</b>
<b>150</b>	<b>20.9302</b>	<b>18.6047</b>	<b>13.9535</b>	<b>17.82947</b>	<b>3.552369</b>	<b>2.050961</b>
<b>200</b>	<b>6.9767</b>	<b>25.5814</b>	<b>16.2791</b>	<b>16.27907</b>	<b>9.30235</b>	<b>5.370714</b>
<b>250</b>	<b>11.6279</b>	<b>30.2326</b>	<b>11.6278</b>	<b>17.82943</b>	<b>10.74146</b>	<b>6.201583</b>

**TABLE 3.7: CONCENTRATION AND PERCENTAGE RADICAL SCAVENGING ACTIVITY OF EXTRACT**

<b>CONCENTRATION</b>	<b>% RSA</b>			<b>MEAN % RSA</b>	<b>STANDARD DEVIATION</b>	<b>STANDARD ERROR OF MEAN</b>
<b>10</b>	<b>13.9535</b>	<b>18.6047</b>	<b>16.2791</b>	<b>16.2791</b>	<b>2.3256</b>	<b>1.342686</b>
<b>20</b>	<b>13.9535</b>	<b>23.2558</b>	<b>20.9302</b>	<b>19.37983</b>	<b>4.841066</b>	<b>2.794991</b>
<b>50</b>	<b>2.3256</b>	<b>6.9767</b>	<b>11.6279</b>	<b>6.976733</b>	<b>4.65115</b>	<b>2.685343</b>
<b>100</b>	<b>- 16.2791</b>	<b>-9.3023</b>	<b>-16.2791</b>	<b>-13.9535</b>	<b>4.028057</b>	<b>2.3256</b>
<b>150</b>	<b>- 20.9302</b>	<b>- 34.8837</b>	<b>-44.186</b>	<b>-33.3333</b>	<b>11.70516</b>	<b>6.75798</b>
<b>200</b>	<b>- 111.628</b>	<b>- 65.1163</b>	<b>-62.7907</b>	<b>-79.845</b>	<b>27.54938</b>	<b>15.90564</b>
<b>250</b>	<b>- 81.3953</b>	<b>- 113.954</b>	<b>-72.093</b>	<b>-89.1473</b>	<b>21.98056</b>	<b>12.69048</b>



**FIGURE 3.6: GRAPH OF TOTAL ANTIOXIDANT CAPABILITY OF STANDARD AND EXTRACT**

**TABLE 3.8: TABLE FOR DETERMINATION OF HALF-MAXIMAL INHIBITORY CONCENTRATION (IC<sub>50</sub>)**

<b>SAMPLE</b>	<b>CONCENTRATION (μg/mL)</b>	<b>% RSA</b>
<b>ASCORBIC ACID</b>	<b>10</b>	<b>23.2558</b>
	<b>20</b>	<b>24.0310</b>
	<b>50</b>	<b>25.5814</b>
	<b>100</b>	<b>19.3799</b>
<b>EXTRACT</b>	<b>10</b>	<b>16.2791</b>
	<b>20</b>	<b>19.3798</b>
	<b>50</b>	<b>6.9767</b>
	<b>100</b>	<b>-13.9535</b>

