IN VITRO ANTIOXIDANT CAPACITY OF ETHANOL EXTRACT OF *Luffa cylindrica* AND ITS HPLC PROFILE

BY

EREDEMI URINRIN OFEGOR

(BMS2009043)

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICINE

UNIVERSITY OF BENIN

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) DEGREE IN MEDICAL BIOCHEMISTRY.

MAY, 2024

CERTIFICATION

We the undersigned hereby certify that EREDEMI URINRIN OFEGOR (BMS2009043) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

Signed

....

Dr. (Mrs.) N. Eluehike (Date)

(Project Supervisor)

....

Dr. F. E. Olumese (Date)

(Head of Department)

....

External Examiner (Date)

DEDICATION

I dedicate this project work to God Almighty and to my loving family, friends and mentors, who have been my unwavering pillars of support throughout this incredible journey of obtaining my B.Sc. in Medical Biochemistry, from the prestigious University of Benin, Edo State. Your constant encouragement, guidance and belief in me have been invaluable. This achievement is a testament to your unconditional love and support.

ACKNOWLEDGEMENTS

My eternal gratitude goes to God Almighty for his guidance and wisdom during this research work. I also want to express sincere gratitude to Dr. (Mrs.) Eluehike for her invaluable guidance, and to my group members, Ekameze David and Nwadike Justin for their knowledge and steadfast assistance towards the completion of this project work.

TABLE OF CONTENTS

TITLE PAGE……………………..……………………………………………………………i
CERTIFICATION ii

DEDICATION iii

ACKNOWLEDGEMENTS iv

LIST OF FIGURES vii

ABSTRACT viii

CHAPTER ONE 1

INTRODUCTION 1

1.1 BACKGROUND OF STUDY 1

1.2 OBJECTIVE OF STUDY 2

1.3 AIM OF STUDY 2

CHAPTER TWO 3

LITERATURE REVIEW 3

2.1 OVERVIEW OF *Luffa cylindrica* 3

2.1.1 BOTANICAL DESCRIPTION 4

2.1.2 GEOGRAPHICAL SOURCE 5

2.1.3 TAXONOMIC CLASSIFICATION 5

2.2 PHYTOCHEMISTRY OF *Luffa cylindrica* 6

2.3 USES OF *Luffa cylindrica* 9

2.3.1TRADITIONAL USES 9

2.3.2 BIOCHEMICAL AND ETHNOMEDICINAL USES 9

2.4 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) PROFILE 10

2.4.1 ITS PRINCIPLE OF OPERATION 11

2.4.2 APPLICATIONS OF HPLC 13

2.5 FREE RADICALS AND ANTIOXIDANTS: FRIENDS OR FOES? 13

2.5.1 ANTIOXIDANT CLASSIFICATION 15

2.6 IN-VITRO ANTIOXIDANT ASSAYS 19

2.6.1 DPPH RADICAL SCAVENGING ASSAY 19

2.6.2 FRAP (FERRIC ION REDUCING ANTIOXIDANT POWER) ASSAY 21

2.6.3 TOTAL ANTIOXIDANT CAPACITY (TAC) 24

2.6.4 NITRIC OXIDE SCAVENGING ACTIVITY 25

2.6.5 HYDROXYL RADICAL SCAVENGING ACTIVITY 26
CHAPTER THREE 28
MATERIALS AND METHODOLOGY 28
3.0 SUBJECT OF STUDY 28
3.1 APPARATUS 28
3.2 EQUIPMENTS USED 29
3.3 CHEMICALS AND REAGENTS USED 29
3.4 PLANT COLLECTION 30
3.5 PREPARATION OF THE EXTRACT 30
3.6 STOCK PREPARATION 30
3.7 EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY 30
3.7.1 NITRIC OXIDE RADICAL SCAVENGING ASSAY 30
3.7.2 DPPH RADICAL SCAVENGING ASSAY 31
3.7.3 FERRIC ION REDUCING ANTIOXIDANT POWER (FRAP) ASSAY 32
3.7.4 REDUCING POWER ASSAY 34
3.7.5 HYDROXYL FREE RADICAL SCAVENGING ACTIVITY 34
3.8 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) PROFILE 35
CHAPTER FOUR 37
RESULTS 37
4.1 HYDROXYL RADICAL SCAVENGING ASSAY 37
4.2 NITRIC OXIDE SCAVENGING ASSAY 39
4.3 DDPH RADICAL SCAVENGING ASSAY 41
4.4 FRAP ASSAY 42
4.5 TOTAL ANTIOXIDANT CAPACITY (TAC) 43
4.6 HPLC PHYTOCHEMICAL PROFILE 44
CHAPTER FIVE 46
5.1 DISCUSSION 46
5.2 CONCLUSION 48
REFERENCES 49

LIST OF FIGURES

Fig 2.1 *Luffa cylindrica* plant.

Fig 2.2 Schematic representation of an HPLC unit.

Fig 2.3 Classification of Antioxidants.

Fig 2.4 The reaction of DDPH free radical with an Antioxidant.

Fig 4.1 Hydroxyl radical scavenging activity of *Luffa cylindrica.*

Fig 4.2 Nitric Oxide scavenging activity of *Luffa cylindrica*.

Fig 4.3 DDPH radical scavenging activity of *Luffa cylindrica.*

Fig 4.4 FRAP assay of *Luffa cylindrica.*

Fig 4.5 Total Antioxidant Capacity of *Luffa cylindrica.*

Fig 4.6 HPLC profiling of *Luffa cylindrica.*

ABSTRACT

Luffa cylindrica is a popular, medicinal vine belonging to the Curcubitaceae family that reproduces from seeds. Commonly called loofah, *Luffa cylindrica* is used in treating pains, backaches, rheumatoid arthritis, fever, syphilis, dysentery and tumours. *Luffa cylindrica* fruit extracts, using ethanol was studied to explore its In-vitro antioxidant activity and HPLC profile. DDPH (α, α-diphenyl-β-picrylhydrazyl) radical, FRAP (Ferric reducing antioxidant power), Hydroxyl radical, and Nitric oxide scavenging activity were assayed to determine the antioxidant capacity of *Luffa cylindrica*. Also, bioactive compounds were determined using High Performance Liquid Chromatography (HPLC). Phytochemical screening of the ethanoic extract of the *Luffa cylindrica* fruit extracts indicated the presence of steroids, flavonoids, terpenoids, glycosides, alkaloids and phenolic compounds. Quercetin was the most predominant compound present.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Free radicals are reactive, unstable, molecular byproducts of oxidation in the human body, that have been linked to a variety of diseases including (heart disease and certain cancers), triggered by damaged cell components, including proteins, lipids and DNA (Caito *et al.,* 2015). The protective mechanism of antioxidants serve to scavenge (eliminate) free radicals which can damage cells, DNA and proteins, contributing to various health issues as a result of their reactive, unstable states (Valko *et al.,* 2016). Antioxidants donate electrons to these free radicals, calming their activity (Sies, 1997). Very common sources of these antioxidants are Vitamins A, C and E, and Carotenoids. Other nutritional supplements have been discovered with the ability to dampen oxidative stress, including- Vitamin D, Glutathione, Selenium, Curcumis in turmeric, Reservatrol in grape skin and seeds, and Myo-inositol. However, these antioxidant supplements are not as proficient as the natural antioxidants in fruits and vegetables such as *Luffa cylindrica.*

Luffa cylindrica is found in many parts of the globe, and widely cultivated in different parts of Asia and West Africa. Common names include tori, sponge gourd, loofah; and it is recognized in traditional medicine for the treatment of varied human ailments including snake bites, convulsions, tetanus, asthma, cramps, nephritis, fever, dysentery, amongst others (Oboh *et al.,* 2009).

The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretary products in it (Sati *et al.,* 2010), such as carbohydrates, tannins, flavonoids, steroids, alkaloids, and phenolic compounds. The most commonly found polyphenolic compounds in plant extracts are the phenolic acids, flavonoids and tannins (Naik *et*

al., 2006). Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom. These compounds together with other phenolic structures of plant origin have been reported as scavengers of Reactive Oxygen Species (ROS) and are seen as promising therapeutic drugs for free radical pathologies (Chang *et al.,* 2007). Most flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immuno-modulatory properties (Wang *et al.,* 2012; Balasundram *et al.,* 2006), which may be due to the presence of gallic acid, ellagic acid, quercetin, tannin acid, vanillin, resorcinol, catechin, etc. Medicinal plants containing these phenolic compounds have been used for centuries as local treatment for many diseases. However, pre-clinical studies for assessing the pharmacognostical, phytochemical, toxic and biological properties of any herbal drug are very essential before its clinical administration, for efficacy.

1.2 OBJECTIVE OF STUDY

The main objective of this research is to determine the phenolic chemical compounds present in *Luffa cylindrica* by High Performance Liquid Chromatography (HPLC).

1.3 AIM OF STUDY

The aim of this research work is to evaluate the In-vitro antioxidant activity of the ethanol extract of *Luffa cylindrica* and its HPLC profile.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF *Luffa cylindrica*

Luffa cylindrica is an annual, medicinal herb belonging to the Curcubitaceae family. Having a rough texture, it climbs by the means of branched tendrils. It reproduces from seeds. Its stout, angled or prostate stem is green and pentagonal, and could be without hairs, or slightly hairy. The leaves are alternate and digitately-lobed. The lobes are finely toothed (denticulate), acute and acuminate. The petiole is up to 10cm long, with soft, dense hairs. The inflorescence is an auxiliary cyme on a long stalk. The flowers have a yellow corolla, with the male and female flowers occurring separately on the same plant. The female flower bears a long, hairy ovary below the sepals. The ovary later elongates and develops into the fruit. The fruit is cylindrical, long (15-50cm), smooth, with a fibrous endocarp that contains many flat seeds (Mazali and Alves, 2005).

Fig 2.1 *Luffa cylindrica* plant

(Mazali and Alves, 2005)

2.1.1 BOTANICAL DESCRIPTION

Luffa cylindrica is an annual, climber or trailer. Tendrils slightly pubescent, 3-6-fid. Stem 5-angled, finely hairy to glabrous. Leaves palmately 5-lobed, dark green, orbicular-cordate, 8-25 cm across, lobes triangular, lanceolate, acute apiculate, entire or sinuate, scabrous. Petiole 5-15 cm long. Flowers bright yellow, pedicellate, 5-6 cm across; male racemose, racemes axillary, 12-25 cm long, 15-20-flowered, female flowers in the same axil as males. Protract fleshy, ovate, with 3-7 glistening glands on the upper surface. Calyx tube short, broadly campanulate, slightly pubescent; lobes triangular-lanceolate, longer than tube. Petals obovate-cuneiform, 2.5-3.5 cm long, 1- 2.5 cm broad, obtuse. Stamens 3-5, filaments 6-8 mm long. Ovary cylindrical, finely

appressed hairy. Fruit cylindrical and fusiform, 20-50 cm long, 6-10 cm across, smooth. Seeds dull black, elliptic-ovoid, 10-12 mm long, 6-8 mm broad, with 1 mm wide margin (Ali Esmail, 2019).

2.1.2 GEOGRAPHICAL SOURCE

Luffa cylindrica is a sub-tropical vegetable, requiring either warm summer or frost temperatures when grown in temperate regions. It is an annual climber, with a long history of cultivation in parts of Africa and Asia for its spongy endocarp. It is difficult, however, to trace accurately the origin of the Luffa species. Indo-Burma is reported to be the center of diversity for sponge gourd. The main commercial production countries are China, Korea, India, Japan and Central America (Partap *et al.,* 2012)

2.1.3 TAXONOMIC CLASSIFICATION

- Kingdom: Plantae
- Subkingdom: Viridiplantae
- Infrakingdom: Streptophyta
- Superdivision: Embryophyta
- Division: Tracheophyta
- Subdivision: Spermatophyta
- Class: Magnoliopsida
- Superorder: Rosanae
- Order: Cucurbitales
- Family: Cucurbitaceae

- Genus: Luffa
- Species: cylindrica

(Ali Esmail, 2019).

2.2 PHYTOCHEMISTRY OF *Luffa cylindrica*

Previous studies indicated that the leaves, seeds and fruits of *Luffa cylindrica* are rich in carbohydrates, protein, fiber, fats, amino acids and minerals including sodium, potassium, iron, phosphorus, calcium, zinc, manganese, chromium, copper and magnesium [Onigemo *et al.,* (2020), Ogunyemi *et al.,* (2020)].

One of these studies revealed that very moderate amounts of potassium and sodium are present in the seed, in approximate concentrations of 13.9 mg/100 g and 8.2 mg/100 g respectively. Chromium was also revealed to be present, but in a low concentration of 0.25 mg/100 g (Kamatenesi-Mugisha *et al.,* 2007).

A preliminary phytochemical screening test carried out on the leaf and seed extracts revealed that both extracts contain saponin, alkaloids and cardiac glycosides. In the seed extract however, steroidal rings were detected while anthraquinones, tannins and phlobatinnins were not detected in either extracts (Oyetayo *et al.,* 2007). In the plant extract, reducing carbohydrates, flavonoids, tannins, saponins and glycosides were revealed to be present (Gandhamalla *et al.,* 2018).

Another phytochemical screening carried out on the methanol extract of the leaf extract of *Luff*a cylindrica indicated that they contain carbohydrates, sterols, saponins, flavonoids, alkaloid and phenols, while resins, tannins, terpenes, balsams and anthraquinones were not found (Aboh *et al.,* 2020). Yet again, a preliminary phytochemical screening of aqueous methanol extract of *Luffa*

cylindrica leaves also revealed the presence of sugar molecules including glucose, fructose and galactose; and amino acids such as phenylalanine, glycine and tyrosine (Howlader *et al.,* 2013). Phytate and oxalate were also found in the methanol extract of flowers and leaves of *Luffa cylindrica* (Aladejimokun *et al.,* 2014).

The result of the screening carried out on the methanol extract of the leaf extract of *Luffa cylindrica* indicated antioxidant potential in the leaf extract via enhanced scavenging of DPPH and superoxide radicals in a dose-dependent fashion (Tripathi *et al.,* 2016*)*. It also scavenged hydrogen peroxide, nitric oxide, and hydroxyl radicals. Similarly, a previous study on the ethanol extract of the fruit of *Luffa cylindrica* earlier reported a strong antioxidant activity against DPPH radical (Du *et al.,* 2006).

Some of these phytochemicals are discussed below;

- **FLAVONOIDS**: Flavonoids are hydroxylated phenolic substances, an important class of plant secondary metabolites, synthesized in response to a microbial infection. Having a polyphenolic structure, which is found in fruits, vegetables and certain beverages (Panche *et al.,* 2015), their activities are structure dependent. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization (Kumar & Pandey, 2013).

  More than 4,000 flavonoids have been identified within the major flavonoid classes which include flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones. Flavonoids are absorbed from the gastrointestinal tracts of humans and animals and are excreted either unchanged or as flavonoid metabolites in the urine and feces (Cook and Samman, 1996).

- **SAPONINS**: Saponin is another major group of plant secondary metabolites that are widely distributed throughout the plant kingdom. These biomolecules can be divided into two main classes represented by triterpenoid and steroid glycosides whose structure varies according to the number of sugar units attached in different positions. Although saponins have been historically considered as anti-nutritional factors, recent studies have indicated that some saponin stereoisomers may show a number of pharmacological activities, such as anti-tumor, antioxidative, anti-inflammatory, antidiabetic, and neuro-protective activities (Nguyen *et al.,* 2020).

  11 main classes of saponins were distinguished: dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids. The dammaranes, lupanes, hopanes, oleananes, ursanes, and steroids are further divided into 16 subclasses, because their carbon skeletons are subjected to fragmentation, homologation, and degradation reactions (Vincken *et al.,* 2007).
- **ALKALOIDS**: Alkaloids are natural products of microbial, plant and animal origin. They differ widely in size and structure, and are responsible for the beneficial effects of traditional medicines such as cinchona bark, but also the harmful effects of poisons such as ergot, they have a reputation as both Nature's curse and blessing (Cushnie *et al.,* 2014). Alkaloids can act as defense compounds in plants, being efficient against pathogens and predators due to their toxicity. Toxic effects, in general, depend on specific dosage, exposure time, and individual characteristics, such as sensitivity, site of action, and developmental stage. At times, toxicity effects can be both harmful and beneficial depending on the ecological or pharmacological context (Matsuura *et al.,* 2014).

2.3 USES OF *Luffa cylindrica*

Luffa cylindrica has diverse uses ranging from traditional uses in rural areas to ethnomedicinal uses to curb a number of conditions. The uses of *Luffa cylindrica* are elucidated below;

2.3.1TRADITIONAL USES

Luffa cylindrica was widely applied in packing medium, shoes mats, sound proof linings, bath sponges, utensil cleaning sponges, adsorbent for removal of heavy metal (such as nickel, lead, chromium, copper, etc.) in waste water, and immobilization matrix for plant, algae, bacteria and yeas. It was used traditionally for the treatment of asthma, intestinal worms, sinusitis, chronic bronchitis pain, carbuncles, abscesses, inflammation, heat rashes of children in summer, bowels or bladder hemorrhage, hemorrhoids, jaundice, menorrhagia, haematuria, leprosy, spleenopathy, as anthelmintic, carminative, emmenagogue, galactagogue and as antiseptic. The fruit pulp of *Luffa cylindrica* was used to induce hemostasis, resolve phlegm and clear fever in traditional Korean medicine (Ali Esmail, 2019).

2.3.2 BIOCHEMICAL AND ETHNOMEDICINAL USES

The leaves and fruits of *Luffa cylindrica* serves as a part of diet in most parts of Africa. They can be consumed raw or cooked, but the young flowers and stems have to be steamed lightly before consumption. The seeds can be roasted, and the kernel of the seeds eaten as snacks; or they can be used to produce oil which is used in the local treatment of boils, leprosy, dysentery, and skin diseases. *Luffa cylindrica* is used internally for rheumatism, backache, internal hemorrhage, chest pains as well as hemorrhoids because they contain a variety of phytochemicals that have the ability to heal wounds and kill bacteria [Bassey *et al.,* (2015), Partap *et al.,* (2012)]. The seeds possess alcalase or tryptic protein hydrolysates that are useful for treating diabetes and hypertension (Arise *et al.,* 2019).

In Ghana, the liquefied fiber of Loofa fruit is employed in the filtration process of water and palm wine. In Togo, the leaf is used in treating oedema and malaria. In Guinea, the fruit pulp is used to treat inflammation and tumors. In Uganda, the leaf is employed during labor to aid childbirth. In Egypt, the seeds play a role in managing diabetes (Achigan-Dako *et al.,* 2011).

The loofah fruit can also be dried, and the fibers would be used to produce sponges that is used in skincare to exfoliate the skin and stimulate blood circulation. Its fruits have been revealed to exhibit anthelmintic, carminative, laxative, depurative, emollient, expectorant, and tonic properties. It is used for dropsy, nephritis, chronic bronchitis and lung complaints. It is also applied to the body in putrid fevers and jaundice, and in the treatment of fever, syphilis, tumors, bronchitis, splenopathy and leprosy (Oboh *et al.,* 2009).

The presence of 462 NBS-LRR genes in the mature fruit of *Luffa cylindrica*, which play a major role in nucleic acid biochemistry, is the reason for its higher stress resistance than in other cucurbitaceous species. Hence, it is often used as stalk in bitter melon and bitter gourd to increase crop yields, combat soil-borne diseases, and improve flooding tolerance (Gandhamalla *et al.,* 2018).

The seed oil is used in several cosmetic products including sunscreens, anti-aging creams, moisturizers, sunless tanners, facial cleansers and sunscreens. This is because its antifungal, anti-infammatory and anti-tumor properties make it toxic to skin cancer (Sangh *et al.,* 2012).

2.4 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) PROFILE

High Performance Liquid Chromatography (HPLC), is an analytical technique that is used to identify and quantify the components of a mixture or sample, which have been dissolved into

liquid solutions. This separation technique requires high pressure pumps to deliver mixtures of various solvents, called the mobile phase, which flows through the system, collecting the sample mixture on the way, delivering it into a cylinder, called the column, filled with solid particles, made of adsorbent material, called the stationary phase (Kazakevich and Lobrutto, 2007).

It is used in many laboratory and clinical processes, as it is a reliable way of ensuring product purity. HPLC is employed in the production of pharmaceutical and biological products, Urinalysis to detect doping, in research and also medical purposes (Bayne *et al.,* 2017). While HPLC can produce extremely high quality (pure) products, it is not always the primary method used in the production of bulk drug materials (Siddiqui *et al.,* 2013). The European pharmacopoeia emphasized that HPLC is used in only 15.5% of syntheses. However, it plays a role in 44% of syntheses in the United States pharmacopoeia (United States Pharmacopoeia, 2004).

2.4.1 ITS PRINCIPLE OF OPERATION

HPLC relies on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (adsorbent). A little volume of the already prepared sample (commonly microliters), is passed into the stream of mobile phase permeating through the column. The molecules of the sample travel at different speeds through the pump, depending on the chemical composition of the sample, into the stationary phase. The specific intermolecular interactions between the molecules of a sample and the stationary phase determines its Retention time (that is, the time at which an analyte rises up (elutes) out of the column). As a result, various molecules of the sample elute at different times. After leaving the column, a sensory unit (e.g UV detector) recognizes the analytes, then converts and records the signals it receives on a data processing

system (computer software) and the output is shown on a chromatogram. A Chromatogram is a graph that relates the signal intensity to time or volume, showing culminates, which represents the constituents of the analyte. Every sample appears in its retention time on the chromatogram, having area proportional to its amount. After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit or to the waste [Meyer, (2010), Mukthi Thammana, (2016)].

In general, a HPLC system comprises of: a solvent reservoir, an infusion pump, an injection valve, a chromatographic column, a sensory unit and a data processing unit. The pump delivers the mobile phase (eluent) at high pressure and constant speed through the HPLC system. A constant flow from the pump is essential to keep the movement and noise of the signal very low. The injection valve presents the sample into the mobile phase.

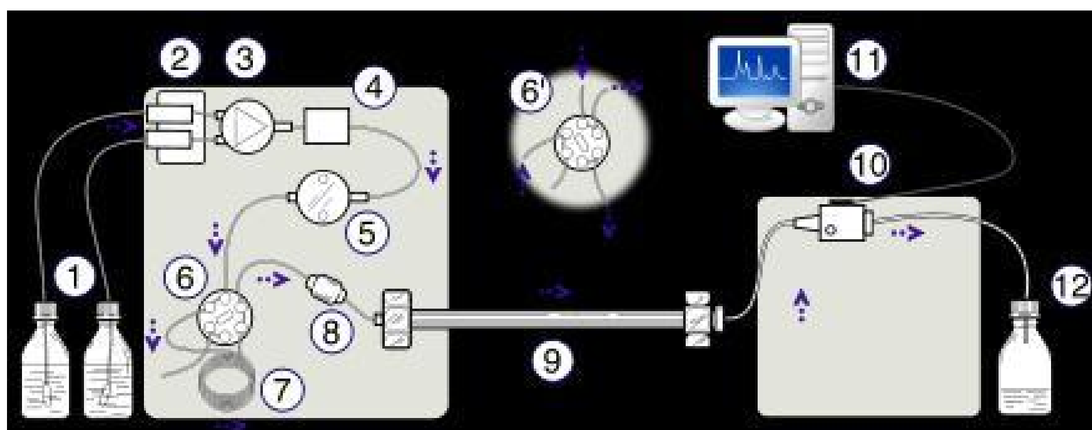


Fig 2.2 Schematic representation of an HPLC unit (1) Solvent reservoirs, (2) Solvent degasser, (3) Gradient valve, (4) Mixing vessel for delivery of the mobile phase, (5) High-pressure pump, (6) Switching valve in “inject position”, (6’) Switching valve in “load position”, (7) Sample injection loop, (8) Pre-column (guard column), (9) Analytical column, (10) Detector (i.e., IR, UV), (11) Data acquisition, (12) Waste or fraction collector.

(Kazakevich and Lobrutto, 2007)

2.4.2 APPLICATIONS OF HPLC

HPLC is employed in the production of pharmaceutical and biological products, Urinalysis to detect drugs, and medical research purposes (Bayne *et al.,* 2017). The most common and convenient method of drug detection has been an immunoassay, as it covers a wide range of drugs (Pesce *et al.,* 2010). Because HPLC is a method of measuring (and possibly improving) purity, it is insufficient in identifying the complexity of these drugs. Therefore, HPLC in this context is often performed in conjunction with mass spectrometry to reduce the sole need of standardizing the HPLC procedures (Tsai *et al.,* 2013). Using liquid chromatography-mass spectrometry (LC-MS) instead of gas chromatography-mass spectrometry (GC-MS) bypasses the necessity for acetylating agents, which can be a burdensome extra step (Weinmann *et al.,* 2000). LC-MS has been used to detect a variety of agents like doping agents, drug metabolites, glucuronide conjugates, amphetamines, opioids, cocaine, BZDs, ketamine, LSD, cannabis, and pesticides [Kolmonen *et al.,* (2007), Pelander *et al.,* (2003)].

In Research, HPLC can be used to detect concentrations of potential antidiabetic, anti-fungal and asthma drugs (Nobilis *et al.,* 2007). This technique is reliable, especially when multiple species in collected samples is under observation and study; but it requires standard experimental runs to identify species.

2.5 FREE RADICALS AND ANTIOXIDANTS: FRIENDS OR FOES?

Free radicals are the natural byproducts of chemical processes, such as metabolism (Valko *et al.,* 2016). A free radical is any molecular species that can exist on its own, and contains an unpaired electron in the atomic orbital. Free radicals attack macromolecules (including proteins, DNA and

lipids) in the body, leading to cell damage and imbalances in body homeostasis. They include Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), Hydroxyl radical (-OH), Hydrogen peroxide (H2O2), and Peroxynitrite (ONOO-). Many radicals are unstable and highly reactive. Seeking to pair their unpaired electron, free radicals react swiftly with other molecules, influencing cellular processes. (Halliwell *et al.,* 2015).

Antioxidants are a diverse group of compounds that play a crucial role in neutralizing or reducing the harmful effects of free radicals and oxidative stress in the body (Hamid *et al;* 2010). They serve as a defense mechanism against the damage caused by reactive oxygen species (ROS) and other free radicals (Das and Roychoudhury, 2014). Antioxidants neutralize free radicals by giving up some of their own electrons. In making this sacrifice, they act as a natural "turn-off" switch for the free radicals. This helps break a chain reaction that can affect other molecules in body cells.

Hence, the most effective and natural method of fighting these free-radical-related diseases is the consumption of fruits and vegetables rich in antioxidants, as part of diet. The presence of phytochemicals such as polyphenols, carotenoids, and vitamin E and C in these antioxidant foods greatly promote health (Hamid *et al.,* 2010). Phenolic compounds however, are commonly found in both edible and non edible herbs, cereals, fruits, vegetables, oils, spices, and other plant materials (Miliauskas *et al.,* 2004).

Antioxidants lower oxidative stress and the effects of cell damage (such as DNA mutations and fatal transformations). Epidemiological studies proved antioxidants' ability to contain the effects of reactive oxygen species activity, and diminish the incidence of cancer and other degenerative diseases (Nandi *et al.,* 2019). Nevertheless, mainly at sustained free radical action, the defense system's capacity against ROS can be overwhelmed, leading to disease occurrence. The first

identified types of antioxidant defense systems developed against oxidative damage, are those that prevent reactive oxygen species occurrence, and those that block and capture radicals that are formed. These systems present in aqueous and membrane cell compartments can be enzymatic and nonenzymatic. Another important antioxidant system of the cell is represented by repair processes that remove the damaged biomolecules, before their aggregation enables alteration of cell metabolism. The repair systems intervention consists in repairing oxidatively damaged nucleic acids by specific enzymes, removing oxidized proteins by proteolytic systems, and repairing oxidized lipids by phospholipases, peroxidases or acyl transferases. It has been assumed that the decay of the repair systems leads to more aging and age related diseases than moderate changes in the antioxidants defense potential against ROS occurrence (Sugamura and Keaney, 2011).

2.5.1 ANTIOXIDANT CLASSIFICATION

A breakdown of different classifications of antioxidants is listed below.

Based on Origin:

- Endogenous Antioxidants: These are produced within the body and include enzymatic antioxidants like suuperoxide dismutase (SOD), catalase, and glutathione peroxidase (Marrazzo *et al.,* 2014).
- Exogenous or Dietary Antioxidants: from food and supplements. They include vitamins (like vitamin C and E), minerals (such as selenium and zinc), polyphenols, carotenoids, and flavonoids (Aguilar *et al.,* 2016).

Based on Chemical Structure:

- Vitamins: Antioxidant vitamins include vitamin C (ascorbic acid), vitamin E (tocopherols and tocotrienols), and vitamin A (in the form of beta-carotene) (Traber and Stevens, 2011).

- Minerals: Certain minerals like selenium, zinc, and copper are components or cofactors for antioxidant enzymes (Khalili-Tilami *et al.,* 2018).
- Polyphenols: This is a diverse group of compounds found in plants, including flavonoids, phenolic acids, and stilbenes, known for their antioxidant properties (Williamson, 2017).

Based on Solubility:

- Water-soluble Antioxidants: These antioxidants dissolve in water and function in watery environments inside and outside cells. Examples include Vitamin C and some polyphenols (Traber and Stevens, 2011).
- Fat-Soluble Antioxidants: These antioxidants dissolve in lipids and primarily protect cell membranes from oxidative damage. Vitamin E is a notable fat-soluble antioxidant (Rayman, 2000).

Based on Action or Mechanism:

- Chain-Breaking Antioxidants: donates an electron to interrupt the propagation phase of free radical formation, effectively breaking the chain reaction, and preventing further generation of ROS by removing peroxides. E.g a- tocopherol, caffeic acid, ascorbic acid (Halliwell and Gutteridge, 2015).
- Preventive Antioxidants: Prevent the formation of free radicals by chelating metals or inhibiting the enzymes involved in the generation of free radicals (Rayman, 2000).
- Enzymatic Antioxidants: superoxide dismutase (SOD), glutathione peroxidase and catalase directly neutralize free radicals within cells (Halliwell and Gutteridge, 2015).

Based on Function:

- Primary Antioxidants: Act directly as free radical scavengers, neutralizing them and preventing oxidative damage (Nandi *et al.,* 2019).
- Secondary Antioxidants: Work indirectly by regenerating or recycling other antioxidants. For instance, vitamin C can regenerate vitamin E, maximizing its antioxidant potential (Traber and Stevens, 2011).

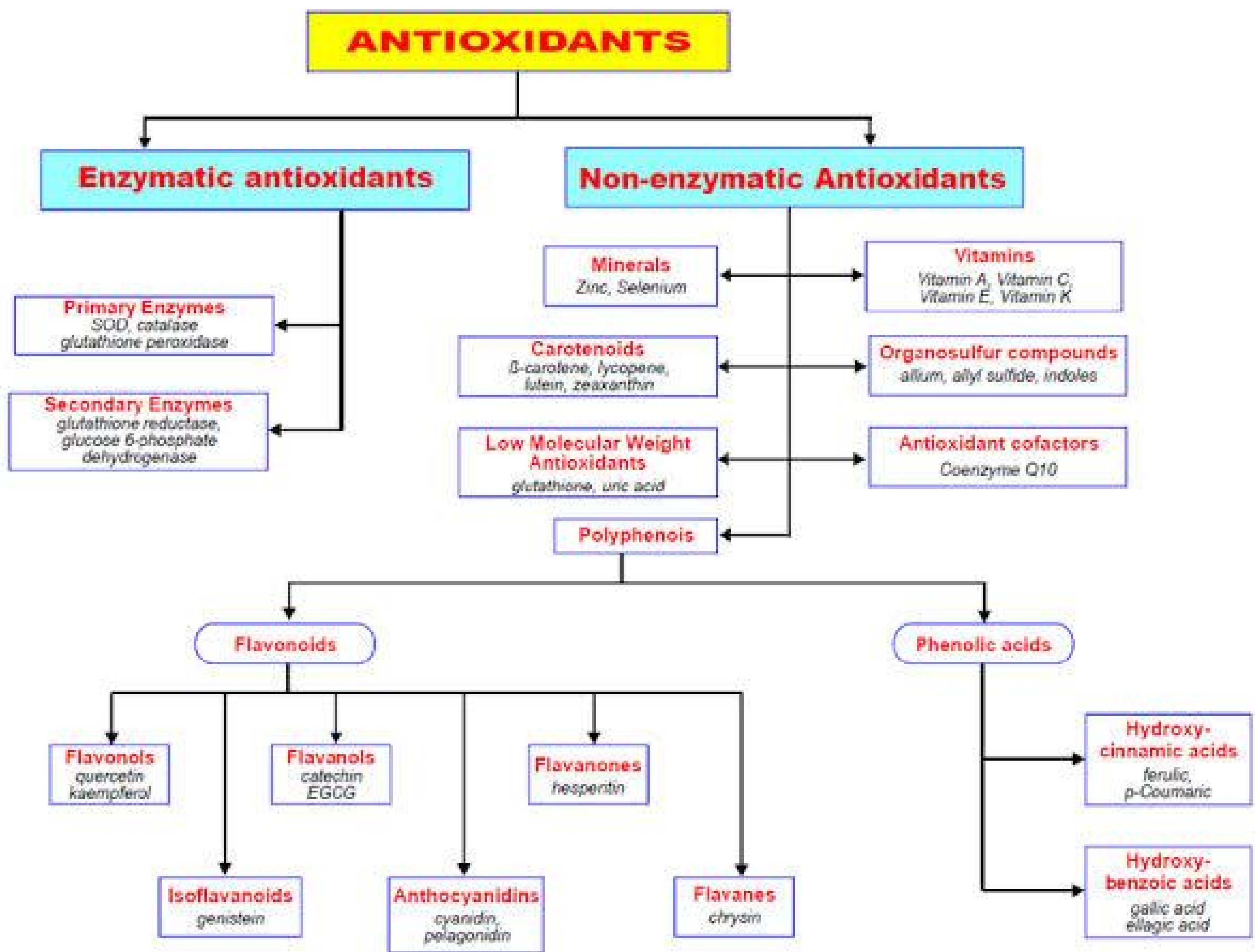


Fig 2.3 Classification of antioxidants

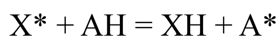
(Flieger, 2021)

2.6 IN-VITRO ANTIOXIDANT ASSAYS

2.6.1 DPPH RADICAL SCAVENGING ASSAY

Blois (1958) developed the DDPH assay using cysteine as an antioxidant, to accurately measure the scavenging capacity of antioxidants towards a stable free radical, DDPH (α, α-diphenyl-β-picrylhydrazyl; C18H12N5O6, M = 394.33). A hydrogen atom is donated from the antioxidant, to reduce the unpaired nitrogen in DDPH, producing hydrazine (Contreras-Guzman and Strong, 1982).

DDPH unlike most free radicals, undergo a phenomenon called delocalization, observed in metals and in aromatic and conjugated organic compounds, where an electron is able to bind several atoms instead of just two. This produces a deep violet color, with an absorbance of 517-520 nm in ethanol. When an antioxidant is mixed with DDPH solution, the deep violet color is lost as DDPH is reduced. The loss of the initial coloration is proportional to the number of electrons taken up in the process.



$$X^* + AH = XH + A^*$$

XH is the reduced DDPH and A* is the resulting free radical formed in this first step. A* undergoes further reactions which determines the final stoichiometry.

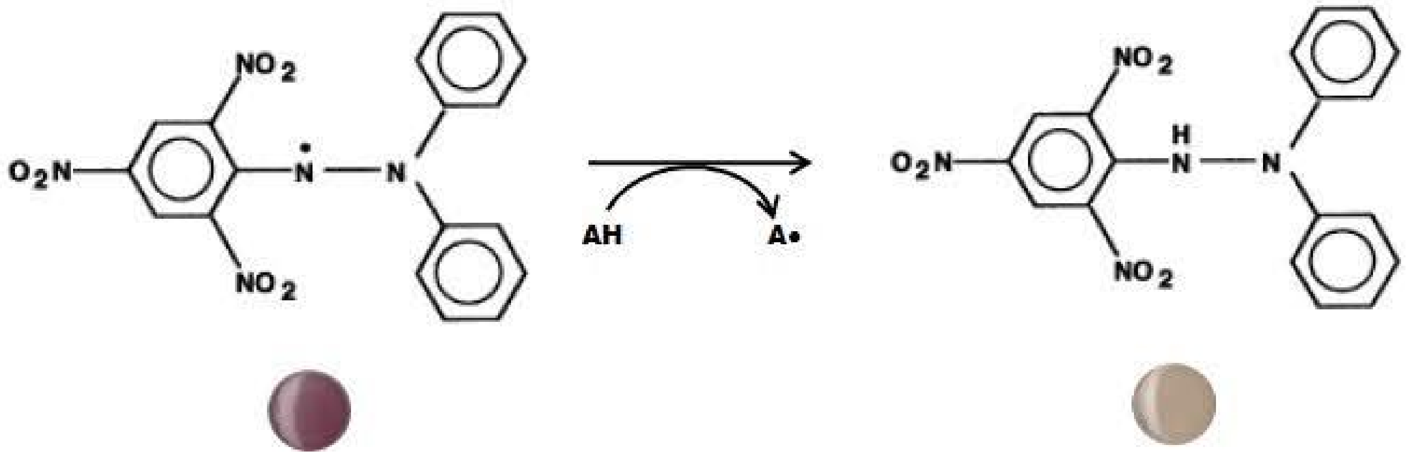


Fig 2.4 The reaction of DDPH free radical with an antioxidant.

(Contreras-Guzman and Strong, 1982)

The DDPH assay is simple and inexpensive, and has been successful in vast applications: to measure the antioxidant potential of different foods; quantitate antioxidant in biological systems if the sample under study is in solid or liquid form; to evaluate the total antioxidant capacity (Prakash, 2001) and free radical scavenging activity of fruit and vegetable juices (Sendra *et al.,* 2006), wheat grain, vegetables, conjugated linoleic acids, herbs, edible seed oils, and flours in several different solvent systems including ethanol, aqueous acetone, methanol, aqueous alcohol and benzene (Yu 2001; Parry *et al.,* 2005); to evaluate the antioxidant potential of cysteine, glutathione, ascorbic acid, tocopherol and polyhydroxy aromatic compounds (Masahiro *et al.,* 2005). DPPH method may be utilized in aqueous and nonpolar organic solvents and can be used to examine both hydrophilic and lipophilic antioxidants (Prior *et al.,* 2005), and foods containing neutral antioxidants.

This assay is advantageous over other antioxidant assays, as DDPH is allowed to react with the whole sample, and provides ample time for the radical to react with weak antioxidants (Prakash, 2001). To prevent the risk of molecular degradation at high temperatures, the antioxidant's scavenging activity of the radical is evaluated at room temperature.

The only disadvantage of this assay is that the DDPH radical may be sensitive to some Lewis bases, oxygen and solvents (Ancerewicz *et al.,* 1998). DPPH can only be soluble in organic solvent and the interference of absorbance from the sample compounds could be a problem for the quantitative analysis (Arnao 2000). Also, DPPH radical interacts with other radicals and the time response curve to reach the steady state is not linear with different ratios of antioxidant/DPPH (Brand-Williams *et al.,* 1995; Sanchez-Moreno *et al.,* 2002). The absorbance of DPPH in methanol and acetone decreases under light (Min 1998). In alcoholic mediums, proteins are precipitated. Hence, the DDPH assay fails to evaluate the antioxidant activity of plasma.

2.6.2 FRAP (FERRIC ION REDUCING ANTIOXIDANT POWER) ASSAY

The FRAP assay was introduced by Iris Benzie and J. J. Strain of the Human Nutrition Research Group at the University of Ulster, Coleraine. This assay evaluates the antioxidant's ability to reduce ferric ion (Fe3+)-ligand complex to the intensely blue-colored ferrous (Fe2+) complex in an acidic medium, using trolox as a standard, at an absorbance of 593 nm (Hegerman *et al.,* 1998). The FRAP assay is based on the formation of O-Phenanthroline-Fe(2+) complex, and the disruption of this complex when chelators are present.

The experimental runs of ferric reducing antioxidant power (FRAP) assay are standardized to maintain iron solubility and, more importantly, drive electron transfer. This will increase the

redox potential, causing a shift in dominant reaction mechanism Antioxidant activity and results are expressed as micromolar Fe^{2+} equivalents or relative to an antioxidant standard (Antolovich *et al.,* 2002).

The first FRAP assay performed by Benzie and Strain used tripyridyltriazine (TPTZ) as the iron-binding ligand, but alternative ligands are now being employed for ferric binding, such as ferrozine for ascorbic acid–reducing power evaluation (Molina-Diaz *et al.,*1998). Many recent studies on this antioxidant assay used potassium ferricyanide as ferric reagent, with the latter production of Prussian blue. This end product which can be quantified spectrophotometrically, indicates the reducing power of the antioxidants tested. Prussian blue may be produced in two different ways, but each has the same result. Antioxidants can either reduce the Fe^{3+} in the solution to Fe^{2+}, which binds the ferricyanide to yield Prussian blue, or reduce the ferricyanide to ferrocyanide, which binds the free Fe^{3+} in the solution and forms Prussian blue (Berker *et al.,* 2010).

In this ferricyanide FRAP assay, the Prussian blue may precipitate, forming a suspension and staining the measuring cuvette. Therefore, the time to add Fe^{3+} ($FeCl_3$) is essential and may introduce error to the resulting interpretation. In order to stabilize the Prussian blue against precipitation, Berker *et al.* (2010) proposed addition of the surfactant sodium dodecyl sulfate, while adjusting the optimal pH to 1.7 to maintain the redox activity of ferric ion without hydrolysis. The authors suggested that this modification also allows evaluation of antioxidants whose redox potential do not exceed that of the Fe^{3+}/Fe^{2+} in a conventional FRAP assay (i.e., ferric reagent too weak to oxidize the antioxidants), such as thiol-type nonenzymatic antioxidants. The FRAP assay was further modified by choosing water/acetone as a solvent in the absence of solubility enhancer (RMCD) to enable simultaneous measurement of both hydrophilic and

lipophilic antioxidants, the latter of which was restricted by conventional FRAP assay (Berker *et al.,* 2012).

Although it was formerly developed for measuring the reducing power in plasma, it is now widely employed in evaluating the antioxidant potential of different body fluids, food and herbs. Pulido *et al.* (2000) reported that FRAP results may vary depending on the analysis time as observed for the reaction between antioxidants and Fe^{3+}, which ranged from several minutes to several hours. Therefore, a single-point absorption end point may not represent a complete reaction, since different antioxidants require different reaction times for detection (Prior *et al.,* 2005).

With the recent adoption and development of electrochemical signaling methods, the FRAP assay now give more accurate results. A coulometric titration method has been developed to determine the FRAP value of different antioxidant materials (Abdullin *et al.,* 2002, Ziyatdinova *et al.,* 2011, Ziyatdinova *et al.,* 2014). Here, an antioxidant reacts with coulometric titrants (electro generated ferricyanide ions), and the quantity of electricity consumed for the titration till the end point (when initial current is resumed) serves as the indicator for reducing power of the antioxidant (Ziyatdinova *et al.,* 2014). This modified method of the FRAP assay had better sensitivity and reliability, and is a very simple test for reducing power evaluation.

An analysis conducted by Brainina *et al.,* (2012) showed that a shift of potential in the $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ mediator system is indirectly correlated with the reducing power of the antioxidant present. More specifically, at certain potentials (higher than 0.35 V), the oxidation process takes place only forming $K_3[Fe(CN)_6]$, and the concentration of $K_4[Fe(CN)_6]$ is entirely from the reaction of $K_3[Fe(CN)_6]$ with antioxidant and is proportional to the reducing power of the antioxidant. This chronoamperometric method has been proposed for assessing

antioxidant activity of biological samples owing to its very low detection limit (e.g., 2×10^{-6} M for ascorbic acid).

Generally, the FRAP antioxidant assay, as an ET-based nonradical method, is argued to have little or no similarity to the radical quenching process (HAT mechanism) occurring in lipid systems, and it has poor correlation with other antioxidant activity measurements. It is, therefore, suggested that this assay could be used in combination with other methods in distinguishing dominant mechanisms for different antioxidants (Prior *et al.,* 2005).

In nutritional science, the FRAP assay is widely employed in evaluating the "total antioxidant content" of various foods, and absorption of antioxidants from cooked and processed foods including soya milk, cocoa and tea (Pulido *et al.,* 2000).

In the agri-food industry, this assay is employed in quality control, to check how various factors (including genetic differences, farming seasons, growth factors and storage) affect the "total antioxidant content" of foods. For example, total antioxidant content of blueberries of the same cultivar grown in the same field can vary by up to 25% depending on the harvesting year, and variation of up to 47% in total antioxidant content is seen in different cultivars grown in the same area and harvested in the same year (Decker *et al.,* 2005).

2.6.3 TOTAL ANTIOXIDANT CAPACITY (TAC)

TAC is defined as the ability of a compound to inhibit the oxidative degradation of lipids (Roginsky and Liss, 2005). Lipid peroxidation involves the oxidative deterioration of lipids with unsaturation. This peroxidation, called the initiation process, begins with the formation of conjugated dienes and trienes, known as primary oxidation products due to the abstraction of a

hydrogen atom. Subsequently, a propagation process is carried out that consists of the reaction of the deprotonated species derived from the lipids with O2, leading to the formation of peroxyl radicals (ROO•). The high energy of free radicals promotes the abstraction of hydrogen atoms from neighboring fatty acids. This leads to the formation of hydroperoxides that promotes the formation of new R• radicals. The latter radicals react with each other to produce stable molecules of the R-R and ROOR type (Collomb and Spahni, 1996). To encourage the antioxidant activity of a chemical compound, it is necessary to inhibit the peroxidation of a fatty acid emulsion; linoleic acid is generally used as a model. The hydroperoxides derived from linoleic acid subsequently react with Fe2+, causing the oxidation of this ion to produce Fe3+. The Fe3+ ions form a complex with thiocyanate (SCN−), and this complex has a maximum absorbance at 500 nm (Guclu *et al.,* 2014). This complex is used to measure the peroxide value. The ferric thiocyanate method is used to measure the peroxide value in edible oils. To avoid errors in the determination of the peroxide value, it is important to avoid the presence of oxygen in the reaction medium and this can be achieved by bubbling nitrogen (Milardovic *et al.,* 2007).These authors found that the results of the thiocyanate assay also depend on the solvent, reducing agent and type of hydroperoxides present in the sample.

2.6.4 NITRIC OXIDE SCAVENGING ACTIVITY

Nitric oxide (NO) and reactive nitrogen species (RNS) are free radicals that are derived from the interaction of NO with oxygen or reactive oxygen species (Tsai *et al.,* 2007). Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide (Amaze *et al.*, 2011). Nitric oxide (NO) is generated from amino acid L-arginine by the enzymes in the vascular endothelial

cells, certain neuronal cells, and phagocytes (Nagmoti *et al.,* 2011). Low concentrations of NO are sufficient in most cases to effect the physiological functions of the radical. As a result, prolonged exposure to the radical are associated with various cancers and inflammatory disorders, such as ulcerative colitis, multiple sclerosis, arthritis and Type 1 diabetes. NO is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities (Bhaskar and Balakrishnan, 2009). The toxicity of NO increases greatly when it reacts with the superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^{-}$) (Amaeze *et al.,* 2011). Nitric oxide has been shown to be directly scavenged by flavonoids, especially Quercetin (Lakhanpal and Rai, 2007). In vitro quenching of NO radical is one of the methods that can be used to determine antioxidant activity (Amaeze *et al.,* 2011). The procedure is based on the principle that at physiological pH, sodium nitroprusside produces nitric oxide in aqueous solution and this interacts with O2 to produce NO3- using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions (Ebrahimzadeh *et al.,* 2010).

2.6.5 HYDROXYL RADICAL SCAVENGING ACTIVITY

Hydroxyl radicals are reactive oxygen species (ROS) formed during the metabolic process in living organisms (Ma *et al.,* 2020). When this radical enters a cell, it swiftly reacts with different molecules within the cell, causing damages and imbalances within the living organism.

Hydroxyl radicals are formed when hydrogen peroxide reacts with ferrous ions (Fenton reaction), but the ferrous ion hydrogen peroxide system cannot be used in hydroxyl radical scavenging assay because many antioxidants are metal chelators, thus interfering with this assay. Halliwell *et al.* (1987) described an organic Fenton reaction in which a mixture of tetrachlorohydroquinone (a

major metabolite of the widely used biocide pentachlorophenol) and hydrogen peroxide hydroxylates salicylic acid yielding 2,3-dihydroxybenzoic and 2,5-dihydroxybenzoic acid, which can be measured using high-performance liquid chromatography with electrochemical detection. This reaction is inhibited by antioxidants that scavenge the radical, and metal chelators have no effect on this inhibition. A new fluorometric method was developed by Ou *et al.* (2002) to evaluate hydroxyl radical scavenging capacity using fluorescein as a probe. Hydroxyl radicals were formed by cobalt (II)-mediated Fenton-like reaction, by the hydroxylation of p-hydroxybenzoic acid. The fluorescence decay curve of fluorescein was monitored in the absence and presence of an antioxidant, and hydroxyl radical scavenging capacity was calculated by subtracting the area under the curve (AUC) of fluorescence observed with blank sample from the AUC of the sample containing antioxidant. Gallic acid was used as standard, and values were expressed as gallic acid equivalent. In this assay, intrinsic absorption of hydrogen peroxide in the UV region at 230 nm was used, and because an antioxidant successfully scavenges hydrogen peroxide, absorption at 230 nm was reduced (Gulaboski *et al.,* 2013).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.0 SUBJECT OF STUDY

This study was conducted at the Medical Biochemistry department, in the University of Benin.

3.1 APPARATUS

These materials were used during the research study;

i. *Luffa cylindrica*
ii. Knife
iii. Bowls
iv. Masking tape
v. Aluminum foil
vi. Stirring rod
vii. Nose mask
viii. Gloves
ix. Filter paper
x. Measuring cylinder
xi. Conical flask
xii. Cheesecloth
xiii. Separating funnel
xiv. Plain bottles
xv. Glass rod
xvi. Beakers (100ml and 250ml)
xvii. Weight balance

xviii. Glass jar

xix. Test tubes

xx. Test tube racks

xxi. Tissue

xxii. Detergent

3.2 EQUIPMENTS USED

i. Blender

ii. Refrigerator

iii. Spectrophotometer

iv. pH meter

3.3 CHEMICALS AND REAGENTS USED

i. Ethanol

ii. Distilled water

iii. Ascorbic acid

iv. Acetate buffer

v. 2,4,6-tripyridyls-triazine (TPTZ)

vi. Hydrochloric acid (HCl)

vii. Ferric chloride solution

viii. Sodium nitroprusside

ix. Griess reagent

x. Potassium ferricyanide

xi. Phosphate buffer

xii. Trichloroacetic acid

xiii. 1, 10-phenanthroline

xiv. Hydrogen peroxide

xv. Ferrous sulphate (FeSO4)

3.4 PLANT COLLECTION

The *Luffa cylindrica* fruits were collected from a demolished building in Okpanam town, Asaba, Delta State on 27th December, 2023 and were verified at the Plant Biology and Biotechnology department, in University of Benin, Edo State by Professor Adewale, and a voucher number of UBH-L587 was obtained. The fruits were then dried at room temperature for three weeks, and ground into fine powder by a blender at the Department of Pharmacy. Finally, the extract was stored for three days at room temperature for further use.

3.5 PREPARATION OF THE EXTRACT

The extraction process was according to Alam *et al.* (2002). The fine powder was weighed in the laboratory, with a result weight of 518g. Using a ratio of 100g:500ml, the powdered fruit was soaked in 2,600ml of ethanol. The jars were sealed and kept for three days with consistent stirring. The extract was then filtered using a cheesecloth and filter papers placed in separating funnels. The filtrate obtained had a volume of 1.2ml and was freeze-dried.

3.6 STOCK PREPARATION

0.1g of the extract was placed in a plain bottle, and diluted with 10ml of methanol.

3.7 EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY

3.7.1 NITRIC OXIDE RADICAL SCAVENGING ASSAY

Nitric oxide (NO) scavenging ability was measured using Griess-Ilosvay's reagent.

- **Principle**

At physiological pH, sodium nitroprusside produces nitric oxide in aqueous solution and this interacts with O2 to produce NO3-. Scavengers of NO compete with O2 resulting in decreased production of NO (Marcocci *et al.,* 1994).

- **Procedure**

Sodium nitroprusside (10 millimole per liter) in phosphate buffer saline was mixed with varying concentrations of the extract and incubated at 25ºC for 150 minutes. The samples were added to Griess reagent (1% sulphanilamide, 2% H3PO4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance was determined at 540 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control.

- **Calculation**

The percentage of inhibition was calculated using the formula below:

Radical scavenging activity(%)= [(A control-A test)/(A control)] ×100

Where:

A control is the absorbance of the control (without extract) and,

A test is the absorbance in the presence of the extract/standard.

3.7.2 DPPH RADICAL SCAVENGING ASSAY

A modified procedure as described by Jain *et al.* (2008) was used to estimate the DPPH radical scavenging ability of the extracts.

- **Principle**

The principle of this assay involves the antioxidant reducing the violet DPPH radical through a hydrogen atom transfer mechanism. This stabilizes the DPPH radical, resulting in a color change to stable pale yellow DPPH molecules. The change can be measured using a UV-Vis spectrophotometer at an absorbance range of 517-520 nm.

- **Procedure**

A solution of 0.1 millimolar DPPH in methanol was prepared and 0.1 milliliter of this solution was mixed with 3.0 milliliter of extracts in methanol containing 0.01-0.2 milligram/milliliter of the extract. The reaction mixture was thoroughly mixed and left for 30 minutes in a dark area. The absorbance of the mixture was read at 517 nm using a spectrophotometer. Ascorbic acid served as the standard.

- **Calculation**

DPPH radical scavenging activity(%) = $[(A0-A1)/A1] \times 100$

Where:

A0 was the absorbance of DPPH radical + methanol;

A1 was the absorbance of DPPH radical + sample extract or standard.

3.7.3 FERRIC ION REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

A modified procedure of Benzie and Strain (1996) was utilized for the ferric reducing antioxidant power (FRAP) assay.

- **Principle**

The principle of this assay is based on the ability of the extracts to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)- TPTZ) at low pH. The deep blue colour of Fe (II)-TPTZ is read spectrophometrically at 593 nm.

- **Procedure**

1.5 milliliter of freshly prepared FRAP solution (25 milliliter of 300 millimolar acetate buffer pH 3.6, 2.5 milliliter of 10 millimolar 2,4,6-tripyridyls-triazine (TPTZ) in 40 millimolar Hydrochloric acid, and 2.5 milliliter of 20 millimolar ferric chloride solution was mixed with 1milliliter of the extracts at concentration of 1.0 milligram per ml. The reaction mixtures were incubated at 37ºC for 30 minutes and absorbance was measured at 593 nm. FeSO4 was used for the calibration curve and ascorbic acid was used as the positive control. FRAP values (expressed as mg Fe (II) per gram of the extract) and those of the extracts were then extrapolated from the standard curve.

- **Calculation**

FRAP value = $[(A1 – A0)/(Ac – A0)] \times 2$

Where:

Ac is the absorbance of the positive control.

A1 is the absorbance of the sample.

A0 is the absorbance of the blank.

3.7.4 REDUCING POWER ASSAY

The method of Lai *et al.* (2001) was used to determine the reducing power of the extracts. 1 milliliter of varying concentrations of extracts (0.1-1.0 milligram per milliliter) in water was mixed with 2.5 milliliter of 0.2molar phosphate buffer, pH 6.6 and 2.5 milliliter of 1 percent potassium ferricyanide. After incubating at 50ºC for 20 minutes, 2.5 milliliter of trichloroacetic acid (10 percent) was added to stop the reaction. 2.5 milliliter of distilled water and 0.5 milliliter of 0.1percent FeCl3 were then added and the absorbance determined spectrometrically at 700 nm. Higher absorbance values indicated higher reducing power. Vitamin C was used as a positive control.

3.7.5 HYDROXYL FREE RADICAL SCAVENGING ACTIVITY

The modified procedure of Chen *et al.* (2013) was utilized to determine the hydroxyl radical scavenging ability of the extracts.

- **Principle**

This assay quantifies the degradation product of 2-deoxyribose by its condensation with thiobarbituric acid.

- **Procedure**

A reaction mixture containing 1 milliliter of 1, 10-phenanthroline (0.75 millimolar), 1.5 milliliter of 0.75 millimolar FeSO4 and 3.8 milliliter of 0.2 molar Phosphate buffered solution (PH 7.4) was mixed with 1milliliter of sample extracts (100-500microgram per ml) and 1milliliter of 0.01% (volume per volume) H202 and the volume was made up to 10 milliliter with distilled water., and the absorbance was measured at 536 nm after incubation at 37 ºC for 60 minutes.

- **Calculation**

The scavenging effect was calculated using the following equation:

Scavenging effect(%) = [(A2-A1)/(A0-A1)] ×100

Where:

A2 and A1 are the absorbance with or without sample.

A0 is the absorbance without sample and H_2O_2.

3.8 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) PROFILE

The HPLC profiling of *Luffa cylindrica* was determined according to Bayne *et al.* (2017).

- **Principle**

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column).

- **Procedure**

A small volume of the sample, usually measured in microliters, is introduced into the mobile phase stream flowing through the column. Based on the chemical structure of the analyte, the sample molecules move at different speeds through the stationary phase. Each molecule’s velocity is determined by the analyte’s composition. The specific interactions between the sample molecules and the adsorbent dictate their retention time, which is the time it takes for a particular analyte to elute from the column. Consequently, different components of a sample elute at varying times. A detection unit, such as a UV detector, identifies the analytes as they exit the column. The signals are then converted and recorded by data management software, producing a graphical output called a chromatogram.

- **Calculation**

Assay (%w/w) = [At x Ws x P x (100 – LOD of std)] / [As x Wt x (100 – LOD of sample)],

Where:

At = Area of the sample,

As = Area of the Standard,

Ws = Weight of the standard,

Wt = Weight of the Sample,

P = Potency or Assay of standard.

CHAPTER FOUR

RESULTS

4.1 HYDROXYL RADICAL SCAVENGING ASSAY

Figure 4.1 illustrates the hydroxyl radical scavenging activity of *Luffa cylindrica* in mg/ml across various concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/ml), compared to the standard, Ascorbic acid. **Table 4.1** shows the IC50 value, indicating the concentration at which the hydroxyl radical scavenging activity is approximately 50%. The results demonstrate that there is no significant difference between the standard and *Luffa cylindrica.* Therefore, *Luffa cylindrica* can be utilized as an antioxidant.

Fig 4.1

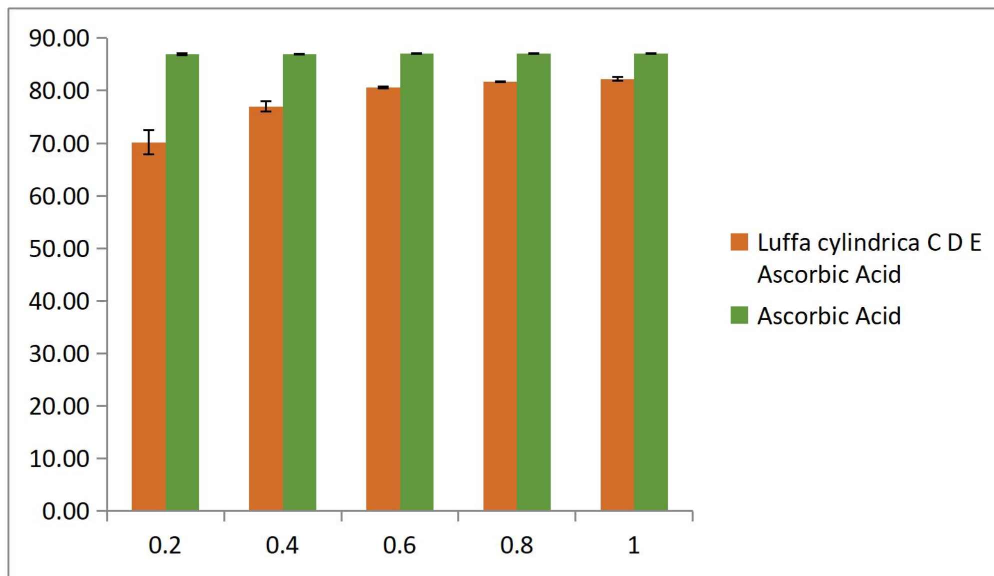


Table 4.1: IC_{50} of hydroxyl radical scavenging activity of *Luffa cylindrica* (mg/ml)

	Ascorbic Acid	*Luffa cylindrica*
hydroxyl	0.40	0.35

4.2 NITRIC OXIDE SCAVENGING ASSAY

Figure 4.2 displays the results from the nitric oxide assay of *Luffa cylindrica* at various concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) compared to the standard, Ascorbic acid. **Table 4.2** presents the IC50 values for the nitric oxide scavenging activity of *Luffa cylindrica* in comparison to Ascorbic acid. The results clearly show a significant difference between the nitric oxide scavenging activity of the standard and the sample. Consequently, *Luffa cylindrica* is not suitable for nitric oxide scavenging.

Fig 4.2

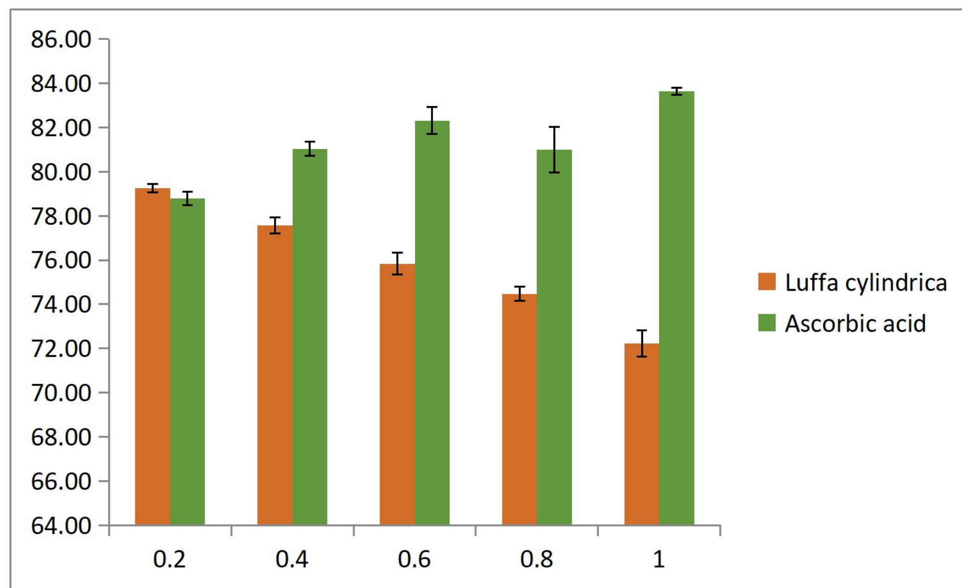


Table 4.2: IC_{50} of Nitric Oxide assay of *Luffa cylindrica* (mg/ml)

	Ascorbic Acid	*Luffa cylindrica*
Nitric Oxide	0.06	2.31

4.3 DDPH RADICAL SCAVENGING ASSAY

Figure 4.3 shows the results from the DPPH radical scavenging assay of *Luffa cylindrica* at different concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) compared to the standard, Ascorbic acid. **Table 4.3** presents the IC50 values for the DPPH radical scavenging activity of *Luffa cylindrica* in comparison to Ascorbic acid. The results indicate a significant difference between the DPPH radical scavenging activity of the standard and the sample. Therefore, *Luffa cylindrica* is not suitable as an antioxidant based on the DPPH assay.

Fig 4.3

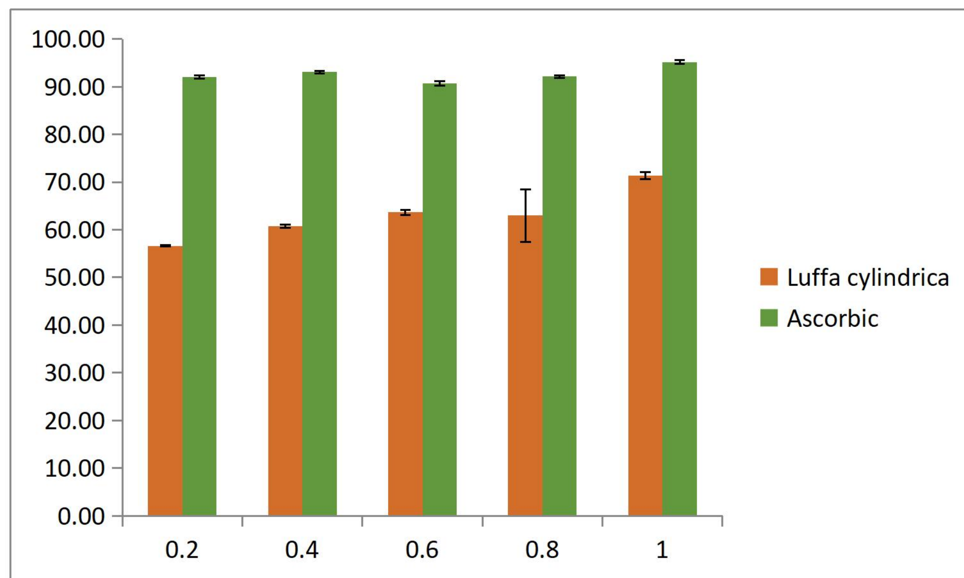


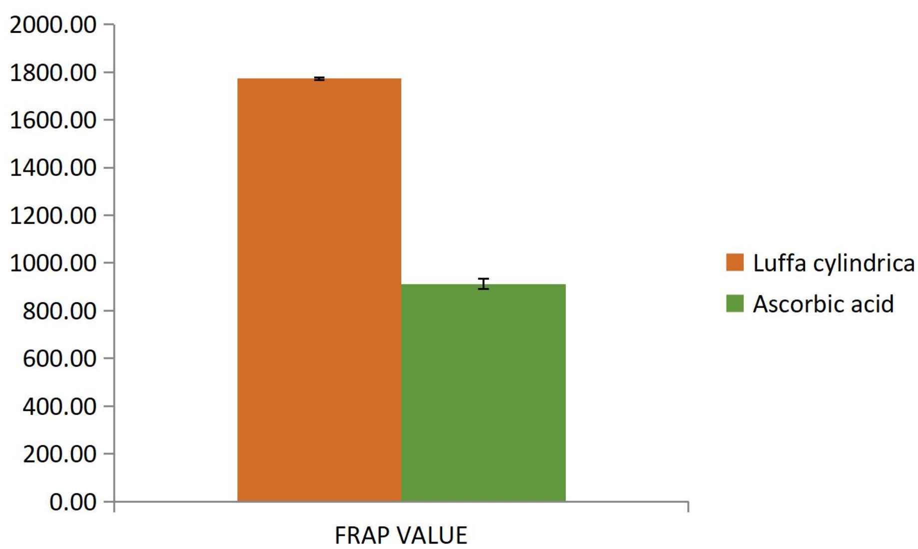
Table 4.3: IC_{50} of DDPH radical scavenging assay of *Luffa cylindrica* (mg/ml)

	Ascorbic Acid	*Luffa cylindrica*
DPPH	1.27	3.71

4.4 FRAP ASSAY

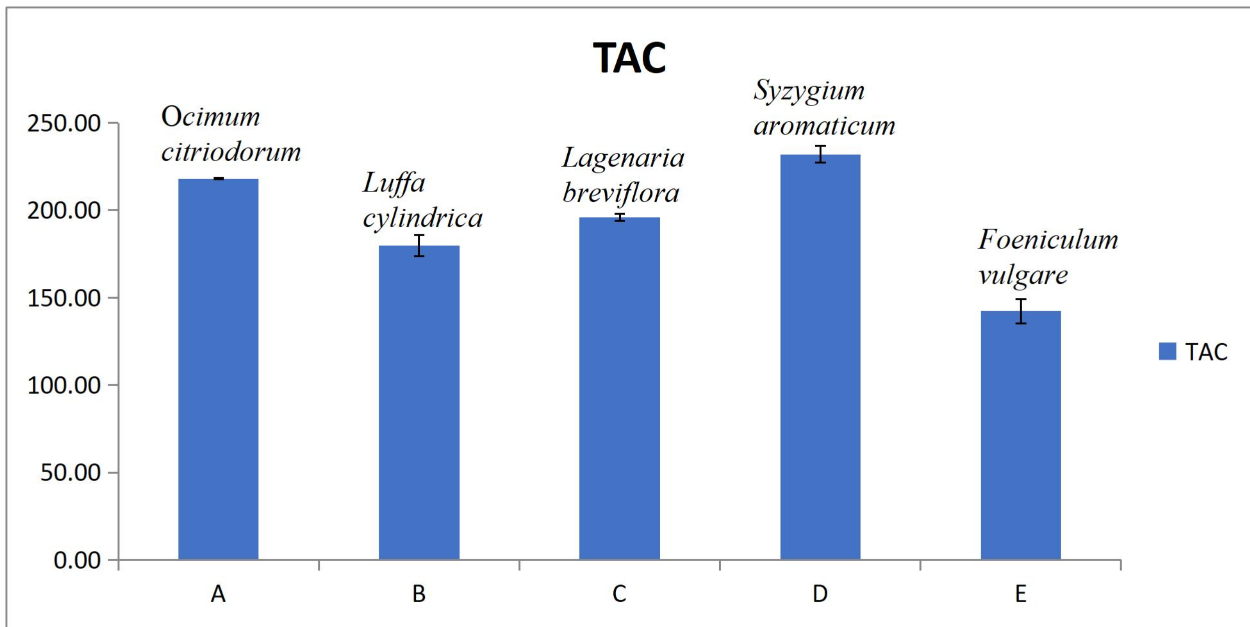
Figure 4.4 depicts the Ferric Reducing Antioxidant Power (FRAP) of *Luffa cylindrica* compared to the standard, Ascorbic acid. The graph clearly shows that the FRAP value of *Luffa cylindrica* is higher than that of the standard. Therefore, it can be concluded that *Luffa cylindrica* has greater ferric reducing antioxidant power compared to Ascorbic acid.

Fig 4.4



4.5 TOTAL ANTIOXIDANT CAPACITY (TAC)

Figure 4.5 illustrates the total antioxidant capacity (TAC) of *Luffa cylindrica* in comparison to other ethanol extracts of plants used in the study. The results show that *Luffa cylindrica* has a relatively high TAC, though *Syzygium aromaticum* exhibits the highest TAC value among the plant extracts.



4.6 HPLC PHYTOCHEMICAL PROFILE

The table below shows the phytochemicals present in the ethanol extract of *Luffa cylindrica*, the amount present, and its retention time. From the table it was seen that Quercetin was present in the highest amount which is likely to be responsible for its antioxidant properties. Other dominant phytochemicals present include; Kaempferol, Ellagic acid, Luffacyclin, P-Coumaric acid, and Luteolin. Other phytochemicals- Caffeic acid, Lutein, Catechol, Catechin, Echinocystic acid, Cinnamic acid, Rutin, Alpha-Luffin, Myrecetin, Apigenin, Naringenin, Ferulic acid, Vitexin and Diosmin are found in moderate quantities.

Table 4.1: HPLC profile of compounds present in ethanol extract of *Luffa cylindrica*

Compounds	Retention	Height	Amount(ppm)
Chlorogenic Acid	1.266	22.465	437.6225
Ellagic acid	2.750	13.476	940.3130
Hydroxybenzoic Acid	4.016	6.783	81.8215
P-Coumaric Acid	4.450	12.292	605.8170
Caffeic Acid	5.466	4.824	261.0990
Lutein	6.483	6.469	173.231
Catechol	7.333	6.298	61.2680
catechin	7.950	6.704	100.7250
Echinocystic Acid	8.816	4.461	63.1560
Cinnamic Acid	9.350	4.674	87.0705
Quercetin	11.050	136.841	6932.7940
Kaempferol	12.166	42.608	2044.3267
Luffacyclin	13.700	19.887	799.8030
Rutin	14.733	4.532	395.8435
Alpha-Luffin	15.700	10.175	294.0955
Myrecetin	16.266	7.684	67.1740
Luteolin	17.616	8.385	454.0275
Apigenin	18.033	9.344	228.2085
Naringenin	18.500	8.795	70.0515
Ferulic Acid	19.816	6.129	68.4880
Vitexin	20.500	4.542	71.1305
Diosmin	21.133	3.289	66.9765

Values represents the amount of active compounds present in the ethanol extract of *Luffa cylindrica*

CHAPTER FIVE

5.1 DISCUSSION

Antioxidants are crucial compounds that can protect the body from damage caused by free radical-induced oxidative stress. The body contains various free radical scavenging antioxidants, many of which come from dietary sources such as fruits, vegetables, and teas (Souri *et al.,* 2008). Research by Saliu *et al.* (2020) and Bhavsar *et al.* (2023) has demonstrated that the methanol extract of *Luffa cylindrica* has antioxidant properties.

This study aims to elucidate the antioxidant activity of ethanol extracts of *Luffa cylindrica* by evaluating its performance in various assays, including DPPH radical scavenging, hydroxyl radical scavenging, ferric reducing antioxidant power, total antioxidant capacity, and nitric oxide scavenging assays.

The hydroxyl radical is a highly reactive oxygen-centered radical generated from the interaction of various hydroperoxides with transition metal ions. It can damage proteins, DNA, polyunsaturated fatty acids in membranes, and other biological molecules upon contact (Aruoma, 1994). This radical is notorious for initiating lipid peroxidation by abstracting hydrogen atoms from membrane lipids. The results depicted in Figure 4.1 indicate that the ethanol extract of *Luffa cylindrica* exhibits hydroxyl radical scavenging activity similar to that of the standard (ascorbic acid), although the control showed slightly higher activity than the extract. Table 4.1 presents the IC50 value for the hydroxyl radical scavenging activity of the ethanol extract of *Luffa cylindrica,* revealing a minor difference of approximately 0.05 compared to the standard value.

Nitric oxide is a free radical produced in mammalian cells that plays a crucial role in regulating various physiological processes. However, excessive production of nitric oxide is linked to

several diseases (Lalenti et al., 1993). Inhibitors of nitric oxide have been found to offer therapeutic benefits in managing inflammation and tissue damage associated with inflammatory diseases. As observed in Figure 4.2, the activity of the ethanol extract of *Luffa cylindrica* decreases with increasing concentration, whereas the activity of the standard remains relatively consistent.

The results from the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay depicted in Figure 4.3 show that the value for ascorbic acid is notably higher compared to the ethanol extracts of *Luffa cylindrica* at the same concentrations. Table 4.3 further indicates a significant difference in the IC50 value between the extract and the standard, ascorbic acid. The DPPH value increases as the concentration rises from 0.2 mg/ml to 1 mg/ml, consistent with findings from studies by Saliu *et al.* (2020) on various extracts of *Luffa cylindrica* leaves and Sharma *et al.* (2012) on methanol extracts of *Luffa cylindrica.*

The findings from the ferric reducing antioxidant power (FRAP) assay depicted in Figure 4.4 indicate that the FRAP value of the ethanol extract of *Luffa cylindrica* was notably greater than that of ascorbic acid. This difference is likely attributed to the presence of polyphenols in *Luffa cylindrica.*

All the results discussed above were found to correspond closely with results from researches carried out by Saliu et al. (2020), Sharma *et al.* (2012) and Bhavsar *et al.* (2023), however methanol extract was used in these studies.

The total antioxidant capacity of the ethanol extract of *Luffa cylindrica* was recorded in Fig 4.5 with a value of 179.86mg/ml which is higher than the total antioxidant capacity of methanol extract of *Luffa cylindrica* reported by Saliu *et al.* (2012) to be 166.46mg/ml, this indicates that

ethanol extract of *Luffa cylindrica* has a higher TAC value than the methanol extract of *Luffa cylindrica* thus attributing to its total antioxidant activity.

HPLC profiling was used to explore the phytochemicals present in *Luffa cylindrica.* From Table 4.6, it was seen that Quercetin was the predominant phytochemical present, and is likely to be responsible for its antioxidant properties. Other phytochemicals including: Kaempferol, Ellagic acid, Luffacyclin, and Luteolin were also present in relatively high amounts. Caffeic acid, Lutein, Catechol, Catechin, Echinocystic acid, Cinnamic acid, Rutin, Alpha-Luffin, Myrecetin, Apigenin, Naringenin, Ferulic acid, Vitexin and Diosmin were found in moderate quantities. This further proves the research conducted by Tsai *et al.* (2013) that suggests that *Luffa cylindrica* is very rich in phytochemicals.

5.2 CONCLUSION

The findings of this research indicate that *Luffa cylindrica* fruits possess significant potential as a natural antioxidant source, highlighting their importance as therapeutic agents in combating or delaying degenerative diseases associated with oxidative stress.

REFERENCES

Abdullin, I. F., Turova, E. N., Budnikov, G. K., Ziyatdinova, G. K., and Gajsina, G. K. (2002). Electrogenerated bromine – Reagent for determination of antioxidant capacity of juices and extracts. *Zavodskaya Laboratory Diagnostic Material,* 68: 12–15.

Aboh, I.M., Fidelis, S., Oladosu, O.P., Adeshina, G.O., Olayinka, B.O., and Olonitola, S.O. (2020). Antifungal potentials of *Luffa cylindrica* ethyl acetate leaf extract. *Journal of Phytopharmacology,* 9 (3): 178-185.

Achigan-Dako, E.G., N'danikou, S., and Vodouhê, R.S., (2011). Brink, M., and Achigan-Dako, E.G. (editors) (2012). *PROTA* (Plant resources of tropical Africa; No. ISBN 9789290814818.

Aguilar, T.A.F., Navarro, B.C.H., and Perez, J.A.M., (2016). Endogenous antioxidants: a review of their role in oxidative stress. *Food Chemistry,* 3: 20-22.

Aladejimokun, A.O., Adesina, I.A., Falusi, V.O., and Edagbo, D.E. (2014). Comparative study of antimicrobial potency and phytochemical analysis of methanolic extracts of the leaf and flower of *Luffa cylindrica. Journal of Natural Sciences Research,* 4(8):7-10.

Alam, S., Khalil, S., Ayub, N., and Rashid, M. (2002). In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. *International Journal of Agriculture and Biology.* 4: 454–458.

Ali Esmail Al-Snafi (2019). Nutritional value and pharmacological importance of citrus species grown in Iraq. *International Journal of Current Pharmaceutical Research,* 11 (6), 1-10.

Amaeze, O.U., Ayoola, G.A., Sofidiya, M.O., Adepoju-Bello, A.A., Adegoke, A.O., and Coker, H.A.O. (2011). "Evaluation of antioxidant activity of *Tetracarpidium conophorum*, Hutch & Dalziel leaves," *Oxidative Medicine and Cellular Longevity,* Article ID 976701, pp 1-7.

Ancerewicz, J., Miglavaca, E., Carrupt, P.A., Testa, B., Bree, F., Zinin, R., Tillement, J.P., Labidelle, P., Goyot, S.D., Chauvet-Monges, A.M., Crevent, A., and Le Ridant, A. (1998). Structure property relationship of trimetadizine derivatives and model compounds as potential antioxidants. *Free Radical Biology and Medicine,* 25 (1):113-120.

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., and Robards, K. (2002). Methods for testing antioxidant activity. *The Analyst,* 127: 183–198.

Arise, R.O., Idi, J.J., Mic-Braimoh, I.M., Korode, E., Ahmed, R.N., and Osemwegie, O. (2019). In vitro Angiotesin-1-converting enzyme, α-amylase and α-glucosidase inhibitory and antioxidant activities of *Luffa cylindrica* seed protein hydrolysate. *Heliyon*, 5:16-34.

Arnao, M.B. (2000). Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practice case. *Trends in Food Science and Technology,* 11 (11):419-421.

Aruoma, O.I. (1994). Nutrition and health aspects of free radicals and antioxidants. *Food Chemtoxicol,* 32: 671-683.

Balasundram, N., Sundram, K., and Samman, S. (2006). Analytical, Nutritional and Clinical Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry,* 99(1): 191-203.

Bassey, A., Essien, E., Okokon, J., and Alalade, I. (2015). Wound healing, phytochemical and antimicrobial properties of *Luffa cylindrica* seed extracts. *International Journal of Pharmaceutical Science Drug Resource,* 7: 340-344.

Bayne, Shirley, Carlin, and Michelle (2017). Forensic Applications of High Performance Liquid Chromatography (1st Edition). *CRC Press.* ISBN 9780429251962.

Benzie, Iris, F.F., and Strain, J.J. (1996). "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay". *Analytical Biochemistry.* 239 (1): 70-76.

Berker, K. I., Demirata, B., and Apak, R. (2012). Determination of total antioxidant capacity of lipophilic and hydrophilic antioxidants in the same solution by using ferric-ferricyanide assay. *Food Analytical Methods,* 5: 1150-1158.

Berker, K. I., Gueclue, K., Tor, I., Demirata, B., and Apak, R. (2010). Total antioxidant capacity assay using optimized Ferricyanide/Prussian blue method. *Food Analytical Methods,* 3: 154–168.

Bhaskar, H.V., and Balakrishnan, N. (2009). "In vitro antioxidant property of laticiferous plant species from Western Ghats Tamilnadu, India." *International Journal of Health Research,* 2(2): 163-170.

Bhavsar, S., Modi, N., and Thakor, M. (2023). Evaluation of antioxidant potential and phytochemical characterization of peel extracts of *Luffa acutangula* and *Luffa cylindrica*. *International Association of Biologicals and Computational Digest*, 2(1): 307–315.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181: 1199-1200.

Brainina, K. Z., Varzakova, D. P., and Gerasimova, E. L. (2012). A chronoamperometric method for determining total antioxidant activity. *Journal of Analytical Chemistry,* 67: 364–369.

Brand-Williams, W., Cuvelier, M.E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 228: 25-30.

Caito, S.W., and Aschner, M., (2015). Mitochondrial redox dysfunction and environmental exposures. *Antioxidants and Redbox signaling,* 23(6): 578-595.

Chang, H.C., Huang, G.J., Agrawal, D.C., Kuo, C.L., Wu, C.R., and Tsay, H.S. (2007). Antioxidant activities and polyphenol contents of six folk medicinal ferns used as Gusuibu. *Botanical Studies,* 48: 397-406.

Chen, G., Farh, J. L., Campbell-Bush, E. M., Wu, Z., and Wu, X. (2013). Teams as innovative systems: Multilevel motivational antecedents of innovation in R&D teams. *Journal of Applied Psychology,* 98: 1018-1027.

Collomb, M., and Spahni, M. (1996). Review of the methods for determining lipid oxidation products, in particular lipids from milk products. *Food Chemistry,* 25: 3–24.

Contreras-Guzman, E.S., and Strong, F.C. (1982). Determination of Tocopherols (Vitamin E) by reduction of cupric ion. *Journal of the Association of Official Analytical Chemists,* 65: 1215-1222.

Cook, N.C., and Samman, S. (1996) Flavonoids-Chemistry, Metabolism, Cardioprotective Effects, and Dietary Sources. *The Journal of Nutritional Biochemistry,* 7: 66-76.

Cushnie, T.P., Benjamart Cushnie, and Andrew J. Lamb (2014). *International Journal for Antimicrobial agents,* 44(5): 377-386.

Das, K., and Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers In Environmental Science,* 2: 53-55.

Decker, E. A., Warner, K., Richards, M. P., and Shahidi, F. (2005). Measuring antioxidant effectiveness in Foods. *Journal of Agricultural and Food Chemistry,* 53: 4303-4310.

Du, Q., Xu, Y., Li, L., Zhao, Y., Jerz, G., and Winterhalter, P. (2006). Antioxidant constituents in the fruits of *Luffa cylindrica. Journal of Agricultural and Food Chemistry,* 54(12): 4186-4190 .

Ebrahimzadeh, M.A., Nabavi, S.M., Bahramian, F., and Bekhradnia, A.R. (2010). "Antioxidant and free radical scavenging activity of *H.officinalis, L.angustifolius, V.odorata, B.hyrcana and C.speciosum,*" *Pakistan Journal of Pharmaceutical Sciences,* 23(1): 29-34.

Flieger, J., Flieger, W., Baj, J., and Maciejewski, R., (2021). Antioxidants: Classification, natural sources, activity/capacity measurements, and usefulness for the synthesis of non-particles. *Materials* 14(15): 4135.

Gandhamalla, P., Shiva, G.B., Pravalika, R., Ramya, D.M., and Boggula Narender (2018). Plant preliminary phytochemical analysis and thrombolytic screening of *Luffa cylindrica* fruits in an in-vivo study. *International Journal of Innovative Pharmaceutical Sciences and Research (IJIPSR),* 6 (01): 61-74.

Guclu, K., Kibrislioglu, G., Ozyurek, M., and Apak, R. (2014). Development of a fluorescent probe for measurement of peroxyl radical scavenging activity in biological samples. *Journal of Agricultural and Food Chemistry,* 62: 1839–1845.

Gulaboski, R., Mirceski, V., and Mitrev, S. (2013). Development of a rapid and simple voltammetric method to determine total antioxidative capacity of edible oils. *Food Chemistry,* 138, 116-121.

Halliwell, B. (1987). Oxidants and Human Diseases: Some New Concepts. *FASEB Journal,* 1: 358-364.

Halliwell, B., and Gutteridge, J.M., (2015). Free radicals in biology and medicine. *Oxford University Press, USA*.

Hamid, K., Saha, M.R., Urmi, K.F., Habib, M.R., and Rahman, M.M. (2010). Screening of different parts of the plant *Pandanus odorus* for its antioxidant activity. *International Journal of Applied Biology and Pharmaceutical Technology,* 1: 1364-1368.

Hegerman, A. E., Riedl, K. M., Jones, G., Sovik, K. N., Rechard, N. T., Hartzfeld, P. W., and Reichel, T. L. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry,* 46: 1887–1892.

Howlader, A.H., Iqbal, Shamim, S.M., Sirajul, I., and Quader, M.A. (2013). Phytochemical constituents of some vegetables. *Dhaka University Journal of Science,* 61(2): 147-151.

Ibewike, G. C., Abiodun, O. G., Bohlin, and Ogungbamila, F. O. (1997). Anti-inflammatory activity of selected Nigerian medicinal plants. *Nigerian Journal of Natural Products and Medicine,* 1: 10-14.

Jain, P.K., Huang, X., El-Sayed, I.H., and El-Sayed M.A (2008). Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. *Accounts of Chemical Research,* 41: 1578-1586.

Kamatenesi-Mugisha, M., Makawiti, D.W., Oryem-Origa, H., and Nganga, J. (2007). The oxytocic properties of *Luffa cylindrica* and *Bidens pilosa*, traditionally used medicinal plants from western Uganda. *The African Journal of Ecology,* 45(3): 88-93.

Kazakevich, Y., and Lobrutto, R. (2007). HPLC for pharmaceutical scientists, *Willey-Verlag,* 3(92): 470-471.

Khalili Tilami, S., and Samples, S., (2018). Nutritional value of fish: lipids, proteins, vitamins and minerals. *Reviews in Fisheries Science and Aquaculture,* 26(2): 243-253.

Kolmonen, Marjo, Leinonen, Antti, Pelander, Anna, Ojanperä, and Ilkka (2007). "A general method for doping agents in human urine by solid phase extraction and liquid chromatography/time-of-flight mass spectrometry". *Analytica Chimica Acta,* 585 (1): 94-102.

Kumar, S., and Pandey, A.K. (2013). Chemistry and biological activities of flavonoids: an overview. *Scientific World Journal,* 162750.

Lai, C.S., Fisher, S.E., Hurst, J.A., Varga-Khadem, F., and Monaco, A.P. (2001). A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature,* 413: 519-523.

Lakhanpal, P., and Rai, D.K. (2007). "Quercetin: a versatile flavonoid," *Internet Journal of Medical Update,* 2(2): 22-37.

Lalenti, A., Lanaro, A., and Moncada, S. (1993). Modulation of acute inflammation by endogenous nitric oxide. *The European Journal of Pharmacology,* 211: 177-182.

Ma, L., Xie, W., Li, D., Shi, L., Mao, Y., and Xiong, Y. (2020). Effect of SARS-CoV-2 infection upon male gonadal function: a single center-based study. *Journal of Clinical Medicine,* 9: 419.

Marcocci, L., Maguire, J .J., Droyl-Lefaix, M. T., and Packer, L. (1994). The nitric oxide scavenging properties of Ginkgo biloba extract. *Biochemical and Biophysical Research Communications,* 201:748-755.

Marrazzo, G., Barbagallo, I., Galvano, F., Malaguarnera, M., Gazzolo, D., Frigiola, A., D'Orazio, N., and Li Volti, G., (2014). Role of dietary and endogenous antioxidants in diabetes. *Critical Reviews in Food Science and Nutrition,* 54(12): 1599-1616.

Masahiro, N., Masahiro, K., Minemitsu, N., Akio, K., and Yoshimi, N. (2005). Non-reductive Scavenging of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) by Peroxyradical: A Useful Method for Quantitative Analysis of Peroxyradicals. *Chemical and Pharmaceutical Bulletin,* 53(6): 714-716.

Matsuura, H.N., Rau, M.R., and Fett-Neto, A.G. (2014). Oxidative stress and production of bioactive monoterpene indole alkaloids: biotechnological implications. *Biotechnology Letters,* 36: 191-200.

Mazali, I.O., and Alves, O.L. (2005). Morphosynthesis: high fidelity inorganic replica of the fibrous network of loofa sponge (*Luffa cylindrica*). *Anais da Academia Brasileira de Ciências*, 77(1): 25-31.

Milardovic, S., Kerekovic, I., and Rumenjak, V. (2007). A flow injection biamperometric method for determination of total antioxidant capacity of alcoholic beverages using bioenzymatically produced ABTS. *Food Chemistry,* 105: 1688-1694.

Miliauskas, G., Veinstone, P.R., and Beek, T.A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry,* 85 :231-237.

Min, D.B., and Akoh, K. (1998). Lipid oxidation of edible oil. Food Lipids: Chemistry, Nutrition and Biotechnology. *New York: Marcel Dekkar,* Pp. 283-296.

Molina-Diaz, A., Ortega-Carmona, I., and Pascual-Reguera, M. I. (1998). Indirect spectrophotometric determination of ascorbic acid with ferrozine by flow-injection analysis. *Talanta,* 47: 531-536.

Mukthi Thammana (2016). Review on High performance liquid chromatography. *Food Chemistry,* 5 (2):22-28.

Nagmoti, D.M., Khatri, D.K., and Juvekar, A.R. (2011). "Antioxidant activity and free radical-scavenging potential of Pithecellobium dulce seed extracts." *Free Radical and Antioxidants,* 2(2): 37-43.

Naik, G.H., Priyadarsini, K.I., and Hari, M. (2006). Free radical scavenging reactions and phytochemical analysis of Triphala. *Current Science,* 90: 1100-1105.

Nandi, A., Yan, L.J., Jana, C.K., and Das, N. (2019). Role of catalase in oxidative stress-and age associated degenerative diseases. *Oxidative medicine and cellular longevity,* Pp. 36-104.

Nguyen, L.T., Farcas, A.C., Socaci, S.A., Tofana, M., Diaconeasa, Z.M., and Pop, O.L. (2020). An overview of Saponins–a bioactive group. *Bulletin UASVM Food Science and Technology,* 77 (1): 25-36.

Nobilis, Voprsalová, Marie, Kolárová, Lenka, Holcapek, and Michal (2007). "Metabolic profiling of a potential antifungal drug, 3-(4-bromophenyl)-5-acetoxymethyl-2,5-dihydrofuran-2-one, in mouse urine using high-performance liquid chromatography with UV photodiode-array and mass spectrometric detection". *Journal of Chromatography,* 853 (1-2): 10-19.

Oboh, I.O., and Aluyor, E.O. (2009). *African Journal of Agricultural Research,* 4 (8):684-688.

Ogunyemi, T. C., Ekuma, C. M., Egwu, J. E., and Abbey, D. M. (2020). Proximate and mineral composition of sponge gourd (*Luffa cylindrica*) seed grown in South-Western Nigeria. *Journal of Scientific Research and Reports,* 26(4): 61-67.

Onigemo, M.A., Dairo, F.A., and Oso, Y.A. (2020). Amino acids profile of loofah gourd, *Luffa cylindrica* seeds subjected to different heat processing methods. *Nigerian Journal of Animal Production,* 47(2): 280-288.

Osai, V. (1998). The transition challenges of herbal drug. *Nigerian Journal of Natural Products and Medicine,* 2: 16-18.

Ou, B., Hampsch-Woodill, M., Flanagan, J., Deemer, E.K., Prior, R. L., and Huang, D. (2002). Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. *Journal of Agricultural and Food Chemistry,* 50: 2772-2777.

Oyetayo, F.L., Oyetayo, V.O., and Ajewole, V. (2007). Phytochemical profile and antibacterial properties of the seeds and leaf of the Luffa plant (*L. cylindrica*). *Journal of Pharmacology and Toxicology,* 2: 586-589.

Panche, A., Chandra, S., and Diwan, A. (2015) Alzheimer's and current therapeutics: a review. *Asian Journal for Pharmaceutical Clinical Research,* 8: 14-19.

Parry, J., Su, L., Luther, M., Zhou, K.Q., Yurawecz, M.P., Whittaker, P., and Yu, L.L. (2005). Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. *Journal of Agricultural and Food Chemistry,* 53: 566-573.

Partap, S., Kumar, A., Sharma, N.K., and Jha, K.K. (2012). *Luffa cylindrica*: An important medicinal plant. *The Journal of Natural Product and Plant Resources,* 2 (1): 127-134.

Pelander, Anna, Ojanperä, Ilkka, Laks, Suvi, Rasanen, Ilpo, Vuori, and Erkki (2003). "Toxicological screening with formula-based metabolite identification by liquid chromatography/time-of-flight mass spectrometry". *Analytical Chemistry,* 75 (21): 5710-5718.

Pesce, Amadeo, Rosenthal, Murray, West, Robert, West, Cameron, Crews, Bridgit, Mikel, Charles, Almazan, Perla, Latyshev, and Sergey (2010). "An evaluation of the diagnostic accuracy of liquid chromatography-tandem mass spectrometry versus immunoassay drug testing in pain patients". *Pain Physician,* 13 (3): 273-281.

Prakash, A. (2001). