CHEMICAL CHARACTERIZATION AND ANTIOXIDANT PROPERTIES OF ETHANOL-WATER LEAF EXTRACTS OF *Justicia secunda* AND *Jatropha tanjorensis*

BY

KEKE, COLLINS OBINNA, B.Sc. (UNIBEN)

(20184141108)

A THESIS SUBMITTED TO THE

POST GRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN BIOCHEMISTRY

OCTOBER, 2023

CERTIFICATION

This is to certify that this thesis entitled "**CHEMICAL CHARACTERIZATION AND ANTIOXIDANT PROPERTIES OF ETHANOL-WATER LEAF EXTRACTS OF *JUSTICIA SECUNDA* AND *JATROPHA TANJORENSIS"*** was carried out by **KEKE COLLINS OBINNA**.

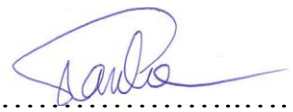
Prof. L.A. Nwaogu — **Date**
(Supervisor)

Dr. C.U. Igwe — **Date**
Supervisor)

Prof. L.A. Nwaogu — **Date**
(Head of Department)

Prof. C. Alisi — **Date**
(Dean, School of Biological Sciences)

Prof. B.O. Esonu — **Date**
(Dean, Post Graduate School)



Prof. S.C. Udedi — **Date**
(External Examiner)

DEDICATION

This work is dedicated to God Almighty for His endless kindness and blessings and to my late father, Mr. Polycarp Mgechaonuwa Keke and my late grandmother Mrs. Regina Ogboko Keke.

ACKNOWLEDGEMENT

I owe a debt of immense gratitude to my wonderful supervisors, Prof. L.A Nwaogu who also doubles as the Head of department Biochemistry, FUTO and Dr. C.U. Igwe. I consider them great scholars, mentors and fathers. They gave me every support I needed. Again, I was privileged to pass through great scholars who played significant role in molding me in the course of this master`s degree programme. These lecturers of mine are indeed wonderful; Prof. C.O. Ibegbulam, Prof. A.C. Ene, Prof. C.S Alisi, Prof. K.M.E. Iheanacho, Prof. R.N Nwaogulkpe, Prof. (Mrs.) G.N. Okwu, Prof. C. O Ujowundu, Dr. (Mrs.) Onuoha, late Prof. N. Nwachukwu and late Dr. K.O. Igwe. I also recognize and appreciate the invaluable contributions of other lecturers of the department.

I wish to also extend my gratitude to my beloved mother, Deaconess Peace A. Keke and my siblings, Mr. Stanley Uzoma Keke, Mr. Sixtus Chinonyerem Keke, Miss Precious Ogechi Keke and Chibuike Godswill Keke. The prayers and encouragements of my pastors, Rev. Dr. Okechukwu Nwauju, Pastor Chinyere Ejinwa, and Deaconess Blessing Onuoha are highly appreciated.

I also want to appreciate all my M.Sc. and Ph.D colleagues for the camaraderie and corporation we had while running the programme.

My thanks will not be complete without mentioning my darling wife, Barr. (Mrs.) Judith Ngozichinyere Obinna Keke for her support, love and beautiful distraction at the tail end of this programme. Thanks a million, my darling.

Finally, all glory and honour be ascribed to God Almighty for his endless mercies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study evaluated the chemical compositions, antioxidant and toxicological properties of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*. Proximate compositions were assessed using standard methods, while minerals, amino acids and phytochemical compositions were evaluated with the aid of atomic absorption spectrophotometer, amino acid analyzer and gas chromatography-mass spectrometer respectively. Antioxidant vitamins, total phenol, flavonoid contents, and free radical scavenging properties were also determined, while toxicological evaluation was via brine shrimp lethality assay. Both plants exhibited significant nutrient contents based on proximate and mineral analyses. Histidine and phenylalanine were the most abundant essential amino acids, while glycine and proline were the most prevalent non-essential amino acids in *J. secunda* and *J. tanjorensis* respectively. Notable amounts of phytochemicals were observed, with *Jatropha tanjorensis* having higher levels of sapogenin, anthocyanin, and anti-nutrient factors. GC-MS analysis revealed 62 and 52 bioactive compounds in *J. secunda* and *J. tanjorensis* respectively. *J. secunda* had higher levels of antioxidant vitamins C, E, and A, while *J. tanjorensis* demonstrated higher total phenolic and flavonoid contents. *J. tanjorensis* also displayed higher DPPH and ABTS radical scavenging activities, while *J. secunda* showed better FRAP. The brine shrimp lethality test indicated that the ethanol-water leaf extract of *J. secunda* exhibited higher toxicity (LC_{50}; $7.59 \times 10^2 \pm 0.03$µg/ml) compared to *J. tanjorensis* (LC_{50}; $9.89 \times 10^3 \pm 0.03$ µg/ml). The study has confirmed the forkloric claim of high nutritive and medicinal value of *J. secunda* and *J. tanjorensis* leaves. It showed that the plants contain significant amounts of bioactive compounds and antioxidants with free radical scavenging potentials but calls for caution in their use especially with *J. secunda* that had significant toxic effect on brine shrimp.

Keyword: Phytochemistry, bioactive compounds, Proximate, amino acids, antioxidants, Brine shrimp lethality assay, *Jatropha tanjorensis, Justicia secunda.*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Natural products such as medicinal plants and herbs have long been used as sources of traditional medicine around the world (Heinrich, 2000). Plants are therefore arguably one of the most important resources nature has bequeathed man and animals (Shakya, 2016). Plants have been used for medicinal purposes for thousands of years, and their therapeutic benefits have been recognized by many cultures around the world (Khan, 2014). Dependence on these plants as sources of bioactive phytoconstituents and natural antioxidants to counteract the dangerous effects of free radicals generated in the body has tremendously increased in recent times due to their roles in boosting the immune system against COV-19 (Akbari *et al*., 2022).This dependence on medicinal plants such as *Justicia secunda* and *Jatropha tanjorensis* increased even further during the COVID-19 pandemic especially among developing countries and Africa where the cost of drugs and medical care is expensive as private individuals and families sought for alternative options to combat the pandemic (Luo *et al*., 2020; Khadka *et al*., 2021). According to a WHO survey, about 80% of people rely on traditional plants for basic health care (Gangal *et al.*, 2020). These medicinal plants are known to contain various physiologically active substances such as alkaloids, saponin, flavonoids, terpenpoids and phenolic compounds more commonly known as phytochemicals (Rabizadeh *et al*., 2022). These phytochemicals are secondary metabolites produced by plants which exhibit a range of biological activities (Koche *et al*., 2016). The ceaseless study of these secondary plant metabolites has yielded ground-breaking results in the area of pharmacology and drug development (Nwaogu *et al*., 2007). For instance, drugs such as vincristine, vinblastine, atropine, digoxin, quinidine, quinine, morphine and codeine just to mention but a

few have all been developed from plants (Rates, 2009; Abelson, 1990). These plant-based chemicals or secondary metabolites have been found to exhibit various pharmacological properties, including anti-inflammatory, anti-cancer, anti-viral, antimicrobial and anti-oxidant activities (Zhu *et al*., 2018).

Due to the presence of these bioactive substances, there is therefore an ever-increasing interest in studying the phytochemistry of medicinal plants and their potential applications in the management and treatment of various diseases. Studies have also shown that these phytonutrients confer significant antioxidant ability to medicinal plants making them excellent sources of natural antioxidants. Antioxidants are compounds that inhibit the oxidation of other molecules by neutralizing free radicals (Gulcin, 2020). Chronic oxidative stress has been implicated in the development and progression of chronic diseases such as diabetes, cancer, heart diseases and neurodegenerative disorders like Parkinson`s disease and Alzheimer`s disease.

Therefore, the search for bioactive plant compounds and natural antioxidants from plant sources has continued to attract huge attention due to their potential as alternative therapeutic agents.

1.2 Statement of Problem

The potential health benefits of natural antioxidants have led to a surge in the exploration of plant-based resources. However, the antioxidant properties of *Justicia secunda* and *Jatropha tanjorensis*, despite their potential, remain underexplored due to a lack of comprehensive chemical characterization. The dearth of information about their proximate and mineral compositions, anti-nutrient factors, amino acid profiles, phytochemical compositions, and antioxidant potentials limits their potential applications. Moreover, the safety profile of these plants, a crucial factor for substances intended for consumption or topical use, is yet to be

thoroughly investigated. This study seeks to address these knowledge gaps, paving the way for the potential use of these plants in health and industry.

1.3 Justification of Study

Justicia secunda and *Jatropha tanjorensis* have been traditionally used for medicinal purposes. By analyzing their phytochemistry and antioxidant properties, this study will provide scientific evidence to support their traditional uses and identify potential bioactive compounds that may be used in the development of drugs or natural products for various therapeutic purposes. This study will also examine the presence of anti-nutrient factors in the plants which can interfere with nutrient absorption and contribute to nutrient deficiencies. This information can be used to develop methods for reducing the anti-nutrient contents of the plants and improving their nutritional value. Furthermore, this study will examine the toxicity of the two plant extracts using brine shrimp lethality assay. Information derived from this preliminary study can be used to determine the safety of the plants for human uses and identify potential risks associated with their consumption.

1.4 Aim of study

The aim of the study was to determine the chemical characterization and antioxidant properties of the ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*.

1.5 Specific objectives

The specific objectives of the study are to:

i. Determine the proximate compositions (ash, moisture, crude fibre, fat, protein and carbohydrate contents) of the plants.
ii. Determine the mineral composition (Na, K, Ca, Mg, Cu, Zn, Fe and Mn) of the plants
iii. Determine the anti-nutrient factors (tannins, phytate, oxalate, alkaloid, cyanogenic glycosides, phenolic acids) of the plants

iv. Determine the amino acid profile of the plants

v. Assess the qualitative and quantitative phytochemical compositions of the plants.

vi. Assess the antioxidant potentials (antioxidant vitamins, total phenolic content, total flavonoids, DPPH, ABTS, ferric reducing antioxidant power) of the plants.

vii. Toxicological evaluate the plants' extracts using brine shrimp lethality assay (BSLA).

CHAPTER TWO

LITERATURE REVIEW

2.1 *Justicia secunda* Vahl

Justicia secunda Vahl also known as St John`s bush is a perennial herbaceous plant and of the family "acanthaceae" (Yamoah *et al*., 2020). Justicia consists of about 600 species and is the largest member of the "acanthaceae" family (Gomez-Verjan *et al*., 2012; Khan *et al*., 2017). *J. secunda* Vahl is of South American origin, but is now grown in tropical and sub-tropical countries of the world such as Nigeria, Ghana, Congo, Cameroon and Cote di`voire (Mpiana *et al*., 2010; Koffi *et al*., 2013; Yamoah *et al*., 2020). It grows up to 1 to 5 meters in height (Osioma & Hamilton-Amachree, 2017; Arogbodo, 2020) and usually grows luxuriantly in humid soils near rivers or creeks mostly in tropical and sub-tropical regions of the world (Herrera-Mata & Rosas-Romero, 2002). *J. secunda* is known by different names among different countries, tribes and ethnicities. It is called “Sanguinaria" in Venezuela (Carrington *et al*., 2012), “Housimani" which literally means a plant that gives blood in Benin (Osioma & Hamilton-Amachree, 2017) "Blood root" in Bardodos (Carrington *et al*., 2012). In Akan language of Ghana, *J. secunda* is known as "Yahowafo mogyra duro" which means "Jehovah`s Witnesses blood tonic (Yamoah *et al*., 2020). The Igbos of South-Eastern Nigeria calls it “Obara bu ndu" which means blood is life or "ogwu obara" meaning blood tonic while the Yorubas call it "ewe eje" meaning blood leaf or "ewe ajeri" which means Jehovah`s witness’s leaf. It is called "Asindiri" or "Ohawaazara” in Niger Delta region of Nigeria by the Ogbia people of Otuoke-Otuaba of Bayelsa State. Just as in South-Eastern Nigeria, *Justicia secunda* vahl is also common in Democratic Republic of Congo and Cote di`voire where it is used in the treatment of anemia by Jehovah`s witnesses who are well known for their blunt refusal of blood transfusion (Mpiana *et al*., 2010).

2.2 Scientific classification of *Justicia secunda* Vahl

Blood root is scientifically classified (USDA, 2009) as belonging to the following:

Kingdom : Plantae

Clade : Tracheophytes

Order : Lamiales

Family : Acanthaceae

Sub-family : Acanthoideae

Genus : Justicia

Species : Secunda

Botanical Name : *Justicia secunda Vahl*

Figure 2.1: *Justicia secunda* Vahl leaves with flowers

2.3 Geographical distribution and botanical description of Justicia

Justicia is the largest and most complex of the family "acanthaceae"(Gomez-Verjan *et al*., 2012). A majority of them grow in tropical and subtropical regions of the world (Gomez-Verjan *et al*., 2012). According to Ezcurra (1999b) about 300 species and 400 species are native to American continent and Asia respectively. Columbia is a home to about 85 species (Leonard, 1951) while nearly 75 species are found in Mexico (Daniel, 1993). According to Joergensen & LeonYanez (1999) there are 27 species in Ecuador, 28 species in Argentina (Ezcurra, 1993a) while 50 species are native to Peru (Brako & Zaruchi, 1993). Justicia are perennial plants or subshrubs that grow in erect or scandent forms (Kitadi *et al*., 2019). Justicia leaves have cystoliths and are petiolate, with a leaf border that is normally whole. Inflorescences appear in the form of spikes or panicle cimas and the species hardly possess solitary or axillary flowers (Kitadi *et al*., 2019). Their bracts and bracteoles are frequently visible and imbricate. Justicia species are distinguished by their bilabial corolla, which has a posterior lip with two lobes, an anterior lip that is three lobed, two stamens, a capsule with four seeds, and a basal sterile part (Kitadi *et al*., 2019).

2.3.1 Justicia Species from Asia

Ezcurra (1999b) reported that about 400 species of Justicia are native to Asia. The chemical and pharmacological properties of those Asian Justicia have been widely studied. Examples of such species are: *J. adhatoda* (*Adhatoda vasica*), *J. procumbrens, J. ciliata, J. extensa, J. tranquebariensis, J. glauca, J. flava, J. prostate* (mainly found in India), *J. simplex* (India), *J. insulinarias* (native to India), *J. gendarussa* (India) (Gomez-verjan *et al*., 2012). Among the Asian species, *J. adhatoda* also known as *Adhatoda vasica* (Claeson *et al*., 2002) stands out as most promising and important of this genus (Gomez-Verjan *et al*., 2012). In traditional

Hindu medicine, it is known as the "Malabar tree"(Claeson *et al*., 2002) and as "Vasaka" in Sanskrit (WHO, 1990).

According to Atal (1980), *J. adhatoda* is popular in Ayurdevic medicine and Unani medicine among Indians since nearly 2000 years. Because of its medicinal properties, it is included in the "Manuel of Traditional Medicine in primary healthcare", and is recommended for both youth and adults for the treatment of bleeding hemorrhoids, cough, phlegm and asthma (WHO, 1999).

2.3.2 African species of Justicia

Justicia hyssopifolia which is common to the Canary Island is native to Africa and has abundance of arilnaftalen lignin called "ellenoside". It contains two lignans named J1 and J2 (Navarro *et al*., 2004a). Ellenoside in *J. hyssopifolia* has the ability of inhibiting intestinal motility *in vitro* (Navarro *et al*., 2006b) and possess CNS depressant activity (Navarro *et al*., 2004a).

2.3.3 American species of Justicia

Though about 300 species of Justicia are found within the American continent (Ezcurra, 1999b), studies have shown that only 3 species namely: *Justicia pectoralis, Justicia spicigera and Justicia secunda* have been chemically and pharmacologically studied (Gomez-Verjan *et al*., 2012).

2.3.4 Justicia pectoralis

Justicia pectoralis is native to American tropics and found mainly in the mainland of South and Central America (Gomez-verjan *et al*., 2012). Brazilians call it" Chamba" while Cubans call it "Tilo or Tila". Its commonest ethnomedicinal use in Brazil is as sedative, though it has also been used as expectorant hence the name "pectoral".

2.3.5 Justicia spicigera

Justicia spicigera is native and common to the South and Central of Mexico and to Guatemala. It is known as “Mutile" in Mexican traditional medicine where it is majorly used against dysentery, because of its antimicrobial, parasitic activity and its effect on intestinal motility. *Justicia reptans* is yet another species of Justicia native to Guatemala and only one study has been done on this species (Alcami *et al*., 2008).

2.3.6 *Justicia secunda* Vahl

According to Gomez-Verjan *et al* (2012), *Justicia secunda* Vahl is commonly found in Central America and Northern South America where it is used in traditional medicine to treat kidney stone. *Justicia secunda* vahl is now grown in tropical and subtropical regions of Africa including Nigeria, Ghana, Cameroon, Cote D’Ivoire and Democratic Republic of Congo (Koffi *et al*., 2013; Kitadi *et al*., 2019). *J. secunda* aqueous leaf extracts is characteristically pink to purple, due to its abundance of polyphenols such as tannins or anthocyanins (Osima & Hamilton-Amachree, 2017).

2.4 Phytochemistry of Justicia

Phytochemical screening of different species of *Justicia secunda* Vahl revealed the abundance of polyphenols such as alkaloids, quinones, flavonoids, tannins, and leuco-anthocyanins (Mpiana *et al*., 2010a). Mpiana and colleagues in another study, reported the presence of saponins, sterols, anthocyanins and terpenes in three species of justicia namely *J. tenella, J. gendarussa* and *J. insularis* (Mpiana *et al*., 2010b). According to the study, alkaloids were only found in *J. tenella* while tannins and leuco-anthocyanins were present in both *Justicia insularis* and *Justicia gendarussa* (Mpiana *et al*., 2010b). In fact Mpiana *et al*, (2010) attributed the antisickling activity of *Justicia secunda* Vahl to the presence of anthocyanins. Presence of coumarins, glycosides, amines, amino acids, stigmasterol, lupeol

and aromatic amines have also been reported (Bedoya *et al*., 2008; Kitadi *et al*., 2019). Arogbodo (2020) also reported the presence of the following phytochemicals in *Justicia secunda* leaf extract - saponins (2.33 ± 0.30 mg/g), tannins (0.55 ± 0.01 mg/g), glycosides (1.58 ±0.05 mg/g), steroids (2.65 ±0.02 mg/g), terpenoids (14.81 ± 0.04 mg/g) and flavonoids (0.48 ± 0.01 mg/g). Ajuru *et al*, (2021) compared the phytochemicals found in the root of *Justicia secunda* to those found in the root of *Justicia carnea*. According to their findings, *Justicia carnea* contained more tannin and saponin, while *Justicia secunda* contained more flavonoid, phytate, alkaloid, and cyanogenic glycoside (Ajuru *et al*., 2021).

2.5 Ethnomedicinal uses of *Justicia secunda* Vahl

Different cultures and regions around the world use *Justicia secunda* Vahl to treat a variety of diseases and conditions. It is used in traditional medicine in Central and Northern South America to treat kidney stones (Gomez-Verjan *et al*., 2012). It is used as an anti-pyretic in Venezuela, where it is known as "Sanguinaria" (Herrera-Mata *et al*., 2002). It is used in Columbia to treat a variety of infectious diseases, including glycemic disorders (Rojas, 2006). It is used to treat menstrual pain and non-specific infections in Trinidad and Tobago (Lans, 2007b), and to treat snake bites and dysentery in hunting dogs (Lans *et al*., 2001a). According to Kone *et al*, the leaves of *J. secunda* are used to treat anemia, wounds, and abdominal pain in the lower regions of the body (Kone *et al*., 2012). Leaf decoctions of the plant are consumed in South Eastern Nigeria, the Democratic Republic of Congo, and Cote d'Ivoire to improve hematocrit count (Mpiana *et al*., 2010).

2.6 Pharmacologically active compounds from different species of justicia

Many pharmacologically active compounds have been isolated from different species of justicia. From *Justicia adhatoda* which is also known as *adhatoda vasica*, many compounds

have been isolated such as vasicine (a quinazoline alkaloid) (Dymock *et al*., 1890), vasicinone, vasicinol, adhatodine, peganine, anisotine, adhatodinine and adhavasinone (Huq *et al*., 1967; Willaman & Li, 1970; Atal, 1989; Chowdhury &Bhattacharyya, 1987; Bhat *et al*., 1978). Studies have also revealed presence of certain essential flavonoids such as quercetin, kaempferol and astragaline in the flowers of *Justicia adhatoda*. α-Amyrin (a triterpenoid) was also found to be contained in the flowers (Huq *et al*., 1967).

One of the pharmacological studies by D`Cruz *et al.* (1979) found that essential oil from the aerial parts of *Justicia adhatoda* has a relaxing effect on tracheal vascular smooth muscle of guinea pig. Modak *et al*. (1966) and Dhar et *al.* (1968) also reported that ethanol extract of aerial portions of the plant produced hypoglycemic action in mice and rabbits. Study by Muller and others further revealed that methanol extract of the whole plant has anti-asthmatic and anti-allergic activities (Muller *et al*., 1993). Infusions of the aerial portions have been demonstrated to have activity on isolated bacteria from gingivitis sufferers (Patel *et al*., 1984). Nevertheless, studies by Naqvi *et al* (1991) reported no antimicrobial activity on yeasts, filamentous fungi, gram (+) and gram (-) bacteria. Anti-helminthic activity of ethanol extract of the root has been demonstrated (Lateef *et al*., 2003). Of all the isolated compounds, the alkaloid "Vasicine" is the most studied and most promising. *In vivo* and *in vitro* studies have shown that, this compound has considerable bronchodilator action (Atal, 1980). Semi-synthesis was employed to create the substance, bromhexine and its main metabolic product in man, ambroxol. The latter of which had strong mucolytic action. Both compounds are now widely used in a variety of medicinal preparations (Grange *et al*., 1996). Vasicine has also been demonstrated to trigger rhythmic contractions in human Myometer, a similar effect to oxytocin (Atal, 1980). Pharmacokinetic and pharmacodynamic studies on vasicine have revealed that it has a half-life of 5-7 minutes when administered intravenously and 1.5-2 hours

when administered intramuscularly. It is metabolized in the liver to vasicinone and other metabolites, and is excreted primarily in the urine (Atal, 1980).

Compounds isolated from *Justicia procumbrens* are; neojusticine-A, neojusticine-B, taiwanine -E as well as juticidines-A, B, C and D (Ohta & Munakata, 1970). These compounds have demonstrated different pharmacological activities. It has been shown that neojusticine-A can cause platelet aggregation (Chen *et al*., 1996) whereas in rats, neojusticine-B has antiarrhythmic action (Lin and Zhong, 1982).

Amongst the most exciting areas of research in these species is antiviral activity, as the lignans justicidine-A, B, C, and D; justicidinosides A, B, and C; and the dyphiline apioside isolated from aerial portions of *J. procumbrens* have exhibited remarkable activity against vesicular stomatitis virus. Furthermore, significant cytotoxic activity in rabbit lung cells was discovered (Asano, 1996).The following have been isolated from *Justicia simplex:* simplexoline, sesamoline, sesamine, asarinine, b-sitosterol (Ghosal *et al*, 1979a), 3-arylnaftalide lignans, justicines C and E (Sastry *et al., 1979*).Further studies by Ghosal *et al.* (1980b) resulted in the isolation of justicisoline, simplexoside and justicisaponin (a triterpenic saponine) which demonstrated a significant anti-fertility effect in rats. In *J. insularinarias* which is also an Indian species, the following compounds were isolated; b-sitosterol and aromatic amines (Chakravarty *et al.*, 1982). *J. genderussa* ethanol extracts have been proven in studies to have anti-arthritic properties in two separate physiological models (Paval, 2009). Many species of Justicia of Asian origin such as *J. tranquebariensis* have had arytetarline, (+)-laricireasinol and (+)- medioresinol isolated from them (Raju & Pillai,1989).

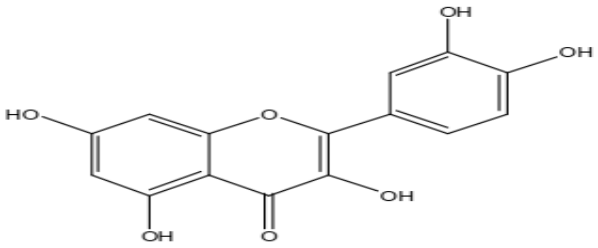
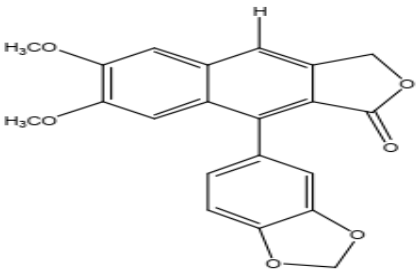
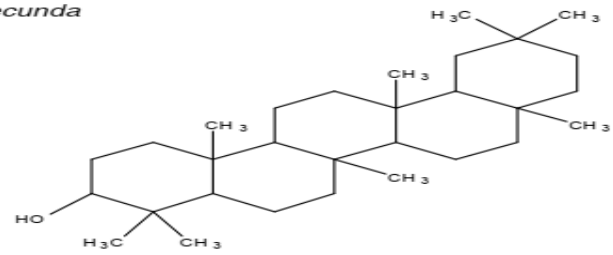
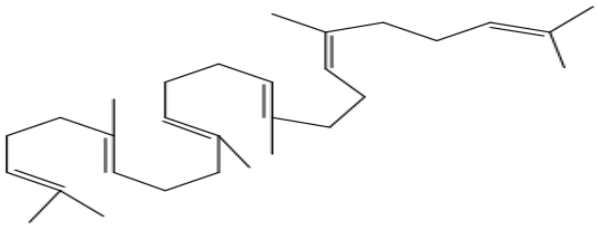
Justicia glauca contains justiciresinol (Subbaraju *et al*., 1991) and jusglacucinol (Rajendrian & Subramanian, 1991). From *J. spicigera*, kampferitrine (Euler & Alam, 1982) and O-

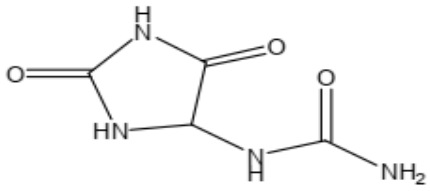
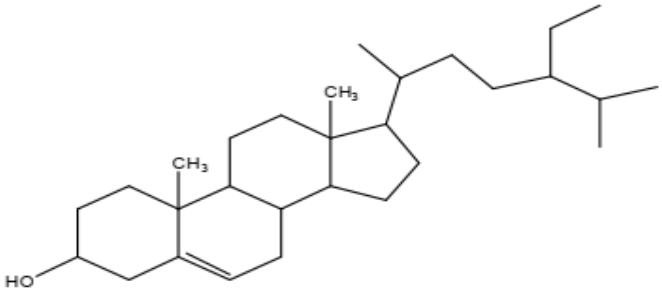
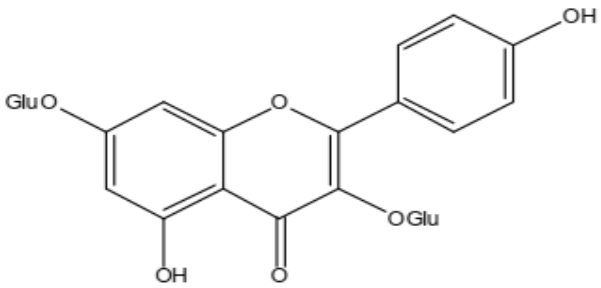
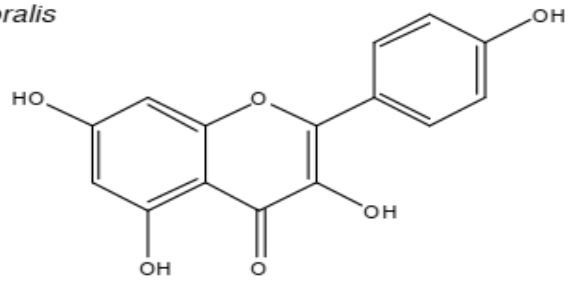
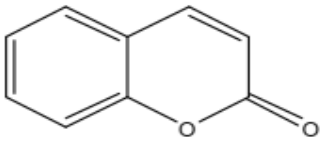
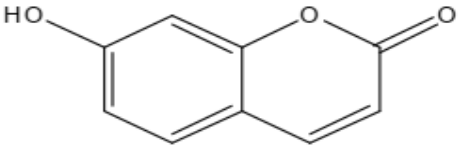
sitosteryl-3-b-glucoside, allantoin and cryptoxanthin (Dominguez *et al.,* 1990) have been isolated. From *J. pectoralis*, the following have been isolated- coumarine, dihydrocoumarine, umbelliferone (Olveira *et al.,* 2000), glycosylflavone (Joseph *et al*., 1988).

Table 1: Some important pharmacologically active compounds isolated from different species of Justicia

Isolated Compound	Species	Reference
4,6-dipheny-2 pyrimidinilamine	*J.secunda*	Herrera-Mata *et al.*, 2002
Carboxylic and Eicosanoid acid	*J.secunda*	Herrera-Mata *et al.*, 2002
Glycosylflavone	*J. pectoralis*	Joseph *et al*., 1988
Coumarines, dihydrocoumarine	*J. pectoralis*	Olveira *et al.,* 2000
Umbelliferine, 1,2-benzopyrene	*J. pectoralis*	Olveira *et al.,* 2000
Kampferitrine	*J. spicigera*	Euler&Alam,1982
O-sitosteryl-3-b-glucoside, Allantoin and Cryptoxanthin	*J. spicigera*	Dominguez *et al*., 1990
Arytetarline	*J.tranquebariensis*	Raju & Pillai,1989
(+)-Laricireasinol	*J.tranquebariensis*	Raju & Pillai,1989
(+)-medioresinol	*J.tranquebariensis*	Raju & Pillai,1989
Justiciresinol	*J. glauca*	Subbarauju *et al*., 1991
Jusglacucinol	J.*glauca*	Rajendrian&Subramania,1991
Justicinol, helioxantine, (+)-Isolariciresinol	*J.flava*	Olaniyi, 1980
Vasicine	*J.adhatoda*	Dymock *et al*, 1980
Vasicinone	*J.adhatoda*	Huq *et al*., 1967
Vasicinol	*J.adhatoda*	Willaman & Li,1970
Neojusticine-A and B	*J.procumbrens*	Ohta & Munakata, 1970
Juticidines-A,B C&D	*J.procumbrens*	Ohta & Munakata, 1970
Elenoside	*J.hyssopifolia*	Navarro *et al*., 2004a
Procumbrenoside	*J.procumbrens*	Day *et al*.,2002
Retoquinesine	*J .prostate*	Ghosal *et al.*,1979a
Taiwanine –E	*J.procumbrens*	Ohta & Munakata, 1970
Dyphiline	*J.procumbrens*	Fukamiya *et al*., 2002

Table 2: Structures of some pharmacologically active compounds isolated from Justicia

		Quercetine	Olveira *et al.*, 2000
		Justicidiane B	Joseph *et al.*, 1988
J. secunda		Taraxerol	Herrera-Mata *et al.*, 2001
		Escualene	Herrera-Mata *et al.*, 2001

J. spicigera		Alantoine	Domínguez *et al.*, 1990
		~~b~~-sitosterol	Domínguez *et al.*, 1990
		Kampferitrine	Alam *et al.*, 1982
J. pectoralis		Kaempferol	Olveira *et al.*, 2000
		1,2-benzopirone	de Vries *et al.*, 1988 Olveira *et al.*, 2000
		Umbeliferone	de Vries *et al.*, 1988 Olveira *et al.*, 2000

2.7 Volatile bioactive components of *Justicia secunda* vahl

Justcia secunda is rich in different volatile bioactive components with many industrial and medicinal purposes. Odokwo (2020) carried out a study to investigate volatile bioactive components of *Justicia secunda* . The study identified and characterized twenty-four volatile phyto-constituents present in leaves and stem of *Justicia secunda* . The study showed the presence of four (4) sesquiterpenes, four (1) sesquiterpenoids, two (2) monoterpenoids and seventeen (17) non-terpenoids (Odokwo, 2020). The constituents of *Justicia secunda* Vahl's oil are primarily C-10 monoterpenoids and the C-15 class (sesquiterpenes) and their derivatives (sesquiterpenoids). Terpenes and their derivatives contained the following volatile constituents: Patchoulene, guaia-1(10), longifolene (3aR, 4R, 7R)-1,4,9,9-tetramethyl-3,4,5,6,7,8-hexahydro-2H-3a, γ-elemene, 7 methanoazulen-2-one and 5(1H)-azulenone,11-diene (Odokwo, 2020). This was the first study that ever reported the presence of these bioactive phyto-constituents in the leaves and stem of *Justicia secunda* that is native to Bayelsa State of Nigeria (Odokwo, 2020).

Hamilton-Amachree and Uzoekwe (2017) also reported for the first time the GC-MS analysis of the chemical components present in the essential oil extracted by hydro-distillation from fresh leaves of *Justicia secunda* . Twenty-five compounds were detected from the study. According to the findings, the oil is high in polyunsaturated or polyenoic fatty acid methyl esters. The following compounds were found to be the most abundant: 9, 12, 15-Octadecatrienoic acid methyl ester (36.56%), 9-Octadecenamide (7.12%), E-14-Hexadecenal (6.3%), Trifluoroacetoxyhexadecane (6.00%), 1-Heptadecene (5.68%), Hexadecanoic acid, methyl ester (Methyl Palmitate) (5.06 %), Phytol acetate, methyl ester (4.33 %), 9, 12-octadecadienoic acid (3.40%).

2.8 Prominent medicinal properties of *Justicia secunda* Vahl

2.8.1 Anti-hypertensive and anti-diabetic properties

Pierre *et al*, (2011) evaluated the anti-hypertensive activity of total aqueous extract of *Justicia secunda* . The study revealed that total aqueous extract of *J. secunda* has a dose-dependent reduction of adrenaline (ADR) induced hypertension in rabbits (*Oryctolagus cuniculus*), suggesting the anti-hypertensive potentials of a total aqueous extract of *J. secunda* (Pierre *et al*., 2011). Abo *et al*, (2016) in a different study reported hypotensive and anti-hypertensive effect of total aqueous extract of *J. secunda* in rabbits. Numerous studies have also reported the antidiabetic activities of *J. secunda*. Marles and Farnsworth, (1995) reported antidiabetic properties of *Justicia secunda* and its active constituents. In Trinidad and Tobago, *J. secunda* has been reported to be used in traditional medicine for the treatment of diabetes and its symptoms (Mahabir and Gulliford, 1997; Otaiza *et al*, 2006). Another study that supported the anti-diabetic potentials of *J. secunda* was that by Theiler *et al*, (2016). The study screened for α- glucosidase inhibitors in *J. secunda* using HPTLC bioautography. The study showed the α-glucosidase inhibiting potentials of extracts and fractions of *J. secunda*. Mea et al, (2017) in yet another study, reported the hypoglycaemic and anti-hyperglycaemic effects of *J. secunda* in Wistar rats.

2.8.2 Anti-anaemic, haematinic and anti-sickling properties

Justicia secunda and some other Justicia species have been reported to possess anti-anaemic, haematinic and anti-sickling properties. *Justicia secunda* is commonly seen around compounds and dwellings of Jehovah`s witnesses in Nigeria, Ghana and Congo due to their doctrinal rejection of blood transfusion in anaemic conditions, hence they resort to using decoctions of *J. secunda* as haematinics. Teklehaymanot, (2000) reported the anti-anaemic activity of decoction of whole plant of *J. secunda* as traditionally used in Suriname and Congo.

N`guessan *et al* (2010) in another study reported the anti-depressant and anti-anaemic activity of infusion of *J. secunda* leaves. Yamoah *et al*, (2020) induced anaemia in adult Sprague-Dewley rats using phenylhydrazine (PHZ) and treated the rats with water, ethyl acetate and methanol extracts of *Justicia secunda* . The study revealed a significant increase in the RBCs and Hb concentration in the rats treated with methanol and water extracts of *J. secunda.* The water extract showed the most significant increase ($P<0.001$) in the number of RBCs and Hb concentration compared with the vehicle-treated (normal saline) PHZ induced anaemic rats. The study indicated that water extract of *J. secunda* leaves possess excellent haematinic properties (Yamoah *et al*, 2020). According to Anyasor *et al,* (2020) extracts of *J. secunda* reduce plasma levels of liver function parameters. In the study, they investigated the haematological effects of *J. secunda* leaf extracts on carbon tetrachloride-induced toxicity in rats. Two different extracts: cold extracts of *J. secunda* leaves and hot extracts of *J. secunda* leaves were used. Findings from the study revealed that 100 and 200 mg/kg hot extracts of *J. secunda* leaves significantly reduced levels of plasma lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase and total bilirubin following CCl_4 induced hepatotoxicity when compared to the untreated rats (Anyasor *et al*., 2020). The study further showed that white blood cell, hematocrit, red blood cell, platelet, hemoglobin and procalcitonin levels of the hot-extract of *J. secunda* treated animals were comparable to the control animals. The study therefore suggested that hot extract of *J. secunda* has the potential of normalizing hematological characteristics (Anyasor *et al*., 2020). Mpiana *et al,* (2009) reported the anti-sickling activity of *J. secunda.* Mpiana *et al*, (2010) further reported the anti-sickling activity of three other species of Justicia from Kisangani (D.R Congo). The richness of *Justicia secunda* in different phytochemicals has before now been implicated for its anti-sickling and anti-anaemic properties without the knowledge of the particular phytochemical

involved until in 2010, when Mpiana *et al,* investigated and found anthocyanin to be responsible for its anti-sickling activity. They went further to provide molecular basis for the anti-sickling properties of anthocyanin from *J. secunda.* In the study, Itano, Emmel and osmotic fragility tests were employed to test the effects of anthocyanin extracts from *J. secunda* leaves on haemoglobin S solubility and sickle cell membrane stability. From the study, anthocyanins from *J. secunda* were once more confirmed to have anti-sickling activity. Treated SS red blood cells recovered a normal typical biconcave shape with a radius of 3.3 ± 0.3µm comparable to that of normal erythrocyte (Mpiana *et al*, 2010). These findings suggested the essential role of anthocyanin extract in inhibiting polymerization of haemoglobin S and stabilization of red blood cell membrane (Mpiana *et al*, 2010).

2.8.3 Hepatoprotective activity

Hot extract of *J. secunda* has been reported to possess a better hepatoprotective activity in carbon tetrachloride (CCl_4) induced hepatotoxicity in rats compared to cold extracts of *Justicia secunda* leaves (Anyasor *et al*., 2020). Another study by Aimofumeh *et al*. (2020) investigated the effects of *J. secunda* leaf fraction on acetaminophen-induced oxidative damage in rat liver. The investigation revealed that animals treated with ethyl acetate leaf fraction of *J. secunda* had lower plasma hepatic enzymes, serum C-reactive proteins, and oxidized low-density lipoprotein levels, while hepatic catalase, superoxide dismutase, and reduced glutathione levels were higher than in the untreated control. Four (4) hepatoprotective compounds were also found in the ethyl acetate leaf extract *of J. secunda* using GC-MS.

2.8.4 Antioxidant and anti-inflammatory properties

Many studies have reported the antioxidant and anti-inflammatory properties of *J. secunda*. In 2017, Osioma and Hamilton-Amachree compared the phytochemicals and *in vitro* antioxidant properties of methanol leaf extract of *J. secunda*. The *J. secunda* leaf extraction

was done using 0.1% of hydrogen chloride (HCl) in methanol and 1% of HCl in methanol. Their findings revealed higher quantitative concentrations of phlobatannin, anthocyanins and phenol in 0.1% HCl extract as compared to 1% HCl extract. The antioxidant scavenging efficacy showed that higher percentage inhibition was observed from the 0.1% HCl methanolic extract of *J. secunda* in its ability to scavenge hydrogen peroxide, superoxide and 1, 1-diphenylpicrylhydrazyl anion (Osioma and Hamilton-Amachree, 2017). Onoja *et al*, (2016) had also reported antioxidant and anti-inflammatory activities of methanolic extract of *J. secunda* leaf. The antioxidant activity was evaluated using 2, 2-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) photometric assay. The extract produced a concentration dependent increase in antioxidant activities in both FRAP and DPPH models. The optimum activity of the extract was at 400µg/ml in both FRAP (1.58µm) and DPPH (54.07%) assays (Onoja *et al*., 2016). In 2020, another study by Anyasor *et al* found that hot extract of *Justicia secunda* leaves contained more total flavonoids and phenols than cold extract of *J.secunda* leaves. Hot extracts of *J. secunda* leaf extracts further exhibited significantly greater ferric reducing antioxidant power and thiobarbituric acid and 1,1-diphenyl-2-picrylhydrazyl scavenging activity than cold extract of *J.secunda* leaves (Anyasor *et al*., 2020). Still in 2020, Koffi *et al* carried out a study to investigate the effects of freeze-drying and spray-drying on total phenolics content and antioxidant activity of aqueous extract of *J. secunda* leaves. Their findings revealed higher content of polyphenols and antioxidant capacity in the powdered *J. secunda* extract obtained from freeze-drying than that obtained by spray-drying. The study therefore suggested freeze-drying technology as a better method to preserve polyphenol structure and by extension their antioxidant capacity in aqueous extracts of *J. secunda* leaves (Koffi *et al*, 2020). The anti-inflammatory properties of *J. secunda* were reported by Onoja *et al*, (2016). They investigated the anti-inflammatory

activity of methanolic extract of *J. secunda* leaves using Carrageenan and Formalin-induced paw edema models. According to the study, the extract showed ($P< 0.05$) dose-dependent increase in anti-inflammatory activities. The anti-inflammatory activities of the extract (0.4g/kg) were comparable with pentazocine and aspirin, the reference drugs used in the study (Onoja *et al.*, 2016). Another study by Anyasor *et al*, (2019) evaluated the anti-inflammatory activity of *J. secunda* leaf extracts using *in vitro and in vivo* inflammation models. Findings from the study revealed that methanol extract of *J. secunda* leaves showed sufficient anti-inflammatory actions in the *in vitro and in vivo* models. The study further proposed the anti-inflammatory mechanisms of action of methanol extract of *J. secunda* leaves are through interference with phase 2 inflammatory stressors, stabilization of inflammatory cell membranes and immunomodulatory activity as well as up regulation of cytoprotective genes (Anyasor *et al*., 2019).

2.8.5 Sedative, analgesic and antinociceptive properties

Justicia species possess sedative, analgesic and antinociceptive properties. They generally have CNS effects hence their utilization in management of epilepsy, mental disorders, fever and headaches as a result of their sedative, analgesic and antinociceptive activities (Cano and Volpato, 2004; Gorzalczary *et al*., 2011; Gomez-verjan *et al*., 2012; Khan *et al*., 2017). Onoja *et al*. (2017) reported the antinociceptive activities of methanolic extract of *J. secunda* leaf. In the study, antinociceptive activity was evaluated using acetic acid-induced writhing reflex and tail flick test models. The extract produced a significant ($p<0.05$) dose-dependent increase in antinociceptive activities. They further reported antinociceptive activities of extract of (0.4 g/kg) to be comparable with the reference drugs, pentazocine and aspirin.

2.8.6 Anti-microbial and antibacterial properties

Justicia secunda has been reported to possess antimicrobial and antibacterial activities. Rojas *et al* (2006) in search for alternative antimicrobial agents against nosocomial infections screened ten medicinal plants used in Colombian folkloric medicine. The following microorganisms were used in the study: *Escherichia coli, Bacillus cereus, Streptococcus β hemolytic, Pseudomonas aeruginosa, Staphylococcus aureus* (bacteria) and *Candida albicans* (yeast). The antimicrobial activity of ethanol, hexane and water extracts of *J. secunda* was tested against these microorganisms revealing that ethanol extract of the plants were active against *Staphylococcus aureus* except *J. secunda (*Rojas *et al*., 2006). *J. secunda* presented the lowest minimal inhibitory concentration (MICs) against *Escherichia coli* (0.6µg/ml) compared to gentamycin sulfate (0.9µg/ml). Furthermore, *J. secunda* showed a minimal inhibitory concentration against *Candida albicans* (0.5µg/ml) comparable to nystatin (0.6µg/ml) (Rojas *et al.,* 2006). Among the plants tested, *J. secunda* extract was the most active against *Candida albicans* (Rojas *et al.*, 2006). This suggests that *J. secunda* could be a source of new antimicrobial agents, particularly against *Candida albicans* (Rojas *et al*., 2006). Six years later Carrington *et al.* (2012) in another study investigated antimicrobial activity of methanol and acetone extract of *J. secunda* against *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and clinical strain of *Enterococcus faecalis* which are commonly found in diabetic wounds. The result showed that methanol and acetone extracts of *J. secunda* with an extraction yield of 15.3% and 0.75% respectively yielded no activity within the concentration range against the three bacteria strains tested (Carrington *et al*., 2012). In another recent study, Ayodele *et al* (2020) evaluated the phytochemical composition and *in vitro* antibacterial activity of methanol, aqueous and ethanol leaf extracts of *J. secunda* on these clinical pathogens: *Escherichia coli, Bacillus cereus, Staphylococcus aureus,*

Pseudomonas aeruginosa and Listeria monocytogenes. According to the study, all the extracts of *J. secunda* showed antibacterial activity against all the bacteria tested at 150mg/mL with the exception of aqueous and methanol extracts that showed no activity against *E. coli* (Ayodele *et al*., 2020). The MIC of the aqueous extract was 37.5 mg/mL for *B. cereus* and *L. monocytogenes*, 150 mg/mL for *S. aureus*, and 18.75 mg/mL for *P. aeruginosa*, with no activity against *E. coli*. On all the tested bacteria, the methanol extract exhibited a minimum inhibitory concentration of 18.75mg/mL except *E. coli* which showed no activity. On the other hand, ethanol extract showed minimal inhibition concentration of 75mg/mL on *P. aeruginosa* and on other bacteria at a minimal inhibitory concentration of 18.75mg/mL. This study further shows that *J. secunda* could be promising in the future design of antibacterial drugs (Ayodele *et al*., 2020).

2.9 *Jatropha tanjorensis*

Jatropha tanjorensis (J.L Ellis & Saroja) is a perennial herb belonging to Euphorbiaceae family (Omoregie and Osagie, 2012). The name "Jatropha" is of Greek origin, derived from the words "Jaras and trophe" meaning doctor and food respectively and directly suggesting the therapeutic and nutritional properties of this plant (Kumar and Sharma, 2008; Sabandar *et al*., 2012). Numerous researches have reported fascinating medical and biological constituents and activities of different Jatropha species including *Jatropha tanjorensis* Ellis & Saroja (Sabandar *et al*., 2012). *Jatropha tanjorensis* is native to Central America, though it has now naturalized in various tropical and subtropical countries of the world such as Nigeria, India, North America and Africa (Oyewole and Akangbala, 2011). *J. tanjorensis* is popularly known as "Reverend father`s vegetable" or "Catholic vegetable" (Arun *et al*., 2014). In Nigeria, the leave is commonly known as "Hospital too far" owing to its quick and miraculous hematinic activities in anaemic conditions. *J. tanjorensis* is known by different names among different

ethnic groups and languages of Nigeria. The Igbos of Southern Nigeria call it “Ugu-oyibo (Nwachukwu, 2018) or “Ulo ogwu di anya” which simply and literally translates to “Hospital is too far”. The Yorubas of South Western Nigeria call it “Iyana-Ipaja” or “Lapa-lapa” (Iwalewa *et al*., 2005; Igbinaduwa *et al*., 2011) while the Hausas` call it “Bindazugu” (Oyewole *et al*., 2007; Ebe and Chukwuebuka, 2019). The Tivs of Northern Nigeria call it “Kon-Awambe”.

2.9.1 Scientific classification of *Jatropha tanjorensis* Ellis & Saroja

Scientifically, *J. tanjorensis* is classified as follows

Kingdom : Plantae
Phyllum : Tracheophyta
Class : Magnoliopsida
Order : Malpighiales
Family : Euphorbiaceae
Genus : Jatropha
Species : *Jatropha tanjorensis* (J.L Ellis & Saroja)

Figure 2. 2: *Jatropha tanjorensis* leaves growing in its natural habitat

2.9.2 Botanical description and geographical distribution of *J. tanjorensis*

Jatropha tanjorensis is a sociable and luxuriant shrub. It is a naturally occurring hybrid of *Jatropha carcass* and *Jatropha grossypifolia* (Anwar, 2007). It grows to a height of about 6m,

with spreading branches from stubby twinges and smooth gray bark that oozes whitish latex when incised (Nwachukwu, 2018). It is long-stemmed, thick and dichotomously branched; the branches are hairy when young but hairless when mature (Ellis and Saroja, 1962). The leaves of *J. tanjorensis* are palmate in nature with 3 -5 lobes. *Jatropha tanjorensis* is native to Central America but has spread to many tropical and subtropical nations, including India, Africa, and North America (Prabakaran and Sujatha, 1999). *J. tanjorensis* is a prevalent weed of field crops in Africa, primarily in West African rainforest zones such as Nigeria (Iwalewa and Agana, 2005). It is commonly grown in Southern Nigeria where it serves ornamental and fencing purposes (Oboh and Masodje, 2009) especially around catholic churches and Reverend fathers` lodges earning it the names "Catholic vegetable" and "Reverend father`s vegetable". In India, *J. tanjorensis* is common in the following –Tiruchirappalli, Pudukottai, Thanjavar and Ramanathapuram districts in Tamil Nadu and Pondicherry (Viswanathan *et al*., 2018).

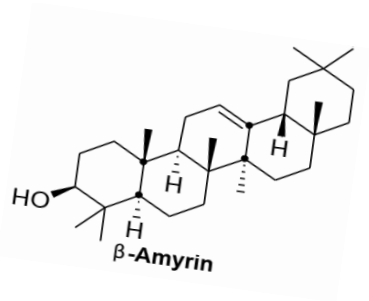
2.9.3 Ethnomedicinal uses of *Jatropha tanjorensis*

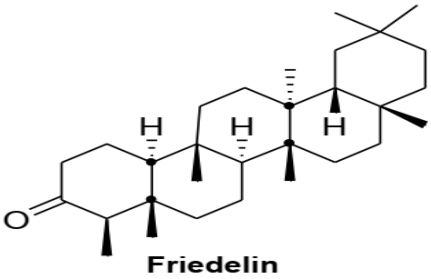
Jatropha species have enormous ethnomedicinal significance. They are used in traditional folklore medicine to cure different ailments in Latin America, Asia and Africa (Heller, 1996) where they have been reported to have minimal side effects (Cowan, 1999). *J. tanjorensis* and many jatropha species are known for their purgative properties which make them essential in the cure of vomiting, retching, dysentery, diarrhea and stomach ache (Sabandar *et al*., 2013). In Nigeria, *J. tanjorensis* is used in the treatment of malaria infection and hypertension (Orhue *et al*., 2008). The leaves are eaten as vegetable as well as applied in diabetes treatment (Olayiwola *et al*., 2004; Mensah *et al*., 2008). *J. tanjorensis* is used in homeopathic medicine for the treatment of leg cramps, diarrhea, colic, cyanosis, collapse cramps and cold sweats (Viswanathan *et al.*, 2018). Its seeds, barks, roots, leaves, fruits, and latex are used to cure a

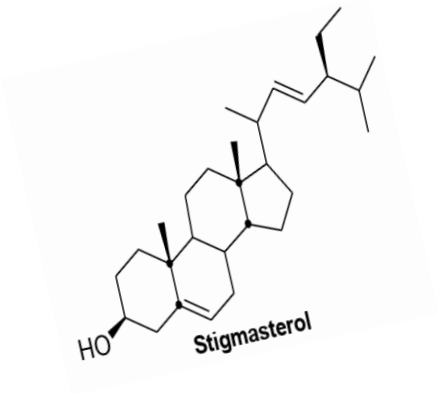
variety of ailments in many parts of the world (Rajore and Batra, 2003). Indians use the juice and crushed leaves of *J. tanjorensis* on wounds and refractory ulcers (Kirtikar and Basu, 2001). In addition, the juice from *J. tanjorensis* has been used successfully to treat ringworm, scabies, and eczema. According to Hartwell (1969), leaves of *J. tanjorensis* are antiparasitic, rubefacient and are applied on hard tumours. The young leaves of *J. tanjorensis* have been reported to possess diuretic, abortifacient, antiseptic, anodyne, depurative cicatrizing, lactogenic,vermifuge, purgative, vulnerary, styptic, emetic and narcotic properties (Viswanathan *et al*., 2018). *J. tanjorensis* latex has been shown to be highly effective against watermelon mosaic virus (Tewar and Shukla, 1982). It is also used topically to treat sores, ulcers, and swollen tongues, as well as stings from bees and wasps (Watt and Brandwijk, 1962; Perry, 1980). Duke and Wain (1981) highlighted its piscicidal, raticidal, and homicidal abilities. Morton (1981) reported that Colombians consume *J. tanjorensis* leaf decoction to treat sexually transmitted infections. Similarly, root decoction of *J. tanjorensis* is used as a mouth wash to treat bleeding gums, toothache and dysentery (Locket *et al*., 2000). One of its main ethnomedicinal uses is its use as a quick haematinic in anemic conditions. It can also be used as an alternative to lettuce in salad preparation as well as in preparation of porridge yam just like fluted pumpkin leaves. According to Albuquerque *et al*., (2006), local people of Perambuco, Alagorinha and Northeastern Brazil drink water diluted with latex of *J. tanjorensis* to treat snake poisoning. *J. tanjorensis* roots are used to ensure a healthy foetus during pregnancy, as well as to treat irregular menstrual cycles and menstrual pain (Van Wyk, 2005). Traditionally, extracts of the leaves have also been reported to treat skin diseases, anemia and cardiovascular diseases (Oduola *et al*., 2005; Iwalewa *et al*., 2005).

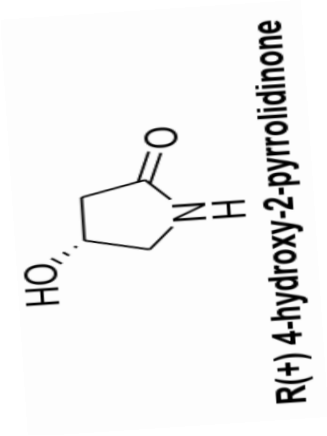
2.9.4 Phytochemistry of *Jatropha tanjorensis*

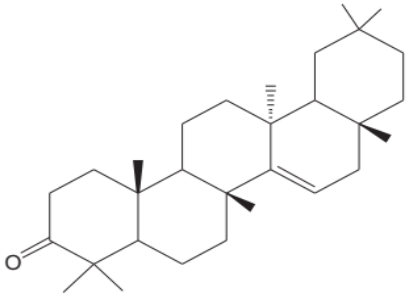
Phytochemical screening of leaves of *J. tanjorensis* revealed the presence of biologically active constituents such as alkaloids, anthraquinones, flavonoids, cardiac glycosides, saponins and tannins (Omoregie and Osagie, 2007; Igbinaduwa *et al*., 2011). These plant based bioactive compounds are known as secondary plant metabolites. They are not essential for plant`s normal growth, reproduction and development. Aside from being employed directly as medications, secondary plant metabolites can also be used as pharmacological probes, templates for synthetic alteration and as drug precursors. A good amount of these plant-based compounds are polyphenols. Polyphenols are a diverse and complex collection of secondary plant metabolites that are vital for plant physiology, with roles in pollination, pigmentation, structure, growth, allelopathy and disease-predator resistance. Polyphenol research has piqued the interest of researchers due to its intriguing antioxidant capacity. It has been reported that they have anti-proliferative, anti-allergic, anti-carcinogenic, antiviral, antioxidant and anti-inflammatory properties. A study by Viswanathan *et al*., (2018) isolated friedelin, β-amyrin, stigmasterol and R-(+) 4- hydroxyl-2-pyrrolidinone with different biological activities. Oladoye *et al.* (2014) later isolated taxaxer-14-en-3-one and taxaxerol.



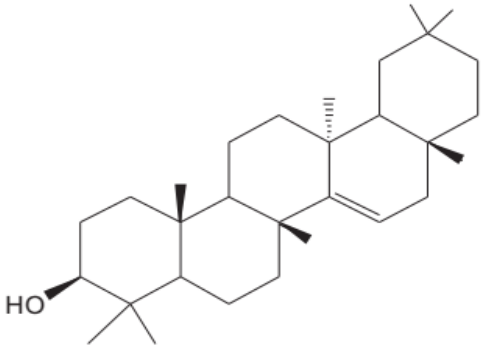








Taraxer-14-en-3-one.



Taraxerol.

Figure 2.3 Chemical structures of bioactive compounds isolated from the leaf of *J. tanjorensis* (Viswanathan *et al*., 2012; Oladoye *et al*., 2014; Viswanathan *et al*., 2018).

2.9.5 Medicinal properties of *Jatropha tanjorensis* (Ellis & Saroja)

2.9.5.1. Antimicrobial and antibacterial activities

Antimicrobial and antibacterial activities of *Jatropha tanjorensis* are among the most studied therapeutic qualities of the plant. Sekran (1998) conducted the first study on the antimicrobial activity of the leaf extract of *Jatropha tanjorensis*. Sekran tested the antimicrobial activities of *J. tanjorensis* ethanol extract on selected gram-positive and gram-negative bacteria. The study showed positive activity against gram-positive *Bacillus cereus, Staphylococcus aureus*, *Bacillus subtilis*, and gram-negative *Mycobacterium phlei* and *Escherichia coli* (Sekran, 1998). Eleven years later, Oboh and Masodje designed a similar study in which they tested the antimicrobial activity of aqueous extract of *J. tanjorensis* leaves against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria and measured their zones of inhibition. Both organisms were sensitive to the extracts, with inhibition zones of 1.6 cm and 1.13 cm for *S. aureus* and *E. coli*, respectively (Oboh and Masodje, 2009). Another remarkable study conducted by Viswanathan *et al* (2012) from India revealed that the antibacterial potency of *Jatropha tanjorensis* was larger than that of *Jatropha curca*. Methanol extract was more effective against *Klebsiella pneumonia, Salmonella paratyphi, Proteus vulgaris, Staphylococcus epidermis*, and *Vibrio alcaligenes* in that investigation (Viswanathan *et al*., 2012). *Aeromonas hydrophila, Proteus vulgaris, Salmonella paratyphi* A, *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* were next, all of which cause skin diseases (Viswanathan *et al*., 2012).

It is worthy of note that, *S. aureus* and *P. aeruginosa* are the most common organisms in leg ulcers and superficial wounds, and they have demonstrated growing resistance to frequently used antibiotics (Valencia *et al*., 2004). Viswanathan *et al* (2012) from the same study further concluded and provided scientific evidence that β-amyrin, friedelin, R (+) 4-hydroxy-2-

pyrrolidinone and stigmasterol present in the methanol extract of *J. tanjorensis* could be responsible for its broad spectrum of antimicrobial activity. The ability of *Jatropha tanjorensis* test extracts to inhibit the causative agents of urinary tract infections such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas mirabilis, Klebsiella pneumoniae* and *Pseudomonas vulgaris* suggested that they could be used in the development of drugs to treat urinary tract infections (Viswanathan *et al*., 2012). Daniyan *et al*. (2018) evaluated the antibacterial activity of *J. tanjorensis* ethanolic and aqueous extracts against *Escherichia coli* and *Staphylococcus aureus* using three different extract concentrations (30, 40, and 50 mg/ml). They discovered more bioactive components in the ethanolic extract of *J. tanjorensis* than in the aqueous extract. The antibacterial activity of the extracts was shown to be dose and bacterial species dependent. *J. tanjorensis* aqueous extract had increased activities on the studied organisms at all concentrations, but *J. tanjorensis* ethanolic extract had no activities on *E. coli* at lower concentrations (Daniyan *et al*., 2018). All concentrations of *Jatropha tanjorensis* ethanolic leaf extract (range = 8.67 ± 3.6 to 15.03 ± 0.95 mm) and *Jatropha tanjorensis* aqueous leaf extract (12.80 ± 0.98 to 20.33 ± 1.53 mm) were susceptible to *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of *Jatropha tanjorensis* aqueous leaf extract was 7.5 mg/ml for both species of bacteria, while the MIC of *Jatropha tanjorensis* ethanolic leaf extract was 15 and 25 mg/L for *S. aureus* and *E. coli*, respectively, while MBC analyses revealed that the leaf extracts were bacteriostatic in activity (Daniyan *et al*., 2018). Anhwange *et al*. examined the antibacterial properties of methanolic and n-hexane extracts of *Jatropha tanjorensis* leaf against *Klebsiella pneumoniae*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* in 2019. They detected cardiac glycosides, reducing sugars, flavonoids, alkaloids, steroids, and saponins in both extracts, with the exception of n-hexane extract, which lacked reducing sugars but contained phlobatannins.

According to the antimicrobial investigation, both extracts had variable degrees of antimicrobial activity (Anhwange *et al.*, 2019). This further lends validity to the previously reported antimicrobial and antibacterial properties of *Jatropha tanjorensis* extracts.

The most recent study on the antibacterial potentials of *Jatropha tanjorensis* was conducted by Babayemi *et al* (2021). They tested the antibacterial activity of *Jatropha tanjorensis* leaf extracts against bacteria associated with clinical wound infections (Babayemi *et al*., 2021). *J. tanjorensis* crude leaf extracts and commercial antibiotics were tested for antibacterial activity against bacterial isolates linked with wound samples using agar well diffusion and disc diffusion procedures, respectively (Babayemi *et al*., 2021). The methanol extract showed the highest zone of inhibition of 33mm against *Staphylococcus aureus*, whereas the cold-water extract had the lowest zone of inhibition of 5mm against *Staphylococcus aureus.* Methanol extract had the maximum zone of inhibition against *Escherichia coli*, ranging from 16mm to 17mm, while cold water extract had the lowest zone of inhibition (3-4mm). Furthermore, *Pseudomonas aeruginosa* had the highest minimum inhibitory concentration (MIC) value of all extracts (100mg/mL), while coagulase–positive *Staphylococcus aureus* had the lowest MIC value (12.5mg/mL). In comparison to other crude extracts and ciprofloxacin, methanol extract exhibited the strongest inhibitory effect against bacteria isolated from wound samples, according to the investigation (Babayemi *et al*., 2021).

2.9.5.2 Anti-anaemic and hematological potentials of *Jatropha tanjorensis*

The anti-anaemic potentials of *Jatropha tanjorensis* appear to be the most popular and well-studied of all the potentials of *Jatropha tanjorensis*. Omoregie and Osagie studied the phytochemical and anti-anaemic effects of *Jatropha tanjorensis* leaf in protein-malnourished rats in 2007. According to the study, the rats were divided into six groups each with eight rats treated as follows: Normal diet (group 1), protein-deficient diet (PDD; group 2), PDD with

10% *J. tanjorensis* leaf supplement (group 3), PDD with iron (fersolate) tablet supplement (group 4), normal diet with 10% *J. tanjorensis* leaf supplement (group 5) and normal diet with iron tablet supplement (group 6) were the six groups (Omoregie and Osagie, 2007). When compared to the control group, the rats fed the leaf-supplemented food had considerably higher levels of haemoglobin, platelets, packed cell volume, total protein, and albumin. When compared to the control rats, the rats fed the PDD exhibited significantly lower levels of these haematological indicators (Omoregie and Osagie, 2007). The liver and kidneys of the rats given the PDD had severe fatty alterations, according to histopathological testing. The liver and kidney histology of the rats fed the leaf-supplemented diet were normal. The leaf's anti-anemic action could be due to an anti-oxidant mechanism. The leaf of *J. tanjorensis* possesses both protective and preventative properties against anemia (Omoregie and Osagie, 2007). Another anti-anaemic study of *Jatropha tanjorensis* leaf was conducted by Idu *et al* (2014) but with rabbits. The animals were divided into five groups (groups A, B, C, D, and E) of three rabbits each for the investigation. The animals in Group A were used as controls and so were not induced with anemia nor administered with *J.tanjorensis* leaf extracts. The animals in groups B, C, and D were induced with anemia and then given varying doses of *J. tanjorensis* leaf extracts. Group E rabbits were induced with anaemia but were not treated with *J. tanjorensis* extract and so served as test control. After treatment with an aqueous extract of *J. tanjorensis* leaves, the researchers discovered a substantial decrease in packed cell volume in groups B, C, and D by day 3 of the study, followed by an increase by day 14 of the investigation. Findings from the study indicated that crude extracts of *J. tanjorensis* reduced the anaemic condition of the treated animals (Groups B, C, and D) as compared to the phenyl hydrazine-induced but untreated Group E animals (Idu *et al*., 2014).

Two significant studies published in 2019 by Nigerian experts demonstrated the effect of *Jatropha tanjorensis* extract on haematological markers in albino Wistar rats. Ebe and Chukwuebuka (2019) of the University of Uyo did the initial study. They investigated the effects of *Jatropha tanjorensis* ethanol root extract on haematological parameters in male albino Wistar rats. The study found that groups administered the extract had a substantial ($P<0.05$) increase in white blood cell (WBC) count, red blood cell (RBC) count, and haemoglobin (HGB) count when compared to the control group. Similarly, there was a substantial ($P <0.05$) rise in haematocrit, plate count, and lymphocyte levels in the treated groups compared to the control (Ebe and Chukwuebuka, 2019). The mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) values in groups II and III were not substantially ($P <0.05$) different from the control group, however group IV was significantly ($P< 0.05$) higher. There was no significant ($P <0.05$) difference between the treated groups and the control group for neutrophil count values. As a result of the findings, the extract may be effective in the treatment and management of anemia, as well as in boosting the immune system in people (Ebe and Chukwuebuka, 2019). The other similar research which reported the effects of *J. tanjorensis* extract on haematological parameters in albino Wistar rats were conducted by Danborno *et al* (2019). They evaluated the effects of aqueous leaf extracts of *Jatropha tanjorensis* on haematological indices in male Wistar rats. A total of 25 male Wistar rats weighing 150-180kg were used for the study. The rats were divided into five groups of five rats each. Group I served as the control, while groups II, III, IV, and V were given 125mg, 250mg, 500mg, and 750mg per kilogram body weight of *Jatropha tanjorensis* leaf extract daily for 14 days, respectively. When compared to the control, the groups treated with 125mg/kg, 250mg/kg, and 500mg/kg aqueous leaf extract of *Jatropha tanjorensis* showed a significant increase in RBC, PCV, Hb, WBC, and PLT

(P<0.05). However, at a greater dose of 750mg/kg of *Jatropha tanjorensis* aqueous leaf extract, there was a decrease in RBC, WBC, PCV, Hb and PLT (P<0.05) as compared to the control (Danborno *et al*., 2019). In all treated groups compared to the control, there were no statistically significant changes in Monocytes, Basophils, Eosinophils, MCHC, MCV, or MCH. Neutrophils were considerably lower in the 750mg/kg group compared to the control group (P> 0.05). According to the findings of this study, the aqueous leaf extract of *Jatropha tanjorensis* leaves may increase haemopoiesis at lower dosages, but higher levels may cause haemolysis (Danborno *et al*., 2019). Ebenyi *et al* (2021) undertook another significant study that investigated the effect of aqueous leaf extracts of *Jatropha tanjorensis* leaf extract on parasitemia and haematological indices in *Plasmodium berghei*-infected mice. The study used a total of forty (40) mice that were infected with *Plasmodium berghei*. For four days, infected mice were divided into five groups of six mice and given varying doses of conventional medicine (artemether/lumefantrine 25mg/kg) and extract (200, 400, and 800mg/kg body weight). Blood was obtained after treatment and used to determine percentage parasitemia, haemoglobin concentration and packed cell volume. Within the first 24 hours and throughout the 14-day study period, no symptoms of neurological, behavioral, or mortality were observed at concentrations of 2,000 and 5,000 mg/kg oral dosages (Ebenyi *et al*., 2021). When compared to the negative control, there was a dose-dependent increase in total haemoglobin (Hb) and packed cell volume (PCV), as well as an increase in weight. When compared to the negative control, the leaf extract of *J. tanjorensis* showed substantial (p<0.05) suppression activity and mean survival time at dosages of 400 mg/kg, 200 mg/kg, and 100 mg/kg. With no symptoms of acute toxicity, the extract of *Jatropha tanjorensis* demonstrated reasonable levels of anti-anemia and antimalarial activity. The result further supported anti-anaemic and blood boosting use of *J. tanjorensis* leaf (Ebenyi *et al*., 2021). Iron (Fe) has been shown to be

abundant in *Jatropha tanjorensis* (Wardaw and Kessel, 2002). The amount of iron available for erythropoiesis is increased due to the high amount or concentration of iron in the extract. The formation of red blood cells and hemoglobin increase as the amount of iron in the body increases.

It is also likely that some of the extract's chemical elements have an erythropoietin-like action on the bone marrow, causing an increase in erythropoiesis rate and, as a result, an increase in packed cell volume and hemoglobin concentration (Falodun *et al*., 2013). This could explain why most studies that looked at the effect of the leaf on haematological indicators found increased packed cell volume and hemoglobin concentration. Extracts of *Jatropha tanjorensis* can thus be used to boost blood levels under physiological situations such as pregnancy and menstruation, when hemoglobin concentration and packed cell volume diminish (Falodun *et al*., 2013). Mabubuike *et al* (2015) also found that methanol extract of *Jatropha tanjorensis* in cyclophosphamide-induced bone marrow suppression in albino Wistar rats exhibited dose-dependent myeloprotective action. This further lends credence to its ethnomedicinal use in the treatment and prevention of anaemia (Madubuike *et al*., 2015).

2.9.5.3 Antidiabetic properties of *Jatropha tanjorensis*

Jatropha tanjorensis has been utilized in ethnomedicine as a diabetes treatment, but there has been no experimental or pharmacological confirmation of this claim until 2004 when Olayiwola *et al* undertook a study to evaluate the antidiabetic potentials of *Jatropha tanjorensis* leaves. The ethanolic extract (JTE) of *Jatropha tanjorensis* was partitioned into chloroform ethylacetate and water concentrated to give the following fractions: *Jatropha tanjorensis aqueous* (JTEA), *Jatropha tanjorensis* ethylacetate (JTEE), and *Jatropha tanjorensis* chloroform (JTEC). A total of 24 Swiss albino Wistar rats were used in the

investigation, which was divided into six experimental groups of four each. Animals in groups 2, 3, 4, 5, and 6 were fasted for 24 hours. JTE was not administered to rats in groups 1, 2, or 5, however it was administered intraperitoneally to rats in groups 3, 4, and 6. Blood samples were taken from the rats' tail veins 30 minutes before extract administration and 30 minutes, 60 minutes, 120 minutes, and 180 minutes following extract administration (Olayiwola *et al*., 2004). Using a phenol in sulphuric acid reaction, blood sugar levels were measured. The study's findings revealed that the extract had no effect on the rats. JTE (2g/Kg, i.p.) significantly lowered blood glucose levels in glucose-laden, fasting rats (62.8 ± 2.8 mg/dL) compared to fasted control (79.55 ± 10.4 mg/dL) and fasted +glucose loaded control group (158.22 ± 23.4 mg/dL). Only the ethylacetate (JTEE) fraction revealed obvious and considerable insulin-release from the INS-1 cells in the in-vitro researches (Olayiwola *et al*., 2004). Insulin secretion was reduced by the JTEC and JTEA fractions. This study confirmed the presence of hypoglycaemic and anti-hyperglyacemic compounds in *J. tanjorensis* leaf. It also revealed pharmacological evidence for the folkloric usage of *J. tanjorensis* leaves in diabetic control (Olayiwola *et al*., 2004). In yet another fascinating work, Ijioma *et al* (2014) investigated the hypoglycemic, hematologic, and hypolipidemic activities of *Jatropha tanjorensis* ethanolic leaf extract (JELE) in alloxane induced diabetic rats. According to the study, the normal control group consisted of 7 normal rats who were given 0.2ml of normal saline. Diabetic rats were placed into four groups of seven individuals each (groups 2-5). The diabetic control group, Group 2, received no treatment. Glibenclamide (5mg/kg) was administered to Group 3, whereas JELE (150 and 300mg/kg) was administered to Groups 4 and 5 (Ijioma *et al*., 2014). All treatments were administered orally and lasted 21 days. All doses of JELE significantly ($P<0.05$) reduced glucose levels in diabetic rats, with 300mg/kg reducing blood glucose from 311.80± 37.10 in diabetic rats to 95.95± 2.90 at the end of 21

days. JELE's hypoglycemic impact was comparable to that of Glibenclamide. Red blood cell (RBC) counts, packed cell volume (PCV), and hemoglobin levels were all considerably higher (P<.05) in treated rats, whereas the elevated WBC level in diabetic rats was reduced (Ijioma *et al*., 2014). Total triglycerides, cholesterol, very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were considerably (P<0.05) lower in diabetic rats, but high-density lipoprotein cholesterol levels increased (HDL-C). These findings reveal that *Jatropha tanjorensis* ethanolic leaf extract (JELE) contains bioactive components with hypoglycemic, hematologic, and hypolipidemic capabilities, suggesting that JELE could be useful in the treatment of diabetes mellitus and its associated anemia and lipid abnormalities (Ijioma *et al*., 2014). Ukubuiwe *et al* (2019) investigated the hypoglycaemic efficacies of leaf and stem extracts of *Jatropha tanjorensis* in alloxane-induced diabetic mice in a different study. Similarly, both aqueous and ethanolic leaf and stem extracts of *Jatropha tanjorensis* demonstrated considerable hypoglycaemic activity, according to the research. At day 10, aqueous stem extract extracts caused the greatest drop in glucose levels. Initial weight loss on day 5 was accompanied by an increase on day 10 post-treatment in all treatments. The study indicated that *J. tanjorensis* leaf and stem extracts have anti-diabetic properties, indicating that they could be used to treat diabetic diseases (Ukubuiwe *et al*., 2019).

2.9.5.4 Pro-antioxidant and antioxidant activities of *Jatropha tanjorensis*

Jatropha tanjorensis has been demonstrated to exhibit pro-antioxidant and antioxidant effects in studies. The pro-antioxidant properties of cold, boiled, and methanolic extracts of nine edible vegetables utilized in South-Western Nigeria, including *Jatropha tanjorensis*, were discovered by Iwalewa *et al* (2005). *J. tanjorensis* demonstrated mild antioxidant activity (10.7-12.1%). However, at higher concentrations of 1 or 5 mg/mL or both, all extracts from the nine edible plants examined, including *J. tanjorensis*, were found to be pro-antioxidant

(Iwalewa *et al*., 2005). Omoregie *et al*. (2011) conducted another study on the antioxidant properties of *Jatropha tanjorensis*. In that study, Omoregie and her colleagues looked at the antioxidant activity of methanol extracts on various indigenous plants including *J. tanjorensis in vitro*, as well as the effect of methanol extracts on nutritionally stressed rats. The extract of *Jatropha tanjorensis* among other plant extracts studied had the highest phenolic concentration, according to the study. The flavonoid content of *Jatropha tanjorensis* leaf extracts were significantly increased ($P<0.05$) (Omoregie *et al*., 2011). The extracts were also tested in vivo in male albino rats that had been subjected to nutritional oxidative stress for 6 weeks on a protein deficient diet (PDD), while the other rats were on a regular diet. When compared to the control, rats on a protein-deficient diet exhibited significantly lower levels of Catalase (CAT) , superoxide dismutase (SOD), Vitamin C, Vitamin E and enhanced lipid peroxidation in their liver and kidney tissues ($P<0.05$). In comparison to the PDD group, supplementing the PDD diet with several extracts, including that of *Jatropha tanjorensis* resulted in significantly higher ($P<0.05$) levels of CAT, SOD, vitamin E, and vitamin C, as well as reduced lipid peroxidation. Similarly, giving the extracts to normal rats resulted in normal levels of these parameters ($p>0.05$) when compared to the control (Omoregie *et al*., 2011). The findings therefore revealed that different plant leaves and *Jatropha tanjorensis* have different levels of strong antioxidant activity in vitro and in vivo (Omoregie *et al*., 2011). Another study by Madubuike *et al*., 2015 further supported the antioxidant potentials of *Jatropha tanjorensis*. The researchers used an in vitro 2, 2 diphenyl picrylhydrazine (DPPH) spectrophotometric assay and an in vivo assessment of serum malondialdehyde, superoxide dismutase, and catalase activity to assess the antioxidant capacity of a methanolic extract of *Jatropha tanjorensis*. In the DPPH spectrophotometric test, the extract (50–400 µg/ml concentration) demonstrated a concentration-dependent, significant ($P<0.05$) antioxidant

effect. When compared to the control group, 400 mg/kg of the extract resulted in a substantial ($P< 0.05$) increase in serum superoxide dismutase and catalase activity, as well as a significant drop in malondialdehyde levels (Madubuike *et al*., 2015). These findings further supported that *J. tanjorensis* possess antioxidant properties. In a more recent work, Ajah *et al* (2021) discovered that *Jatropha tanjorensis* methanol leaf extract contains bioactive constituents exhibiting free radical scavenging action. The total phenolic content of methanol extract of *Jatropha tanjorensis* leaf assessed by Folin-Ciocalteau reagent in units of gallic acid equivalent was 11.35 ± 0.82 mgGAE/g, according to the findings. The flavonoid content of the plant sample was 15.91 ± 1.60 mgQCE/g when calculated as Quercetin equivalent. The total phenolic content of the plant extract was related to its antioxidative action. The lowering potency of the radical scavenging activity increased in a dose-dependent manner. At 200µg/ml, the minimum NO inhibitory activity was 8.88 ± 0.63, while at 800µg/ml, the maximum activity was 32.70 ± 2.71. At 200µg/ml, the minimum percentage H_2O_2 radical inhibitory activity was 8.30±0.88, while at 800µg/ml, the highest activity was 22.80 ± 2.28. The scavenging effect of the lipid peroxide radical was likewise increased in a concentration-dependent manner. The findings suggested that *Jatropha tanjorensis* leaf has antioxidant capabilities and could be used as a free radical inhibitor (Ajah *et al*., 2021)

2.9.5.5 Anti-malarial and anti-plasmodial activity of *Jatropha tanjorensis*

In their study titled, *in vitro* anti-plasmodial activity and cytotoxicity of leaf extracts from *Jatropha tanjorensis*, Omoregie and Sisodia (2011) found that *Jatropha tanjorensis* has intriguing anti-plasmodial activity. *Jatropha tanjorensis* leaves were extracted in three different extract forms: ethanolic, aqueous, and hydro-ethanolic (50:50 v/v) extracts, according to the study. On extracts, high performance thin layer chromatography (HPTLC) was used for quality control. A 3D7 chloroquine sensitive clone of *Plasmodium falciparum*

NF-54 isolate was used to test antiplasmodial activity in vitro (Omoregie and Sisodia, 2011). The 48-hour microassay approach was used to determine parasite growth inhibition, with the extracts dissolved in either DMSO or water. The positive control was chloroquine, whereas the negative control was medium containing solely parasitized red blood cells. The cytotoxicity of extracts against non-cancerous Vero cell lines (C-1008 African green monkey kidney fibroblasts) was assessed using doxorubicin as the standard cytotoxic agent (Omoregie and Sisodia, 2011). The chemical fingerprint spectra of the extracts revealed many peaks that were more prominent in the ethanolic extract than in the other extracts. When compared to the other extracts, the ethanolic extract showed the strongest antiplasmodial activity (IC_{50}; 10.86 ± 1.52 µg/ml), cytotoxicity (IC_{50}; 86.8 ± 4.8 µg/ml), and the lowest selectivity index (8.0). The findings supported local reports that *Jatropha tanjorensis* leaves has antiplasmodial properties and can be used to cure malaria infection (Omoregie and Sisodia, 2011).

2.9.5.6 Cytotoxicity effects of *Jatropha tanjorensis*

The same study on *Jatropha tanjorensis* anti-plasmodial activity also found that hydro-ethanolic, ethanolic, and aqueous extracts of *Jatropha tanjorensis* had little or no cytotoxic effects on non-cancerous Vero cell lines, with the exception of the moderate cytotoxic activity shown by just the ethanolic extract (IC50 Vero = 86.8 ± 4.8 µg/ml) which was still less toxic than the standard cytotoxic drug doxorubicin (IC50 Vero = 1.8 ± 0.42 µg/ml) (Omoregie and Sisodia, 2011). The cytotoxic potentials of pyrrolidinone from the leaves of *Jatropha tanjorensis* were reported by Viswanathan *et al*., (2018) in a different investigation. The study used standard procedure to investigate the cytotoxic activity of methanol and R (+) 4-hydroxy-2- pyrrolidinone of *J. tanjorensis* in vivo and in vitro utilizing four cancer cell lines: HEP-2, B16F10, A549, and NRK 49F. The in vivo cytotoxic effect of the methanol fraction of *J. tanjorensis* was investigated in mice using B16F10 melanoma cells. Simultaneous injection

of methanol fractions at doses of 100 and 200 mg/kg, as well as R (+) 4- hydroxy-2-pyrrolidinone per oral, was used (Viswanathan *et al*., 2018). The results of the in vitro testing demonstrated that the methanol extract and R (+) 4-hydroxy-2-pyrrolidinone had stronger cytotoxic effect against all four cell types. With an IC50 value of 43.24g/ml, the Caucasian male larynx epithelium cornicing Hep-2 was shown to be more sensitive. The activity of the other cell line was lower, as seen by the relatively high IC50 value. The results of the in vivo study demonstrated that R (+) 4-hydroxy-2- pyrrolidinone increased life span significantly more than the methanol fraction (Viswanathan *et al*., 2018).

2.9.5.7 Antiviral activity of *Jatropha tanjorensis*

Various extracts of *Jatropha tanjorensis* have been reported to possess varying degrees of antiviral activity. This fascinating finding was described in a study by Esimone *et al* (2008). A recombinant lentiviral (HIV-1) vector expressing a luciferase reporter gene was used for the rapid screening of potential anti-HIV medicinal plants in the study titled, single cycle vector based antiviral screening assays for high throughput evaluation of potential anti-HIV medicine plants; a pilot study on some Nigerian herbs (Esimone *et al*., 2008). The vector could only replicate once (improving safety), and the tropism was boosted by pseudo typing with a Vesicular Stomatitis Virus glycoprotein (VSV-G) envelope (Esimone *et al*., 2008). The water (JTW), dichloromethane (JTD) and n-hexane (JTH) extracts of *Jatropha tanjorensis*, ethylacetate (NLE), the water (NLW), dichloromethane (NLD), butanol (NLB) and n-hexane (NLH) extracts of *Nymphaea lotus*, as well as extracts of *Morinda lucida* (ML), *Garcinia kola* (GK), and *Chenopodium ambrosioides* (CH). A typical anti-HIV medicine, nevirapine, was used as a control. While GK, ML, and CH had no antiviral activity, the n-hexane and dichloromethane extracts of *J. tanjorensis* (JTD, JTH, NLD, and NLH) had varying degrees of antiviral activity, with JTD showing the greatest activity. The antiviral activity of JTW,

NLW, NLB, and NLE was very weak. The *J. tanjorensis* dichloromethane (JTD) extract was fractionated according to bioassays, yielding four strong anti-HIV fractions: JTD10, JTD11, JTD12, and JTD13. The suggested single-cycle vector-based antiviral screening assay for medicinal plants is extremely fast, safe, inexpensive, and repeatable (Esimone *et al*., 2008).

2.9.5.8 Spermatogenic, testicular toxicity and increased serum testosterone effect of *Jatropha tanjorensis*

According to Osuchukwu *et al* (2016), *Jatropha tanjorensis* exhibits spermatogenic effect. The impact of an aqueous extract of *J. tanjorensis* on the testes of adult Wistar rats was studied. The study used a total of twenty-four (24) male Wistar rats. The rats were divided into three experimental groups, each with eight rats. The control group was Group I, while the treatment groups were Groups II and III. The rats in group I were given free access to food and water. Group II rats were fed the same usual diet as group I rats, but they were additionally given the extract in two different dosages. For 14 days, group IIA received 500mg of the extract per kilogram of body weight (mg/kg b.wt), while group IIB received 1000mg/kg b.wt. The spermatozoa in the seminiferous tubules of those in group IIA treated with 500mg of the extract showed a little rise, whilst those in group IIB treated with 1000mg for 2 weeks exhibited a greater increase in sperm cells (Osuchukwu *et al*., 2016). The testes of those in group IIIA treated with 500mg for 3 weeks had a greater increase in sperm cells and the lumen of the seminiferous tubules was almost completely enclosed by the tail of the spermatozoa, whereas those in group IIIB treated with 1000mg for 3 weeks had a major increase in sperm cells and the lumen of the seminiferous tubules were almost totally enclosed by the tail of the spermatozoa. The study therefore concluded that when taken for a short length of time, *Jatropha tanjorensis* may have the capacity to enhance spermatogenesis, in a dose-dependent manner (Osuchukwu *et al*., 2016). Iroanya *et al* (2018) found in another investigation that

ethanolic leaf extract of *Jatropha tanjorensis* has the capacity to impact sperm shape at high doses. The study found indications of acute sperm deformation at a high dose of 800mg/kg. However, such severe sperm abnormalities were not found at lower doses, implying that the ethanolic leaf extract of *J.tanjorensis* produced sperm deformity only at high concentrations (Iroanya *et al*., 2018). Their findings corroborated those of Osuchukwu *et al* (2016). The effect of *J. tanjorensis* on male reproductive parameters was taken a notch further by Bassey *et al* (2019) by studying the effect of ethanolic leaf extract of *Jatropha tanjorensis* on testicular microstructure and hormonal profile of male albino Wistar rats following the administration of highly active antiretroviral therapy (HAART) (Zidovudine, Lamivudine and Nevirapine [ZLN]) (Bassey *et al*., 2019). According to the study, the testicular histo-architecture of the test groups exhibited moderate aberrations in the seminiferous tubules, with significantly ($P<0.05$) higher testosterone compared to the control. Finally, the concurrent administration of ZLN and JT has the potential to be testiculotoxic (Bassey *et al*., 2019).

2.9.5.9 Hypolipidaemic effect of *Jatropha tanjorensis*

Methanolic extracts of *Jatropha tanjorensis* have been reported to possess hypolipidemic effects on albino Wistar rats as reported by Oyewole and Akingbala (2011). For the study, 24 albino rats weighing between 130 and 150g were separated into four groups. Group 1 was the control group, whereas Groups 2, 3, and 4 received different doses of methanolic leaf extract of *Jatropha tanjorensis* daily for 14 days (Oyewole and Akingbala, 2011). Serum lipid profile measurements in rats given the extract revealed a substantial ($P<0.05$) drop in the mean values of total cholesterol, total lipids, and LDL cholesterol, whereas HDL cholesterol and triglycerides levels were not significantly different from the control. The study concluded that *Jatropha tanjorensis* leaf extract contains bioactive components capable of decreasing blood

cholesterol levels and may be effective for the treatment of cardiovascular diseases induced by hyperlipidemia (Oyewole and Akingbala, 2011).

2.9.5.10 Hepatoprotective and cardioprotective effect of *Jatropha tanjorensis*

Oxidants and free radicals represent a significant threat to the liver, the body's primary detoxifying mechanism. Many researches have been undertaken to investigate the hepatoprotective potentials of *J. tanjorensis* extracts in order to counteract the damage caused by the release and accumulation of free radicals. One of such researches was conducted by Madubuike *et al* (2015). He and his colleagues evaluated the hepatoprotective activity of methanolic extract of *Jatropha tanjorensis* in carbon tetrachloride-induced hepatotoxicity. According to the study, liver toxicity was induced in albino rats using carbon tetrachloride and the methanolic extracts of *Jatropha tanjorensis* was orally administered to the rats at doses of 200mg/kg and 400mg/kg for 14 days while using the standard hepatoprotective drug silymarin as a reference drug (Madubuike *et al.*, 2015). Both doses of *J. tanjorensis* significantly ($P< 0.05$) reduced serum levels of Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), and Aspartate aminotransferase (AST). When compared to the negative control rats, the higher dose (400 mg/kg) of the extract considerably ($P< 0.05$) reduced total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) and significantly ($P< 0.05$) increased high density lipoprotein cholesterol (HDL-C). When compared to the negative control, the lower dose (200 mg/kg) significantly ($p< 0.05$) reduced the levels of TG and LDL-C but had no effect on the levels of TC, HDL-C, and VLDL-C in the treated groups. The plant's activities were dosage – dependent and equivalent to silymarin's. Iroanya *et al* (2018) observed that ethanolic leaf extract of *Jatropha tanjorensis* displayed hepatoprotective effect against acetaminophen-induced liver injury in Swiss albino rats in a separate investigation. In yet another similar study Ebe *et al* (2019) observed a marked

reduction in three serum liver enzyme markers (AST, ALT and ALP) in albino rats treated with ethanolic extract of *J. tanjorensis*, this further buttress previous researches that leaf extract of *Jatropha tanjorensis* has the potentials of protecting the liver against oxidative damage. *J. tanjorensis* is not only hepatoprotective but cardioprotective as well. This was reported by Uchenna *et al* (2021). Uchenna and his colleagues evaluated the cardioprotective impact of methanol extract of *Jatropha tanjorensis* leaves on certain cardiac functional biomarkers, endogenous antioxidant activity, and heart pathology in isoprenaline-induced myocardial infarction in albino rats. The study found that a 400mg/kg dose of methanol extract of *Jatropha tanjorensis* significantly ($p<0.05$) reduced CK-MB, Troponin I, LDH, hsCRP, and MDA compared to the negative control (Uchenna *et al*., 2021).There was also a substantial ($p<0.05$) rise in catalase, SOD, GPx, and GSH levels in the treated groups, with the exception of the group given 600mg/kg of the extract, which had a significant reduction in catalase levels when compared to the normal and other groups. They concluded that the extract at 400mg/kg dose was more effective in preventing cardiomyocyte damage, despite moderate changes in heart histoarchitecture (Uchenna *et al*., 2021). In addition to having hepatoprotective activity, extracts of *J. tanjorensis* have been reported to possess wound healing activity thus their application on wounds and refractory ulcers (Viswanathan *et al*., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and instruments

The following equipment were used in this study: UV-Visible Spectrophotometer (Genesys, 10UV,/ 10-S, USA), Atomic Absorption Spectrophotometer (Agilent Technologies, FS240 AA, USA) , Gas Chromatography (Buck, Model 910, and Model 530, USA), GC Systems (GC-7890A/MS-5975C, Agilent Technologies C.A ,USA), Gas chromatography column- HP 88 capillary column (100m x 0.25µm film thickness) Rotary Evaporator (Heidolph Rotavapor, Germany), Flame ionization detector, Centrifuge, Cyclomixer, Homogenizer, and Refrigerator. Other laboratory apparatus used are volumetric flask, Water bath, separatory funnel, conical flask, Micro pipette, pasture pipette, test tubes, filter paper (Whatman No.1), measuring cylinder and electric blender (ES-BL-090/350W/220-240V/50Hz /China).

3.1.2 Chemicals and reagents

Chemicals and reagents used were of analytical grade and were purchased from BDH and Sigma-Aldrich, USA.

3.1.3 Collection of plant materials

Fresh leaf samples of *J. secunda* and *J. tanjorensis* were collected from a garden at Ichi-Ukwu autonomous community in Isiala Ngwa South, Abia State, Nigeria. The leaves were identified and botanically authenticated by Mr. Iwueze Francis Onyemauche, a taxonomist with the department of Forestry and Wildlife, Federal University of Technology, Owerri. Samples of *J. secunda* and *J. tanjorensis* with voucher numbers FUTO/FWT/ERB/2021/63 and

FUTO/FWT/ERB/2021/62 respectively were deposited in the herbarium of department of Forestry and Wildlife Federal University of Technology, Owerri.

3.1.4 Plant preparation and extraction

The fresh leaves of *Justicia secunda* and *Jatropha tanjorensis* were washed with distilled water and air-dried for 3 weeks. The dried leaves were double blended into fine powder using electric blender (ES-BL-090/350W/220-240V/50Hz /China) and stored in a sterile polythene bag until further use.

3.2 Methods

3.2.1 Extraction method

Soxhlet extraction was used and the extraction solvent was ethanol-water (Et-OH) (80:20v/v). About 100g of each powdered leaf sample was placed in the Soxhlet apparatus. The extraction was carried out using 1000mL of 80% V/V of Et-OH for 4 hours. After the extraction process, the suspensions were filtered and concentrated using a rotary evaporator (Heidolph Rotavapor, Germany).

3.2.2 Determination of proximate composition

3.2.2.1 Ash content

The ash content was determined using the procedure given by AOAC (Williams, 1984).

Procedure

The weight of an empty platinum crucible was measured after it had been cleansed and dried. One gram of the sample was weighed into the platinum crucible and placed in a muffle furnace at 550°C for three hours. After burning, the sample was cooled in a desiccator and weighed. Ash content (%) was calculated using the formula below:

$$\frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where;

W1 = weight of empty platinum crucible

W2 = weight of platinum crucible and sample before burning

W3 = weight of platinum and ash.

3.2.2.2 Moisture content

The moisture content was determined using AOAC method as described by (Williams, 1984).

Procedure

A petri-dish was washed and dried in the oven. Approximately 1g of the sample was weighed into the petri dish. Before drying, the weight of the petri dish and sample was recorded. The petri dish and sample were put in the oven and heated at 105^0C for 2 hours and the result noted. And heated for another 1 hour until a fixed result is obtained and the weight was noted. The drying process was repeated until a constant weight was achieved.

Moisture content (%) was determined thus:

$$\%\ Moisture\ content = \frac{W_3 - W_1}{Weight\ of\ sample} \times \frac{100}{1}$$

Where:

w1 = weight of petri-dish and sample before drying

W2 = weigh of petri-dish and sample after drying

3.2.2.3 Crude fibre

The Crude fibre was determined using AOAC method described by (Williams, 1984)

Procedure

About 2g of the extract was defatted with petroleum ether and boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution. The

resulting solution was filtered through fine linen and washed with boiling water until the washings were no longer acid to litmus. The residue was transferred to a beaker and boiled for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 10ml. The final residue was filtered through a thin but closed pad of washed and ignited asbestos in a Gooch crucible. The Gooch crucible and its contents were dried in an electric oven and weighed. The contents of the crucible were further incinerated, cooled and weighed. The lost in weight after incineration x 100 represents the percentage of crude fibre.

$$\%\ Crude\ fibre = \frac{Weight\ of\ fibre}{Weight\ of\ sample} \times \frac{100}{1}$$

3.2.2.4 Crude protein

The Crude protein was determined using the procedure given by AOAC (Williams, 1984)

Procedure

In order to prevent the sample from striking the flasks' side walls, 0.5g of sample was carefully weighed into each 30ml Kjeldahl flask before the flasks were sealed and shaken. The Kjeldahl catalyst mixture was then added in the 0.5g sample. The mixture was carefully heated in a digestion rack over fire until it formed a clear solution. The clear solution was then left to stand for 30 minutes before being cooled. To avoid caking, 100ml of distilled water was added after cooling, and 5ml was transferred to the Kjeldahl distillation apparatus, followed by 5ml of 40% NaOH. A 100ml receiver flask containing 5ml of 2% boric acid and an indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methylene blue was placed under the distillation apparatus's condenser so that the tap was about 20cm inside the solution, and distillation began immediately until 50 drops reached the receiver flask, after which it was titrated to pink using 0.01N hydrochloric acid.

Calculations

% Nitrogen =Titre value x 0.01 x 14 x 4

% Protein = % Nitrogen x 6

3.2.2. 5 Crude fat

The Soxhlet fat extraction method, as described by (AOAC, 1990) was used to determine crude fat Continuous extraction of the samples in a Soxhlet apparatus for 6 hours with a non-polar organic solvent such as petroleum ether was used for this procedure.

Procedure

Clean 250ml boiling flasks were dried in an oven at 105-110°C for approximately 30 minutes before being placed in a desiccator and permitted to cool. The labeled and cooled boiling flasks were correspondingly weighed. The boiling flasks were filled with about 300ml of petroleum ether (boiling point 40-60°C). The extraction thimble was lightly plugged with cotton wool. The Soxhlet apparatus was assembled and allowed to reflux for about 6hrs.The thimble was then carefully removed and petroleum ether collected in the top container of the set –up and drained into a container for re-use. Flask was removed and dried at 105-110°C for 1hr when it was almost free of petroleum ether and finally transferred from the oven into a desiccator and allowed to cool and then weighed

$$\%fat = \frac{wt\ of\ flask + oil - wt\ of\ flask}{Weight\ of\ sample} \times \frac{100}{1}$$

3.2.2.6 Carbohydrate content

Total Carbohydrate content of the leaf samples was determined using differential method as described by Muller & Tobin (1980). This method is used after all other proximate components have been determined. In this method, the sum of all the percentages of all the

other proximate components was subtracted from 100 (Muller & Tobin, 1980) as shown below:

$$Total\ Carbohydrates\ (\%) = 100\% - (\%Protein + \%Moisture + \%Ash + \%Fat + \%Fibre)$$

3.2.3 Determination of mineral composition using atomic absorption spectrometry (AAS)

The mineral compositions were assessed using FS240AA Agilent Atomic Absorption Spectrophotometer using the method described by APHA, (1998).

Principle

Atomic Absorption Spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Because metals have distinct absorption wavelengths, a source lamp made of that element is used, keeping the method relatively free of spectral or radiational interferences. The amount of energy absorbed in the flame by the characteristic wavelength is proportional to the concentration of the element in the sample. Sample digestion was done according to Adrian, (1973) as follows: approximately 2g of the dried sample was weighed into a digestion flask and 20ml of the acid mixture (65ml conc HNO_3, 80ml Perchloric acid, 20ml conc H_2SO_4) was added. The flask was heated until a clear digestion was achieved. The digest was diluted with distilled water to the 100ml mark.

Procedure for Mineral Determination

The AAS was used to aspirate the sample into the flame and atomized only when the light beam in the AAS was directed through the flame to the detector, which then measured the amount of light absorbed by the atomized element in the flame. (APHA, 1998). The quantity of energy absorbed in the flame is related to the mineral element concentration in the sample.

3.2.4 Determination of amino acid composition

The amino acid composition of the leaf samples was determined using HPLC as described by (Gonzalez-Castro *et al*., 1997). A Spectra Physics (San Jose, CA) HPLC apparatus with an 8700 XR ternary pump, a 20-µL Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, an 8440 XR UV-vis detector, and a 4290-integrator linked via Labnet to a computer running on WINner 8086 software was used (operating system, MS.DOS version 3.2). A 250 x 4.6 mm column packed with 5 µm Spherisorb C1 8 (Sugelabor, Madrid, Spain) was used for separation.

Derivatization procedure

The method used was a modification of Elkin *et al* (1985) method. Pipetting a standard solution (5, 10, 15, or 20 µL) or 50 µL of sample solution into a 10- 5-mm tube and drying in vacuo at 65°C. The residue was treated with 30 µL of methanol-water-Phenylisothiocianate (2:2:1 [v/v]) and then evaporated in vacuum at 65°C. The derivatizing reagent methanol-water-Phenylisothiocianate (7:1:1:1 [v/v]) was then added in 30 µL increments, agitated, and allowed to stand for 20 minutes at ambient temperature. Each tube received a phosphate solution containing 5% acetonitrile. Finally, the solvents were extracted using a nitrogen stream, and the tube was sealed and stored at 4°C until further analysis. Each tube received 150 µL of diluent containing 5mM sodium phosphate and 5% acetonitrile prior to injection.

Chromatographic procedure

Chromatography was performed at a constant temperature of 30°C using the gradient elution method described below. Eluant A was an aqueous buffer made by adding 0.5 mL/L Triethylamine to 0.14M sodium acetate and titrating it to pH 6.20 with glacial acetic acid; eluant B was an acetonitrile-water (60:40 [v/v]) solution.

3.2.5 Qualitative phytochemical screening

The leaf samples were subjected to qualitative phytochemical analysis to identify the presence of alkaloids, saponins, flavonoids, tannins, resins, glycosides and terpenoids using standard analytical techniques.

3.2.5.1 Wagner`s reagent test for alkaloids

Principle

Alkaloids under acidic condition and at room temperature react with iodine and potassium iodide to give reddish brown precipitate in the presence of Wagner's reagent.

Procedure

The filtrate was pipetted into a test tube with 1.0 ml. Wagner's reagent was then pipetted into the test tube in an additional 1.0 ml, carefully mixed, and checked for color change. A reddish-brown precipitate indicated the presence of alkaloids (Evans, 2002).

3.2.5.2 Test for glycosides

To 10 cm^3 of 50% H_2SO_4, 1.0 ml of the extract was added, and the mixture was heated for 5 minutes in boiling H_2O. It was mixed with 10 cm^3 of Fehling's solution, which was made up of 5cm3 of each solution A and B. A brick- red precipitate indicating presence of glycosides was observed (Hikino *et al*., 1984).

3.2.5.3 Salkowiski test for terpenoids

Concentrated H_2SO_4 (3ml) was carefully added to produce a layer after 5.0ml of each extract was carefully added to 2ml of chloroform (Numex, India). A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids (Edeoga *et al*., 2005).

3.2.5.4 Frothing test for saponins

In a water bath, 2g of the powdered sample was boiled in 20ml of distilled water and filtered.

To produce stable, enduring foam, 10ml of filtrate was combined with 5ml of distilled water and rapidly shaken. Three drops of olive oil were added to the mixture, which was then rapidly shaken before being observed. The formation of an emulsion after the addition of 3 drops of olive oil yielded a positive result (Edeoga *et al*., 2005).

3.2.5.5 Alkali test for flavonoids

Principle

Flavonoids at room temperature and under alkaline pH forms observable precipitate.

Procedure

A test tube was pipetted with 1.0 ml of the extract. The same test tube was then pipetted with 1.0 ml of diluted NaOH solution, stirred, and observed for color change. Formation of precipitate showed positive test (Trease and Evans, 1989).

3.2.5.6 Ferric chloride ($FeCl_3$) test for tannins

About 0.5g of each sample was stirred with distilled water of about 10ml and then filtered. To 2ml of the filterate, few drops of 1% ferric solution was added. Formation of green, blue-black or blue-green precipitate showed positive test (Evans, 2002).

3.2.5.7 Precipitation test for resins

Using 15ml of 90% ethanol, 0.2g of the powdered leaf samples were extracted. In a beaker, the alcoholic extract was then combined with 20ml of distilled water. Precipitate formation yielded a positive result (Ojukwu and Ibekwe, 2018).

3.2.6 Quantitative phytochemical and anti-nutrient analyses using Gas Chromatography with Flame Ionization Detector (GC-FID)

3.2.6.1 Extraction of phytochemicals

After weighing and placing 1g of sample in a test tube, 15ml of ethanol and 10ml of 50% m/v potassium hydroxide were added. The test tube was allowed to react in a 60°C water bath for

60 minutes. The reaction product in the test tube was transferred to a separatory funnel after the reaction time. The tube was successfully washed with 20ml of ethanol, 10ml of cold water, 10ml of hot water, and 3ml of hexane, all of which were transferred to the funnel. This extract was combined and washed three times with a 10ml aqueous solution of 10% v/v ethanol. The solvent was evaporated after the solution was dried with anhydrous sodium sulfate. The sample was solubilized in 1000µl of pyridine of which 200µL was transferred to a vial on the Gas Chromatography machine for quantitative phytochemical analysis.

3.2.6.2 Quantification using GC-FID

The phytochemical analysis was carried out on a BUCK M910 Gas chromatography fitted with a flame ionization detector (FID) (Ogbuagu *et al*., 2019). A 15-meter RESTEK MXT-1 column (15m x 250µm x 0.15µm) was used. The injector temperature was 280°C, with a splitless injection of 2µL of sample and a linear velocity of 30cms^{-1}, and the carrier gas was Helium 5.0 Psi at a flow rate of 40 ml/min. The oven initially operated at 200°C but was heated to 330°C at a rate of 30°C per minute for 5 minutes. The detector was set at 320°C. The ratio of the area and mass of the internal standard to the area of the identified phytochemicals was used to determine the phytochemicals (Ugoeze *et al*., 2020). The concentration of the various phytochemicals contained in the extract was expressed as µg/g.

3.2.7 GC-MS assisted bioactive constituents' analysis

Bioactive compounds of the leaves extracts were analyzed as described by Enemor *et al*., (2019) using GC–MS. using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) fitted with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). An electron ionization system using high energy electrons was used for spectroscopic detection by GC–MS (70 eV). The carrier gas was pure helium gas (99.995% purity) at a flow rate of 1 mL/min. The initial

temperature was maintained at 50–150 °C, with a 3 °C/min increase rate and a holding time of about 10 minutes but was later raised to 300 °C at a rate of 10 °C/min. In a splitless mode, 1 µL of the prepared 1% extracts diluted with respective solvents was injected. The relative quantity of bioactive compounds present in each sample of the extract was stated as a percentage based on peak area generated in the chromatogram.

3.2.7.1 Identification of chemical constituents

Bioactive components extracted from the plant extracts were identified using GC retention time on an HP-5MS column and spectral matching with computer software data from standards (Replib and Mainlab data from GC–MS systems) (Enemor *et al*., 2019).

3.2.8 Antioxidant determination

3.2.8.1 Total phenolic content

The total phenolic content of the leaf samples was determined as described proposed by Mallick and Singh (1980).

Principle

In the Folin-Ciocalteau reagent, phenols react with phosphomolybdic acid to produce a blue-colored complex in alkaline medium that can be measured spectrophotometrically at 650nm.

Procedure

In a 10x volume of 80% ethanol, 0.5g of each leaf sample was homogenized. For 20 minutes, the homogenate was centrifuged at 10,000rpm. The residue was extracted again with 80% ethanol. The supernatants were collected and evaporated until dry. After that, the residue was dissolved in a known volume of distilled water. Pipettes were used to extract various aliquots, and the volume in each tube was filled to 3.0ml with distilled water. After adding 0.5ml of Folin-Ciocalteau reagent, the tubes were placed in a boiling water bath for one minute. After

cooling the tubes, the absorbance was spectrometrically measured using Genesys 10-S, USA at 650nm against a reagent blank. Standard catechol solutions (0.2-1ml) corresponding to 2.0-10µg concentrations were also treated in the same manner as described above. The phenol concentration was expressed in mg/g tissue.

3.2.8.2 Estimation of total flavonoids

To sample and estimate flavonoids in the leaf samples, the method proposed by Cameron *et al.* (1943) was used.

Principle

Flavonoids react with vanillin to produce a coloured product, which can be spectrophotometrically measured.

Extraction of flavonoids

Initially, 0.5g of leaf samples were analyzed with a 2:1 ratio of methanol to water, and then with a 1:1 ratio. The samples were vigorously agitated and allowed to sit overnight. The supernatants were collected and measured in volume. This supernatant was concentrated before being used in the assay.

Procedure

The sample was pipetted out in a known volume and evaporated to dryness. After adding 4.0ml of vanillin reagent, the test tubes were then for 15 minutes heated in a boiling water bath. The standard at different concentration was also treated in the same way. The optical density was spectrophotometrically determined at 340nm using Genesys 10-S, USA. The concentration of flavonoids in the different leaf samples was determined using a standard curve. Flavonoids were measured in mg/g sample.

3.2.8.3 Determination of antioxidant vitamins

3.2.8.3.1 Vitamin C (ascorbic acid) content

The spectrophotometric method described by Roe and Keuther (1943) was used to measure vitamin C.

Principle

When absorbate is treated with activated charcoal, it is converted into dehydroascorbate, which then reacts with 2, 4-dinitrophenyl hydrazine to yield osazones. When these osazones are dissolved in sulphuric acid, they produce an orange-colored solution whose absorbance can be determined spectrophotometrically at 540nm.

Extraction of vitamin C

From 1g of leaf sample, ascorbate was extracted with 4% TCA and diluted to 10ml using same. After centrifuging at 2000rpm for 10 minutes, the supernatant was treated with a pinch of activated charcoal, vigorously shaken with a cyclomixer, and kept for 5 minutes. Centrifugation was used to remove the charcoal particles, and aliquots were utilized for the estimation.

Procedure

Standard ascorbates in concentrations ranging from 0.2 to 1.0ml, as well as 0.5 to 1.0ml of supernatant, were used. With 4% TCA, the volume was increased to 2.0ml. All of the tubes received 0.5ml of DNPH reagent, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated for 3 hours at 370 degrees Celsius, which then resulted in the formation crystals of osazones. Cold 2.5ml of 85% sulphuric acid was used to dissolve the crystals. After adding sulphuric acid, DNPH reagent and thiourea were introduced to the blank. After cooling the tubes in ice, the absorbance was measured in a spectrophotometer at

540nm. An electronic calculator set to linear regression mode was used to create a standard graph. The ascorbate concentration in the sample was calculated and expressed in mg/kg of sample.

3.2.8.3.2 Vitamin E (tocopherol) content

Each leaf sample was homogenized into 2.5 g in 50 ml of 0.1N sulfuric acid, then permitted to sit for the night. The contents of the flask were vigorously shaken before being filtered through Whatman No.1 filter paper. Estimation was done using aliquots of the filtrate.

Procedure

Separate 1.5ml portions of the sample, the standard, and the 1.5ml of water were pipetted into three stoppered centrifuge tubes. Each tube received 1.5 ml of each substance before being thoroughly mixed and centrifuged. The xylene (1.0ml) layer was transferred using a second stoppered tube. Each tube received 1.0ml of dipyridyl reagent, which was thoroughly mixed. The mixture (1.5ml) was pipetted into a cuvette, and the extinction was measured calorimetrically at 460nm as described by Lowry et *al*., (1983).

3.2.8.3.3 Beta–carotene content

Beta-carotene content of the leaf samples were estimated using the method described by Zakaria *et al*. (1979).

Principle

Beta-carotene can be sampled in leaf samples using petroleum ether and then measured at 450nm and 503nm respectively.

Procedure

To avoid photolysis of carotenoids after saponification, the experiment was conducted in the dark. In water bath at 60°C for 30 minutes, 0.5g of each sample was homogenized and saponified with 2.5ml of 12% alcoholic potassium hydroxide. The saponified sample was

mixed thoroughly before being moved to a separating funnel comprising 10- 15ml of petroleum ether. The lower aqueous layer was then moved to a different separating funnel, and the topmost petroleum ether layer, which contained the carotenoids, was collected. The process was repeated till the aqueous layer lost its color. To remove excess moisture from the petroleum ether sample, a small quantity of anhydrous sodium sulphate was added. The petroleum ether sample's final volume was recorded. Using petroleum ether as a blank, the absorbance of the yellow color was spectrometrically measured using (Genesys 10-S, USA) at 450nm and 503nm.The amount of Beta carotene and lycopene was calculated using the formulae:

$$Amount\ of\ Beta\ Carotene = \frac{A450 \times Volume\ of\ the\ sample}{Weight\ of\ sample} \times 4$$

3.2.8.4 Antioxidant assay

3.2.8.4.1 α, α-diphenyl-β-picrylhydrazyl (DPPH) scavenging activity

The potential of the natural antioxidants in the leaves to scavenge the stable free radical DPPH was assessed using the method described by Mensor *et al*. (2001).

(DPPH)

Procedure

The leaf samples were combined with 0.5ml of a 0.1mM DPPH methanolic solution and 0.48ml of methanol in a volume of 20µL**.** For 30 minutes, the mixture was left to react at room temperature. Methanol served as the blank, DPPH in methanol served as the positive control, and butylated hydroxytoluene (BHT) served as the reference. A spectrophotometer was used to measure the discoloration of the purple color after 30 minutes of incubation at 518nm (Genesys 10-S, USA). The following formula was used to calculate radical scavenging activity:

$$Scavening\ activity\ \% = \frac{100 - A518(sample) - A518(blank)}{A518(blank)} \times \frac{100}{1}$$

3.2.8.4.2 2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS) scavenging activity

The antioxidant activity of the leaf samples was investigated using the ABTS (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay developed by Shirwaikar *et al*. (2006). ABTS radical cations (ABTS+) were created by reacting an ABTS solution (7mM) with 2.45mM ammonium persulphate. Before using, the reaction mixture was kept in the dark at room temperature for about 12-16 hours. Aliquots (0.5ml) of each of the two leaf samples were added to 0.3ml of ABTS solution before being diluted to 1ml with ethanol. The absorbance was measured in a spectrophotometer at 745nm (Genesys 10-S, USA) and the percentage inhibition was calculated by using the formula;

$$Inhibition\ (\%) \frac{(Control - test)}{Control} \times \frac{100}{1}$$

3.2.8.4.3 Ferric Ion Reducing Antioxidant Power (FRAP)

The extracts' reducing property was determined using the method described by Pulido *et al*. (2000).

Principle

The assay's principle is the estimation of ferric degradation product via condensation with the extract.

Procedure

Mix 0.25 ml of extract, 0.25 ml of 200 mM sodium phosphate buffer, pH 6.6, and 0.25 ml of 1% potassium ferrocyanide. After incubation at 50°C for 20 minutes, 0.25 mL of 10% trichloroacetic acid was added and centrifuged at 2000 rpm for 10 minutes, 1 mL of the

supernatant was mixed with 1 mL distilled water and 0.2 mL ferric chloride, and the absorbance was measured at 700 nm.

3.2.9 Toxicological test using brine shrimp lethality assay

The brines shrimp lethality assay was done using the method described by Meyer *et al.* (1982) with little modifications.

Principle

The brine shrimp lethality assay is a preliminary in vitro bioassay that evaluates the toxicity of chemicals and natural products such as plant extracts using brine shrimp nauplii, a tiny crustacean (*Artemia salina*). In brine medium, the procedure measures the LC50 values of active compounds and extracts in µg/ml. The activities of wide range of known bioactive constituents in the test sample results in toxicity to the brine shrimp larvae.

Procedure

Brine shrimp eggs (*Artemia salina* Leach) were obtained from Department of Pharmacognosy, University of Ibadan, Oyo State, Nigeria. Two grams (2.0g) of the brine shrimp eggs were transferred and allowed to hatch in natural sea water obtained from bar beach, Ikoyi Lagos containing 3.8g/L salt. The larvae (Nauplii) were put in a filtered sea water for 48h at 25°C under constant aeration and illumination so as to ensure survival and maturity before use (Simorangkir *et al*., 2017; Ogbole *et al*., 2017). After the 48h, eggs that have hatched into a nauplii (larvae) were used as test animals. Stock solutions (10mg/mL) of the two plant extracts were made and diluted serially in clean text tubes of 10mL volume to obtain five final concentrations (1000µg/mL, 500 µg/mL, 100 µg/mL ,10 µg/mL and 1.0 µg/mL). Ten nauplii (larvae) were collected with the aid of a pipette and added to the serially diluted ten solutions. The test was carried out in triplicate. The negative control consisted of ten nauplii per tube in sea water without plant extracts (Ogbole *et al*., 2017) while thymol 1% aqueous solution was

the positive control (Basto *et al*., 2009). After the 24h incubation at 25°C, a magnifying lens was used to count the number of dead larvae and the percentage mortality was calculated. Larvae were considered dead only if they did not move for few seconds after pricking with a sharp object during observation.

3.2.10 Statistical analysis

Analyses were done in triplicates and results expressed as mean ± SD. Data was analyzed by ANOVA using GraphPad Prism version 5.0. The LC_{50} was calculated using probit analysis and the data was determined by the best fit line method, while percentage mortality was calculated using mean survival of treated and untreated brine shrimp larvae.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

Table 4.1 Proximate composition (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*

Parameter (%)	***Justicia secunda***	***Jatropha tanjorensis***	**T-value**	**P-value**	**Comment**
Moisture	6.37 ± 0.09	5.32 ± 0.07	15.95	<0.0001	significant
Fibre	4.62 ± 0.04	5.22 ± 0.05	16.23	<0.0001	significant
Ash	8.89 ± 0.07	9.79 ± 0.09	13.67	0.0002	significant
Fat	8.45 ± 0.03	7.65 ± 0.04	27.71	<0.0001	significant
Protein	10.15 ± 0.10	9.80 ± 0.07	4.966	0.0077	significant
Carbohydrate	61.52 ± 0.12	62.22 ± 0.14	6.575	0.0028	significant

Values are mean ± standard deviation of triplicate determinations.

Table 4.1 shows the proximate composition of the ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.* There were significantly ($p<0.05$) higher contents of moisture (6.37 ± 0.09%), fat (8.45 ± 0.03%), and protein (10.15 ± 0.10%) in *J. secunda* compared to *J. tanjorensis,* while *J. tanjorensis* showed higher contents of fibre (5.22 ± 0.05%), ash (9.79 ± 0.09%) and carbohydrate (62.22 ± 0.14).

Table 4.2: Mineral composition (mg/kg dry weight) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*

Parameter (mg/kg dry weight)	***Justicia secunda***	***Jatropha tanjorensis***	**T-value**	**P-value**	**Comment**
Sodium	6.48 ± 0.10	6.99 ± 0.12	5.655	0.0048	Significant
Potassium	8.49 ± 0.08	8.58 ± 0.10	1.217	0.2904	Not Significant
Calcium	5.68 ± 0.07	5.99 ± 0.09	4.709	0.0092	Significant
Magnesium	9.39 ± 0.10	8.48 ± 0.08	12.31	0.0003	Significant
Copper	0.79 ± 0.03	0.67 ± 0.04	4.157	0.0142	Significant
Zinc	0.79 ± 0.04	0.67 ± 0.05	3.246	0.0315	Significant
Iron	1.99 ± 0.08	1.67 ± 0.06	5.543	0.0052	Significant
Manganese	0.57 ± 0.02	0.53 ± 0.03	1.922	0.1270	Not Significant

Values are mean ± standard deviation of triplicate determinations

Table 4.2 shows the result of the mineral analysis of the plants' extracts. There is significantly ($p<0.05$) higher contents of sodium (6.99 ± 0.12 mg/kg), and calcium (5.99 ± 0.09 mg/kg) in *J. tanjorensis* in comparison with *J. secunda*. *J. secunda* generally had higher contents of the microelements compared to *J. tanjorensis.* No significant ($p>0.05$) difference was observed in the potassium and manganese contents of the two plant leaves.

Table 4.3: Amino acids composition (µg/g) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*

Amino acids (µg/g)	*Justicia secunda*	*Jatropha tanjorensis*	T-value	P-value	Comments
Essential Amino Acids					
Valine (Val)	4.75 ± 0.03	4.74 ± 0.03	0.4083	0.7040	Not Significant
Threonine (Thr)	3.57 ± 0.08	2.98 ± 0.03	11.96	0.0003	Significant
Isoleucine (Ile)	2.85 ± 0.04	4.74 ± 0.07	40.60	<0.0001	Significant
Leucine (Leu)	2.65 ± 0.09	2.98 ± 0.03	6.025	0.0038	Significant
Lysine (Lys)	3.85 ± 0.04	3.74 ± 0.09	1.934	0.1252	Not Significant
Methionine (Met)	1.50 ± 0.03	1.54 ± 0.02	1.922	0.1270	Not Significant
Phenylalanine (Phe)	3.74 ± 0.09	5.78 ± 0.04	35.88	<0.0001	Significant
Histidine (His)	4.76 ± 0.08	2.89 ± 0.05	34.33	<0.0001	Significant
Tryptophan (Trp)	1.79 ± 0.09	1.24 ± 0.05	9.253	0.0008	Significant
Non-Essential Amino Acids					
Glycine (Gly)	4.85 ± 0.03	2.44 ± 0.09	44.00	<0.0001	Significant
Alanine (Ala)	3.84 ± 0.07	2.38 ± 0.02	34.74	<0.0001	Significant
Serine (Ser)	2.57 ± 0.04	3.84 ± 0.09	22.33	<0.0001	Significant
Proline (Pro)	3.69 ± 0.09	4.78 ± 0.04	19.17	<0.0001	Significant
Aspartate (Asp)	2.89 ± 0.03	2.21 ± 0.08	13.79	0.0002	Significant
Glutamate (Glu)	2.75 ± 0.03	4.69 ± 0.04	67.20	<0.0001	Significant
Arginine (Arg)	2.74 ± 0.05	3.49 ± 0.06	16.63	<0.0001	Significant
Tyrosine (Tyr)	2.92 ± 0.02	2.97 ± 0.09	1.369	0.2428	Not Significant
Cystine (Cys)	2.74 ± 0.03	1.44 ± 0.06	33.57	<0.0001	Significant

Table 4.3: Amino acids composition (µg/g) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.* A total of eighteen amino acids were identified in each of the plants. The highest essential amino acid found in *Justicia secunda* was valine followed by histidine, lysine and phenylalanine while phenylalanine, valine, isoleucine and lysine were the highest occurring essential amino acids in *Jatropha tanjorensis*. The highest non-essential amino acids (NEAA) identified in *Justicia secunda* was glycine, followed by alanine, proline and aspartate while proline, glutamate, arginine and tyrosine were the most abundant in *Jatropha tanjorensis*. *J. tanjorensis* has a higher (30.63±0.41 µg/g) distribution of essential amino acid as opposed to (29.46 ± 0.57 µg/g) observed in *J. secunda*. Conversely *J.secunda* had a total amount of non-essential amino acid as (28.99 ±0.39 µg/g) as against 28.24±0.57µg/g) seen in *J. tanjorensis*

4.4: Phytochemical screening of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*

Parameters	***Justicia secunda***		***Jatropha tanjorensis***	
	Observation	**Remark**	**Observation**	**Remark**
Alkaloid	++	High	++	High
Saponin	+	Low	++	High
Flavonoids	++	High	++	High
Tannin	++	High	++	High
Resin	+	Low	+	Low
Cardiac glycoside	+	Low	++	High
Terpenoids	+	low	+	Low

Key ++ = High + =Low

Table 4.4 shows the result of phytochemical screening of ethanol-water leaf extracts of *J. secunda* and *J. tanjorensis*. The table shows high amount of alkaloid, saponin, flavonoids, tannin and cardiac glycoside in *J. tanjorensis* as against low saponin, cardiac glycoside, terpenoids and resin in *J.secunda.* Terpenoids and resin were low in both plants.

Table 4.5: Composition (µg/ml) of phytochemicals and antinutrient factors in ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*

Component (µg/ml)	***Justicia secunda***	***Jatropha tanjorensis***	**T-value**	**P-value**	**Comment**
Sapogenin	6.76 ± 0.24	14.13 ± 0.39	27.88	<0.0001	Significant
Naringenin	4.09 ± 0.09	26.21 ± 0.23	155.10	<0.0001	Significant
Anthocyanin	6.68 ± 0.75	11.35 ± 0.51	8.918	0.0009	Significant
Epihedrine	0.01 ± 0.30	4.67 ± 0.82	9.244	0.0008	Significant
Dihydrocytisine	8.07 ± 0.82	8.38 ± 0.48	0.5651	0.6022	Not Significant
Kaempferol	4.72 ± 0.51	6.09 ± 0.39	3.696	0.0209	Significant
Cynogenic glycoside	5.13 ± 0.54	7.83 ± 0.25	7.859	0.0014	Significant
Aphylidine	5.55 ± 0.23	12.77 ± 0.40	27.10	<0.0001	Significant
Steroid	4.35 ± 0.39	15.75 ± 0.19	45.52	<0.0001	Significant
Tannin	18.88 ± 0.28	26.12 ± 0.15	39.48	<0.0001	Significant
Flavonones	6.58 ± 0.27	9.04 ± 0.17	13.35	0.0002	Significant
Catechin	3.97 ± 0.16	24.40 ± 0.06	207.10	<0.0001	Significant
Flavone	7.08 ± 0.50	7.36 ± 0.35	0.7946	0.4713	Not Significant
Proanthocyanidin	6.21 ± 0.71	8.16 ± 0.19	4.595	0.0101	Significant
Ribalinidine	9.14 ± 0.34	18.27 ± 0.16	42.08	<0.0001	Significant
Spartein	2.76 ± 0.22	4.34 ± 0.18	9.627	0.0007	Significant
Cardiac glycoside	3.00 ± 0.45	10.34 ± 0.43	20.43	<0.0001	Significant
Phytate	4.15 ± 0.59	7.74 ± 0.11	10.36	0.0005	Significant
Oxalate	2.53 ± 0.37	3.22 ± 0.15	2.993	0.0402	Significant
Ammodendrine	5.99 ± 0.28	22.36 ± 0.11	94.25	<0.0001	Significant

Table 4.5 shows the phytochemical composition (µg/ml) and antinutrient factors in ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.* A higher amount of phytochemicals and anti-nutrient factors such as tannin, phytate and oxalate was observed in *J. tanjorensis* compared to *J. secunda.* A significant ($P<0.05$) difference was observed in all the phytochemicals and antinutrient factors except in dihydrocystisine and flavone ($p>0.05$) between the two plant leaves.

Table 4.6a-4.6f: GCMS-identified bioactive components of ethanol-water leaf extracts of *Justicia secunda* (arranged according to their Retention time (Min), % Report (percentage abundance), molecular formula and molecular weight)

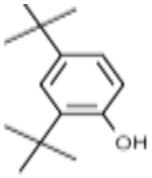
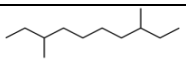
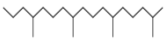
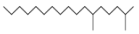
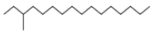
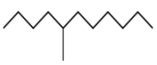
S/N	RT (min)	Bioactive compound	Formula	MW	% Report	Structure	Biological activities
		Phenol					
1	22.130	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	8.08		Anticancer, antibacterial Antiproliferative, Antifungal, Antimicrobial, cytotoxic Antioxidant, Antiinflammatory antiviral, antimalarial, insecticidal,nematocidal and phytopathogenic
		Alkane					
2	9.430	Decane,3,8-dimethyl	$C_{12}H_{26}$	170	7.72		No reported activity
3	9.816	Heptadecane,2,6,10,14-tetramethyl	$C_{21}H_{44}$	296	4.72		No reported activity
4	10.268	Heptadecane,2,6-dimethyl	$C_{19}H_{40}$	268	4.51		No reported activity
5	8.876	Hexadecane,3-methyl	$C_{17}H_{36}$	240	3.38		No reported activity
6	9.737	Dodecane	$C_{12}H_{26}$	170	3.16		Antioxidant, antimicrobial and anti-cancer
7	9.622	Undecane	$C_{11}H_{24}$	156	2.93		Anti-inflammatory , Anti-allergic, Anti-fungal & anti-microbial
8	8.980	Nonane	C_9H_{20}	128	1.84		Antifungal & antibacterial
9	15.706	Tridecane	$C_{13}H_{28}$	184	1.82		Antimicrobial & Antioxidant
10	10.506	Undecane,5-methyl	$C_{12}H_{26}$	170	1.70		No reported activity

Table 4.6b

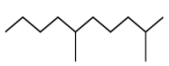
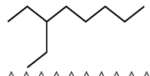
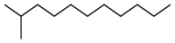
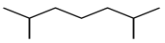
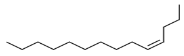
11	9.138	2,6-Dimethyldecane	$C_{12}H_{26}$	170	1.69		No reported activity
12	10.635	Octane,3-ethyl	$C_{10}H_{22}$	142	1.52		No reported activity
13	10.579	Tricosane, 2-methyl	$C_{24}H_{50}$	338	1.30		Antifungal & antibacterial
14	9.059	Decane	$C_{10}H_{22}$	142	1.19		
15	21.104	Undecane, 2-methyl	$C_{12}H_{26}$	170	0.88		Antifungal, antibacterial & antioxidant activity
16	23.597	Hexadecane	$C_{16}H_{34}$	226	0.57		Antibacterial Antimicrobial & antioxidant
17	26.072	Heptane, 2,6-dimethyl-	C_9H_{20}	128	0.41		Induced morphological changes in human skin and monkey kidney cells Exhibits weak antibacterial activity
		Alkene					
18	28.325	1-octadecene	$C_{18}H_{36}$	252	2.66		Antibacterial, antioxidant, Anticancer.
19	23.440	Z-8-Hexadecene	$C_{16}H_{32}$	224	2.35		Antimicrobial
20	18.283	4-tetradecene, (Z)	$C_{14}H_{28}$	196	1.31		No reported activity
21	32.181	1-Docosene	$C_{22}H_{44}$	308	1.03		Antimicrobial
22	34.796	1- Hexacosene	$C_{26}H_{52}$	364	0.41		Cytotoxic activity

Table 4.6c

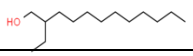
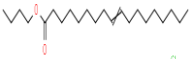
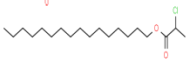
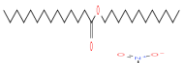
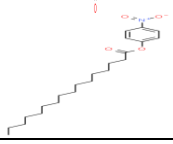
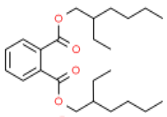
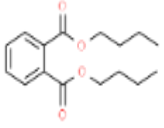
23	23.743	Cetene	$C_{16}H_{32}$	224	0.31		Antioxidant & antitumour ,
24	28.553	3-Eicosene, (E)-	$C_{20}H_{40}$	280	0.15		Antioxidant,cytotoxic antimicrobial, anti-hyperglycemic,insecticide -al activity
		Fatty Acid					
25	32.843	Oleic acid	$C_{18}H_{34}O_2$	282	0.61		Antimicrobial, Antifungal, Inhibitor of 5-α reductase, Allergenic, anti-inflammatory, anti-androgenic, cancer preventive, anemiagenic, anti-alopecic, propecic anti-leukotriene-D4, choleretic, dermatitigenic, hypocholesterolemic, insectifuge,
26	33.550	Hexacosanoic acid	$C_{26}H_{52}O_2$	396	0.20		Antimicrobial & antifungal
		Fatty alcohol					
27	19.743	1-Hexacosanol	$C_{26}H_{54}O$	382	0.15		Antitumour activity
28	8.521	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	214	1.41		Anti-helminthic
		Fatty Acid Ester					
29	10.159	Carbonic acid, nonyl vinyl ester	$C_{12}H_{22}O_3$	214	3.73		No reported activity
30	9.938	Carbonic acid nonyl prop-1-en-yl ester	$C_{13}H_{24}O_3$	228	1.24		probable antibacterial activity
31	5.250	Butyl 9-octadecenoate	$C_{22}H_{42}O_2$	338	1.22		No reported activity
32	33.375	2-Chloropropionic acid, hexadecyl ester	$C_{19}H_{37}ClO_2$	332	1.08		No reported activity
33	34.116	Tetradecanoic acid, dodecyl ester	$C_{26}H_{52}O_2$	396	0.36		No reported activity
34	7.137	Hexadecanoic acid, 4-nitrophenyl ester	$C_{22}H_{35}NO_4$	377	0.32		Has cosmetic uses
		Phthalate Ester					
35	34.543	Bis (2-ethylhexyl) Phthalate	$C_{24}H_{38}O_4$	390	1.50		Antimicrobial Anticancer & anti-tumour Cytotoxic
36	30.527	Dibutylphthalate	$C_{16}H_{22}O_4$	278	1.31		Antimicrobial, Antifouling ,Antifungal Antimalarial

Table 4.6d

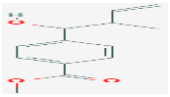
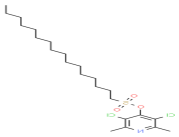
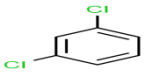
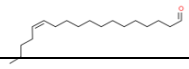
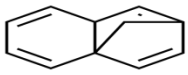
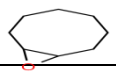
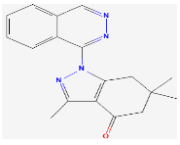
		FAME					
37	30.069	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.02		Promotes aortic dilation, Promotes membrane autolysis, Inhibits phagocytosis and influences nitric oxide production in some cells
38	31.551	9,12-Octadecanoic acid, methyl ester, (E,E)	$C_{19}H_{34}O_2$	294	1.43		Antifungal Anticancer, anti-oxidant, anti-acne, anti-eczemic, anti-histamine, Anti-inflammatory, insectifuge, nematicide, hepatoprotective and hypercholesterolemic
39	25.513	Benzoic acid, 4-(3-hydroxy-3-methyl-1-butynyl)-,methyl ester	$C_{13}H_{16}O_3$	220	0.32		No reported activity
		Pyridine derivative					
40	8.612	1-Hexadecanesulfonic acid,3,5-dichloro-2,6-dimethyl-4-pyridyl ester	$C_{23}H_{39}Cl_2NO_3S$	480	2.07		Anti-microbial and antioxidant
		Chlorobenzene					
41	7.425	Benzene,1,3-dichloro	$C_6H_4Cl_2$	147	1.33		Toxic to aquatic organisms like bluegill
		Aldehyde					
42	6.885	9-octadecenal, (Z)	$C_{18}H_{34}O$	266	1.11		Antimicrobial
43	12.584	E-14-Hexadecenal	$C_{16}H_{30}O$	238	0.95		Antioxidant & antibacterial
44	37.248	13-Octadecenal, (Z)-	$C_{18}H_{34}O$	266	0.33		
		Bicyclic Organic Compound					
45	15.873	1,4-Methanonaphthalene,1,4-dihydro	$C_{11}H_{10}$	142	0.84		Antimicrobial, antibacterial
46	16.361	9-Oxabicyclo[6.1.0] nonane	$C_8H_{14}O$	126	0.53		Probable inhibitory effect against acetylcholinesterase Antibacterial, anti-fungal & nematicidal activity
		Heterocyclic Organic Compound					
47	30.886	Indazole-4-one,3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro	$C_{18}H_{18}N_4O$	306	0.60		No reported activity

Table 4.6e

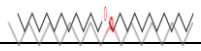
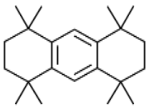
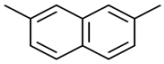
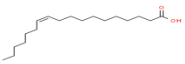
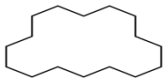
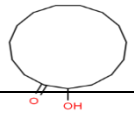
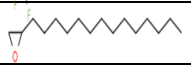
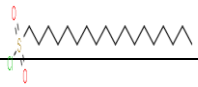
		Ether					
48	7.788	Octadecane,1-(ethenylo xy)	$C_{20}H_{40}O$	296	0.59		Antisepsis
49	20.899	Dodecane, 1,1'-oxybis-	$C_{24}H_{50}O$	354	0.16		No reported activity
		Dialkyl Ether					
50	14.888	Decyl octyl ether	$C_{18}H_{38}O$	270	0.57		No reported activity
		Poly aromatic Hydrocarbon(PAH)					
51	29.799	Anthracene, 1,2,3, 4,5, 6,7,8-octa hydro-1,1,4, 5,5,8,8-octamethyl-	$C_{22}H_{34}$	298	0.47		No reported activity
52	18.963	Naphthalene, 2,7-dimethyl-	$C_{12}H_{12}$	156	0.33		No reported activity
		MUFA					
53	5.455	Erucic acid	$C_{22}H_{42}O_2$	338	0.44		Regulates mesenchymal stem cell differentiation into osteoblasts and adipocyte Broad-spectrum antiviral activity against IAV Antiinflammatory/Pro-inflammatory amplification effect. Inhibits NF-κB and p38 MAPK
54	29.928	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	0.20		Antibacterial May down regulate gluconeogenesis and liver fat accumulation

Table 4.6f

		Cycloalkane					
55	23.978	Cyclohexadecane	$C_{16}H_{32}$	224	0.30		insecticidal, antioxidant, anticancer
		Hydrazide					
56	10.992	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298	0.26		No reported activity
		Chloroalkane					
57	17.473	Hexadecane, 1-chloro	$C_{16}H_{33}Cl$	260	0.26		No reported activity
		Ketone					
58	25.958	Cyclopentadecanone, 2-hydroxy	$C_{15}H_{28}O_2$	240	0.26		Antimicrobial
		Acyl fluoride					
59	13.191	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3$	338	0.24		Antifungal activity
		Epoxide					
60	17.804	Oxirane,tetradecyl	$C_{16}H_{32}O$	240	0.23		No reported activity
		Monoterpenoid Alcohol					
61	19.354	Citronellol	$C_{10}H_{20}O$	156	0.23		Antifungal, cytotoxic, antiproliferative
		Sulfonyl Chloride					
62	11.563	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	353	0.19		No reported activity

FAME: Fatty acid methyl ester, MUFA: Monounsaturated fatty acid, PAH: Polyaromatic hydrocarbon

Table 4.7a-4.7f: GCMS-identified bioactive components of ethanol-water leaf extract of *Jatropha tanjorensis* (arranged according to their Retention time (Min), % Report (percentage abundance), molecular formula and molecular weight

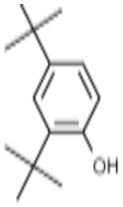
S/N	RT (Min)	Bioactive compound	Formula	MW	% Report	Structure	Biological activities
		Phenol					
1	22.133	2,4-Di-tert-butyl phenol	$C_{14}H_{22}O$	206	7.74		Anticancer, antibacterial Antiproliferative, Antifungal Antimicrobial, cytotoxic Antioxidant Antiinflammatory antiviral, antimalarial, insecticidal, nematocidal and phytopathogenic
2	25.514	2-Chloro-4-fluoro phenol, TBDMS derivative	$C_{12}H_{18}ClFOSi$	160	0.23		No activity reported
		Alkane					
3	9.428	Docosane, 2,21- dimethyl	$C_{24}H_{50}$	338	8.16		No activity reported
4	9.815	Undecane	$C_{11}H_{24}$	156	4.88		Anticancer Anti-inflammatory & Anti-allergic Anti-fungal & anti-microbial
5	9.553	Decane	$C_{10}H_{22}$	142	2.19		Antifungal & antibacterial
6	9.138	Tricosane,2-methyl	$C_{24}H_{50}$	338	1.84		No reported activity
7	15.705	Tridecane	$C_{13}H_{28}$	184	1.56		Antimicrobial
8	18.476	Tetradecane	$C_{14}H_{30}$	198	1.24		Antimicrobial, Diuretic & Antituberculosis

Table 4.7b

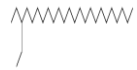
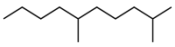
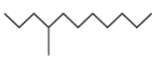
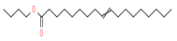
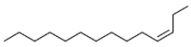
9	9.937	Tetracosane, 3-ethyl	$C_{26}H_{54}$	366	1.24		No reported activity
10	12.803	Dodecane	$C_{12}H_{26}$	170	1.22		Antioxidant Antimicrobial
11	10.578	2,6-Dimethyldecane	$C_{12}H_{26}$	170	1.14		No reported activity
12	11.464	Undecane, 5-methyl-	$C_{12}H_{26}$	170	0.24		No reported activity
		Fatty Acid Ester					
13	33.375	2-Chloropropionic acid, hexadecyl ester	$C_{19}H_{37}ClO_2$	332	1.25		No reported activity
14	5.253	Butyl 9-octadece noate	$C_{22}H_{42}O_2$	338	0.93		No reported activity
15	34.110	Eicosanoic acid, isobutyl ester	$C_{24}H_{48}O_2$	368	0.38		No reported activity
16	31.022	9-Methyl-10,12-hexadecadien-1-ol-acetate	$C_{19}H_{34}O_2$	294	0.34		No reported activity
17	23.597	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	268	0.32		Antibacterial Antioxidant
		Alkene					
18	28.090	1-octadecene	$C_{18}H_{36}$	252	2.40		Antibacterial, Antioxidant Anticancer
19	23.441	Hexadecene	$C_{16}H_{32}$	224	2.07		Antibacterial Antimicrobial and antioxidant
20	18.282	Tetradecene, (Z)	$C_{14}H_{28}$	196	1.18		Antimicrobial

Table 4.7c

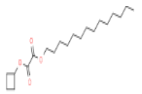
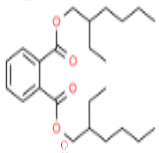
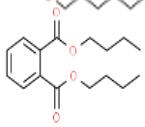
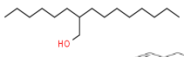
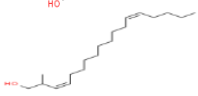
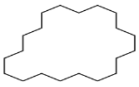
21	32.181	1-Docosene	$C_{22}H_{44}$	308	1.02		Antimicrobial
		Ester					
22	8.669	Oxalic acid, cyclobutyl tetradecyl ester	$C_{20}H_{36}O_4$	340	1.12		No reported activity
		Phthalate Ester					
23	34.544	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	1.33		Antimicrobial Anticancer & anti-tumour Cytotoxic
24	30.527	Dibutylphthalate	$C_{16}H_{22}O_4$	278	0.88		Antimicrobial, Antifouling Antifungal Antimalarial
		FAME					
25	31.588	Trans-13 octa decenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	1.70		Hypocholesterolemic 5-alpha reductase inhibitor Anemiagenic, Insectifuge, Sickle erythrocyte membrane stabilizing ability
26	31.550	8,11-octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	1.57		No reported activity
27	31.764	Methyl stearate	$C_{19}H_{38}O_2$	298	0.72		Antidiarrheal Cytotoxic & Antiproliferative .Antifungal, antioxidant,anticancer
		Fatty alcohol					
28	10.158	1-Decanol, 2-hexyl	$C_{16}H_{34}O$	242	5.09		No reported activity
29	26.626	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	0.29		Insecticidal, herbicidal, pesticidal and Pheromonal activity
		Cycloalkane					
30	34.796	Cycloeicosane	$C_{20}H_{40}$	280	0.36		No reported activity

Table 4.7d

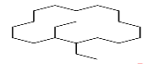
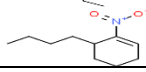
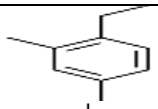
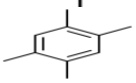
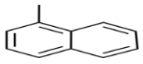
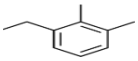
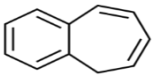
31	30.704	Cyclohexadecane,1,2-diethyl	$C_{20}H_{40}$	280	1.79		No reported activity
32	26.072	Cyclohexane,6-butyl-1-nitro	$C_{10}H_{17}NO_2$	183	4.36		No reported activity
		Ether					
33	8.875	Octadecane,1-(ethenyloxy)	$C_{20}H_{40}O$	306	3.84		Antisepsis
34	10.268	Allyl n-octyl ether	$C_{11}H_{22}O$	170	2.61		No reported activity
		Oxime					
35	9.619	Hydroxylamine, O-decyl	$C_{10}H_{23}NO$	173	2.90		Antimicrobial
		Aromatic Hydrocarbons					
36	10.638	Benzene, 1-ethyl-2-4-dimethyl	$C_{10}H_{14}$	134	2.67		No reported activity
37	10.505	Benzene,1,2,4,5-tetramethyl	$C_{10}H_{14}$	134	2.15		No reported activity
38	15.872	Naphthalene, 1-methyl	$C_{11}H_{10}$	142	0.77		No reported activity
39	11.571	Benzene, 1-ethyl-2,3-dimethyl-	$C_{10}H_{14}$	134	0.33		No reported activity
40	16.352	Benzocycloheptatriene	$C_{11}H_{10}$	142	0.15		No reported activity

Table 4.7e

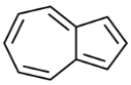
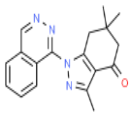
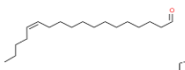
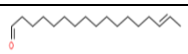
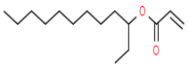
41	6.884	**Fluorinated Organic compounds** Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338	1.22		Antifungal
42	7.425	**Chlorinated Aromatic Hydrocarbons** Benzene,1,4-dichloro	$C_6H_4Cl_2$	147	1.20		Pesticide
43	12.595	**Bicyclic aromatic hydrocarbon** Azulene	$C_{10}H_8$	128	1.21		Antifungal Antimicrobial
44	32.601	**Fatty Acid** Oleic acid	$C_{18}H_{34}O_2$	282	0.86		Antimicrobial, Antifungal Inhibitor of 5-α reductase Allergenic, anti-inflammatory, anti-androgenic, cancer preventive, anemiagenic, anti-alopecic, propecic anti-leukotriene-D4, choleretic, dermatitigenic, hypocholesterolemic, insectifuge
45	8.613	**Alcohol** 1-Hexadecanol,2-methyl	$C_{17}H_{36}O$	256	0.94		Antioxidant, Antimicrobial
46	30.887	**Heterocyclic compound** Indazol-4-one, 3, 6, 6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-	$C_{18}H_{18}N_4O$	306	0.62		No reported activity
47	37.258	**Aldehyde** 13-octadecenal, (Z)	$C_{18}H_{34}O$	266	0.60		Antibacterial
48	14.889	9-octadecenal, (Z)	$C_{18}H_{34}O$	266	0.56		Antimicrobial

Table 4.7f

49	28.327	E-15- Heptacenal	$C_{17}H_{32}O$	252	0.32		AntibacterialAnti-inflammatory
		Hydrazide					
50	11.329	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298	0.36		No reported activity
		Fatty Acid Derivative					
51	33.999	3-(Prop-2-enoyloxy) dodecane	$C_{15}H_{28}O_2$	240	0.32		Propesic
		Epoxide					
52	11.123	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	0.32		No reported activity

FAME: Fatty acid methyl ester, MUFA: Monounsaturated fatty acid

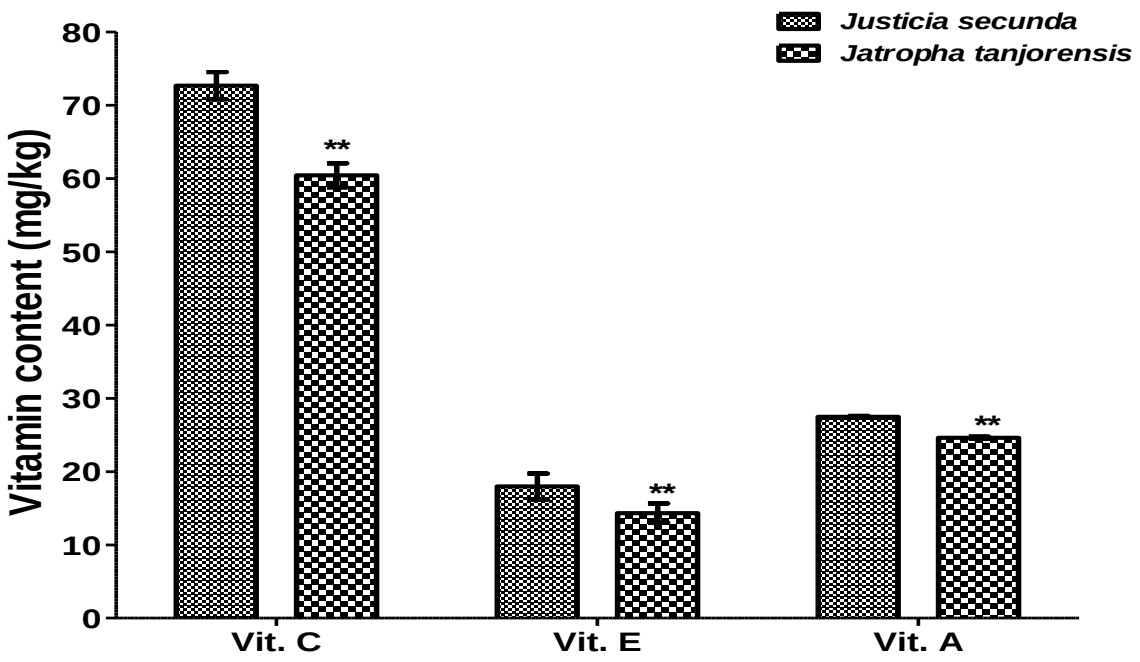


Figure 4.1: Antioxidant vitamin contents (mg/kg) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.* Bars are mean ± standard deviation of triplicate determinations. **Vitamin values of *Jatropha tanjorensis* significantly ($p<0.05$) lower than corresponding vitamin values of *Justicia secunda* .

Table: 4.8 Total phenolic content (mg/ml) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*

Extract Conc. (mg/ml)	*Justicia secunda*	*Jatropha tanjorensis*	T-value	P-value	Comment
0	0.00 ± 0.00	0.00 ± 0.00	-	-	-
10	3.39 ± 0.07	4.54 ± 0.02	27.36	<0.0001	Significant
20	4.60 ± 0.04	4.98 ± 0.03	13.16	0.0002	Significant
40	4.84 ± 0.08	6.05 ± 0.07	19.72	<0.0001	Significant
80	4.48 ± 0.06	6.09 ± 0.07	30.25	<0.0001	Significant

Table 4.8 shows total phenolic content (mg/ml) of ethanol-water leaf extracts of *J. secunda* and *J. tanjorensis.* The result shows a significant difference (P<0.05) in total phenolic content of the two plant extracts. At all concentration, *J. tanjorensis* showed higher and a concentration dependent increase in total phenolic content.

Table 4.9: Total flavonoid content (mg/ml) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*

Extract Conc. (mg/ml)	*Justicia secunda*	*Jatropha tanjorensis*	T-value	P-value	Comment
0	0.00 ± 0.00	0.00 ± 0.00	-	-	-
10	10.69 ± 0.14	8.63 ± 0.19	15.12	0.0001	Significant
20	11.87 ± 0.15	11.49 ± **0.1**6	3.001	0.0399	Significant
40	10.93 ± 0.18	13.79 ± 0.13	22.31	<0.0001	Significant
80	11.59 ± 0.16	12.48 ± 0.17	6.603	0.0027	Significant

Table 4.9 shows total flavonoid content (mg/ml) of ethanol-water leaf extracts of *J.secunda* and *J.tanjorensis.* The result shows a significant difference (P<0.05) in total flavonoid content of the two plant extracts. At extract concentrations of 10mg/ml and 20mg/ml *J.secunda* showed higher total flavonoid contents of 10.67±0.14mg/ml and 11.87 ±0.15mg/ml. The highest total flavonoid content was observed in *J.tanjorensis* at extract concentrations of 40 and 80mg/ml.

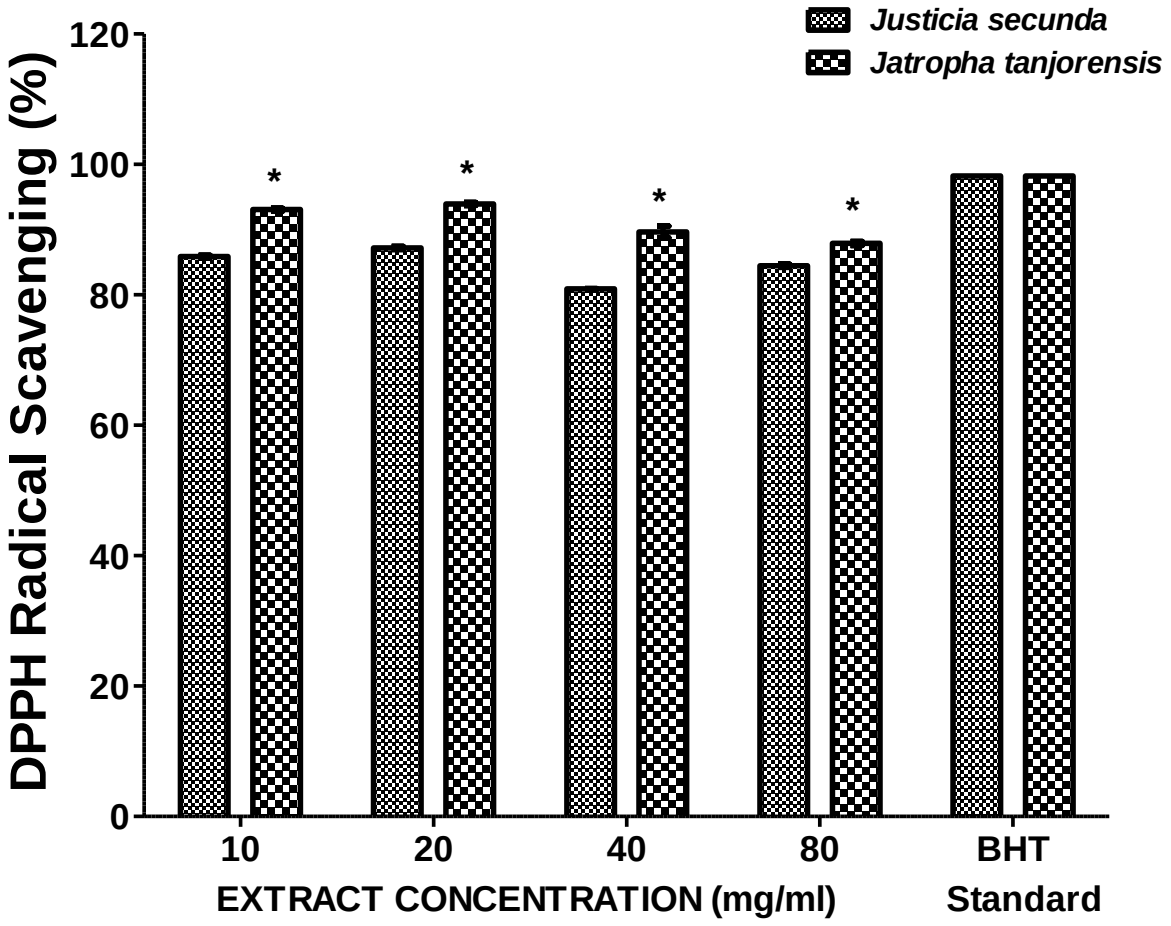


Figure 4.2: DPPH Radical Scavenging activity (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.* Bars are mean ± standard deviation of triplicate determinations. * DPPH values of *Jatropha tanjorensis* significantly ($p<0.05$) higher than corresponding *Justicia secunda* DPPH values.

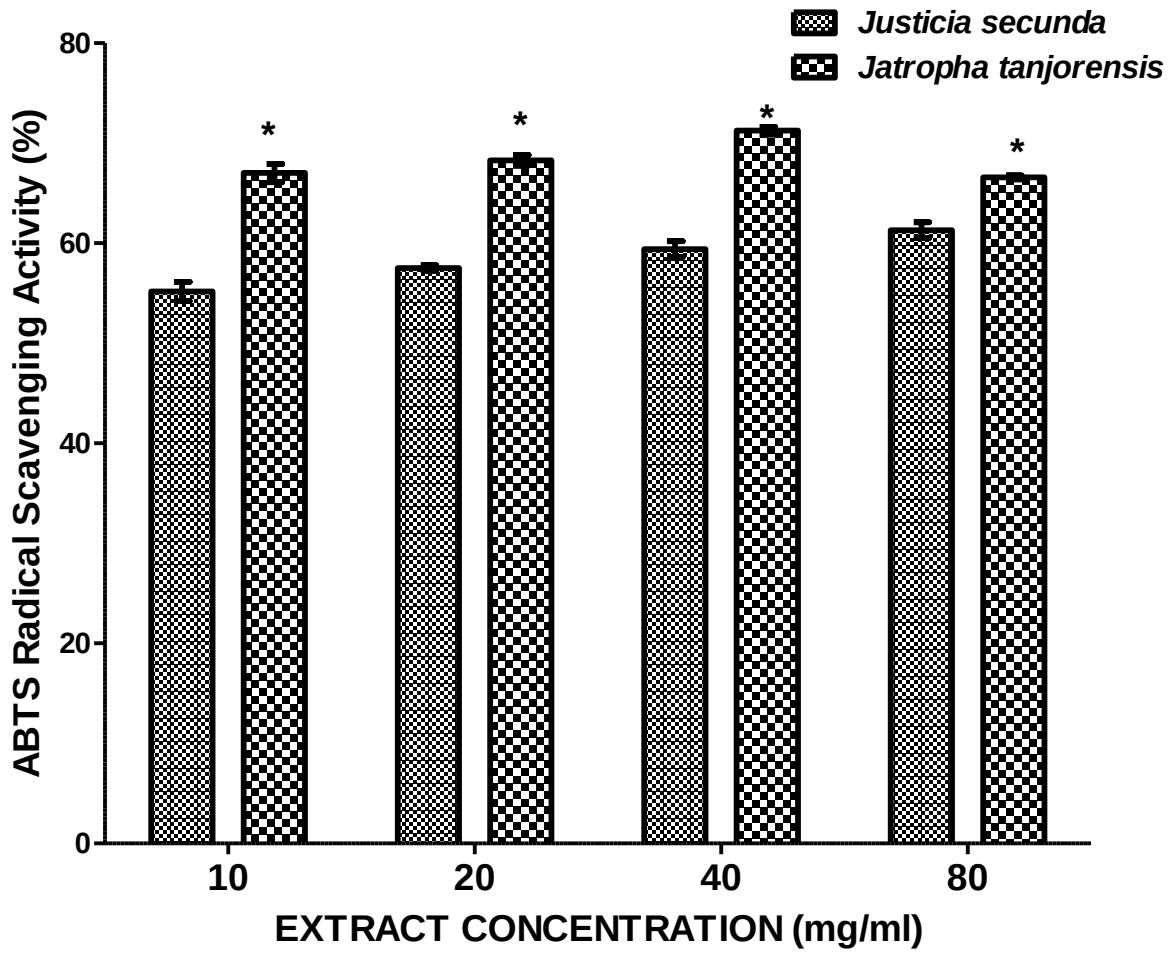


Figure 4.3: ABTS Radical Scavenging activity (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*. Bars are mean ± standard deviation of triplicate determinations. * DPPH values of *Jatropha tanjorensis* significantly ($p<0.05$) higher than corresponding *Justicia secunda* DPPH values

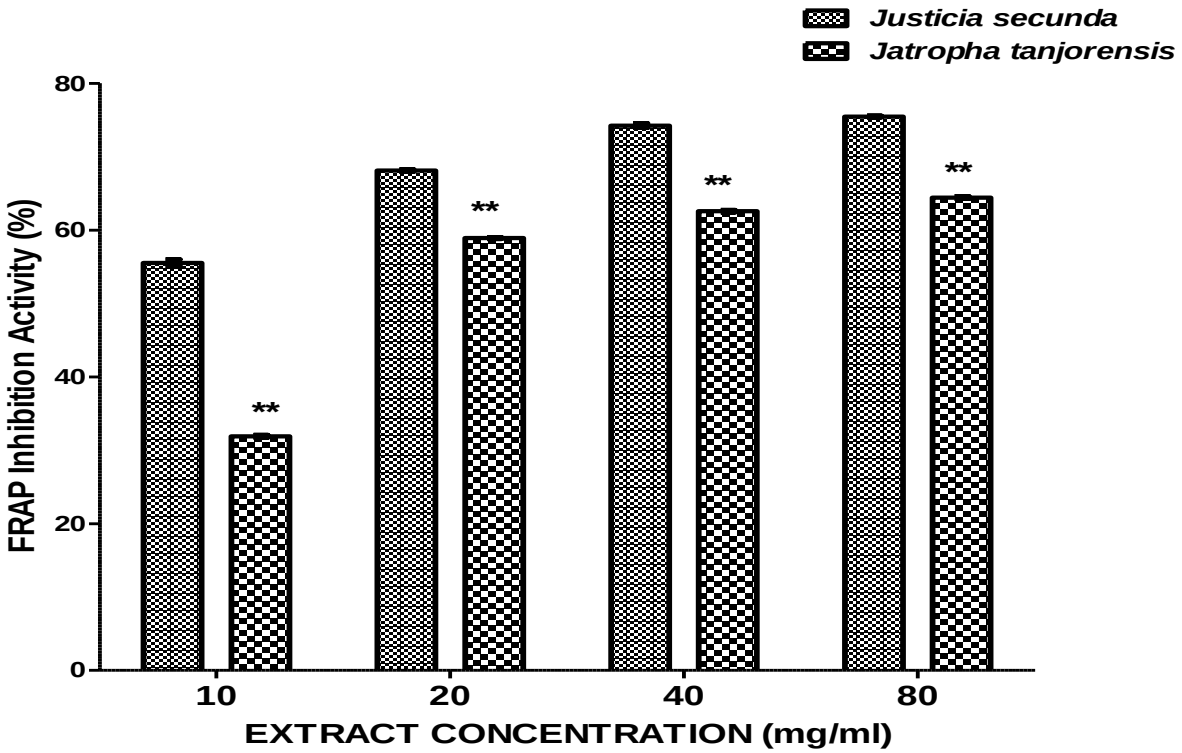


Figure 4.4: FRAP Radical Scavenging activity (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*. Bars are mean ± standard deviation of triplicate determinations. ** FRAP values of *Jatropha tanjorensis* significantly ($p<0.05$) lower than corresponding *Justicia secunda* FRAP values.

Table 4.10: Brine shrimp lethality (% mortality) effect of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.*

Extract Concentration (µg/ml)	***Justicia secunda***	***Jatropha tanjorensis***	**T-value**	**P-value**	**Comment**
1	13.31 ± 0.03	39.79 ± 0.03	1081.0	<0.0001	Significant
10	27.62 ± 0.03	48.98 ± 0.04	739.9	<0.0001	Significant
100	37.88 ± 0.03	57.25 ± 0.02	930.5	<0.0001	Significant
500	58.30 ± 0.03	71.13 ± 0.02	616.3	<0.0001	Significant
1000	71.08 ± 0.03	83.47 ± 0.02	595.2	<0.0001	Significant
LC50 value	7.59 (x 10^2) ± 0.03	9.89 (x 10^3) ± 0.03	93.90	<0.0001	Significant
Inference	Low toxicity	Non toxic	-	-	-

Table 4.10 shows the result of brine shrimp lethality (%mortality) effect of ethanol – water leaf extracts of *J. secunda* and *J. tanjorensis*. There is a significant difference ($p<0.05$) in % Mortality effect of the extracts at different concentration. *J. secunda* showed an LC50 value of 7.59 (x 10^2) ± 0.03 as against 9.89 (x 10^3) ± 0.03 observed in *J. tanjorensis.*

4.2 DISCUSSION

The result of the proximate composition of ethanol-water leaf extract of *Justicia secunda* and *Jatropha tanjorensis* (Table 4.1.1) indicated there were significant ($p< 0.05$) differences in the percentage moisture, fibre, ash, fat, protein and carbohydrate composition of the leaves. The results showed that *Justicia secunda* has higher moisture (6.37%), fat (8.45%), and protein (10.15%) while *Jatropha tanjorensis* has higher ash (9.79%) and fibre contents (5.22%). Both leaves are rich sources of carbohydrates with *Jatropha tanjorensis* having a slightly higher percentage content (62.22%) as against 61.52% seen in *Justicia secunda* . The results showed that for both leaves, the main nutritional constituents followed this order: carbohydrate > protein > ash > fat > moisture > fibre. Just like this findings, Arogbodo, (2020) and Onochie *et al*., (2020) had reported carbohydrate as the highest nutritional constituents of *Justicia secunda* leaves. Conversely, our study found a higher percentage of carbohydrate than in the cited reported. Ajuru *et al*. (2021) reported fibre and ash as the major nutritional constituents of *Justicia secunda* root. As against our findings that carbohydrate (62.22%) was the major nutritional constituent of ethanol-water leaf extract of *Jatropha tanjorensis*, Idu *et al*, (2014) reported protein (41.65%) and fat/oil (36.73%) as the major nutritional constituents in the leaves of *Jatropha tanjorensis.* In another study, Nwachukwu (2018) and Anhwange *et al*., (2019) reported moisture content (81.62% and 80.59% respectively) as the major nutritional constituents of *Jatropha tanjorensis* leaves. Results our study generally suggest that both leaves could serve as good dietary sources of carbohydrate to humans and omnivorous animals, providing a cheap and good source of energy. Carbohydrate is the primary constituent of all normal diets and provides most of the energy required of the body. Carbohydrates yield glucose on hydrolysis which serves as an important and immediate source of energy and may be stored in the liver and muscles to be mobilized and utilized when needed

by the body (Raven *et al*., 1999; Okeke *et al*., 2008). The protein contents were 10.15 ± 0.10% in *Justicia secunda* and 9.80 ± 0.07% in *Jatropha tanjorensis* leaves. Suffice it to say that both leaves are not excellent sources of protein. This is in accordance to Pearson, (1976) who stated that plant foods are considered good sources of protein only if more than 12% of their caloric value is made up of protein. Nevertheless, both leaves could still be fair sources of dietary or protein supplements, especially for vegetarians, people who live in rural areas, and people who have protein deficient diseases (Mgbeje *et al*., 2019). Proteins remain essential biomolecules and are a major constituent of the body. Their roles are vital and highly diversed and seen in numerous physiological processes such as cell division and growth (Sholey *et al*., 2003), as a part and pro-immune system component (Kelley *et al*., 2017), required in enzyme, blood plasma and hormone synthesis (Mathew *et al*., 2021). According to Igile *et al*. (2013), it is an essential part of human diet required for the replacement of dead tissues and for the supply of energy and sufficient amount of required amino acids. (Arogbodo, (2020) and Onochie *et al.*, (2020) have earlier reported a lower fibre content of 0.60% and 6.51% respectively in leaves of *Justicia secunda* . The highest fibre content of 55.18% was reported in the roots of *Justicia secunda* by Ajuru *et al*. (2021). On the other hand, fibre contents of 2.80% and 3.46% were reported by Nwachukwu, (2018) and Idu *et al*. (2014), while Anhwange *et al*. (2014) reported a higher fibre content of 7.15%. In humans, dietary fibers have a significant impact on blood cholesterol levels (Schweizer and Würsch, 1991). Studies have shown that compared to matched controls, vegetarians who consume high-fibre diets possess lower plasma total cholesterol, LDL cholesterol, and HDL cholesterol (Knuiman and West, 1982). After ten years of observation, men who consumed more fiber were less likely to develop atherosclerosis and certain other degenerative diseases than those who consumed

less fiber (Kromhout *et al*., 1982). Dietary fiber is essential for regular bowel movements, the avoidance of piles and prevention of constipation (Asaolu *et al*., 2012).

Justicia secunda and *Jatropha tanjorensis* leaves have low fat content. Because the leaves are low in fat, they can be included in the diets of individuals that aim to lose weight. The findings indicated that the leaves are poor sources of plant-derived fats. Fats and oil are essential in human health as they serve as sources of energy and components of biological membranes (Michelle *at al*., 1993). Fats are secondary plant products that yield more energy per gram than carbohydrate. Dietary fats are crucial owing to their high calorific value as well as the essential fatty acids and fat-soluble vitamins that are found within fat of natural foods. Fats and oils are beneficial in the production and repair of essential cell components, as well as in blood pressure regulation (Dutta, 2003).

When the organic matter in a food sample is completely oxidized or ignited, the inorganic residue left over is referred to as ash. A food's ash content is important because it represents a measure of the food's overall mineral composition (Vunchi *et al*., 2011). As a result, the ash content of the leaves is determined by the mineral elements present in the leaves. The ash contents obtained were 8.89 ± 0.07 and 9.79 ± 0.09% for *J. secunda* and *J. tanjorensis* respectively. Ash contents of 9.81% in *Justicia carnea* and 16.75% in *Justicia secunda* were reported by Ajuru *et al*. (2021). Earlier, Onochie *et al*. (2020) had reported an ash content of 12.83% in *Justicia secunda* . In similar studies, Idu *et al*. (2014), Nwachukwu (2018) and Anhwange *et al*., (2019) found ash contents of 4.71%, 0.98%, and 5.06% in *Jatropha tanjorensis* leaves. It should be noted that their findings were significantly lower than those of the present study. Dietary ash has been shown to be important in creating and maintaining the blood system's acid-alkaline balance, in addition to controlling hyperglycemia (Gokani *et al*., 1992).

The moisture contents were 6.37 ± 0.09% in *Justicia secunda* leaves and 5.32 ± 0.07% in *Jatropha tanjorensis* leaves. Moisture content of food is an indicator of its water activity (Olutiola *et al*., 1991). The amount of moisture in a product determines its stability and vulnerability to microbiological contamination and spoilage (Uraiha and Izuagbe, 1990). Because both leaves have low moisture content, they can apparently be stored for long duration of time without deterioration.

Results of the mineral composition analysis showed a significant difference ($p<0.05$) in sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) composition of both leaves whereas, there is no significant difference ($p>0.05$) in potassium (K) and manganese (Mn) contents of the leaves. Among these mineral elements identified, Na, K, Ca and Mg are macro minerals while Cu, Zn, Fe and Mn are essential trace elements or minerals. Both leaves contain more macro minerals/elements than the trace minerals/elements. The result of the mineral composition of the leaves of *J. secunda and J. tanjorensis* obtained is not comparable to that of Onochie *et al*. (2020) who had earlier reported a higher mineral composition in leaves of *Justicia secunda* except for Mg which is higher in the present study. Another higher mineral composition in *J. secunda* was reported by Anhwange *et al*., (2019). Their findings indicated the highest amount of calcium (98.10 mg/kg) reported so far in *J.secunda*, followed by iron (35.34 mg/kg). They went further to report potassium and zinc as 13.73 and 13.83 mg/kg respectively, while Mg, Mn, and Cu were 5.85, 6.83 and 0.76 mg/kg respectively. In yet another investigation, Ogunbamowo *et al*., (2020) reported mineral compositions lesser than the findings of this study, both in macro minerals and micro minerals. Okunade and Adesina, (2014) reported higher Na (9.33mg/100g), Mg (33.30 mg/100g), K (110.30 mg/100g) but lesser Ca (1.38± mg/100g), Fe (1.22 mg/100g), and Zn (0.01 mg/100g) in the leaves of *Jatropha tanjorensis.*

Sodium and potassium are complementary macrominerals. Sodium is an important intracellular cation component, while potassium remains an essential extracellular cation

component (Asaolu *et al*., 2012). As one of the principal electrolytes in the blood, sodium contributes to the contraction of muscles, maintenance of acid-base balance, the transmission of nerve impulses, and the control of plasma volume (Akpanyung, 2005; Ghanghro, 2019). The RDA for sodium is 500 mg/day. Low plasma sodium occurs due to clinical conditions such as trauma, cachexia, overuse of diuretics. Anorexia nervosa, congestive heart failure, ulcerative colitis, liver disease and diarrhea are all conditions that can lead to loss of sodium. Thus, the leaves of *J. secunda* and *J. tanjorensis* can be relied upon to supply fair amount of sodium as a means of dietary supplementation.

Potassium concentrations in both leaves of *J. secunda* and *J. tanjorensis* were 8.49 and 8.58 mg/kg respectively. Just like sodium, potassium plays pivotal roles in transmission of nerve impulses and contraction of skeletal muscle (Toaheed *et al*., 2017).

Calcium was found to be 5.68mg/kg in *J. secunda* and 5.99 mg/kg in *J. tanjorensis*. Calcium promotes blood clotting, muscle contraction and relaxation, vitamin B12 absorption, and strong teeth and bones (Toaheed e*t al*., 2017). Controlling calcium levels is crucial for both insulin release and resistance (Ozcan and Tabas, 2016). Calcium balance is disrupted in diabetics, which plays a role in the abnormal cell control of skeletal muscles, cardiac muscles, erythrocytes, and platelets (Dubey *et al*., 2020). According to Pittas *et al*. (2007), alterations in vitamin D and calcium concentrations are associated with the onset of type 2 diabetes (T2DM). High amounts of cytosolic calcium have been linked to insulin resistance in cell culture studies (Henquin, 2011). In addition, a dose-dependent meta-analysis of cohort studies found that calcium in the diet protects against type 2 diabetes (Aune *et al*., 2013; Gijsbers *et al*., 2016).

Magnesium levels were 9.39 mg/kg in *J. secunda* and 8.48 mg/kg in *J. tanjorensis*. Magnesium is utilized in a variety of biological processes, serving as a cofactor in over 300

enzyme systems that govern various of biochemical processes in the body, including protein synthesis, muscles and neuron functions, blood sugar balance, and blood pressure regulation. (Institute of Medicine, 1997; Rude, 2010; Rude, 2012). Additionally, it aids in the synthesis of RNA, DNA and glutathione, an important antioxidant (Romani, 2013). Muscle contraction, nerve impulse conduction, and regular heart rhythm all rely on the active transport of potassium and calcium ions across cell membranes, a process that cannot occur without magnesium (Rude, 2012). The recommended dietary allowance (RDA) for magnesium is 400 mg/day for healthy adult males and 320mg/day for heathy adult females (Institute of Medicine, 1997). Among the best sources of dietary magnesium are; whole grains, spinach, nuts, legumes and seeds (USDA-ARS, 2012). Magnesium deficiency is uncommon but seen majorly because of low dietary intake or alcoholics (Rude, 2012; USDA-ARS, 2012). Studies have also shown that inadequate intake of Mg results in muscle spasm, diabetes, high pressure, migraines, osteoporosis, cerebral infarction (Kobrin and Goldfarb, 1990; Larsson *et al*., 2008; Romani, 2013) and hypocalcemia or hypokalemia in severe cases. Therefore, Mg supplementation through dietary intake of these leaves is recommended.

Copper (Cu) levels observed were 0.79 and 0.67 mg/kg for *J. secunda* and *J. tanjorensis* respectively. Cu is an essential trace mineral in both plants and animals (Al-fartusie and Mohssan, 2017). A minute amount of 150mg of this essential mineral is contained in the human body (Al-fartusie and Mohssan, 2017). In the gut, copper is absorbed and subsequently transferred to the liver coupled to albumin. Cu is transported to various organs after being metabolized in the liver. The majority of the body's Cu reserves are transported to the liver via the protein ceruloplasmin. Numerous enzymes, including, ascorbic acid oxidase, peroxidase monoamine oxidase, catalase, and cytochrome oxidase require copper as an important component. The hematologic and neurological systems need it. It is necessary for

the development of myelin sheaths, the operation of neurological systems and the integration of iron into hemoglobin (Tan *et al*., 2006). The recommended dietary allowance (RDA) for Cu is 2mg/day in healthy adults (National Research Council, Food Nutrition Board,1980). Among the best-known dietary sources of Cu are sunflower seeds, peanuts, almonds, walnuts, mushrooms, barley, wheat, fish and cashews (Roger, 2011). Malabsorption of Cu after gastric bypass surgery and other forms of remote gastrointestinal surgery is the leading cause of Cu deficiency whereas acquired Cu deficiency is usually as a result of dietary deficit frequently seen in malnourished infants with low birth weight, newborns, and young infants (Tan *et al*., 2006). Therefore, decoction of these leaves maybe given as a cheap supplementation especially among indigent rural dwellers.

Results obtained for Zn are similar to those of Cu. Zn is found throughout the entire body system with half seen in the muscle tissue (Zevenhove and Kilipinen, 2001). It's a vital mineral that helps metabolism and cell growth by acting as a cofactor for enzymes. Nearly 300 different enzymes use it (Prasad, 2003; Plum *et al*., 2010; Osredkar and Sustar, 2011). As a component of several enzymes, it is involved in the metabolism of proteins, lipids, energy, and carbohydrates (Al-fartusie and Mohssan, 2017). It plays a crucial role in maintaining a healthy immune system and a strong resistance to infections and promoting radiant skin (Al-fartusie and Mohssan, 2017). Zn acts as a necessary requirement for DNA and protein synthesis, insulin activity, and liver function throughout cell division and development. Zn is also crucial for metabolism in ovaries and testes (Prasad, 2003; Plum *et al*., 2010; Osredkar and Sustar, 2011; Burjonrappa and Miller, 2012). Inadequate dietary intake of zinc may lead to zinc deficiency. It is estimated that almost two billion people in developing countries are zinc-deficient (Prasad, 2003). The RDA for Zn is 8mg/day for women and 11mg/day for men (Wellman, 2009). Good amount of dietary zinc has been found in wheat, brown rice, soybean,

oats, peanuts, walnuts and numerous vitamin mineral supplements such as citrate and sulfate (Ensminger and Ensminger, 1993; Berdanier *et al*., 2007; Wellman, 2009). Severe Zn deficiency results in hair loss, delayed sexual maturation, impotence, hypogonadism in males, delayed healing and taste abnormalities (Heyneman, 1996; Prasad, 2004; Maretm and Sandstead, 2006). Therefore, leaves of *J. secunda* and *J. tanjorensis* are recommended as dietary supplements for such conditions. World Health Organization also recommends Zn supplementation in severe cases of diarrhea and malnutrition (WHO, 2007).

This study reported 1.99 mg/kg of iron in *J. secunda* and 1.67 mg/kg in *J. tanjorensis*. Iron is the most abundant metal in the human body (Al-fertusie and Mohssan, 2017). Fe concentration in the body is roughly 3 to 4 grams, which is virtually equivalent to a concentration of 40 to 50 milligrams of iron per kilogram of body weight (Crichton *et al*., 2008). The most of the iron in the body is housed within hemoglobin. The distinctive red color of blood is attributable to the presence of Fe in the hemoglobin (Wessling-Resnick, 2014). Iron is also a crucial component of myoglobin (Agget, 2012). Iron is essential for development, synthesis of certain hormones, connective tissues, normal cellular functions, growth and development (Murray-kolbe *et al*., 2010; Agget, 2012). Men and post-menopausal women have an iron RDA of 8mg per day, whereas menstruation women have an iron RDA of 18mg per day (Russell *et al*, 2001). This is because of the frequent high blood volume lose during their monthly period. The following are rich dietary sources of iron, seafood, leafy vegetables, chickpeas, black-eyed peas, poultry, beans, liver and red meat. Investigations have revealed that iron in meat is more easily absorbed than that in vegetables (Roger, 2011). Due to the iron content of the leaves, frequent consumption of these leaves is recommended for people suffering from iron-deficient conditions such as anemia as the leaves will provide some dietary portions. Mn is another important trace mineral found in the body in a minute quantity.

About 12mg of Mn can be found in a typical human body. The skeletal system contains roughly 43% of the body's manganese, with the remainder distributed throughout soft tissues such as liver, pancreas, kidneys, brain, and central nervous system (Roger, 2011; Emsley, 2011). Mn aids the body in the formation of bones, sex hormones, connective tissues and blood-clotting factors (Fraga, 2005; Palacios, 2006). Additionally, it is necessary for calcium absorption, blood sugar control, and fat and carbohydrate metabolism (Henn *et al*, 2010; Avila *et al*., 2013). Mn is a part of the antioxidant SOD, which aids in the defense against free radicals (Law *et al*., 1998; Treiber *et al*., 2012). Mn RDA for adults is 2.3mg/day for men and 1.8mg/day for females (Russell *et al*.,2001). Dietary sources include peas, dried beans, bulgur wheat, rye, oats, brown rice, corn, legumes, leafy greens and sea vegetables (Cabrera-Vique and Bouzas, 2009; US Department of Health and Human Services, 2010; Roger, 2011). The leaves of both *J. secunda* and *J. tanjorensis* can be recommended as a dietary supplement in people with impaired glucose tolerance, hypercholesterolemia, dermatitis and infertility (Berdainer, 2001; Soetan *et al*., 2010; Dutta and Mukta, 2012).

Amino acid compositions of ethanol-water leaf extracts of *J. secunda* and *J. tanjorensis* are shown in Table 4.3. There significant ($p<0.05$) differences in the amino acids contents detected in the plants, except in valine, lysine, methionine and tyrosine which showed no significant ($p>0.05$) difference. Among the eighteen amino acids identified, nine belonged to the special class of amino acids known as essential amino acids (EAA) while the remaining nine were of non-essential amino acids (NEAA). The highest essential amino acid found in *J. secunda* was valine followed by histidine, lysine and phenylalanine while phenylalanine, valine, isoleucine and lysine were the highest occurring essential amino acids in *J. tanjorensis*. The highest non-essential amino acids (NEAA) identified in *J. secunda* was glycine, followed by alanine, proline and aspartate while proline, glutamate, arginine and tyrosine were the most abundant in *J.*

tanjorensis. The result obtained in this study were lower in comparison to the values of amino acids of some important medicinal plants that are also used as blood boosters such as the seeds of *Mucuna pruriens* (Fitriyah *et al*., 2021), *Telfairia occidentalis*, *Amaranthus hybridus* and *Solanum aethiopicum* (Adeyeye and Omolayo, 2011; Aja *et al*., 2021). Though, the methionine composition of *A. hybridus and T. occidentalis* as reported by Aja *et al*, (2021) were lesser than the values obtained in the present study. In another study, Najda *et al*. (2013) identified only four amino acids namely; asparagine, proline, cysteine and histidine in *Jatropha curcas* an important members of the *Jatropha* family. Amino acids are essential biomolecules required for protein building in the body. Each amino acids whether essential or non-essential plays a critical role in disease prevention as well as in the transportation of nutrients (Ogunka-Nnoka *et al*., 2020).The amino acid threonine plays a critical role in building muscles and also helps maintain a healthy equilibrium between the brain's various neurotransmitters (Watford, 2008). Isoleucine speeds up protein synthesis and aids in building muscle, both of which are crucial for maintaining stable blood sugar levels (Elango *et al*., 2009; Chmurzynska, 2010). Glutamic acid and alanine protect the heart and blood vessels and give energy to the body's cells (Bruhat *et al*., 2009). Glycine is important for the health of the brain and prostate (Wu *et al*., 2004; Mohammad *et al*., 2017). For a healthy and functional liver and cartilage, methionine is pivotal (Chen *et al*., 2007; Palii *et al*., 2009). Tyrosine functions as a monomeric unit for synthesis of proteins and polypeptide (Wang *et al*., 2008). The amino acid lysine is essential for proper cell division and growth; without it, fatty acid metabolism is disrupted and connective tissues are weakened (Elango *et al*., 2009). In humans, arginine aids in the production of growth hormones and insulin, which are essential for a strong immune system (Wu *et al*., 2004; Tan *et al*., 2009). Phenylalanine on the other hand plays a key role in the processes of glucose synthesis in the brain, insulin secretion, and fat

oxidation (Brosnan *et al*., 2010). Collagen syntheses, as well as the synthesis of adrenaline, creatine, DNA, and RNA, require the amino acids proline and serine (Brasse *et al*., 2009; Brosnan *et al*., 2010). Aspartate was discovered to have a crucial role in RNA and DNA metabolism, immunoglobulin and antibody formation, and other cellular processes. It aids the body in promoting a strong metabolism and in the treatment of depressive symptoms and weariness (Albrecht *et al*., 2010).

Phytochemical screening showed high presence of alkaloids, flavonoids, and tannins in *J. secunda,* while alkaloids, saponin, flavonoids, tannins and cardiac glycosides were seen appreciably in *J. tanjorensis*. On the other hand, low content of saponin, resin, cardiac glycosides and terpenoids were seen *J. secunda* while only resin and terpenoids were low in *J. tanjorensis*. This result is comparable to those of Osioma and Hamilton-Amachree (2017), Arogbodo (2020), Ogunbamowo (2020) and Ajuru *et al*., (2021), and Oyewole and Akingbala, (2011), Omoregie and Osagie (2017) and Igbinaduwa *et al*., (2017) who reported the presence of the same phytochemicals as revealed by this study in various leaf extracts of *J. secunda* and *J. tanjorensis* respectively. These are secondary metabolites isolated from plants that have been shown to provide health benefits for humans, such as the prevention or treatment of disease (Ajuru *et al*., 2017). Besides luring in pollinators and dispersal animals, plants rely on secondary metabolites for structural and mechanical support. In addition to protecting the plants from predators and pathogens, the secondary metabolites also reduce the growth of neighboring plants, which lessen competition, and protects the plant from harmful UV rays (Aziz, 2015). Some of these secondary metabolites and their derivatives are have medicinal properties (Haddad-Kashni *et al*., 2012). Alkaloids for example are a class of plant-friendly compounds (Igwe *et al*., 2010). They have been shown to have a variety of pharmacological properties, including antiasthmatic, anticancer, and antimalarial properties (Kittakoop *et al*.,

2014). While alkaloids can be toxic, they have been shown to have vasodilatory, antihyperglycemic, cholinomimetic, antiarrhythmic (Shi *et al*., 2014), antimicrobial, and analgesic characteristics (Cushnie *et al*., 2014; Robbers *et al*., 2016). The antimicrobial, anti-allergic, antioxidant, hepatoprotective, anti-inflammatory, antiviral and anticancer properties of many plants have been attributed to their flavonoids content (Watson, 2000). In addition, flavonoids inhibit platelet aggregation (Okwu and Emenike, 2006). Tannins are important secondary metabolites of plants with different medicinal importance. Because of their astringent and haemostatic qualities, they help wounds heal faster and reduce mucus membrane inflammation (Ajuru *et al*., 2017). Additionally, tannins have been shown to be powerful and long-lasting antioxidants (Awosika, 1991; Ogunleye and Ibitoye, 2003). Tannins impede digestion of proteins in foods by forming complexes with digestive enzymes (D`Amelia, 1998). Saponins, on the other hand, are a type of potent expectorant that are crucial in the treatment of inflammations of the upper respiratory tract. Anti-fungal and anti-diabetic activities have also been attributed to them (Trease and Evans, 1989; Kamel, 1991). Saponins are characteristically foamy hence known as natural detergents. They have a wide variety of pharmacological effects, including anticancer activity, immunological modulatory effects, and the suppression of cancer cell proliferation. Cholesterol-lowering effects of saponins have also been studied (Jimoh and Oladji, 2005). Terpenoids are thought to be the most prevalent chemicals in natural products, with a variety of medicinal and pharmacological functions such as anti-tumor, anti-inflammatory, antibacterial, antiviral, and hypoglycemic properties (Yang *et al*, 2020). They have also been shown to have wide anti-cancer capabilities (Chen *et al*., 2015).

Cardiac glycosides are steroids that have the capability to operate specifically and strongly on the heart muscles. A minute amount is just potent enough to stimulate healing of a heart that

has been damaged by disease (Morsy, 2017). They are crucial for treating congestive heart failure (Kelly, 1990). Without increasing oxygen consumption, they promote free cardiac contraction. As a result, the myocardium improves its pumping ability and can keep up with the needs of the cardiovascular system (Kelly, 1990). Plants use resins, which are sticky secretions that harden when exposed to air, largely for defense against herbivores and microbes (Langenheim, 1990).

Results of the quantitative phytochemical and anti-nutrient analyses of *J. secunda* and *J. tanjorensis* indicated significant ($p<0.05$) differences in the values of all phytochemicals and anti-nutrients studied except in the values of dihydrocystistine and flavone. The concentrations of all the identified phytochemicals and anti-nutrient factors were higher in *J. tanjorensis* compared to *J. secunda*. The most abundant anti-nutrient factors detected in both leaf extracts are tannin (18.88 ± 0.28 µg/ml, 26.12 ± 0.15 µg/ml), followed by phytate (4.15 ± 0.59 µg/ml, 7.74 ± 0.11 µg/ml) and oxalate (2.53±0.37 µg/ml, 3.22 ± 0.15 µg/ml). Tannins are regarded as anti-nutrients because they cause proteins to clump together, making them hard to be digested therefore reducing the amount of amino acids in the body. This happens when the hydroxyl group of tannins and the carbonyl groups of proteins form reversible or irreversible tannin-protein complexes (Lampart-Szczapa *et al*., 2003; Raes *et al*., 2014). There are two kinds of tannin groups: hydrolysable groups and condensed groups. Hydrolyzable tannin groups are easily broken down in ruminant animals during digestion. Substantial quantities of potentially toxic compounds are produced as a byproduct of the breakdown process (Kumar, 1992). Goats can tolerate the highly concentrated tannins found in legume fodder and certain seeds, while cattle and sheep are extremely susceptible to them (Bhattarai *et al*., 2016; Smeriglio *et al*., 2017; D`Mello, 2000). Ingestion of tannins lead to production of a variety of digestive enzymes which consequently results in reduced protein digestion

(Joye, 2019). Another important anti-nutrient factor identified in the leaf extracts is phytate. The phytate content of the leaf extracts were 4.15 ± 0.59 and 7.74 ± 0.11 µg/ml respectively. With reference to Onimawo and Akubor (2012), the levels of phytate detected in both leaf extracts falls outside the tolerable range. Onimawo and Akubor (2012) had stated that ingestion of 2.5g or more of phytic acid per day has been reported to cause a decrease in bioavailability of certain mineral elements such as calcium, zinc and iron. The result obtained from this study is lower than that of Ajuru *et al* (2021) who reported higher values of tannin, phytate and cyanogenic glycosides in ethanol leaf extracts of *J. carnea* and *J. secunda.* Nwachukwu (2018) had also reported a higher value of phytate in *J. tanjorensis.* Phytates also reduce the bioavailability of magnesium. Phytates have been linked to the elimination of phosphorus and the production of indigestion and flatulence in humans (Groff *et al*., 1995). The concentration of oxalate found in *J. secunda* and *J. tanjorensis* were 2.53 ± 0.37 and 7.74 ± 0.11 µg/ml respectively. An insoluble salt is formed when oxalate is present in food, which both irritates the tongue and prevents the body from absorbing divalent metallic cations like calcium and iron (Ola and Oboh, 2000; Ladeji *et* al., 2004). In the case of calcium, it prevents calcium from performing its usual biochemical and physiological functions, such as maintaining healthy bones and teeth, acting as a cofactor in enzyme reactions, clotting factors, and nerve impulse transmission. In addition, eating foods high in oxalate can lead to hyperoxaluria, which increases the likelihood of developing kidney stones (Chai and Liebman, 2004; Hassan and Umar, 2004; Hassan *et al*, 2007). The results obtained from this investigation therefore showed that both leaf extracts contain important phytochemicals with diversed medicinal properties but *J. tanjorensis* has higher contents of the anti-nutrient factors - tannins, phytates and oxalates. Generally, however, the concentrations of these antinutrient

components in the leaves are within the permissible limits so insufficient to cause toxicity when ingested except for phytate.

The results of the GC-MS phytochemical analysis of the ethanol-water leaf extracts of *J. secunda* and *J. tanjorensis* are presented in Tables (4.6a-4.6f) and (4.7a-4.7f) respectively. GC-MS analysis of *J. secunda* revealed sixty-two (62) prominent peaks indicating sixty-two bioactive compounds. The major compounds were; 2,4-Di-tert-butylphenol (8.08%), decane,3,8-dimethyl (7.72%), heptadecane,2,6,10,14-tetramethyl (4.72%), heptadecane,2,6-dimethyl (4.51%), carbonic acid, nonyl vinyl ester (3.37%), hexadecane,3 methyl (3.38%), and dodecane (3.16%). The least abundant bioactive compounds detected in *J. secunda we*re 3-eicosene (0.15%), dodecane, 1,1`-oxybis (0.16%), 1-octadecanesulphonyl chloride (0.19%), hexacosanoic acid (0.20%), cis-vaccenic acid (0.20%), citronello (0.23%), oxirane, tetradecyl (0.23%), trifluoroacetoxy hexadecane (0.24%), and cyclopentadecanone, 2-hydroxyl- (0.26%). Most of the identified bioactive compounds were alkanes, fatty acid esters, aromatic hydrocarbons, alkenes, fatty acid methyl esters and cycloalkanes. Many of these bioactive compounds have been reported to show diverse biological activities. 2, 4-Di-tert-butylphenol for example, has been reported to possess the following biological activities; anticancer (Nair *et al.*, 2020; Seenivasan *et al*., 2022), anti-proliferative, anti-fungi, cytotoxic (Chawawisit *et al*., 2015b; Seenivasan *et al*., 2022), antioxidant (Belght *et al*., 2016), anti-inflammatory, and antiviral (Sang *et al*., 2012) effects. Dodecane has antioxidant activity (Nandhini *et al*., 2015), while undecane has anticancer (Van Duuren, 1976), anti-inflammatory, anti-allergic (Choi *et al*., 2020) and anti-fungal properties (Jeong-Ho *et al*., 2008; Sasidharam and Menon, 2010).1-Octadecene has been investigated to possess antibacterial, antioxidant and anticancer properties (Mishra and Sree, 2007; Lee *et al*., 2007). The result from the GC-MS analysis of *J. secunda* is comparable with that of Hamilton-

Amachree and Uzoekwe (2017) who reported the presence of 2-4-Di-tert-butylphenol, E-14-hexadecenol, hexadecanoic acid methyl ester and trifluoroacetoxy hexadecane in essential oil of *J. secunda*. The most abundant compound according to their report was 9,12,15-octadecatrienoic acid, methyl ester (36.65%), followed by 9-octadecenamide (7.12%), E-14-hexadecenal (6.3%), trifluoroacetoxy hexadecane (6.00%) and 1-heptadecene (5.68%). In similar study, bis (2-ethylhexyl) phthalate (52.52%), diethyl phthalate (28.71%) and (3aR, 4R, 7R)-1,4,9,9-tetramethyl -3,4,5,6,7,8-hexahydro-2H-3a,7-methanoazulen-2-one (6.71%) were the most abundant compounds detected (Odokwo and Onifade, 2020).

On the other hand, GC-MS analysis of *Jatropha tanjorensis* showed fifty-two peaks indicating 52 bioactive compounds. The major compounds detected were: docosane, 2,21-dimethyl (8.16%), 2,4-Di-tert-butylphenol (7.74%), 1-decanol, 2-hexyl (5.09%), undecane (4.88%), cyclohexane, 6-butyl-1-nitro (4.36%), and octadecane, 1-(ethnyloxy) (3.84%). The least abundant bioactive compounds were benzocycloheptatriene (0.15%), 2-chloro-4-fluorophenol, TBDMS derivative (0.23%), undecane, 5-methyl (0.24%), 2-methyl-z-z-3, 13-octadecadienol (0.29%), oxirane, tetradecyl (0.32%), 3-(Prop-2-enoyloxy) dodecane (0.32%), E-15-heptacenal (0.32%), carbonic acid, eicosyl vinyl ester (0.32%), benzene,1-ethyl-2,3-dimethyl (0.33%), 9-methyl-10,12-hexadecadien-1-ol-acetate (0.34%) and stearic acid hydrazide (0.36%). Habila *et al*. (2021) had earlier reported dodecanoic acid, 1,2,3-propanetriyl ester (22.93%) was most abundant in 70% ethanol leaf extract of *J. tanjorensis* followed by methoxyacetic acid, tridecyl ester (21.77%) and carbamic acid, N-(3-chloro-4-methoxyphenyl)-,glycidyl ester (14.56%), while in 98% ethanol leaf extract of *J. secunda*, lauric anhydride (73.54%) was the most abundant compound followed by 1H-1,2,3,4-Tetrazole-1-propanamide, N-(2-pyrazinyl) (5.85%) and docosanoic acid nonyl ester (5.26%). The authors further reported that 2-heptanol,4-methyl-(27.28%), octane, 2-methyl- (18.19%)

and 3-prop-2-enoyloxy dodecane (17.65%) were the most abundant phytocompounds in GC-MS analysis of aqueous leaf extract of *J. tanjorensis*. Interestingly none of these compounds were detected in the present study.

Table 4.7a-4.7f revealed that alkanes, alkene, fatty acid esters, fatty acid methyl esters and aldehyde were the most abundant groups of compounds detected. Many of the identified bioactive compounds have been reported to possess many biological activities as also shown in the table. Z-8 hexadecane has been reported to possess antimicrobial activity (Strobel *et al.*, 2010), 1-hexadecanesulfonic acid, 3,5-dichloro-2,6-dimethyl-4-pyridyl ester has antimicrobial and antioxidant properties (Hamad, 2015; Bichi and Abdulmumin, 2022). Studies have demonstrated antifungal and antibacterial properties of nonane (Povlovic *et al*., 2011). Tridecane has also been reported to possess antimicrobial and antioxidant activities (Duke, 1992; Faridha *et al*., 2016). Other bioactive compounds in *J. tanjorensis* with known biological activities are bis(2-ethylhexyl)phthalate which has antimicrobial, anticancer, antitumor and cytotoxic properties (Habib and Karim, 2009; Habib and Karim, 2012; Thenmozhi and Rajan, 2015); ethyl-1-dodecanol that is anti-helminthic (Dulara *et al*., 2017); and 9-12 octadecanoic acid methyl ester which has been shown to have antifungal, anticancer, antioxidant and anti-acne properties (Rajalakshmi and Mohan, 2016; Kumar *et al*., 2017; Abdurrahman and Cai-Xiab, 2020).

Analysis of antioxidant vitamins content of the plants showed vitamins' C, E and A concentrations of 72.65 ± 1.85, 17.96 ± 1.78 and 27.47 ± 0.14 mg/kg for *J. secunda* and 60.43 ± 1.63, 14.33 ± 1.33 and 24.61 ± 0.16 mg/kg respectively. The result shows that *J. secunda* and *J. tanjorensis* have high concentrations of the vitamins with *J. secunda* having more of these essential antioxidant vitamins. High contents of these vitamins observed in both leaves is an indication of their high antioxidant properties (Wasagu *et al*., 2013). Vitamin C also

known as L-ascorbic acid is seen in most living systems as ascorbate (Sies *et al*., 1992). Vitamin C is most abundant in the following parts of the human body: spleen, liver, blood cells, pituitary gland, brain, ovaries, adrenal glands, extracellular fluid surrounding the eye, and lungs. Vitamin C plays a vital role in hydroxylation reactions necessary for collagen formation and carnitine synthesis as well as the facilitation of iron absorption (Levine, 1986). It further acts as a chain-breaking antioxidant in lipid peroxidation (Doba *et al*., 1985; Niki *et al*., 1985). Specifically, in groups with low ascorbate, such as smokers, dietary ascorbate has been shown to defend sperm from endogenous oxidative DNA damage, which could decrease sperm quality and raise the chance of genetic abnormalities (Fraga *et al*., 1981). The body's own production of free radicals can be detrimental to genetic information stores including DNA, then enzymes and lipid membranes (Halliwell and Gutteridge, 1985) but vitamin C's antioxidant properties can help protect the body from these free radical damage (Bendich *et al*., 1986). Tocopherol, or vitamin E, is the principal lipid-soluble, chain-breaking antioxidants, notwithstanding its modest concentration in membranes (Olson, 1988). It is well-known as the immediate line of defense against lipid peroxidation, as its free radical-neutralizing activity in bio membranes shields polyunsaturated fatty acids in cell membranes from damage caused by free radicals (Horwitt, 1986; Van Gossum *et al*., 1988). In order to prevent and treat cancer, vitamin E is crucial (Pecker, 1991). In addition to scavenging free radicals, vitamin E boosts the body's immunological response when consumed in large amounts (Pecker, 1991). Additionally, it influences the stomach's transformation of nitrites to nitrosamines (Watson and Leonard, 1986). There are many different forms of vitamin A, including retinols, retinals, and retinoic acids (Polcz and Barbul, 2019). It is acknowledged as being crucial for maintaining healthy cells and tissues (Ross, 2010). The most well-known use of vitamin A is for maintaining vision. Additionally, it is essential for immunity, growth,

wound healing, and embryological development (Polcz and Barbul, 2019). Therefore, the richness of *J. secunda* and *J. tanjorensis* particularly in vitamins C and A is an indication of their importance as dietary sources of essential antioxidant vitamins which possess diverse biological and health benefits.

The results of the total phenolic contents (TPC) of *J. secunda* and *J. tanjorensis* are presented in Table 4.9. There are significantly ($p<0.05$) high TPC in the plants which increased significantly with increase in extract concentration. Meanwhile at all extract concentrations, *J. tanjorensis* showed a higher TPC than *J. secunda*. Phenolic compounds are the primary secondary metabolites that hugely contribute to the multifarious medicinal properties of medicinal plants such as anti-carcinogenic, anti-microbial, anti-inflammatory, anti-allergic and anti-viral (Atik and Mohammedi, 2011). A number of epidemiological investigations have found a correlation between eating foods high in flavonoids and phenolic compounds with powerful antioxidant activities and a lower risk of developing diabetes, cardiovascular disease, neurodegenerative illness, and cancer (Adebooye *et al*., 2008). Plant polyphenolics in human diets have been reported as the most predominant antioxidants (Dutta and Ray, 2020). Scientists have found that overall phenolic levels correlate positively with the antioxidant properties of plant extracts (Sun *et al*., 2002; Kauer and Kapoor, 2002). Hence plants with the high total phenolic contents have potentials for greater antioxidant activity (Cai *et al*., 2006; Igwe *et al.,* 2021). Besides antioxidant activity, total phenolic content has been reported to correlate with mainly, the position and number of primary - OH group as well as molecular structure (Cai *et al*., 2006).

As was observed with TPC, total flavonoid contents (TFC) of both *J. secunda* and *J. tanjorensis* were appreciably high and TFC values increased with increase in extract concentration. Flavonoids have huge pharmacological importance. Numerous derivatives of

flavonoids have been reported to exhibit anti-allergic, anti-viral, anti-inflammatory and antibacterial properties (Dicarlo *et al*., 1999; Montoro *et al*., 2005). The majority of oxidizing molecules with the inclusion of singlet oxygen and other free radicals linked to a number of diseases have been demonstrated to be effectively neutralized by flavonoids (Bravo, 1998). Flavonoids work as antioxidants because they have functional OH groups that get rid of free radicals and bind to metal ions (Sarian *et al*., 2017; Governa *et al*., 2018). Antioxidant activity of flavonoids is important in both prevention and treatment of diabetes and its complications. This is because, flavonoids can stop formation of reactive oxygen species (ROS) by inhibiting enzymes or by chelating trace elements involved in free radical production (Hajiaghaalipour *et al*., 2015). They can also get rid of ROS by scavenging them and stopping enzymes that make ROS, like microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and others (Dabeek and Marra, 2019). The richness of these leaves in flavonoids especially *Jatropha tanjorensis* explains their antioxidant properties.

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a fast, easy and widely used method to determine the antioxidant activity of foods, natural compounds or extracts (Baliyan *et al*., 2022). The DPPH radical scavenging activity of the extracts was expressed as a percentage, which represents the ability of the extracts to reduce the stable DPPH radical to a non-radical form. The higher the percentage, the higher the antioxidant activity of the extract. Figure 4.2 showed the DPPH radical scavenging activity (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis* at various concentrations (10, 20, 40, and 80 mg/ml) compared to a reference antioxidant, butylated hydroxytoluene (BHT). It further indicated that both *Justicia secunda and Jatropha tanjorensis* extracts have significant antioxidant activity, as evidenced by their high percentage inhibition of the DPPH radical at all concentrations tested. The highest percentage inhibition was observed for

Jatropha tanjorensis extract at 93.95% at 20 mg/ml concentration, while the lowest percentage inhibition was observed for *Justicia secunda* extract at 80.90% at 40 mg/ml concentration. The results therefore suggest that *Jatropha tanjorensis* extract has stronger DPPH radical scavenging activity than to *Justicia secunda* extract. This is in consonance with earlier observed higher TPC and TFC levels in *Jatropha tanjorensis.* It is important to note that the reference compound, BHT, showed the highest percentage inhibition of the DPPH radical at 98.24%, indicating high antioxidant activity compared to the plant extracts. In a similar study, Onoja *et al* (2017) reported that methanolic leaf extract of *J. secunda* caused a concentration dependent increase in percentage antioxidant activity, resulting in a rise of antioxidant activity from 7.22% at 25µg/ml concentration to 54.07% at 400 µg/ml concentration. Osima and Hamilton-Amachree (2017) carried out yet another related study that investigated the phytochemical and *in vitro* antioxidant properties of methanolic leaf extracts of *J. secunda* using DPPH, H_2O_2 and superoxide anion. Their study also revealed that *J. secunda* possess free radical scavenging activities, an activity which they attributed to the plant`s richness in phytochemicals such anthocyanins, phenols and flavonoids.

The ABTS assay is yet another commonly used method to evaluate the antioxidant activity of plant extracts. The results of the present study indicate that the percentage of ABTS radical scavenging activity increased with increasing concentration of the extract for both plant species. At all concentrations tested, *Jatropha tanjorensis* extract exhibited higher ABTS radical scavenging activity than *Justicia secunda* extract. The p-values indicate that the differences between the ABTS radical scavenging activity of the two plant species at each concentration tested are statistically significant. This means that the difference in ABTS radical scavenging activity between the two plant species is not due to chance or random

variation. Overall, these results suggest that *Jatropha tanjorensis* leaf extract has stronger antioxidant activity than *Justicia secunda* leaf extract, as measured by the ABTS assay.

Figure 4.4 shows the results of FRAP inhibition activity (%) of ethanol-water leaf extracts of the two different plant species, *Justicia secunda* and *Jatropha tanjorensis*, at different concentrations (10, 20, 40, and 80 mg/ml). The results indicate that the percentage of FRAP inhibition activity increased with increasing concentration of the extract for both plant species. However, unlike the DPPH and ABTS assay results, *Jatropha tanjorensis* extract showed lower FRAP inhibition activity than *Justicia secunda* extract at all concentrations tested. *Justicia secunda* has better FRAP inhibition activity than *Jatropha tanjorensis*, implying that it has a greater capacity to contribute electrons to lower the ferric ions in the process. Overall, these results suggest that *Justicia secunda* leaf extract has stronger FRAP inhibition activity than *Jatropha tanjorensis* leaf extract, as measured by the FRAP assay.

Results of the brine shrimp lethality (%) mortality effect of ethanol-water leaf extracts *Justicia secunda* and *Jatropha tanjorensis* show that both extracts exhibited a concentration-dependent increase in lethality to the brine shrimp. At the lowest concentration tested (1µg/ml), *J. secunda* and *J. tanjorensis* extracts induced 13.31% and 39.79% mortality respectively. At higher concentration (10µg/ml to 1000µg/ml), the percentage mortality increased for both extracts with *J. tanjorensis* extracts exhibiting higher lethality than *J. secunda*. Between the extracts, *J. secunda* had a lower LC_{50} value of 7.59 ($x10^2$ µg/ml) compared to the higher LC_{50} value of 9.89 ($x10^3$µg/ml) obtained from *J. tanjorensis* indicating that former is more toxic than the later. According to Nguta and Mbaria (2013), lower LC_{50} value correlates with higher cytotoxicity of plant extracts. LC50 is the number that shows the concentration of toxic substances that kill up to 50% of the test organisms at a given time (Rasy *et al*., 2020). Toxicity evaluation of plant extracts using brine shrimp lethality assay is

usually determined using the Clarkson`s toxicity index which stipulates that LC_{50} of > 1000 µg/ml indicates non-toxic, 500-1000 µg/ml (low toxicity), 100-500 µg/ml (medium toxicity) and 0-100 µg/ml (high toxicity) (Clarkson *et al*., 2004).

Apparently, the present study is the first to evaluate the toxicity of *J. secunda and J. tanjorensis* using brine shrimp lethality assay. Though this method has been used by Patel and Zaveri (2012) and Maskat and Hussain (2012) to evaluate cytotoxicity of different extracts of *Justicia genderussa* and methanol extract of *Justicia adhatoda* respectively. Methanolic fraction of leaf extract of *J. genderussa* showed higher cytotoxicity than n-hexane and water fractions of the leaf and root extracts of the plant (Patel and Zaveri, 2012). According to Meskat and Hussain (2012), the n-hexane, ethyl acetate and chloroform soluble fractions of methanolic crude extracts of *Justicia adhatoda* exhibited significant cytotoxicity. Similarly, Akhter *et al*., (2018) studied n-hexane extract of *Jatropha pandurifolia* using brine shrimp lethality assay. Their result showed that *J. pandurifolia* showed a concentration dependent increase in lethality, exhibiting a significant cytotoxicity with LC_{50} of 4.67 µg/ml as against 0.27 µg/ml of Vincristin sulphate.

Brine shrimp lethality assay is considered a useful tool for preliminary evaluation of toxicity. It has also been employed in the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials (Barahona and Sanchez-Fortun, 1999).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The results obtained from this study have shown the richness of *Justicia secunda* and *Jatropha tanjorensis* in phytochemicals and essential plant-based nutrients. Both plants showed substantial amounts of alkaloids, tannins and flavonoids. *Jatropha tanjorensis* showed higher content of saponin and cardiac glycoside. Anti-nutrient factors such as phytate and oxalate where also detected in higher quantity in *Jatropha tanjorensis* than in *Justicia secunda* . Both plants contain significant amounts of carbohydrate, amino acids and a fair content of important mineral elements such potassium, magnesium and sodium.

GC-MS analysis revealed 62 and 52 bioactive compounds in *J. secunda* and *J. tanjorensis* respectively with diverse biological activities. From the results of the antioxidant vitamins composition, both plants particularly *J. secunda* could be relied upon as excellent dietary sources of vitamins C, E and A. High total phenolic and flavonoid contents were noted in the plants and were seen to be directly related to their observed high antioxidant activity. The observed significant DPPH and ABTS radicals scavenging activities, and FRAP inhibition activity of both plants justify them as good sources of natural antioxidants. Hence, the plants can be considered as good sources of natural antioxidants to counteract the dangerous effects of free radicals that the body is exposed to.

The results of brine shrimp lethality test indicate that the leaf extracts of *J. secunda* and *J. tanjorensis* showed LC_{50} values 7.59 ($x10^2$) ± 0.03 µg/ml and 9.89 ($x10^3$) ± 0.03 µg/ml respectively. Therefore, given the observed LC_{50} values, ethanol-water leaf extract of *J. tanjorensis* is non-toxic, while that of *J. secunda* showing lower LC_{50} value has low toxicity to the brine shrimp.

5.2 Contribution to knowledge

This research has contributed the following to the body of knowledge:

Identification of new bioactive compounds: Using GC-MS and other techniques, the phytochemical study of the two plant extracts has led to the discovery of new bioactive chemicals that might be investigated further for possible therapeutic applications.

Antioxidant activity evaluation: Investigating the antioxidant activity of the two plant extracts using various assays such as FRAP, ABTS, and DPPH scavenging activities has aided in identifying the plants with the most potent antioxidant activity, which can then be used to develop nutraceuticals and functional foods.

Determination of nutritional value: The determination of the amino acid composition, mineral content, and anti-nutrient factors of the plant extracts has provided valuable information on the nutritional value of the plants, which could be useful for the formulation of animal feed and human diets.

Assessment of toxicity: The evaluation of the toxicity of the plant extracts using the Brine shrimp lethality assay has provided information on the safety of the plant extracts, which is important for their potential use in medicine and food.

Comparative analysis: The comparative analysis of the phytochemical and antioxidant profiles of the two plant extracts has provided insights into the similarities and differences in their chemical composition and biological activities, which could be useful for their classification, conservation, and utilization.

5.3 Recommendation

This study has gone a step further in providing good information on the phytochemistry, antioxidant and toxicological properties of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*. For more study on these essential medicinal plants, the following are hereby recommended:

1. An investigation into the in vivo antioxidant properties of the ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis* in animal models of oxidative stress. This would provide more information on the potential health benefits of these plant extracts in the context of preventing or mitigating oxidative damage in living organisms.
2. Conduct of a comprehensive phytochemical analysis of the ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis* using more advanced analytical techniques such as HPLC or LC-MS/MS. This would enable the identification and quantification of a wider range of phytochemical compounds and elucidation of their potential synergistic effects.
3. Conduct further toxicological studies to determine the safety profile of the plant extracts beyond the brine shrimp lethality assay. This could include acute and sub-chronic toxicity studies in animal models, as well as assessing the potential genotoxic and mutagenic effects of the extracts.
4. Exploration of the potential applications of the plant extracts in the development of functional foods, nutraceuticals, or cosmeceuticals. This could involve investigating the stability, bioavailability, and sensory properties of the extracts, as well as their potential to improve the nutritional or functional properties of food products or skincare formulations.

REFERENCES

(AOAC) Association of Official Analytical Chemists International (1990). Official Methods of Analysis, 15th edn (edited by. K. Helrich) Pp. 1028–1039. Arlington, VA: Association of Official Analytical Chemists Inc.

(AOAC)Association of Analytical Chemists (1984). Standard Official Methods of Analysis of the Association of Analytical Chemists. 14th edition, S.W Williams (Ed), Washington, D C., p. 121.

Abdurrahman, I., and Cai-Xiab, Y. (2020). Isolation and characterization of fatty acid derivatives from the stem barks of *Albizia Amara* (Fabaceae), Sudanese Medicinal Plant. *Chemical Methodologies*, *4*(4), 369-377.

Abelson, P. H. (1990). Medicine from plants. *Science*, *247*(4942), 513-514.

Adebooye, O. C., Vijayalakshmi, R., and Singh, V. (2008). Peroxidase activity, chlorophylls and antioxidant profile of two leaf vegetables (*Solanum nigrum L.* and *Amaranthus cruentus* L.) under six pretreatment methods before cooking. *International journal of food science & technology*, *43*(1), 173-178.

Adeyemi, M. A., Ekunseitan, D. A., Abiola, S. S., Dipeolu, M. A., Egbeyale, L. T., and Sogunle, O. M. (2017). Phytochemical analysis and GC-MS determination of *Lagenaria breviflora* R. fruit. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(7), 1045-1050.

Adeyeye, E. I., & Omolayo, F. O. (2011). Chemical composition and functional properties of leaf protein concentrates of *Amaranthus hybridus* and *Telfairia occidentalis*. *Agric. Biol. JN Am*, *3*, 499-511.

Adrian, W. J. (1973). A comparison of a wet pressure digestion method with other commonly used wet and dry-ashing methods. *Analyst*, *98*(1164), 213-216.

Ai, H. W., Kang, Y. X., Cao, Y., & Zheng, C. J. (2014). Antifungal Properties and Chemical Analysis of Essential Oil from *Vitex negundo* Seeds.

Aimofumeh, E. O., Anyasor, G. N., and Esiaba, I. (2020). *Justicia secunda* Vahl leaf fraction protects against acetaminophen-induced liver damage in rats by alleviating oxidative stress and enhancing membrane-bound phosphatase activities. *Asian Pacific Journal of Tropical Biomedicine*, *10*(11), 479.

Aja, P. M., Ale, B. A., Ekpono, E. U., Nwite, I., Aja, L., Asouzu, N. C., and Njoku, A. (2021). Amino acid profiles of *Solanum aethiopicum, Amaranthus hybridus,* and *Telfairia occidentalis*, common leafy vegetables in Nigeria. *Science Progress*, *104*(3), 00368504211032079.

Ajah, O., Onyedikachi, U. B., Alaebo, P. O., Odo, C. E., Godwin, O. K., and Omodamiro, O. D. (2021). Methanol leaf extract of *Jatropha tanjorensis* Ellis and Saroja Possess Phytoconstituents with free radical scavenging activity. *fudma journal of sciences*, *5*(3), 286-293.

Ajoku, G. A., Okwute, S. K., and Okogun, J. I. (2015). Isolation of hexadecanoic acid methyl ester and 1, 1, 2-ethanetricarboxylic acid-1-hydroxy-1, 1-dimethyl ester from the calyx of green *Hibiscus Sabdariffa* (Linn). *Natural Products Chemistry & Research*.

Ajuru, M. G., Kpekot, A. K., Omubo, J., and Morrison, I. (2021). Comparative Study of Proximate and Phytochemical Analysis of the Roots of *Justicia carnea* Lindi. and *Justicia secunda* Vahl. *Nigerian Annals of Pure and Applied Sciences*, *4*(1), 22-33.

Ajuru, M. G., Williams, L. F., and Ajuru, G. (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences*, *5*(5), 198-205.

Akbari, B., Baghaei- Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant- derived natural antioxidants in reduction of oxidative stress. *BioFactors*, *48*(3), 611-633.

Akhter, A., Rahman, M. S., and Ahsan, M. (2008). Preliminary antimicrobial and cytotoxic activities of n-hexane extract of *Jatropha pandurifolia*. *Latin American Journal of Pharmacy*, *27*(6), 918-21.

Akpanyung, E. O. (2005). Proximate and mineral element composition of bouillon cubes produced in Nigeria. *Pakistan journal of nutrition*, *4*(5), 327-329.

Albrecht, J., Sidoryk-Węgrzynowicz, M., Zielińska, M., and Aschner, M. (2010). Roles of glutamine in neurotransmission. *Neuron glia biology*, *6*(4), 263-276.

Albuquerque, U. P., Monteiro, J. M., Ramos, M. A., & de Amorim, E. L. C. (2007). Medicinal and magic plants from a public market in northeastern Brazil. *Journal of ethnopharmacology*, *110*(1), 76-91.

Al-Fartusie, F. S., and Mohssan, S. N. (2017). Essential trace elements and their vital roles in human body. *Indian J Adv Chem Sci*, *5*(3), 127-136.

Alves, R. R., and Rosa, I. M. (2007). Biodiversity, traditional medicine and public health: where do they meet. *Journal of ethnobiology and ethnomedicine*, *3*(1), 1-9.

American Public Health Association (1998). 3111B, Direct Air- Acetylene Flame Method, Standard Methods for the examination of metals in blood 20th Edition, APHA, AWWA, WEF.

Amine, A. H., and Mehta, D. R. (1959). Bronchodilator alkaloid from *Adhatoda vasica*. *Nature*, *184*, 1317.

Amudha, M., and Rani, S. (2014). Assessing the bioactive constituents of *Cadaba fruticosa* (L.) Druce through GC-MS. *International Journal of Pharmaceutical Sciences Pharm Sci*, *6*(2), 383-5.

Anhwange, B. A., Agbidye, I. G., Kyenge, B. A., and Ngbede, P. O. (2019). Phytochemical Screening, Antimicrobial Activities and Nutritional content of *Jatropha tanjorensis* Leaves. *Nigerian Annals of Pure and Applied Sciences*, *2*, 108-113.

Anwar, F., Latif, S., Ashraf, M., and Gilani, A. H. (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(1), 17-25.

Anyasor, G. N., Moses, N., and Kale, O. (2020). Hepatoprotective and hematological effects of *Justicia secunda* Vahl leaves on carbon tetrachloride induced toxicity in rats. *Biotechnic & Histochemistry*, *95*(5), 349-359.

Anyasor, G. N., Okanlawon, A. A., and Ogunbiyi, B. (2019). Evaluation of anti-inflammatory activity of *Justicia secunda* Vahl leaf extract using in vitro and in vivo inflammation models. *Clinical Phytoscience*, *5*(1), 1-13.

Arogbodo, J. O. (2020). Evaluation of the Phytochemical, Proximate and Elemental Constituents of *Justicia secunda* M. Vahl Leaf. *Evaluation*, *5*(5).

Arun, P. K., Rajesh, S. S., Sundaram, S. M., Sivaraman, T., and Brindha, P. (2014). Structural characterizations of lead anticancer compounds from the methanolic extract of *Jatropha tanjorensis*. *Bangladesh Journal of Pharmacology*, *9*(4), 452-65.

Asano, J., Chiba, K., Tada, M., and Yoshii, T. (1996). Antiviral activity of lignans and their glycosides from *Justicia procumbens*. *Phytochemistry*, *42*(3), 713-717.

Asaolu, S. S., Adefemi, O. S., Oyakilome, I. G., Ajibulu, K. E., and Asaolu, M. F. (2012). Proximate and mineral composition of Nigerian leafy vegetables. *Journal of food Research*, *1*(3), 214.

Atal, C. K. (1980). Chemistry and pharmacology of vasicine. *A New Oxytocic and Abortifacient, Regional research laboratory, Canal Road, Jammu-Tawi*, 125-6.

Atik, F. A. W. Z. I. A., and Mohammedi, Z. O. H. R. A. (2011). Impact of solvent extraction type on total polyphenols content and biological activity from *Tamarix aphylla (L.) Karst. International Journal of Pharma Bioscience*, *2*(1), 609-15.

Atmaca, U., Daryadel, S., Taslimi, P., Çelik, M., and Gülçin, İ. (2019). Synthesis of β-amino acid derivatives and their inhibitory profiles against some metabolic enzymes. *Archiv der Pharmazie*, *352*(12), 1900200.

Aune, D., Norat, T., Romundstad, P., and Vatten, L. J. (2013). Dairy products and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. *The American journal of clinical nutrition*, *98*(4), 1066-1083.

Avila, D. S., Puntel, R. L., and Aschner, M. (2013). Manganese in health and disease. *Interrelations between essential metal ions and human diseases*, 199-227.

Awosika, F. (1991). Local medicinal plants and health of consumers. *Clin. Pharm. Herbal Med*, *9*, 28-29.

Ayodele, A. E., Odusole, O. I., and Adekanmbi, A. O. (2020). Phytochemical screening and in-vitro antibacterial activity of leaf extracts of *Justicia secunda* Vahl on selected clinical pathogens. *MicroMedicine*, *8*(2), 46-54.

Aziz, M. A. (2015). Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of *Microcos paniculata* barks and fruits. *Journal of Integrative Medicine*, *13*(3), 173-184.

Babayemi, O. O., Oke, E. A., and Bayode, M. T. (2021). Antibacterial activity of *Jatropha tanjorensis leaf* extracts against bacteria associated with wound infections from the clinical setting. *Nusantara Bioscience*, *13*(2).

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., and Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, *27*(4), 1326.

Balunas, M. J., and Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life sciences*, *78*(5), 431-441.

Barahona, M. V., and Sanchez-Fortun, S. (1999). Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. *Environmental Pollution*, *104*(3), 469-476.

Bartolome, A. P., Villaseñor, I. M., and Yang, W. C. (2013). *Bidens pilosa* L. (Asteraceae): botanical properties, traditional uses, phytochemistry, and pharmacology. *Evidence-based complementary and alternative medicine*, *2013*.

Bassey, E. I., Edagha, I. A., Aquaisua, A. N., and Archibong, A. F. (2019). Testicular Microstructure and Hormonal Profile Following HAART Administration: The Role of *Jatropha tanjorensis*. *Journal of Basic and Clinical Reproductive Sciences*, *8*(2).

Bedoya, L. M., Álvarez, A., Bermejo, M., González, N., Beltran, M., Sanchez-Palomino, S., and Alcamí, J. (2008). Guatemalan plants extract as virucides against HIV-1 infection. *Phytomedicine*, *15*(6-7), 520-524.

Bendich, A., Machlin, L. J., Scandurra, O., Burton, G. W., and Wayner, D. D. M. (1986). The antioxidant role of vitamin C. *Advances in Free Radical Biology & Medicine*, *2*(2), 419-444.

Berdanier, C. D. (2001). Trace Mineral Deficiencies. In *Handbook of Nutrition and Food* (pp. 1527-1552). CRC Press.

Berdanier, C. D., Dwyer, J. T., and Feldman, E. B. (Eds.). (2007). *Handbook of nutrition and food*. CRC press.

Bezerra, K. D. S., and Antoniosi Filho, N. R. (2014). Characterization and quantification by gas chromatography of free steroids in unsaponifiable matter of vegetable oils. *Journal of the Brazilian Chemical Society*, *25*, 238-245.

Bhat, V. S., Nasavatl, D. D., and Mardikar, B. R. (1978). *Adhatoda Vasica*-An ayurvedic medicinal plant. *Indian Drugs*, *15*(62), 6.

Bichi, S. A., and Abdulmumin, Y. (2022). AbdulmuminT. *M, Muhammad IA, Murtala M, DalhatuM. M, Amina L. A, Sarki SI, Danjaji HI, Mustapha RK*, 57-66.

Brako, L., and Zarucchi, J. L. (1993). Catalogue of the flowering plants and gymnosperms of Peru. Catálogo de las angiospermas y gimnospermas del Perú. *Monographs in systematic botany from the Missouri Botanical Garden*, *45*, 1-1286.

Brasse-Lagnel, C., Lavoinne, A., and Husson, A. (2009). Control of mammalian gene expression by amino acids, especially glutamine. *The FEBS journal*, *276*(7), 1826-1844.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews*, *56*(11), 317-333.

Brosnan, J. T., & Brosnan, M. E. (2010). Creatine metabolism and the urea cycle. *Molecular genetics and metabolism*, *100*, S49-S52.

Bruhat, A., Chérasse, Y., Chaveroux, C., Maurin, A. C., Jousse, C., and Fafournoux, P. (2009). Amino acids as regulators of gene expression in mammals: molecular mechanisms. *Biofactors*, *35*(3), 249-257.

Buccafusco, R. J., Ells, S. J., and LeBlanc, G. A. (1981). Acute Toxicity of Priority Pollutants to Bluegill (*Lepomis macrochirus*). *Bulletin of Environmental Contamination and Toxicology*, *26*(1), 446-452.

Burjonrappa, S. C., and Miller, M. (2012). Role of trace elements in parenteral nutrition support of the surgical neonate. *Journal of Pediatric Surgery*, *47*(4), 760-771.

Burns, J. J. (1959). Biosynthesis of L-ascorbic acid; basic defect in scurvy. *The American journal of medicine*, *26*(5), 740-748.

Cabrera-Vique, C., and Bouzas, P. R. (2009). Chromium and manganese levels in convenience and fast foods: In vitro study of the dialyzable fraction. *Food Chemistry*, *117*(4), 757-763.

Cai, Y. Z., Sun, M., Xing, J., Luo, Q., and Corke, H. (2006). Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life sciences*, *78*(25), 2872-2888.

Cameron, G. R., Milton, R. F., and Allen, J. W. (1943). Measurement of flavonoids in plant samples. *Lancet*, *179*(11), 125.

Cano, J. H., and Volpato, G. (2004). Herbal mixtures in the traditional medicine of Eastern Cuba. *Journal of Ethnopharmacology*, *90*(2-3), 293-316.

Carrington, S., Cohall, D. H., Gossell-Williams, M., and Lindo, J. F. (2012). The antimicrobial screening of a Barbadian medicinal plant with indications for use in the treatment of diabetic wound infections. *West Indian Medical Journal*, *61*(9).

Chakravarty, A. K., Dastidar, P. P. G., and Pakrashi, S. C. (1982). Simple aromatic amines from Justicia gendarussa. 13C NMR spectra of the bases and their analogues. *Tetrahedron*, *38*(12), 1797-1802.

Chan, K. (2003). Some aspects of toxic contaminants in herbal medicines. *Chemosphere*, *52*(9), 1361-1371.

Chen, C. C., Hsin, W. C., Ko, F. N., Huang, Y. L., Ou, J. C., and Teng, C. M. (1996). Antiplatelet arylnaphthalide lignans from *Justicia procumbens*. *Journal of natural products*, *59*(12), 1149-1150.

Chen, L., Yin, Y. L., Jobgen, W. S., Jobgen, S. C., Knabe, D. A., Hu, W. X., and Wu, G. (2007). In vitro oxidation of essential amino acids by jejunal mucosal cells of growing pigs. *Livestock Science*, *109*(1-3), 19-23.

Chen, T. C., Da Fonseca, C. O., and Schönthal, A. H. (2015). Preclinical development and clinical use of perillyl alcohol for chemoprevention and cancer therapy. *American journal of cancer research*, *5*(5), 1580.

Chikezie, P. C., & Ekeanyanwu, R. C. (2019). GC-MS and FT-IR analyses of phytocomponents from petroleum ether fraction of leaf extract of *Psidium guajava.* *Research Journal of Medicinal Plant*, *13*(1), 26-31.

Chikezie, P. C., and Ekeanyanwu, R. C. (2020). Phytocomponents from *Anacardium occidentale*, *Psidium guajava*, and *Terminalia catappa* altered membrane osmotic stability of sickle erythrocytes. *Beni-Suef University Journal of Basic and Applied Sciences*, *9*(1), 1-22.

Chmurzynska, A. (2010). Fetal programming: link between early nutrition, DNA methylation, and complex diseases. *Nutrition reviews*, *68*(2), 87-98.

Chowdhury, B. K., and Bhattacharyya, P. (1987). Vasicine and related-compounds. 4. Adhavasinone-A New Quinazolone Alkaloid from *Adhatoda-vasica* Nees. *Chemistry & Industry*, (1), 35-36.

Claeson, U. P., Malmfors, T., Wikman, G., and Bruhn, J. G. (2000). *Adhatoda vasica*: a critical review of ethnopharmacological and toxicological data. *Journal of Ethnopharmacology*, *72*(1-2), 1-20.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., and Folb, P. I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of ethnopharmacology*, *92*(2-3), 177-191.

Claude, A. K. J., Léandre, K. K., and Adou, Y. A. P. O. Hypotensive and Antihypertensive Effects of Total Aqueous Extract of *Justicia secunda* Vahl M. (Acanthaceae) in Rabbits.

Contributors, W. H. O. (2007). The impact of zinc supplementation on childhood mortality and severe morbidity. *World Health Organization, Geneva, Switzerland*.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.

Crichton, R. R., Danielson, B. G., and Geisser, P. (2008). *Iron therapy with special emphasis on intravenous administration*. Uni-Med Verlag.

Cushnie, T. T., Cushnie, B., and Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International journal of antimicrobial agents*, *44*(5), 377-386.

Dabeek, W. M., and Marra, M. V. (2019). Dietary quercetin and kaempferol: Bioavailability and potential cardiovascular-related bioactivity in humans. *Nutrients*, *11*(10), 2288.

D'Amelio Sr, F. S. (1998). *Botanicals: A phytocosmetic desk reference*. CRC Press.

Danborno, A. M., Tarfa, F., Toryila, J. E., Awheela, E. U., and Shekarau, V. T. (2019). The effects of *Jatropha tanjorensis* aqueous leaf extract on haematological parameters in Wistar rats. *Journal of African Association of Physiological Sciences*, *7*(2), 133-137.

Daniel, T. F. (1993a). New and reconsidered Mexican Acanthaceae III, Justicia. *Contr. Univ. Michigan Herb.*, **17:** 133-137.

Daniyan, S. Y., Ukubuiwe, C. C., Ukubuiwe, A. C., Oluwafemi, O. J., and Chukwudi, P. O. (2018). Antibacterial Activities of Leaf Extracts of *Jatropha tanjorensis* Ellis and Saroja (Euphorbiaceae).

Day, S. H., Lin, Y. C., Tsai, M. L., Tsao, L. T., Ko, H. H., Chung, M. I., and Lin, C. N. (2002). Potent Cytotoxic Lignans from *Justicia procumbens* and Their Effects on Nitric Oxide

and Tumor Necrosis Factor-α Production in Mouse Macrophages. *Journal of natural products*, *65*(3), 379-381.

Devika, R., and Kovilpillai, J. (2014). screening and evaluation of bioactive components of tagetes erecta l. by GC-MS analysis. *Asian Journal of Pharmaceutical and Clinical Research*, 58-60.

Dhar, M. L., Dhar, M. M., Dhawan, B. N., Mehrotra, B. N., and Ray, C. (1968). Screening of Indian plants for biological activity: Part I.

Di Carlo, G., Mascolo, N., Izzo, A. A., and Capasso, F. (1999). Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life sciences*, *65*(4), 337-353.

Doba, T., Burton, G. W., and Ingold, K. U. (1985). Antioxidant and co-antioxidant activity of vitamin C. The effect of vitamin C, either alone or in the presence of vitamin E or a water-soluble vitamin E analogue, upon the peroxidation of aqueous multilamellar phospholipid liposomes. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, *835*(2), 298-303.

Domínguez, X. A., Achenbach, H., González, C., and Ferré-D'Amare, A. R. (1990). Estudio químico del muitle (*Justicia spicigera*). *Rev Latinoamer Quím*, *21*, 142-143.

Dubey, P., Thakur, V., and Chattopadhyay, M. (2020). Role of minerals and trace elements in diabetes and insulin resistance. *Nutrients*, *12*(6), 1864.

Duke, J. A., and Wain, K. K. (1981). Medicinal plants of the world. *Computer index with more than*, *85*(000), 3.

Dulara, B. K., Godara, P., and Barwer, N. (2019). In-vivo and In-vitro phytochemical GC-MS analysis of volatile constituents of *Andrographis paniculata* (Burm. f.) Nees. *The Pharma Innovation Journal*, *8*, 255-261.

Durkee, L. H. (1986). *Flora Costaricensis. Family200 Acanthaceae*. Field Museum of Natural History.

Dutta, A.C. (2003). Botany for Degree Students. 6th Edition. Oxford University Press, New Delhi, India, pp. 32-39

Dutta, S. U. M. A. N. A., and Ray, S. A. N. J. I. B. (2015). Evaluations of in vitro free radical scavenging activity of leaf extract fractions of *Manilkara hexandra* (Roxb.) Dubard in relation to total phenolic contents. *Int. J. Pharm. Pharm. Sci*, *7*(10), 296-301.

Dutta, S., and Ray, S. (2020). Comparative assessment of total phenolic content and in vitro antioxidant activities of bark and leaf methanolic extracts of *Manilkara hexandra* (Roxb.) Dubard. *Journal of King Saud University-Science*, *32*(1), 643-647.

Dutta, T. K., and Mukta, V. (2012). Trace elements. *Medicine*, *22*, 352-7.

Dymock, W., Warden, C. J. H., and Hooper, D. (1891). *Pharmacographia Indica: A History of the Principal Drugs of Vegetable Origin, Met with in British India* (Vol. 2). K. Paul, Trench, Trübner & Company, ld.

Ebe, N. U., and Chukwuebuka, E. V. (2019). Effects of ethanol roots extract of *Jatropha tanjorensis* on haematological indices of male albino wistar rats. *European Journal of Biology and Medical Science Research, 7*(1), 7-14.

Ebe, N. U., Effiong, G. S., and Ikot, A. E. (2019). Qualitative phytochemical analysis of ethanol leaves extract of *Jatropha tanjorensis* and its effects on liver function of male albino wistar rats. *International Journal of Biochemistry, Bioinformatics and Biotechnology Studies*, *4*(2), 1-11.

Ebenyi, L. N., Yongabi, K. A., Ali, F. U., Ominyi, M. C., Anyanwu, C. B., Benjamin, E., and Ogbanshi, M. E. (2021). Effect of Aqueous Leaf Extract of *Jatropha tanjorensis* on parasitaemia and haematological parameters in mice infected with Plasmodium ber ghei. *Nigerian Journal of Biotechnology*, *38*(1), 146-153.

Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, *4*(7), 685-688.

Ejoh, A. R., Mbiapo, F. T., and Fokou, E. (1996). Nutrient composition of the leaves and flowers of Colocasia esculenta and the fruits of *Solanum melongena. Plant Foods for Human Nutrition*, *49*(2), 107-112.

Elango, R., Ball, R. O., and Pencharz, P. B. (2009). Amino acid requirements in humans: with a special emphasis on the metabolic availability of amino acids. *Amino acids*, *37*(1), 19-27.

Elkin, R. G., and Griffith, J. E. (1985). Amino acid analysis of feedstuff hydrolysates by cation exchange high performance liquid chromatography. *Journal of the Association of Official Analytical Chemists*, *68*(5), 1028-1032.

Ellis, J.L., & Saroja, T.L. (1962). *Journal of the Bombay Natural History Society Published*: 58: 834-836

Emsley, J. (2011). *Nature's building blocks: an AZ guide to the elements*. Oxford University Press.

Enemor, V., Oguazu, C., Odiakosa, A., and Okafor, S. (2019). Research Article Evaluation of the Medicinal Properties and Possible Nutrient Composition of *Citrullus lanatus* (Watermelon) Seeds. *J. Med. Plant*, *13*(4), 129-135.

Ensminger, M. E., and Ensminger, A. H. (1993). *Foods & Nutrition Encyclopedia, Two Volume Set*. CRC press.

Esimone, C. O., Omobuwajo, R. M. L., Sowemimo, A. A., Proksch, P., & Nabi, G. (2008). Single cycle vector based antiviral screening assays for high throughput evaluation of

potential anti-HIV medicinal plants: a pilot study on some Nigerian herbs. *Phytopharmacology and therapeutic values I*, 49-60.

Evans, W. C. (2002). Trease and Evans Pharmacognosy. 15th edition. *Edinburgh, Saunders, 249*.

Ezcurra, C. (1993a). Acanthaceae. In A. L. Cabrera (editor), Flora (le la Provincia de Jujuy 9: 278-359. Colee 3i. 1NTA, Buenos Aires

Ezcurra, C. (1999). Acanthaceae. En: FO Zuloaga y O. Morrone (eds.), Catálogo de las Plantas Vasculares de Argentina II. *Monographs in Systematic Botany from the Missouri Botanical Garden*, *74*, 1-14.

Falodun, A., Udu-Cosi, A. A., Erharuyi, O., Imieje, V., Falodun, J. E., Agbonlahor, O, and Hamann, M. T. (2013). *Jatropha tanjorensis*-Review of Phytochemistry, Pharmacology, and Pharmacotherapy. *Journal of pharmaceutical and allied sciences*, *10*(3).

Farnsworth, N. R. (1966). Biological and phytochemical screening of plants. *Journal of pharmaceutical sciences*, *55*(3), 225-276.

Fennell, C. W., Light, M. E., Sparg, S. G., Stafford, G. I., and Van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: agricultural and storage practices. *Journal of Ethnopharmacology*, *95*(2-3), 113-121.

Finar, I. L. (1989). Chemistry; Stereochemistry and the chemistry of Natural products, 5th Edn, Vol. 2. Longman group, UK. Pp.517-605.

Fitriyah, H., Anwar, F., and Palupi, E. (2021, April). Morphological characteristics, chemical and amino acids composition of flours from velvet beans tempe (*Mucuna pruriens*), an indigeneous legumes from Yogyakarta. In *Journal of Physics: Conference Series* (Vol. 1869, No. 1, p. 012012). IOP Publishing.

Fongod, A. G. N., Modjenpa, N. B., and Veranso, M. C. (2013). Ethnobotany of Acanthaceae in the Mount Cameroon region. *Journal of Medicinal Plants Research*, *7*(38), 2859-2866.

Fraenkel, G. S. (1959). The Raison d'Etre of Secondary Plant Substances: These odd chemicals arose as a means of protecting plants from insects and now guide insects to food. *Science*, *129*(3361), 1466-1470.

Fraga, C. G. (2005). Relevance, essentiality and toxicity of trace elements in human health. *Molecular aspects of medicine*, *26*(4-5), 235-244.

Fraga, C. G., Motchnik, P. A., Shigenaga, M. K., Helbock, H. J., Jacob, R. A., and Ames, B. N. (1991). Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proceedings of the National Academy of Sciences*, *88*(24), 11003-11006.

Frankel, E. N., German, J. B., Kinsella, J. E., Parks, E., and Kanner, J. (1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *The Lancet*, *341*(8843), 454-457.

Gangal, N., Nagle, V., Pawar, Y., and Dasgupta, S. (2020). Reconsidering traditional medicinal plants to combat COVID-19. *AIJR Preprints*, *34*(1), 1-6.

Ghanghro, A. W. (2019). Daily Intake of Buffalo's Milk and its Contribution in the RDA Values of Some Essential Minerals Required for 1-12 Years Children. *Scientific Journal of Food Science and Nutrition*, *5*(1), 16-19.

Ghosal, S., Banerjee, S., and Frahm, A.W. (1979a): Chemical constituents of *Justicia*. III. Prostalidins A, B, C and retrochinensis: a new antidepressant; 4-aryl-2,3-naphtalide lignans from *Justicia prostrata*. *Chem Ind.*, 23: 854–855.

Ghosal, S., Banerjee, S., Jaiswal, D.K. (1980b). Chemical constituents of *Justicia*. Part 2. New furfurans lignans from *Justicia simplex*. *Phytochemistry* 19: 332–334.

Gijsbers, L., Ding, E. L., Malik, V. S., De Goede, J., Geleijnse, J. M., and Soedamah-Muthu, S. S. (2016). Consumption of dairy foods and diabetes incidence: a dose-response meta-analysis of observational studies. *The American journal of clinical nutrition*, *103*(4), 1111-1124.

Girija, S., Duraipandiyan, V., Kuppusamy, P. S., Gajendran, H., and Rajagopal, R. (2014). Chromatographic characterization and GC-MS evaluation of the bioactive constituents with antimicrobial potential from the pigmented ink of *Loligo duvauceli*. *International Scholarly Research Notices*, *2014*.

Gokani, A., Ibrahim, G., and Shah, H. (1992). Alkaline-ash foods in the dietary management of diabetes mellitus. *Insulin*, *19*, 100.

Gomez-Verjan, J. C., Ricardo, R., and María, I. A. (2012). Chemistry and pharmacology of selected Asian and American Medicinal species of Justicia. *Bioactive Phytochemicals: Perspectives for Modern Medicine*, *1*, 400-418.

González-Castro, M. J., López-Hernández, J., Simal-Lozano, J., and Oruña-Concha, M. J. (1997). Determination of amino acids in green beans by derivatization with phenylisothiocianate and high-performance liquid chromatography with ultraviolet detection. *Journal of Chromatographic Science*, *35*(4), 181-185.

Gorzalczany, S., Marrassini, C., Miño, J., Acevedo, C., and Ferraro, G. (2011). Antinociceptive activity of ethanolic extract and isolated compounds of *Urtica circularis*. *Journal of ethnopharmacology*, *134*(3), 733-738.

Governa, P., Baini, G., Borgonetti, V., Cettolin, G., Giachetti, D., Magnano, A. R., and Biagi, M. (2018). Phytotherapy in the management of diabetes: a review. *Molecules*, *23*(1), 105.

Grange, J. M., Noel, J.C. Snell. (1996). Activity of bromhexine and ambroxol, semisynthetic derivatives of vasicine from the Indian shrub *Adhatoda vasica*, against *Mycobacterium tuberculosis in vitro*. *Journal of Ethnopharmacology*, 50: 49-53.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3), 651-715.

Habib, M. R., and Karim, M. R. (2016). Chemical characterization and insecticidal activity of Calotropis gigantea L. flower extract against *Tribolium castaneum* (Herbst). *Asian Pacific Journal of Tropical Disease*, *6*(12), 996-999.

Habila, M. M., Festus, E. A., Morumda, D., Joseph, I., Chinonso, A. D., and Sunday, A. M. (2021). FTIR and GC-MS analysis of the aqueous and ethanolic extracts of *Jatropha tanjorensis* leaves.

Haddad-Kashani, H., Seyed-Hosseini, E., Nikzad, H., and Aarabi, M. H. (2012). Pharmacological properties of medicinal herbs by focus on secondary metabolites. *Life Science Journal*, *9*(1), 509-520.

Hajiaghaalipour, F., Khalilpourfarshbafi, M., and Arya, A. (2015). Modulation of glucose transporter protein by dietary flavonoids in type 2 diabetes mellitus. *International journal of biological sciences*, *11*(5), 508-524.

Halliwell, B., and Gutteridge, J. M. (1985). Free radicals in biology and medicine.

Hamiltons-Amachree, A., and Uzoekwe, S. A. (2017). GC-MS analysis of oil rich in polyenoic fatty acid methyl esters from leaves of *Justicia secunda* Vahl growing abundantly in the lowland rain forests of the Niger Delta region of Nigeria. *American Journal of Essential Oils and Natural Products*, *5*(4), 1-4.

Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.

Harborne, J. B. (1986). Nature, distribution and function of plant flavonoids. *Plant Flavonoids in Biology and Medicine, Buffalo, New York (USA), 22-26 Jul 1985*.

Harwig, J., and Scott, P. (1971). Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. *Applied microbiology*, *21*(6), 1011-1016.

Hatami, S., Sani, A. M., and Yavarmanesh, M. (2016). Chemical composition and antibacterial activity of organic extra virgin olive oil from Iran. *Nutrition & Food Science*.

Haynes, G. S. (1906). The pharmacological action of Digitalis, Strophanthus, and Squill on the heart. *Biochemical Journal*, *1*(2), 62.

Heinrich, M. (2000). Ethnobotany and its role in drug development. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *14*(7), 479-488.

Heller, J. (1996). Physic nut-*Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. International Plant Genetic Resources Institute, Rome, Italy. *International Plant Genetic Resources Institute, Rome*.

Henn, B. C., Ettinger, A. S., Schwartz, J., Téllez-Rojo, M. M., Lamadrid-Figueroa, H., Hernández-Avila, M. and Wright, R. O. (2010). Early postnatal blood manganese levels and children's neurodevelopment. *Epidemiology (Cambridge, Mass.)*, *21*(4), 433.

Henquin, J. C. (2011). The dual control of insulin secretion by glucose involves triggering and amplifying pathways in β-cells. *Diabetes research and clinical practice*, *93*, S27-S31.

Herrera-Mata, H., and Rosas-Romero, A. (2002). Biological activity of "Sanguinaria" (*Justicia secunda*) extracts. *Pharmaceutical biology*, *40*(3), 206-212.

Hertog, M.G.L., Feskens, E.J.M., Hollmann, P.C.H., Katan, M.B., Kroumhout. D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly study. Lancet 342:1007-1011.

Heyneman, C. A. (1996). Zinc deficiency and taste disorders. *The Annals of pharmacotherapy*, *30*(2), 186-187.

Higashimoto, M., Purintrapiban, J., Kataoka, K., Kinouchi, T., Vinitketkumnuen, U., Akimoto, S., and Ohnishi, Y. (1993). Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand. *Mutation Research Letters*, *303*(3), 135-142.

Hikino, H., Kiso, Y., Wagner, H., and Fiebig, M. (1984). Antihepatotoxic actions of flavonolignans from *Silybum marianum f*ruits. *Planta medica*, *50*(03), 248-250.

Horwitt, M. K. (1986). Interpretations of requirements for thiamin, riboflavin, niacin-tryptophan, and vitamin E plus comments on balance studies and vitamin B-6. *The American journal of clinical nutrition*, *44*(6), 973-985.

Houghton, P. J. (1995). The role of plants in traditional medicine and current therapy. *The Journal of Alternative and Complementary Medicine*, *1*(2), 131-143.

Huq, M. E., Ikram, M., and Warsi, S. A. (1967). Chemical composition of *Adhatoda vasica* Linn. II. *Pak. J. Sci. Ind. Res*, *10*, 224-225.

Idu, M., Igbafe, G., and Erhabor, J. (2014). Anti-anaemic activity of *Jatropha tanjorensis* Ellis & Saroja in Rabbits. *Journal of Medicinal Plants*, *2*(1).

Igbinaduwa, P. O., Usifoh, C. O., and Ugwu, C. C. (2011). Phytochemical analysis and toxicological evaluation of the methanolic extract of *Jatropha tanjorensis* leaf. *Journal of Pharmacy and Bioresources*, *8*(2), 86-91.

Igile, G. O., Iwara, I. A., Mgbeje, B. I. A., Uboh, F. E., and Ebong, P. E. (2013). Phytochemical, proximate and nutrient composition of *Vernonia calvaona* Hook (Asterecea): A green-leafy vegetable in Nigeria. *Journal of Food Research*, *2*(6), 1.

Igwe, C. U., Onyeze, G. O. C., Onwuliri, V. A., Osuagwu, C. G., and Ojiako, A. O. (2010). Evaluation of the chemical compositions of the leaf of S*pondias mombin* Linn from Nigeria. *Australian Journal of Basic and Applied Sciences*, *4*(5), 706-710.

Ijioma, S. N., Okafor, A. I., Ndukuba, P. I., and Akomas, S. C. (2014). Hypoglycemic, hematologic and hypolipidemic activity of *Jatropha tanjorensis* ethanol leaf extract in alloxan induced diabetic rats. *Ann Biol Res*, *5*(7), 25-30.

IOM (Institute of Medicine), (1997) dietary reference ıntakes: Calcium, phosphorus, magnesium, vitamin D and fluoride. *Food and Nutrition Board*, Washington, DC: National Academy Press.

Iroanya, O. O., Egwuatu, T. F., and Chukwudozie, O. (2018). Hepatoprotective effect of the ethanolic extract of *Jatropha tanjorensis* on acetaminophen-induced toxicity in rat model. *Bio-Research*, *16*(1), 995-1001.

Iwalewa, E. O., Adewunmi, C. O., Omisore, N. O. A., Adebanji, O. A., Azike, C. K., Adigun, A. O., and Olowoyo, O. G. (2005). Pro-and antioxidant effects and cytoprotective potentials of nine edible vegetables in southwest Nigeria. *Journal of Medicinal Food*, *8*(4), 539-544.

Jimoh, F. O., and Oladiji, A. T. (2005). Preliminary studies on Piliostigma thonningii seeds: proximate analysis, mineral composition and phytochemical screening. *African Journal of Biotechnology*, *4*(12).

Jorgensen, P. M., and Leon-Yanez, S. (Eds.). (1999). *Catálogo de las plantas vasculares del Ecuador* (Vol. 75, pp. 633-668). Missouri Botanical Garden.

Joseph, H., Gleye, J., Moulis, C., Mensah, L. J., Roussakis, C., and Gratas, C. (1988). Justicidin B, a cytotoxic principle from *Justicia pectoralis*. *Journal of natural products*, *51*(3), 599-600.

Kallner, A. (1982). Vitamin C: man's requirement. In: Vitamin C (Ascorbic Acid). (J.N Counsell and D.H, Horing. Eds) pp 63-73, Applied Science, London

Kamel, J. M. (1991). An extract of the mesocarps of fruits of *Balanite aegyptiaca* exhibited prominent antidiabetic properties in mice. *Chemical and Pharmaceutical Bulletin*, *39*, 1229-1233.

Karakaya, S., El, S. N., Karagözlü, N., & Şahin, S. (2011). Antioxidant and antimicrobial activities of essential oils obtained from oregano (*Origanum vulgare* ssp. hirtum) by using different extraction methods. *Journal of medicinal food*, *14*(6), 645-652.

Kaur, C., and Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science & Technology*, *37*(2), 153-161.

Kedare, S. B., and Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of food science and technology*, *48*, 412-422.

Kelley, K. W., Weigent, D. A., and Kooijman, R. (2007). Protein hormones and immunity. *Brain, behavior, and immunity*, *21*(4), 384-392.

Kelly, R. A. (1990). Cardiac glycosides and congestive heart failure. *The American journal of cardiology*, *65*(10), E10-E16.

Khadka, D., Dhamala, M. K., Li, F., Aryal, P. C., Magar, P. R., Bhatta, S. and Shi, S. (2021). The use of medicinal plants to prevent COVID-19 in Nepal. *Journal of ethnobiology and ethnomedicine*, *17*(1), 1-17.

Khan, I., Jan, S. A., Shinwari, Z. K., Ali, M., Khan, Y., and Kumar, T. (2017). Ethnobotany and medicinal uses of folklore medicinal plants belonging to family Acanthaceae: An updated review. *MOJ Biol Med*, *1*(2), 34-38.

Khatiwora, E., Adsul, V. B., Kulkarni, M., Deshpande, N. R., and Kashalkar, R. V. (2012). Antibacterial activity of Dibutyl Phthalate: A secondary metabolite isolated from *Ipomoea carnea* stem. *J Pharm Res*, *5*(1), 150-152.

Kitadi, J. M., Inkoto, C. L., Lengbiye, E. M., Tshibangu, D. S. T., Tshilanda, D. D., Ngbolua, K. N and Mpiana, P. T. (2021). Mineral Composition of Medicinal Plants Traditionally Used in the Management of Sickle Cell Disease in the Kwilu Province (Congo-Kinshasa).

Kitadi, J. M., Lengbiye, E. M., Gbolo, B. Z., Inkoto, C. L., Muanyishay, C. L., Lufuluabo, G. L., and Mpiana, P. T. (2019). *Justicia secunda* Vahl species: Phytochemistry, Pharmacology and Future Directions: a mini-review. *Discovery Phytomedicine*, *6*(4), 157-158171.

Kittakoop, P., Mahidol, C., and Ruchirawat, S. (2014). Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. *Current topics in medicinal chemistry*, *14*(2), 239-252.

Knuiman, J. T., and West, C. E. (1982). The concentration of cholesterol in serum and in various serum lipoproteins in macrobiotic, vegetarian and non-vegetarian men and boys. *Atherosclerosis*, *43*(1), 71-82.

Kobrin, S. M., and Goldfarb, S. (1990, November). Magnesium deficiency. In *Seminars in Nephrology* (Vol. 10, No. 6, pp. 525-535).

Koche, D., Shirsat, R., and Kawale, M. A. H. E. S. H. (2016). An overview of major classes of phytochemicals: their types and role in disease prevention. *Hislopia J*, *9*(1/2), 1-11.

Koffi, E. N., Le Guernevé, C., Lozano, P. R., Meudec, E., Adjé, F. A., Bekro, Y. A., and Lozano, Y. F. (2013). Polyphenol extraction and characterization of *Justicia secunda* Vahl leaves for traditional medicinal uses. *Industrial Crops and Products*, *49*, 682-689.

Koffi, E., Kassi, A. B. B., Adje, F. A., Lozano, Y. F., and Bekro, Y. A. (2020). Effect of freeze-drying and spray-drying on total phenolics content and antioxidant activity from aqueous extract of *Justicia secunda* leaves. *Trends in Phytochemical Research*, *4*(2), 69-76.

Kris-Etherton, P. M., Harris, W. S., and Appel, L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *circulation*, *106*(21), 2747-2757.

Krishnamoorthy, K., and Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. *International scholarly research notices*, *2014*.

Kromhout, D., Bosschieter, E. B., and Coulander, C. D. L. (1982). Dietary fibre and 10-year mortality from coronary heart disease, cancer, and all causes: the Zutphen Study. *The Lancet*, *320*(8297), 518-522.

Kumar, A., and Sharma, S. (2008). An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): a review. *Industrial crops and products*, *28*(1), 1-10.

Kumar, D. R. N., George, V. C., Suresh, P. K., and Kumar, R. A. (2015). Cancer-specific chemoprevention and anti-metastatic potentials of *Rheum emodi* rhizome ethyl acetate extracts and identification of active principles through HPLC and GC-MS analysis. *Pak J Pharm Sci*, *28*(1), 83-93.

Kumar, R. M., Gayatri, N., Sivasudha, T., and Ruckmani, K. (2017). Profiling of bioactive components present in *Ziziphus mauritiana* Lam for in-vitro antioxidant and in-vivo antiinflammatory activities. *International Research Journal of Pharmacy*, *8*(9).

Kuppuswamy, K. M., Jonnalagadda, B., and Arockiasamy, S. (2013). GC-MC analysis of chloroform extract of *Croton bonplandianum*. *International Journal of Pharma and Bio Sciences*, *4*(4).

Langenheim, J. H. (1990). Plant resins. *American Scientist*, *78*(1), 16-24.

Larsson, S. C., Virtanen, M. J., Mars, M., Männistö, S., Pietinen, P., Albanes, D., and Virtamo, J. (2008). Magnesium, calcium, potassium, and sodium intakes and risk of stroke in male smokers. *Archives of internal medicine*, *168*(5), 459-465.

Lateef, M., Iqbal, Z., Khan, M. N., Akhtar, M. S., and Jabbar, A. (2003). Anthelmintic activity of *Adhatoda vesica* roots. *International journal of Agriculture and Biology*, *5*(1), 86-90.

Law, N. A., Caudle, M. T., and Pecoraro, V. L. (1998). Manganese redox enzymes and model systems: properties, structures, and reactivity. In *Advances in inorganic chemistry* (Vol. 46, pp. 305-440). Academic Press.

Leal, L. K. A. M., Ferreira, A. A. G., Bezerra, G. A., Matos, F. J. A., and Viana, G. S. B. (2000). Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: a comparative study. *Journal of ethnopharmacology*, *70*(2), 151-159.

Lee, J. H., Yang, H. Y., Lee, H. S., and Hong, S. K. (2008). Chemical composition and antimicrobial activity of essential oil from cones of *Pinus koraiensis*. *Journal of microbiology and biotechnology*, *18*(3), 497-502.

Leonard, E. C. (1958). The Acanthaceae of Colombia, III: Subfamily Acanthoideae, completed. *The Acanthaceae of Colombia*.

Levine, M. (1986). New concepts in the biology and biochemistry of ascorbic acid. *New England Journal of Medicine*, *314*(14), 892-902.

Li, Q., Ning, P., Zheng, L., Huang, J., Li, G., and Hsiang, T. (2010). Fumigant activity of volatiles of Streptomyces globisporus JK-1 against *Penicillium italicum* on *Citrus microcarpa*. *Postharvest Biology and Technology*, *58*(2), 157-165.

Liang, X., Huang, Y., Pan, X., Hao, Y., Chen, X., Jiang, H., and Yang, Z. (2020). Erucic acid from *Isatis indigotica* Fort. suppresses influenza A virus replication and inflammation in vitro and in vivo through modulation of NF-κB and p38 MAPK pathway. *Journal of pharmaceutical analysis*, *10*(2), 130-146.

List, P. H., and Hörhammer, L. (Eds.). (1969). *Hagers Handbuch der Pharmazeutischen Praxis: Für Apotheker, Arzneimittelhersteller, Ärzte und Medizinalbeamte: Wirkstoffgruppen II Chemikalien und Drogen (A-AL)*. Berlin, Germany: Springer.

Lohdip A, Oyewale A, Amupitan J. (2014). Isolation and characterization of hexadec-12-enoic acid from the stem of *Chenopodium ambrosioides* (Linn). Journal of Chemical Society of Nigeria, 39: 32-37.

Lopez-Velez, M., Martinez-Martinez, F., and Valle-Ribes, C. D. (2003). The study of phenolic compounds as natural antioxidants in wine.

Lowry, S. E., Robert, L. H., Lehman, I. R., Lefkotz, R.J., Hamher, P. and White, A. (1983).

Luo, H., Tang, Q. L., Shang, Y. X., Liang, S. B., Yang, M., Robinson, N., and Liu, J. P. (2020). Can Chinese medicine be used for prevention of corona virus disease 2019 (COVID-19). A review of historical classics, research evidence and current prevention programs. *Chinese journal of integrative medicine*, *26*, 243-250.

M. P. Romani, (2013) Magnesium in health and disease. In A. Sigel, H. Sigel, R. K. O. Sigel, (Ed.), *Interrelations between Essential Metal Ions and Human Diseases, Metal Ions in Life Sciences,* Vol. 13. Ch. 3. Dordrecht: Springer, p49-79.

Madhuri, S., and Pandey, G. (2009). Some anticancer medicinal plants of foreign origin. *Current science*, 779-783.

Madubuike, K. G., Yusuf, N. O., and Robinson, E. S. (2015). Evaluation of the in vitro and in vivo antioxidant potentials of *Jatropha tanjorensis* methanolic leaf extract. *International Journal Pharmacognosy Phytochemical Research*, *7*(4), 648-52.

Madubuike, K. G., Yusuf, N., and Ibekwe, A. M. (2015). Hepatoprotective activity of methanolic extract of *Jatropha tanjorensis* in carbontetrachloride–induced hepatotoxicity. *Archives of Applied Science Research*, *7*(5), 45-48.

Madubuike, K. G., Yusuf, N.O., and Ahiarakwem, J. K. (2015). Myeloprotective activity of methanolic leaf extract of *Jatropha tanjorensis* in cyclophosphamide induced bone marrow suppression. *Annals of Biological Research*, 6 (4):43-46

Maggi, F., Fortuné Randriana, R., Rasoanaivo, P., Nicoletti, M., Quassinti, L., Bramucci, M., and Vittori, S. (2013). Chemical composition and in vitro biological activities of the essential oil of *Vepris macrophylla* (Baker) I. Verd. endemic to Madagascar. *Chemistry & biodiversity*, *10*(3), 356-366.

Mahabir, D., and Gulliford, M. C. (1997). Use of medicinal plants for diabetes in Trinidad and Tobago. *Revista Panamericana de Salud Pública*, *1*, 174-179.

Malick, C. P., and Singh, M. B. (1980). Plant Enzymology and Histo Enzymology Kalyari"Publishers New Delhi pp, 286.

Mammalian Biochemistry, 3rd edn. McGraw- Hill Book Co. (UK). Pp 666-676

Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*, *79*(5), 727-747.

Maret, W., and Sandstead, H. H. (2006). Zinc requirements and the risks and benefits of zinc supplementation. *Journal of trace elements in medicine and biology*, *20*(1), 3-18.

Marles, R. J., and Farnsworth, N. R. (1995). Antidiabetic plants and their active constituents. *Phytomedicine*, *2*(2), 137-189.

Mathew, J., Sankar, P. and Varacallo, M. (2021). Physiology, blood plasma. In *Stat Pearls [Internet]*. StatPearls Publishing.

McLaughlin, J. L., Chang, C. J., and Smith, D. L. (1991). Bench-top bioassays for the discovery of bioactive natural products: an update. *Studies in natural products chemistry*, *9*, 383-409.

Mea, A., Ekissi, Y. H. R., Abo, K. J. C., and Kahou Bi, G. P. (2017). Hypogglycaemiant and antihyperglycaemiant effect of *Justicia secunda* M. Vahl (Acanthaceae) on Glycaemia in the wistar rat. *International Journal of Development Research*, *7*(06), 13178-13184.

Mensah, J. K., Okoli, R. I., Ohaju-Obodo, J. O., and Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *African journal of Biotechnology*, *7*(14).

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., and Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, *15*(2), 127-130.

Meskat, M., and Hussain, M. M. (2012). Cytotoxic activity of methanolic extracts of *Justica adhatoda*. *International Journal of Pharmaceutical Sciences and Research*, *3*(8), 2655.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. J., and McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica*, *45*(05), 31-34.

Mgbeje, B. I., Umoh, E. U., and Ekpe, O. (2019). Comparative Proximate, Vitamin and Mineral Composition of Leaves of Four Selected Tropical Vegetable Plants Namely: *Ocimum gratissimum, Piper guineense*, *Gongronema latifolium* and *Vernonia amygdalina*. *European Journal of Nutrition and Food Safety*, *10*(1), 84-93.

Michael, A. S., Thompson, C. G., and Abramovitz, M. (1956). *Artemia salina* as a test organism for bioassay. *Science*, *123*(3194), 464-464.

Michelle, A; Hopkins, P.J.J; McLaughlin, C.W; Johnson, S.E; Warner, M.Q; Lahart, D.F and Wright, J.D (1993). Lipids, Human Biology, and Health, Eagle Wood Cliffs, New Jersey, Usa-Prentice Hall, p1176-1180.

Modak, A. T., and Rao, M. R. R. (1966). Hypoglycemic activity of a non-nitrogenous principle from the leaves of *Adhatoda vasica* nees. *Indian J Pharm*, *28*, 105-06.

Mohammed, M. S., Khalid, H. S., Ahmed, W. J., Garelnabi, E. A., and Mahmoud, A. M. (2014). Analysis of anti–inflammatory active fractions of *Tribulus terrestris* by high resolution GC-MS. *Journal of Pharmacognosy and Phytochemistry*, *3*(2), 38-42.

Mohammed, Y.M., Shovon, B., Abida, S., Shukanta, B., Ashish, K.S., Shujit, C.P and Asaduz, Z. (2017). Evaluation of amino acid profile of *Artocarpus heterophyllus* seed and its utilization for development of protein enriched supplementary food. Journal of Noakhali Science and Technology University. 1(1): 77-84.

Montoro, P., Braca, A., Pizza, C., and De Tommasi, N. (2005). Structure–antioxidant activity relationships of flavonoids isolated from different plant species. *Food chemistry*, *92*(2), 349-355.

Morsy, N. (2017). Cardiac glycosides in medicinal plants. *Aromatic and medicinal plants–back to nature. London: Intechopen*, 29-45.

Morton, J. F. (1981). *Atlas of medicinal plants of Middle America: Bahamas to Yucatan*. Charles C. Thomas.

Moswa, J. L., Kapanda, N., Mungende, D. M., Okitolonda, W., Mayangi, M., Mihigo, S., and Mbale, K. (2005, August). Plants as an Important Source of Iron for the Treatment of Anaemia: Case of *Justicia secunda*. In *11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar, August* (pp. 9-12).

Mpiana, P. T., Bokota, M.T., Ndjmele, M.B.L., Mudogo, V., Tshibangu, D.S.T., Ngbolua, K.N., Atibu, E.K., Kwembe, J.T.K., and Makelele, L.K. (2010). Antisickling Activity of Three Species of Justicia from Kisangani (DR Congo): *J. tenella, J. gendarussa and J. insularis*. *International Journal of Biological and Chemical Sciences*, *4*(6).

Mpiana, P. T., Ngbolua, K. N. N., Bokota, M. T., Kasonga, T. K., Atibu, E. K., Tshibangu, D. S., and Mudogo, V. (2010). In vitro effects of anthocyanin extracts from *Justicia secunda* Vahl on the solubility of haemoglobin S and membrane stability of sickle erythrocytes. *Blood transfusion*, *8*(4), 248.

Mpiana, P. T., Ngbolua, K. N., Bokota, M. T., Kasonga, T. K., Atibu, E. K., and Mudogo, V. (2009, August). Antisickling activity of *Justicia secunda* Vahl. In *Drug discovery from African rain forest, The 13th Symposium of the Natural Product Research Network for Eastern and Central Africa, August* (pp. 10-14).

Mueller, A., Antus, S., Bittinger, M., Dorsch, W., Kaas, A., Kreher, B.and Wagner, H. (1993). Chemistry and Pharmacology of the Antiasthmatic Plants *Galphimia glauca, Adhatoda vasica*, and *Picrorhiza kurrooa*. *Planta Medica*, *59*(S 1), A586-A587.

Muller, H. G., and Tobin, G. (1980). *Nutrition and food processing*. Croom Helm.

Murray-Kolbe, L. E., BJIICP, B. J., and Blackman, M. R. (2010). Encyclopedia of Dietary Supplements.

Musa, A. M., Ibrahim, M. A., Aliyu, A. B., Abdullahi, M. S., Tajuddeen, N., Ibrahim, H., and Oyewale, A. O. (2015). Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). *Journal of intercultural ethnopharmacology*, *4*(2), 129.

N'guessan, K., Kouassi, K. H., and Ouattara, D. (2010). Plants used to treat anaemia, in traditional medicine, by Abbey and Krobou populations, in the South of Côte-d'Ivoire. *J Appl Sci Res*, *6*, 1291-1297.

Najda, A., Almehemdi, A. F. and Zabar, A. F. (2013). Chemical composition and nutritional value of jatropha (*Jatropha curcas* L.) leaves. *Journal of Genetic and Environmental Resources Conservation*, *1*(3), 222-227.

Naqvi, S., Khan, M. S. Y., and Vohora, S. B. (1991). Anti-bacterial, anti-fungal and anthelmintic investigations on Indian medicinal plants. *Fitoterapia*, *62*(3), 221-228.

National Research Council, Food Nutrition Board, (1980) Copper. In: *Recommended Dietary Allowances*, Washington, DC: NRC/NAS, p151-154.

Navarro, E., Alonso, S. J., Navarro, R., Trujillo, J., and Jorge, E. (2006). Elenoside increases intestinal motility. *World Journal of Gastroenterology: WJG*, *12*(44), 7143.

Navarro, E., Alonso, S. J., Trujillo, J., Jorge, E., and Pérez, C. (2004). Central nervous activity of elenoside. *Phytomedicine*, *11*(6), 498-503.

Nguta, J. M., and Mbaria, J. M. (2013). Brine shrimp toxicity and antimalarial activity of some plants traditionally used in treatment of malaria in Msambweni district of Kenya. *Journal of ethnopharmacology*, *148*(3), 988-992.

Niki, E., Kawakami, A., Yamamoto, Y., and Kamiya, Y. (1985). Oxidation of lipids. VIII. Synergistic inhibition of oxidation of phosphatidylcholine liposome in aqueous dispersion by vitamin E and vitamin C. *Bulletin of the Chemical Society of Japan*, *58*(7), 1971-1975.

Nwachukwu, C. N. (2018). Nutrient, phytochemical and anti-nutrient evaluation of *Jatropha tanjorensis leaf* (hospital too far). *Journal of Agriculture and Food Sciences*, *16*(2), 36-46.

Nwaogu, L. A., Alisi, C. S., Ibegbulem, C. O., and Igwe, C. U. (2007). Phytochemical and antimicrobial activity of ethanolic extract of Landolphia owariensis leaf. *African Journal of Biotechnology*, *6*(7).

Nwiloh, B. I., Uwakwe, A. A., and Akaninwor, J. O. (2016). Phytochemical screening and GC-FID analysis of ethanolic extract of root bark of *Salacia nitida* L. Benth. *Journal of medicinal plants Studies*, *4*(6), 283-287.

Oboh, F. O., and Masodje, H. I. (2009). Nutritional and antimicrobial properties of *Jatropha tanjorensis leaves. American-Eurasian Journal of Scientific Research*, *4*(1), 7-10.

Odokwo, E. O. (2020). Volatile Constituents of the Leaves and Stem of *Justicia secunda* Vahl. *Communication in Physical Sciences*, *6*(2).

Oduola, T., Avwioro, O. G., and Ayanniyi, T. B. (2005). Suitability of the leaf extract of *Jatropha gossypifolia* as an anticoagulant for biochemical and haematological analyses. *African Journal of Biotechnology*, *4*(7), 679-681.

Ogbuagu, A., Eric, S., Obumselu, F., and Ogbuagu, J. (2019). Qualitative and quantitative phytochemical and physicochemical analyses on the oil and extracts from *Ricinodendron Heudelotii. Chemical and Pharmaceutic Research*, *1*(1), 1-7.

Ogunbamowo, P. O., Olaniyi, M. B., Awotedu, O. L., and Lawal, I. O. (2020, December). Assessment of the foliar micromorphology, phytochemical and mineral composition of *Justicia secunda* Valh leaves. In *Anales de Biología* (Vol. 42, pp. 183-192). Servicio de Publicaciones de la Universidad de Murcia.

Ogunleye, D. S., and Ibitoye, S. F. (2003). Studies of antimicrobial activity and chemical constituents of *Ximenia americana. Tropical Journal of Pharmaceutical Research*, *2*(2), 239-241.

Ohta, K., and Munakata, K. (1970). Justicidin C and D, the 1-methoxy-2, 3-naphthalide lignans, isolated from *Justicia procumbens* L. *Tetrahedron Letters*, *11*(12), 923-925.

Ojukwu, U., and Ibekwe, O. (2018). Phytochemical and antimicrobial screening and nutritional qualities of *ficus sur* (forssk). *Int J Engineering Technologies Management Res*, *5*(10), 35-44.

Okeke, C. U., Izundu, A. I., and Uzoechinda, E. (2008). Phytochemical and proximate study of female pawpaw (*Carica papaya* Linn.) Caricaceae. *Journal of science, Engineering and Technology*, *15*(2), 8207-8216.

Okunade, O. A., and Adesina, K. (2014). Preliminary study on the nutritional, anti-nutritional and elemental composition of bishops vegetable (*Jatropha tanjorensis*) and Cashew shoot (*Anarcadium occidentale)* Leaves. *International Journal of Advanced Research in Chemical Science (IJARCS)*, *1*(7), 43-46.

Okwu, D. E., and Emenike, I. N. (2006). Evaluation of the phytonutrients and vitamin contents of Citrus fruits. *Int. J. Mol. Med. Adv. Sci*, *2*(1), 1-6.

Olaniyi, A.A (1980). *Justicia flava* Vahl: studies on the petroleum ether extract of the leaves, stems and roots. *Niger J Pharm.,* 11: 133–134.

Olayiwola, G., Iwalewa, E. O., Omobuwajo, O. R., Adebajo, A. C., Adeniyi, A. A., and Verspohl, E. J. (2004). The antidiabetic potential of *Jatropha tanjorensis* leaves. *Nigerian Journal of Natural Products and Medicine*, *8*, 55-58.

Oliveira, A. F. M., Xavier, H. S., Silva, N. H., and Andrade, L. H. C. (2000). Chromatographic screening of medicinal Acanthaceae: *Justicia pectoralis* Jacq. and *J. gendarussa* Burm. *Revista Brasileira de Plantas Medicinais, 3*(1), 37-41.

Olson, R. (1988). Inhibition of free radical chain oxidation by alpha-tocopherol and other plasma antioxidants. *Nutr. Rev*, *46*, 206.

Olutiola, P. O., Famurewa, O., and Sonntag, H. G. (1991). An introduction to general microbiology: a practical approach. *Germany: Heidelberger Verlagsanstalt und Druckerei GmbH Heidelberg*.

Omer, E., Elshamy, A., El Gendy, A. N., Cai, X., Sirdaarta, J., White, A., and Cock, I. E. (2016). Cakile maritima Scop. extracts inhibit the growth of some bacterial triggers of autoimmune diseases: GC-MS analysis of an inhibitory extract. *Pharmacognosy Journal*, *8*(4).

Omoregie, E. S., and Osagie, A. U. (2012). Antioxidant properties of methanolic extracts of some Nigerian plants on nutritionally-stressed rats. *Nigerian Journal of Basic and Applied Sciences*, *20*(1), 7-20.

Omoregie, E. S., and Sisodia, B. S. (2012). In vitro antiplasmodial activity and cytotoxicity of leaf extracts of *Jatropha tanjorensis* JL Ellis and Soroja. *Bayero Journal of Pure and Applied Sciences*, *5*(1), 90-97.

Omoregie, E. S., Osagie, A. U., and Iruolaje, E. O. (2011). In vitro antioxidant activity and the effect of methanolic extracts of some local plants on nutritionally stressed rats. *Pharmacologyonline*, *1*, 23-56.

Onoja, S. O., Ezeja, M. I., Omeh, Y. N., and Onwukwe, B. C. (2017). Antioxidant, anti-inflammatory and antinociceptive activities of methanolic extract of *Justicia secunda* Vahl leaf. *Alexandria Journal of Medicine*, *53*(3), 207-213.

Onyeabo, C., Achi, N. K., Ekeleme-Egedigwe, C. A., Ebere, C. U., and Okoro, C. K. (2017). Haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats. *Acta Scientiarum Polonorum Technologia Alimentaria*, *16*(2), 217-230.

Orhue, E.S., Idu, M., Ataman, J.E. and Ebite, L.E (2008). Haematological and Histopathological Studies of *Jatropha tanjorensis* (J.L. Ellis and Soroja) Leaves in Rabbits. *Asian Journal of Biological Sciences, 1: 84-89*

Ornoregie, E. S., and Osagie, A. U. (2007). Phytochemical screening and anti-anaemic effect of *Jatropha tanjorensis* leaf in protein malnourished rats. *Plant Archives*, *7*(2), 509-516.

Osioma, E., and Hamilton-Amachree, A. (2017). Comparative study on the phytochemical and in vitro antioxidant properties of methanolic leaf extract of *Justicia secunda* vahl. *Nigerian Journal of Science and Environment*, *15*(1), 111-117.

Osredkar, J., and Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *Journal of Clinical Toxicology S*, *3*(2161), 0495.

Osuchukwu, I. W., Sakpa, C. L., Ekezie, J., Okeke, C. U., Eke, C. C., and Ezejindu, D. N. (2016). Effect of Leave Extract of *Jatropha tanjorensis* On The Testis Of Wistar Rats. *Journal of Dental and Medical Sciences*, *15*(4), 66-71.

Otaiza, R. G., Arzola, J. C., and Rodríguez, M. C. (2006). Plantas Medicinales de la Mesa de Los Indios, Municipio Campo Elías (Estado Mérida, Venezuela). *Plantula*, *4*(1), 55-68.

Overington, J. P., Al-Lazikani, B., and Hopkins, A. L. (2006). How many drug targets are there?. *Nature reviews Drug discovery*, *5*(12), 993-996.

Oyedoh, E. A., Erumi, G. A., and Akhabue
, C. E. (2020). Comparative study of response surface methodology and artificial neural network for modeling and optimization of extraction process parameters on *Tetrapleura tetraptera*. *Journal of Applied Sciences and Environmental Management*, *24*(2), 313-321.

Oyewole, I. O., Magaji, Z. J., and Awoyinka, O. A. (2007). Biochemical and Toxicological studies of aqueous extract of *Tithonia diversifolia* (Hemsl.) leaves in Wistar albino rats. *Journal of Medicinal Plants Research*, *1*(2), 30-33.

Oyewole, O. I., and Akingbal, P. F. (2011). Phytochemical analysis and hypolipidemic properties of *Jatropha tanjorensis* leaf extract. *European Journal of Medicinal Plants*, *1*(4), 180.

Oyewole, O. I., and Akingbala, P. F. (2011). Phytochemical analysis and hypolipidemic properties of *Jatropha tanjorensis* leaf extract. *European Journal of Medicinal Plants*, *1*(4), 180.

Oyewole, O. I., and Akingbala, P. F. (2011). Phytochemical analysis and hypolipidemic properties of *Jatropha tanjorensis* leaf extract. *European Journal of Medicinal Plants*, *1*(4), 180.

Ozcan, L., and Tabas, I. (2016). Calcium signalling and ER stress in insulin resistance and atherosclerosis. *Journal of internal medicine*, *280*(5), 457-464.

Packer, L. (1991). Protective role of vitamin E in biological systems. *The American journal of clinical nutrition*, *53*(4), 1050S-1055S.

Palacios, C. (2006). The role of nutrients in bone health, from A to Z. *Critical reviews in food science and nutrition*, *46*(8), 621-628.

Palii, S. S., Kays, C. E., Deval, C., Bruhat, A., Fafournoux, P., and Kilberg, M. S. (2009). Specificity of amino acid regulated gene expression: analysis of genes subjected to either complete or single amino acid deprivation. *Amino acids*, *37*(1), 79-88.

Patel, S. S., and Zaveri, M. N. (2012). Cytotoxic activity to find bioactive compound from *Justicia gendarussa* using brine shrimp lethality assay. *Asian Journal of Traditional Medicines*, *7*(3), 102-108.

Patel, V. K., and Venkatakrishna-Bhatt, H. (1984). In vitro study of antimicrobial activity of Adhatoda vasika Linn. (leaf extract) on gingival inflammation--a preliminary report. *Indian journal of medical sciences*, *38*(4), 70-72.

Pauldasan, A., Arockiyaehil, I.T. and Anand, V. G (2020) phytochemical screening and GC-MS studies of *Cyperus compressus* Rottb. Journal of Medicinal Plants Studies; 8: 90-93

Perry, L. M., & Metzger, J. (1980). *Medicinal plants of east and southeast Asia: attributed properties and uses*. MIT press.

Pierre, M., Abrogoua, D. P., Bahi, C., Dano, D. S., Gnahoui, G., and Jerocirc, B. (2011). Evaluation of the antihypertensive activity of total aqueous extract of *Justicia secunda* Valh (Acanthaceae). *African Journal of Pharmacy and Pharmacology*, *5*(16), 1838-1845.

Plum, L. M., Rink, L., and Haase, H. (2010). The essential toxin: impact of zinc on human health. *International journal of environmental research and public health*, *7*(4), 1342-1365.

Polcz, M. E., and Barbul, A. (2019). The role of vitamin A in wound healing. *Nutrition in Clinical Practice*, *34*(5), 695-700.

PoonamKushwaha, Darshan Singh and Ganga Bisht. (2015). Chemical composition of volatile extract of Gnaphaliumluteo-album l. Its and its antibacterial activity. *Journal of Drug Discovery and Therapeutics* Volume 3, Issue 30: 23-28

Prabakaran, A. J., and Sujatha, M. (1999). *Jatropha tanjorensis* Ellis & Saroja, a natural interspecific hybrid occurring in Tamil Nadu, India. *Genetic Resources and Crop Evolution*, *46*(3), 213-218.

Prasad, A. S. (2003). Zinc deficiency: has been known of for 40 years but ignored by global health organisations. *Bmj*, *326*(7386), 409-410.

Prasad, A. S. (2004). Zinc deficiency: its characterization and treatment. *Metal ions in biological systems*, *41*, 103-138.

Pulido, R., Bravo, L., and Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of agricultural and food chemistry*,*48*(8), 3396-3402.

R. K. Rude, (2010) Magnesium. In: P. M. Coates, J. M. Betz, M. R. Blackman, G. M. Cragg, M. Levine, J. Moss, J. D. White, (Ed.), *Encyclopedia of Dietary Supplements*, 2nd ed. New York, NY: Informa Healthcare, p527-537

R. K. Rude, (2012) Magnesium. In: A. C. Ross, B. Caballero, R. J. Cousins, K. L. Tucker, T. R. Ziegler, (Ed.), Modern Nutrition in Health and Disease, 11th ed. Baltimore, Mass: Lippincott Williams & Wilkins, p159-175.

Rabizadeh, F., Mirian, M. S., Doosti, R., Kiani-Anbouhi, R., and Eftekhari, E. (2022). Phytochemical Classification of Medicinal Plants Used in the Treatment of Kidney Disease Based on Traditional Persian Medicine. *Evidence-Based Complementary and Alternative Medicine*, *2022*.

Rahman, M. M., Ahmad, S. H., Mohamed, M. T. M., and Ab Rahman, M. Z. (2014). Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*. *The Scientific World Journal*, *2014*.

Rajalakshmi, K., and Mohan, V. R. (2016). GC–MS analysis of bioactive components of *Myxopyrum serratulum* AW Hill (Oleaceae). *Int J Pharm Sci Rev Res*, *38*(1), 30-5.

Rajendiran, C., Pai, B. R., and Subramanian, P. S. (1991). Lignans of *Justicia glauca* Rottl. *Indian J Chem Sect B*, *30*, 681-683.

Rajore, S., and Batra, A. (2003). *Jatropha curcas* L.: a plant of immense potential value. *Journal of Economic and Taxonomic Botany*, *27*(1), 36-41.

Raju, G. S., and Pillai, K. R. (1989). Lignans from *Justicia tranquebariensis* Linn. *Indian J. Chem*, *28*, 558-561.

Rasyid, M. I., Yuliani, H., and Angraeni, L. (2020, June). Toxicity test LC50 of pineung nyen teusalee Seeds (*Areca catechu*) extract by brine shrimp lethality test (BSLT) Methode. In *IOP Conference Series: Earth and Environmental Science* (Vol. 515, No. 1, p. 012052). IOP Publishing.

Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon*, *39*(5), 603-613.

Raven, P.M., Johnson, G.B. and Madison, W.I. (1999). Biology. 2nd Edition, McGraw-Hill, London, pp. 45-67.

Raymond, S. S., Jonathan, S. J., and Watkins-Pitchford, J. M. (2010). The essence of analgesia and analgesics. *Cambridge University Press*, 82-90.

Rice-Evans, C. A., Miller, N. J., and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.

Rizwan, A. A., Rabiu, K. M., Ayuba, V., and Omolabake, O. O. (2020). Review on *Jatropha tanjorensis* (Hospital too far); Significance as an Anti-Anaemia Plant. *International Blood Research & Reviews*, 1-7.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food chemistry*, *66*(4), 401-436.

Robbers, J. E., Speedie, M. K., and Tyler, V. E. (1996). *Pharmacognosy and pharmacobiotechnology*. Williams & Wilkins.

Roe, J. H., and Kuether, C. A. (1943). The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *Journal of Biological chemistry*, *147*, 399-407.

Roger, M. (2011). The minerals you need.

Rojas, J. J., Ochoa, V. J., Ocampo, S. A., and Muñoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC complementary and alternative medicine*, *6*(1), 1-6.

Ross, A. C. (2010). Vitamin A. In *Bioactive compounds and cancer* (pp. 335-356). Humana Press, Totowa, NJ. DOI: 10.1007/978-1-60761-627-6_16

Russell, R., Beard, J. L., Cousins, R. J., Dunn, J. T., Ferland, G., Hambidge, K., & Yates, A. A. (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *A report of the panel on micronutrients, subcommittees on upper reference levels of nutrients and of interpretation and uses of dietary reference intakes, and the standing committee on the scientific evaluation of dietary reference intakes food and nutrition board Institute of medicine*, 797.

Russo, P., Frustaci, A., Del Bufalo, A., Fini, M., and Cesario, A. (2013). Multitarget drugs of plants origin acting on Alzheimer's disease. *Current medicinal chemistry*, *20*(13), 1686-1693.

Sabandar, C. W., Ahmat, N., Jaafar, F. M., and Sahidin, I. (2013). Medicinal property, phytochemistry and pharmacology of several Jatropha species (Euphorbiaceae): a review. *Phytochemistry*, *85*, 7-29.

Saleem, A., Ahotupa, M., and Pihlaja, K. (2001). Total phenolics concentration and antioxidant potential of extracts of medicinal plants of Pakistan. *Zeitschrift für Naturforschung C*, *56*(11-12), 973-978.

Sarian, M. N., Ahmed, Q. U., Mat So'ad, S. Z., Alhassan, A. M., Murugesu, S., Perumal, V., and Latip, J. (2017). Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based study. *BioMed research international*, *2017*.

Sastry, K. V., Rao, E. V., Pelter, A., and Ward, R. S. (1979). 4-Aryl-2, 3-Naphthalide lignans from *justicia-simplex*. *Indian journal of chemistry section b-organic chemistry including medicinal chemistry*, *17*(4), 415-416.

Sathya, S., Lakshmi, S., and Nakkeeran, S. (2016). Combined effect of biopriming and polymer coating on chemical constituents of root exudation in chilli (*Capsicum*

annuum L.) cv. K 2 seedlings. *Journal of Applied and Natural Science*, *8*(4), 2141-2154.

Satyal, P., Maharjan, S., and Setzer, W. N. (2015). Volatile constituents from the leaves, fruits (berries), stems and roots of *Solanum xanthocarpum* from Nepal. *Natural Product Communications*, *10*(2), 1934578X1501000239.

Scholey, J. M., Brust-Mascher, I., and Mogilner, A. (2003). Cell division. *Nature*, *422*(6933), 746-752.

Schweizer, T. F., and Würsch, P. (1991). The physiological and nutritional importance of dietary fibre. *Experientia*, *47*(2), 181-186.

Sharma, S., Stutzman, J. D., Kelloff, G. J., and Steele, V. E. (1994). Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. *Cancer research*, *54*(22), 5848-5855.

Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. *International journal of herbal medicine*, *4*(4), 59-64.

Shi, Q. I. U., Hui, S. U. N., Zhang, A. H., Hong-Ying, X. U., Guang-Li, Y. A. N., Ying, H. A. N., and Xi-Jun, W. A. N. G. (2014). Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chinese Journal of Natural Medicines*, *12*(6), 401-406.

Shirwaikar, A., Shirwaikar, A., Rajendran, K., and Punitha, I. S. R. (2006). In vitro antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine. *Biological and Pharmaceutical Bulletin*, *29*(9), 1906-1910.

Sies, H., Stahl, W., and Sundquist, A. R. (1992). Antioxidant functions of vitamins: Vitamins E and C, Beta-Carotene, and other carotenoids a. *Annals of the New York Academy of Sciences*, *669*(1), 7-20.

Sleet, R. B., and Brendel, K. (1983). Improved methods for harvesting and counting synchronous populations of Artemia nauplii for use in developmental toxicology. *Ecotoxicology and environmental safety*, *7*(5), 435-446.

Stavric, B. (1994). Quercetin in our diet: from potent mutagen to probable anticarcinogen. *Clinical biochemistry*, *27*(4), 245-248.

Subbaraju, G. V., Kumar, K. K. K., Raju, B. L., Pillai, K. R., and Reddy, M. C. (1991). Justiciresinol, a new furanoid lignan from *Justicia glauca*. *Journal of natural products*, *54*(6), 1639-1641.

Suganthy, M., and Gajendra, C. V. (2019). Phytochemical profiling of *Ailanthus excelsa* leaf extracts using GC-MS. *Journal of Pharmacognosy and Phytochemistry*, *8*(5), 1120-1124.

Sun, J., Chu, Y. F., Wu, X., and Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of agricultural and food chemistry*, *50*(25), 7449-7454.

T. Lockett, Christopher C. Calvert, Louis E. Grivetti, C. (2000). Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, Northeastern Nigeria. *International Journal of food sciences and nutrition*, *51*(3), 195-208.

Takahashi, A., Dohi, H., Egashira, Y., and Hirai, S. (2020). Erucic acid derived from rosemary regulates differentiation of mesenchymal stem cells into osteoblasts/adipocytes via suppression of peroxisome proliferator-activated receptor γ transcriptional activity. *Phytotherapy Research*, *34*(6), 1358-1366.

Tan, B., Yin, Y., Liu, Z., Li, X., Xu, H., Kong, X., and Wu, G. (2009). Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs. *Amino acids*, *37*(1), 169-175.

Tan, J. C., Burns, D. L., and Jones, H. R. (2006). Severe ataxia, myelopathy, and peripheral neuropathy due to acquired copper deficiency in a patient with history of gastrectomy. *Journal of Parenteral and Enteral Nutrition*, *30*(5), 446-450.

Taoheed, A. A., Tolulope, A. A., Saidu, A. B., Odewumi, O., Sunday, R. M., and Usman, M. (2017). Phytochemical properties, proximate and mineral composition of *Curcuma longa* Linn. and *Zingiber officinale* Rosc.: A comparative study. *Journal of Scientific Research and Reports*, *13*(4), 1-7.

Teklehaymanot, T. (2009). Ethnobotanical study of knowledge and medicinal plants use by the people in Dek Island in Ethiopia. *Journal of Ethnopharmacology*, *124*(1), 69-78.

Tewari, J. P., and Shukla, I. K. (1982). Inhibition of infectivity of two strains of watermelon mosaic virus by latex of some angiosperms. *Geobios*.

Theiler, B. A., Istvanits, S., Zehl, M., Marcourt, L., Urban, E., Caisa, L. O. E., and Glasl, S. (2017). HPTLC bioautography guided isolation of α-glucosidase inhibiting compounds from *Justicia secunda* Vahl (Acanthaceae). *Phytochemical Analysis*, *28*(2), 87-92.

Tolbert, B. M. (1985). Metabolism and function of ascorbic acid and its metabolites. *International Journal for Vitamin and Nutrition research. Supplement Internationale Zeitschrift fur Vitamin-und Ernahrungsforschung. Supplement*, *27*, 121-138.

Trease, G. E., and Evans, W. C. (1989). Pharmacognsy. 11th edn. Brailliar Tiridel Can.

Trease, G.E., and Evans, I.C. (1983) Pharmacognosy (12th edn) Bailliere Tindall London. pp: 21-22

Treiber, N., Maity, P., Singh, K., Ferchiu, F., Wlaschek, M., and Scharffetter-Kochanek, K. (2012). The role of manganese superoxide dismutase in skin aging. *Dermato-endocrinology*, *4*(3), 232-235.

Tyler, V. E., Brady, L. R., and Roberts, J. E (1988). Pharmacology.Lea and Ferbiger, Philadelphia, Pp. 85-90

Uchenna, E., Obinna, A., Unegbu Chika, C., and Nnaoma Ikenna, E. (2021). Cardioprotective effect of methanol extract of *Jatropha tanjorensis* leaves in isoprenaline induced myocardial infarction in albino rats: cardiac function biomarkers, antioxidant and heart histoarchitecture evaluation. *World Journal of Pharmaceutical Research* 10(6), 78-107.

Ugoeze, K. C., Oluigbo, K. E., and Chinko, B. C. (2020). Phytomedicinal and Nutraceutical Benefits of the GC-FID Quantified Phytocomponents of the Aqueous Extract of *Azadirachta indica* leaves. *Journal of Pharmacy and Pharmacology Research*, *4*(4), 149-163.

Uka, E., Eghianrunwa, Q. A., and Akwo, V. D. (2022). GC-MS Analysis of Bioactive Compounds IN Ethanol leaves extract of Sphenocentrum jollyanum and their biological activities. *world*, *6*(01).

Ukubuiwe, C. C., Daniyan, S. Y., Ukubuiwe, A. C., Busari, B. M., and Abalaka, M. E. (2019). Hypoglycaemic Efficacies of Leaf and Stem Extracts of *Jatropha tanjorensis* (Euphorbiaceae) in Diabetic Mice. J. *Applied Sci*, *19*(4), 331-336. DOI: 10.3923/jas.2019.331.336

Uraih, N., and Izuagbe, Y. (1990). *Public health, food, and industrial microbiology*. Uniben press.

US Department of Agriculture, Agricultural Research Service, (2012) *USDA National Nutrient Database for Standard Reference, Release 25*, USA: Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, USDA-ARS.

US Department of Health and Human Services. (2010). US Department of Agriculture Dietary Guidelines for Americans, 2010. 2010. *Washington, DC: US Government Printing Office*, *7*.

Van Gossum, A., Kurian, R., Whitwell, J., and Jeejeebhoy, K. N. (1988). Decrease in lipid peroxidation measured by breath pentane output in normals after oral supplementation with vitamin E. *Clinical Nutrition*, *7*(1), 53-57.

Van Wyk, A. S., and Prinsloo, G. (2020). Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. *South African Journal of Botany*, *133*, 54-62.

Van Wyk, B. E., and Wink, M. (2018). *Medicinal plants of the world*. CABI.

Vanhaecke, P., Personae, G., Claus, C., and Gorgeous, P. (1981). Proposal for a short-term toxicity test with Artemia nauplii. *Ecotoxicology and environmental safety*, *5*(3), 382-387.

Varma, S.D., Kumar, S. and Richards, R. D. (1979) Light-induced damage to ocular lens cation pump: Prevention by Vitamin C. *Proc Natl. Acad. Sci*. USA 76: 3504-3506.

Viswanathan M.B., Ananthi J.D.J., Kumar P.S. (2012). Antimicrobial activity and bioactive compounds and leaf extract in *J. tanjorensis.* Fitoterapia 83:1153-1159.

Viswanathan, M. B., Ananthi, J. D., Raja, N. L., and Venkateshan, N. (2018). Wound healing activity of *Jatropha tanjorensis* leaves. *Pharm Res*, *3*(4), 24-30.

Vunchi, M. A., Umar, A. N., King, M. A., Liman, A. A., Jeremiah, G., and Aigbe, C. O. (2011). Proximate, vitamins and mineral composition of *Vitex doniana* (black plum) fruit pulp. *Nigerian Journal of Basic and Applied Sciences, 19*(1).

Wang, J., Chen, L., Li, P., Li, X., Zhou, H., Wang, F., and Wu, G. (2008). Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. *The Journal of nutrition*, *138*(6), 1025-1032.

Wardlaw, G. M., and Kessel, M. W. (2002). *Perspectives in nutrition*. McGraw-Hill.

Wasagu, R. S. U., Shehu, S., and Mode, Y. D. (2013). Comparative proximate composition and antioxidant vitamins contents of two honey varieties (light amber and dark amber) from Sokoto State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, *6*(2), 118-120.

Wasshausen, D. C., and Wood, J. R. I. (2004). Acanthaceae of Bolivia. *Contributions from the United States National Herbarium*, *49*.

Watford, M. (2008). Glutamine metabolism and function in relation to proline synthesis and the safety of glutamine and proline supplementation. *The Journal of nutrition*, *138*(10), 2003S-2007S.

Watson, R. R. (2000). *Vegetables, fruits, and herbs in health promotion*. CRC press.

Watson, R. R., and Leonard, T. K. (1986). Selenium and vitamins A, E, and C: nutrients with cancer prevention properties. *Journal of the American Dietetic Association*, *86*(4), 505-510.

Wei, Y., Zhu, J., and Nguyen, A. (2014). Urinary concentrations of dichlorophenol pesticides and obesity among adult participants in the US National Health and Nutrition Examination Survey (NHANES) 2005–2008. *International journal of hygiene and environmental health*, *217*(2-3), 294-299.

Wei, Z., Bin, N., 2011. GC-MS analysis of the chemical components of the volatile oil from leaves of *Aquilaria sinensis* (Lour.) Gilg. Med. Plant 2, 34-36.

Weir, N. L., Johnson, L., Guan, W., Steffen, B., Djousse, L., Mukamal, K. J., and Tsai, M. Y. (2018). cis-Vaccenic acid is associated with lower HOMA-IR and incident T2D in participants from the MESA cohort. *Diabetes*, *67*(Supplement_1).

Weir, N. L., Johnson, L., Guan, W., Steffen, B., Djousse, L., Mukamal, K. J., and Tsai, M. Y. (2018). cis-Vaccenic acid is associated with lower HOMA-IR and incident T2D in participants from the MESA cohort. *Diabetes*, *67*(Supplement_1).

Wellman, N. S. (2009). *Handbook of clinical nutrition and aging* (p. 442). C. W. Bales, and C. S. Ritchie (Eds.). New York, NY, USA: Humana Press.

Wessling-Resnick, M. (2014). Iron. U: Modern Nutrition in Health and Disease. Ross, A.C, Caballero, B, Cousins, R.J, Tucker, KL, Ziegler RG.

Willaman, J. J., and Li, H. L. (1970). Alkaloid-bearing plants and their contained alkaloids, 1957-1968. *Lloydia*, *33*(3A).

Williams, S. (1984). *Official methods of analysis of the Association of Official Analytical Chemists* (No. BOOK). Association of Official Analytical Chemists, Inc.

Willis, R.J and Kratzing, C.C (1976). Extracelluar ascorbic acid in lung. *Biochemical et Biophysica Acta* 144: 108-117

World Health Organization. (1990). *The use of traditional medicine in primary health care* (No. Regional Health Paper No. 19). WHO Regional Office for South-East Asia.

Wright, M. H., Lee, C. J., Arnold, M. S. J., Shalom, J., White, A., Greene, A. C., and Cock, I. E. (2017). GC-MS analysis of *Tasmannia lanceolata* extracts which inhibit the growth of the pathogenic bacterium *Clostridium perfringens*. *Pharmacognosy Journal*, *9*(5).

Wu, G., Bazer, F. W., Cudd, T. A., Meininger, C. J., and Spencer, T. E. (2004). Maternal nutrition and fetal development. *The Journal of nutrition*, *134*(9), 2169-2172.

Yamoah, A., Adosraku, R. K., Amenu, J. D., Baah, M. K., and Abaye, D. A. (2020). Evaluation of the haematinic activities of extracts of *Justicia secunda* Vahl leaves in red blood cells of laboratory rats. *Journal of Biosciences and Medicines*, *8*(3), 48-57.

Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., and Yu, X. (2020). Advances in pharmacological activities of terpenoids. *Natural Product Communications*, *15*(3),

Yirankinyuki, F. F., Magaji, B., Danbature, W. L., and Abdullah, A. M. (2020). Identification of Active Compounds of *Annona muricata* (Soursop) Leaf Wax Extract Using GC-MS. Archives of Current Research International.20(4): 17-21, 2020; Article no. ACRI.57139 ISSN: 2454-7077

Zakaria, M., Simpson, K., Brown, P. R., and Krstulovic, A. (1979). Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes. *Journal of Chromatography A*, *176*(1), 109-117.

Zevenhoven, R., and Kilpinen, P. (2001). Trace elements, alkali metals. *Control of pollutants in flue gases and fuel gases. Finland: Helsinki University of Technology*, 8-1.

Zheng, W., and Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food chemistry*, *49*(11), 5165-5170.

Zhu, F., Du, B., and Xu, B. (2018). Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Critical reviews in food science and nutrition*, *58*(8), 1260-1270.

APPENDIX

Table: A1: Antioxidant vitamins content of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.*

Component	*Justicia secunda*	*Jatropha tanjorensis*	T-value	P-value	Comment
Vitamin C (mg/kg)	72.65 ± 1.85	60.43 ± 1.63	8.584	0.0010	Significant
Vitamin E (mg/kg)	17.96 ± 1.78	14.33 ± 1.33	2.830	0.0474	Significant
Vitamin A (mg/kg)	27.47 ± 0.14	24.61 ± 0.16	23.30	<0.0001	Significant

Table A2: DPPH Radical Scavenging activity (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.*

Extract Concentration (mg/ml)	*Justicia secunda*	*Jatropha tanjorensis*	T-value	P-value	Comment
10	85.87 ± 0.19	93.08 ± 0.22	42.96	<0.0001	Significant
20	87.21 ± 0.23	93.95 ± 0.24	35.12	<0.0001	Significant
40	80.90 ± 0.08	89.65 ± 0.89	16.96	<0.0001	Significant
80	84.47 ± 0.23	87.88 ± 0.29	15.96	<0.0001	Significant
Reference (BHT)	98.24 ± 0.00	98.24 ± 0.00	-	-	-

Table A3: ABTS Radical Scavenging activity (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.*

Extract Concentration (mg/ml)	*Justicia secunda*	*Jatropha tanjorensis*	T-value	P-value	Comment
10	55.18 ± 0.97	67.02 ± 0.90	15.50	0.0001	Significant
20	57.51 ± 0.29	68.29 ± 0.53	30.91	<0.0001	Significant
40	59.41 ± 0.80	71.25 ± 0.36	23.38	<0.0001	Significant
80	61.31 ± 0.78	66.60 ± 0.19	11.41	0.0003	Significant

Table A4: FRAP inhibition activity (%) of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis*.

Extract Concentration (mg/ml)	*Justicia secunda*	*Jatropha tanjorensis*	T-value	P-value	Comment
10	55.52 ± 0.47	31.90 ± 0.18	81.29	<0.0001	Significant
20	68.10 ± 0.16	58.90 ± 0.11	82.07	<0.0001	Significant
40	74.23 ± 0.31	62.58 ± 0.16	57.84	<0.0001	Significant
80	75.46 ± 0.12	64.42 ± 0.18	88.39	<0.0001	Significant

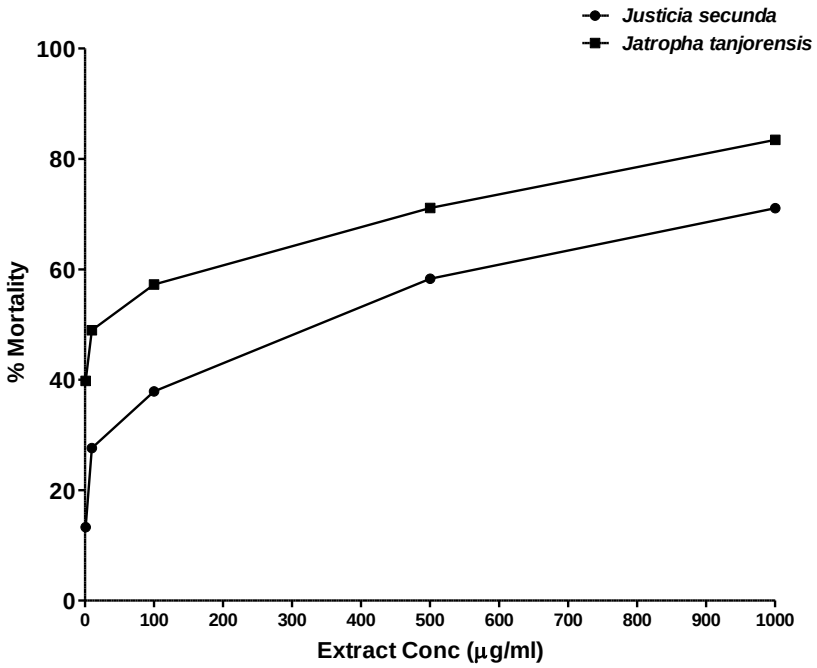


Figure 4.7: Brine shrimp lethality (% mortality) effect of ethanol-water leaf extracts of *Justicia secunda* and *Jatropha tanjorensis.*