### 3.7.2 DETERMINATION OF 50% INHIBITORY CONCENTRATION (IC<sub>50</sub>) VALUES

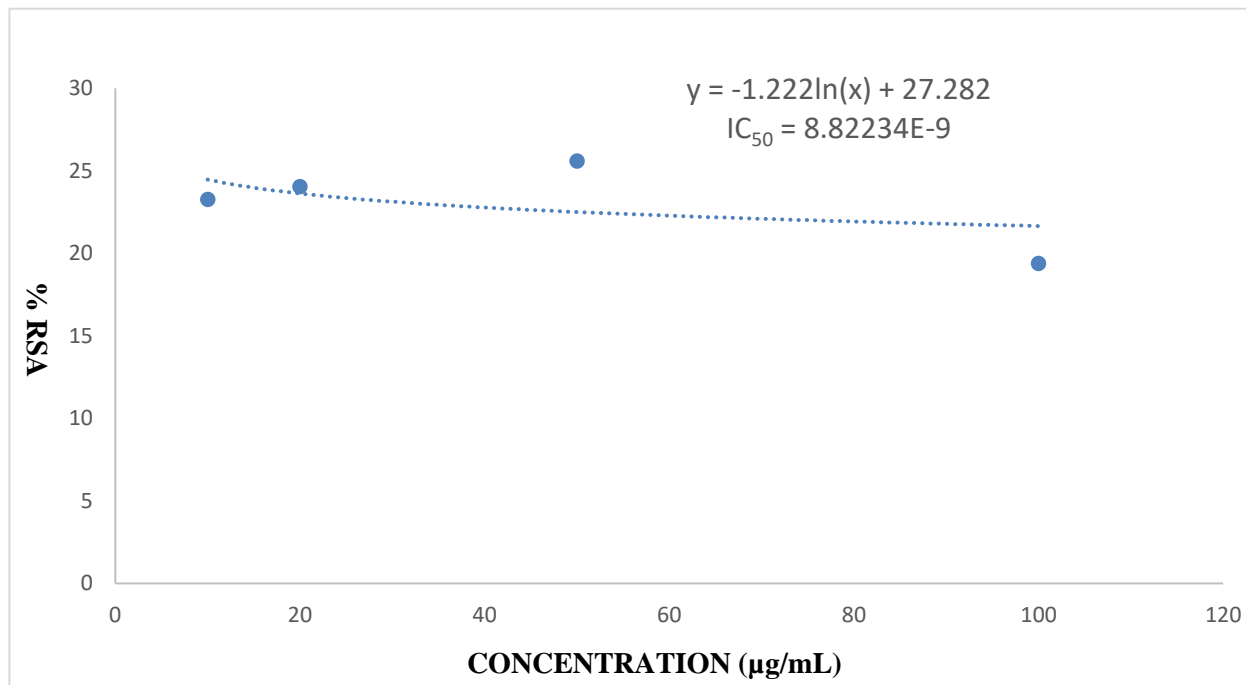


FIG. 3.7: GRAPH TO DETERMINE IC<sub>50</sub> OF ASCORBIC ACID

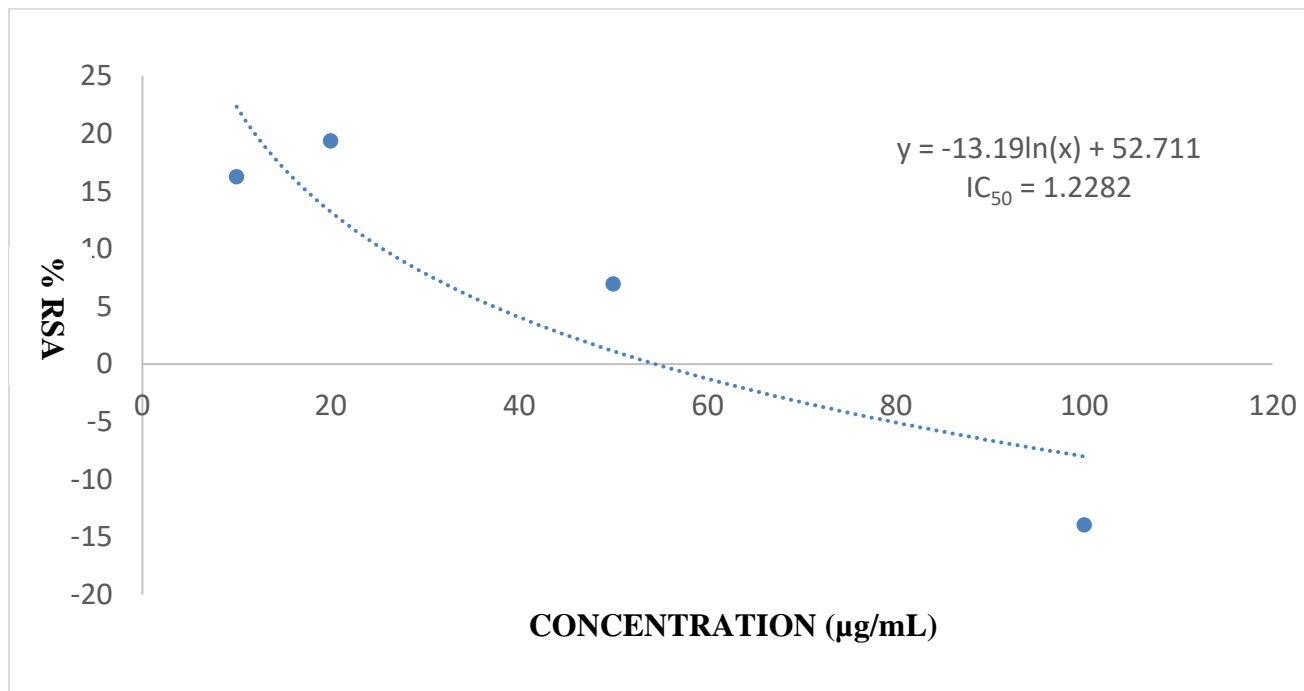


FIG. 3.8: GRAPH TO DETERMINE IC<sub>50</sub> OF METHANOL EXTRACT OF LEAVES OF *Ficus exasperata* Vahl

## CHAPTER FOUR

### DISCUSSION

#### 4.1 PHYTOCHEMICAL CONSTITUENTS

The analysis of the aqueous extract of the powdered leaves of *Ficus exasperata* Vahl, revealed the presence of glycosides, alkaloids, tannins, flavonoids, phenolics, and reducing sugars. Conversely, phytosterols, proteins, anthraquinones, and saponins were absent in the sample. This composition suggests that the plant possesses certain bioactive compounds with potential health benefits while lacking others that are commonly found in different plant species. The absence of specific phytochemicals may be attributed to factors such as plant species variability, extraction method, or environmental conditions during plant growth. This aligns as well as contrasts with the findings of Ajayi *et al.*, (2012) and Adebayo *et al.*, (2009) in which flavonoids, alkaloids and tannins were observed to be present.<sup>[27][28]</sup> According to the works of Adebayo and Ishola (2009), presence of tannins and other observed phytochemicals in the tested plant's part is an indication that the plant is of pharmacological importance.<sup>[28]</sup>

Possible reasons for these results could include variations in the plant's genetic makeup, environmental factors, or the analytical techniques used. It's important to consider that the presence or absence of certain phytochemicals can vary widely among plant species and even within different parts of the same plant.

Among the factors that could affect the result obtained, method of extraction of plant sample is of crucial importance. Method of extraction, boiling, of plant sample may affect results in the following areas:

**Extraction Efficiency:** Boiling with water is a common method for extracting water-soluble compounds from plant materials. Water can efficiently extract various phytochemicals like glycosides, tannins, and flavonoids. However, it may not be as effective in extracting certain non-polar compounds like phytosterols or lipids. The choice of solvent (in this case, water) affects which compounds are extracted.

**Heat Sensitivity:** Some phytochemicals can be heat-sensitive and may degrade or become altered during the boiling process. For example, excessive heat can cause the hydrolysis of glycosides into aglycones, which could be responsible for some of the observed compounds.

**Selectivity:** Boiling with water is selective for water-soluble compounds. This therefore means that non-polar compounds which are almost or completely insoluble in water would not be completely extracted or not extracted at all. Therefore, the absence of phytosterols and proteins in the observed results might be attributed to their lower solubility in water.

**Plant Part:** The choice of plant part for extraction can also influence the results. Different parts of a plant may contain varying phytochemical profiles.

**Extraction Time:** The duration of boiling can affect the extract's composition. Prolonged boiling may lead to the loss or degradation of some phytochemicals.

**Concentration:** The concentration of the extract, which can be influenced by the volume of water used and the time of boiling, affects the detection and quantification of compounds. Very dilute extracts may yield lower concentrations of phytochemicals.

**Extraction Temperature:** The boiling point of water (100°C) can cause compounds to vaporize and be lost in the steam if the extraction is carried out under an open system. The use of a closed

system can help retain volatile compounds. To improve the accuracy and comprehensiveness of results, a combination of solvents with different polarities (e.g., water, methanol, ethanol) for sequential extractions may be considered. This approach can help capture a broader spectrum of phytochemicals from the plant sample. Additionally, optimizing the extraction conditions, such as temperature and duration, can also be crucial in obtaining a representative extract.

#### **4.2 TOTAL POLYPHENOLIC CONTENT**

A negative value for the total phenol content was gotten after its determination which is in contrast to the values of 96.10 (mgGAE/g Extract)  $\pm$  2.70 and 69.20 (mgGAE/g Extract)  $\pm$  0.00 from the works of Anigboro *et al.*, (2019) and Oluwafemi *et al.*, (2014), respectively. The values obtained from the study clearly indicate the presence and abundance of flavonoids which is in accordance with various research and textbooks. This is crucial to the study as flavonoid content is attributed to the antioxidant property and pharmacological attributes of the sample. <sup>[50][48]</sup>

#### **4.4 DPPH FREE RADICAL SCAVENGING ACTIVITY**

The methanol extract derived from powdered *Ficus exasperata* Vahl leaves exhibited a similar DPPH free radical scavenging capacity, consistent with the IC<sub>50</sub> values of 16.34 mg/ml (Anigboro) and 0.635 mg/ml (Akanni). In accordance with the research conducted by Phongpaichit *et al.* (2012), extracts manifesting IC<sub>50</sub> values within the interval of 10 to 50 mg/mL are deemed to exhibit pronounced antioxidant potency. Conversely, extracts showcasing IC<sub>50</sub> values falling between 50 to 100 mg/mL are denoted as possessing a moderate degree of antioxidant activity, while those registering values surpassing 100 mg/mL are identified as demonstrating a relatively modest antioxidant effect. It can therefore be inferred that the methanol extract of leaves of *Ficus exasperata* Vahl showed strong antioxidant activity. <sup>[50][49][1]</sup>

DPPH (2,2-diphenyl-1-picrylhydrazyl) is frequently employed for antioxidant screening in scientific investigations due to its numerous advantages, which include:

**Stability:** DPPH is a stable free radical, ensuring that it can be stored for extended durations without significant degradation. This stability guarantees consistent outcomes and permits the preparation of standard solutions.

**Ease of Utilization:** The DPPH assay is relatively uncomplicated and direct in its execution. It entails the amalgamation of the test compound with a DPPH solution, rendering it suitable for researchers of varying experience levels.

**Prompt Outcomes:** The reaction between antioxidants and DPPH transpires relatively expeditiously, typically within the span of 30 minutes to an hour. This swift reaction enables the efficient screening of numerous samples.

**Alteration of Colour:** DPPH undergoes a colour transformation during the antioxidant reaction, transitioning from a purple hue to yellow. This alteration in colour is readily discernible and quantifiable through the utilization of a spectrophotometer or a colorimeter.

**Quantitative Measurement:** The reduction of DPPH by antioxidants is contingent on dosage, signifying that the extent of colour modification correlates directly with the concentration of antioxidants present. This attribute facilitates the quantitative assessment of antioxidant activity.

**Versatility:** The DPPH assay is adaptable for diverse sample types, encompassing plant extracts, food products, and synthetic antioxidants. Furthermore, it can be harnessed to evaluate the antioxidant potential of both hydrophilic and lipophilic compounds.

**Broad Acceptance:** The DPPH assay is a well-established method that has garnered widespread acceptance within the scientific community. Numerous research endeavors and publications have employed this assay, simplifying the comparison and validation of findings across different studies.

**Cost-Efficiency:** DPPH is reasonably priced in comparison to alternative antioxidant assays, enhancing its accessibility for researchers operating with limited financial resources.

**Relatively Indicative Antioxidant Capacity:** While DPPH does not entirely replicate the intricate oxidative processes that unfold within biological systems, it furnishes a valuable relative gauge of the antioxidant capacity of compounds. Researchers can gauge and contrast the antioxidant activity of diverse samples based on their DPPH scavenging capabilities.

It is noteworthy that the DPPH assay, while advantageous for screening and comparative analyses of antioxidants, represents a simplified model of oxidative occurrences in biological systems. Consequently, researchers frequently complement DPPH assays with other methodologies to attain a more comprehensive understanding of antioxidant behavior within real-life contexts.

#### **4.5 TOTAL ANTIOXIDANT CAPABILITIES (TAC)**

The results obtained from the research shows comparable activity of the methanol extract of powdered leaves of *Ficus exasperata* Vahl with that of Ascorbic acid, a well-known antioxidant commonly used as a positive control in antioxidant assays. The fact that the extract demonstrated activity at par with ascorbic acid suggests its potent antioxidant potential. The extract showed maximum antioxidant capability at 20 µg/mL after which there was a steady decline in %RSA. The research indicates that the extract's maximum antioxidant capability was observed at a

concentration of 20  $\mu\text{g/mL}$ . This means that at this specific concentration, the extract exhibited the highest level of radical scavenging activity (%RSA). This finding suggests that 20  $\mu\text{g/mL}$  is the optimal concentration for harnessing the extract's antioxidant benefits. This observation implies that there may be a concentration-dependent effect, with higher concentrations of the extract potentially resulting in reduced antioxidant efficacy. It is crucial to determine the dose-response relationship to understand the extract's behavior at varying concentrations accurately.

## **CHAPTER FIVE**

### **CONCLUSION**

Overall, these findings suggest that the methanol extract of *Ficus exasperata* Vahl's leaves possesses potent antioxidant properties, which can be equated to the antioxidant activity of ascorbic acid. However, it's crucial to consider the concentration at which this antioxidant activity is most pronounced to optimize its use effectively. Further studies may be needed to explore the underlying mechanisms and to assess the safety and efficacy of the extract for potential therapeutic applications in combating oxidative stress-related conditions.

## REFERENCES

1. "Traditional Medicine: Definitions". World Health Organization. 2008-12-01
2. "WHO traditional medicine strategy: 2014-2023". The World Health Organization. December 2013
3. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary, and alternative medicines : AJTCAM, 10(5), 210–229. <https://doi.org/10.4314/ajtcam.v10i5.2>
4. . Smith-Hall, C., Larsen, H. O., & Pouliot, M. (2012). People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption. Journal of ethnobiology and ethnomedicine, 8, 43. <https://doi.org/10.1186/1746-4269-8-43>Medico-Biowealth of India, Volume- VI; ISBN:978-81-952750-9-0
5. Ahn K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB reports, 50(3), 111–116. <https://doi.org/10.5483/bmbrep.2017.50.3.221>
6. Gershenzon J, Ullah C (January 2022). "Plants protect themselves from herbivores by optimizing the distribution of chemical defenses". Proc Natl Acad Sci USA. 119 (4). Bibcode:2022PNAS..11920277G. doi:10.1073/pnas.2120277119. PMC 8794845. PMID 35084361
7. "[Active Plant Ingredients Used for Medicinal Purposes](#)". United States Department of Agriculture. [Archived](#) from the original on 12 July 2018. Retrieved 18 February 2017.
8. <http://www.jnkvv.org/PDF/11042020204520primaryandsecondarymetabolitesandtheirapplications.pdf>

9. A. Hussein, R., & A. El-Anssary, A. (2019). Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. IntechOpen. doi: 10.5772/intechopen.76139
10. IUPAC, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (1997). Online corrected version: (2006–) "alkaloids". doi:10.1351/goldbook.A00220
11. Manske, R. H. F. (12 May 2014). The Alkaloids: Chemistry and Physiology, Volume 8. Vol. 8. Saint Louis: Elsevier. pp. 683–695. ISBN 9781483222004. OCLC 1090491824
12. Lewis, Robert Alan (23 March 1998). Lewis' Dictionary of Toxicology. CRC Press. p. 51. ISBN 9781566702232. OCLC 1026521889
13. Petrovska B. B. (2012). Historical review of medicinal plants' usage. Pharmacognosy reviews, 6(11), 1–5. <https://doi.org/10.4103/0973-7847.95849>
14. Zhang, J., Onakpoya, I. J., Posadzki, P., & Eddouks, M. (2015). The safety of herbal medicine: from prejudice to evidence. Evidence-based complementary and alternative medicine : eCAM, 2015, 316706. <https://doi.org/10.1155/2015/316706>
15. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. 2004. [https://ris.who.int/bitstream/handle/10665/43034/9241592214\\_eng.pdf](https://ris.who.int/bitstream/handle/10665/43034/9241592214_eng.pdf)
16. Ahmed F, Mueen Ahmed KK, Abedin MZ, Karim AA. (2012) Traditional uses and pharmacological potential of *Ficus exasperata* vahl. Syst Rev Pharm 2012; 3(1) p.15-23
17. Romita Devi, B. L. Manjula, Manish Kumar, Sanjeet Kumar, Sugimani Marndi (2022) Food and medicinal values of some *Ficus* species. Medico-Biowealth of India. Vol(6). p21-26 ISBN:978-81-952750-9-0

18. Koshimizu K, Ohigashi H, Huffman MA, Nishida T, Takasaki H. (1993) Physiological activities and the active constituents of potentially medicinal plants used by wild chimpanzees of the Mahale mountains, Tanzania. *Int J Primatol.* Vol(14). p345-6.
19. Ahmed, Faiyaz; Mueen Ahmed, K. K.; Abedin, Zainul; Karim, Alias A. (2012). Traditional Uses and Pharmacological Potential of *Ficus exasperata* Vahl. *Systematic Reviews in Pharmacy.* Jan-Dec2012, Vol. 3(1), p15-23
20. Ogunleye, D. S., Adeyemi, A. A., & Sanni, A. M. (2003). Hypoglycaemic activities of the stem bark of *Cola acuminata* Vahl and leaf of *Ficus exasperata* (P. Beauv) Schott and Endl. *Nigerian Quarterly Journal of Hospital Medicine*, 13(1), 58-60.
21. Dongfack, M. D. J., Wandji, J., Lallemand, M. C., & Tillequin, F. (2008). New acyl-glucosylsterol with unusual fatty acids from *Ficus exasperata*. *Planta Medica*, 74(09), PB86.
22. Taiwo I A, Adebisin OA, Funmilayo A. (2010) Glycaemic activity of *Ficus exasperata* in fructose-induced glucose intolerance in rats. *Res*;2:80-3.
23. Adewole SO, Adenowo T, Naicker T, Ojewole JA. (2011) Hypoglycaemic and hypotensive effects of *Ficus exasperata* Vahl. (Moraceae) leaf aqueous extract in rats. *Afr J Tradit Complement Altern Med*;8:275-83
24. Abotsi WM, Ozolua E, Ainooson GK, Amo-Barimah AK, Boakye-Gyasi E. (2010) Antiarthritic and antioxidant effects of the leaf extract of *Ficus exasperata* P. Beauv. (Moraceae). *Pharmacognosy.* Vol(2). p89-97
25. Akah PA, Orisakwe OE, Gamaniel KS, Shittu A. (1998) Evaluation of Nigerian Traditional medicines on peptic ulcer. *J Ethnopharmacol.* vol(62). p123-7

26. Kofie W., Osman H., Bekoe S. O. (2015) Phytochemical properties of extracts and isolated fractions of leaves and stem bark of ficus exasperate. World journal of pharmacy and pharmaceutical sciences. Vol. 4(12). p.91-105. ISSN 2278 – 4357
27. Ajayi, O.B., Oluyeye, J.O., Olalemi, O.M., Ilesanmi, T.M. (2012) Nutritional composition, phytochemical screening and antimicrobial properties of the leaf of Ficus exasperata (vahl). Asian Journal of Biological and Life Sciences. Vol. 1(3). p.242-246.
28. E. A. Adebayo, O. R. Ishola, O. S. Taiwo, O. N. Majolagbe, B. T. Adekeye. (2009) Evaluations of the methanol extract of Ficus exasperate stem bark, leaf and root for phytochemical analysis and antimicrobial activities. African Journal of Plant Science Vol. 3 (12), pp. 283-287. ISSN 1996-0824
29. Sonibare MA, Ogunwande IA, Walker TM, Setzer WN, Soladoye MO, Essien E. (2006) Volatile Constituents of Ficus Exasperata Leaves. Natural Product Communications. vol1(9). doi:10.1177/1934578X0600100912
30. Umerie SC, Ogbuagu AS, Ogbuagu JO. (2004) Stabilization of palm oils by using Ficus exasperata leaves in local processing methods. Bioresource Technol;94. p307-10
31. Alli Smith YR, Aluko BT, Faleye FJ. (2018) Antioxidant activity and inhibitory effect of ethanolic extract of Ficus exasperata leaves on pro- oxidant induced hepatic and cerebral lipid peroxidation in albino rats in vitro. International Journal Of Herbal Medicine Vol. 6(3), p. 20-24. ISSN: 2394-0514
32. Sies, H., & Jones, D. P. (2020). Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. Nature Reviews Molecular Cell Biology, 21(7), 363-383.33.

34. Halliwell, B., & Gutteridge, J. M. C. (2007). *Free Radicals in Biology and Medicine*. Oxford University Press
35. Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, 408(6809), 239-247
36. Harman, D. (1956). Aging: A theory based on free radical and radiation chemistry. *Journal of Gerontology*, 11(3), 298-300
37. Halliwell, B. (2007). Free radicals and antioxidants: Updating a personal view. *Nutrition Reviews*, 65(12), 510-512
38. Frei, B., & Higdon, J. V. (2003). Antioxidant vitamins and health: Benefits of taking vitamin C and vitamin E supplements. The Linus Pauling Institute Micronutrient Information Center, Oregon State University
39. Sies, H. (2015). Oxidative stress: A concept in redox biology and medicine. *Redox Biology*, 4, 180-183.
40. Prior, R. L., & Cao, G. (1999). Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. *HortScience*, 34(4), 809-812
41. Helmut SIES, 1993 Strategies for antioxidant defense *European Journal of Biochemistry* 215(2) p. 213-219
42. Darr, D., & Combs, S. (1992). Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *British Journal of Dermatology*, 127(3), 247-253.

43. N. Apostolova, A. M. Cervera, V. M. Victor, S. Cadenas, A. Sanjuan-Pla, A. Alvarez-Barrientos, J. V. Esplugues, K. J. McCreath. (2006) Loss of apoptosis-inducing factor leads to an increase in reactive oxygen species, and an impairment of respiration that can be reversed by antioxidants. *Cell Death Differ.* Vol(13). p(354-357).
44. M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, J. Tesler. (2007). Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. *The International Journal of Biochemistry & Cell Biology*, 39(3), p(44-84).
45. Kim D.O., Being S.W., Lee C.Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81:321-326
46. Ebrahimzadeh M.A., Pourmorad F., Bekhradnia A.R. (2008). "Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran". *African Journal of Biotechnology*. 7(18):3188-3192.
46. Jain A., Soni M., Deb L., Rout S., Gupta V., Krishna K. (2008). "Antioxidant and hepato-protective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. Leaves". *Journal of Ethnopharmacology* .115(1):61-66.
47. D. Moonmun, R. Majumder, A. Lopamudra. (2017). Quantitative Phytochemical Estimation and Evaluation of Antioxidant and Antibacterial Activity of Methanol and Ethanol Extracts of *Heliconia rostrata*. *Indian Journal of Pharmaceutical Sciences*. 79(1): 79-90
48. Ojo, Oluwafemi & Christopher, Akintayo. (2014). Assessment of antioxidant activity of *Ficus asperifolia* Miq aqueous extract -In vitro studies. *The Journal of Phytopharmacology*. 3. 16-21. 10.31254/phyto.2014.3103.

49. Akanni, O. O., Owumi, S. E., & Adaramoye, O. A. (2014). In vitro studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from *Artocarpus altilis*, *Ficus exasperata* and *Kigelia africana*. *Asian Pacific journal of tropical biomedicine*, 4(Suppl 1), S492–S499. <https://doi.org/10.12980/APJTB.4.2014C581>

50. Akpovwehwee A. Anigboro, Oghenetega J. Avwioroko, Oghenenyore A. Ohwokevwo, Beruoritse Pessu, Bioactive components of *Ficus exasperata*, *Moringa oleifera* and *Jatropha tanjorensis* leaf extracts and evaluation of their antioxidant properties, *EurAsian Journal of BioSciences*, vol. 13, 2019 (p)1763-1769

**APPENDIX**

**TABLE A.1: TABLE OF CONCENTRATION OF GALLIC ACID AND ABSORBANCE**

<b>CONCENTRATION (mg/ml)</b>	<b>ABSORBANCE (nm)</b>		
<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>12.5</b>	<b>-</b>	<b>0.157</b>	<b>-</b>
<b>25</b>	<b>0.271</b>	<b>0.253</b>	<b>0.297</b>
<b>50</b>	<b>0.503</b>	<b>0.728</b>	<b>0.655</b>
<b>75</b>	<b>0.634</b>	<b>0.651</b>	<b>0.606</b>
<b>100</b>	<b>0.732</b>	<b>0.849</b>	<b>0.767</b>
<b>150</b>	<b>0.963</b>	<b>1.050</b>	<b>0.976</b>

**TABLE A.2: TABLE OF CONCENTRATION OF GALLIC ACID AND AVERAGE ABSORBANCE.**

<b>CONCENTRATION (mg/ml)</b>	<b>AVERAGE ABSORBSANCE (nm)</b>
<b>0</b>	<b>0</b>
<b>12.5</b>	<b>0.157</b>
<b>25</b>	<b>0.274</b>
<b>50</b>	<b>0.629</b>
<b>75</b>	<b>0.630</b>
<b>100</b>	<b>0.783</b>
<b>150</b>	<b>0.996</b>

**TABLE A.3: TABLE OF 0.5 ML OF ABSORBANCE OF METHANOLIC SOLUTION OF CRUDE.**

<b>EXTRACT (1 mg/ml)</b>	<b>ABSORBANCE (nm)</b>		
	<b>0.112</b>	<b>0.093</b>	<b>0.100</b>

**TABLE A.4: TABLE OF CONCENTRATION QUERCETIN AND ABSORBANCES.**

<b>CONCENTRATION (mg/ml)</b>	<b>ABSORBANCE (nm)</b>		
<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>12.5</b>	<b>0.350</b>	<b>0.359</b>	<b>0.424</b>
<b>25</b>	<b>0.514</b>	<b>0.572</b>	<b>0.561</b>
<b>50</b>	<b>0.821</b>	<b>0.848</b>	<b>0.911</b>
<b>75</b>	<b>1.372</b>	<b>1.972</b>	<b>1.426</b>

**TABLE A.5: TABLE OF CONCENTRATION OF QUERCETIN AND AVERAGE ABSORNBACE.**

<b>CONCENTRATION (mg/ml)</b>	<b>AVERAGE ABSORBANCE (nm)</b>
<b>0</b>	<b>0</b>
<b>12.5</b>	<b>0.378</b>
<b>25</b>	<b>0.549</b>
<b>50</b>	<b>0.860</b>
<b>75</b>	<b>1.590</b>

**TABLE A.6: TABLE OF ABSORBANCE OF EXTRACT**

<b>EXTRACT</b>	<b>ABSORBANCE (nm)</b>		
<b>0.5 ml</b>	<b>-</b>	<b>1.296</b>	<b>1.006</b>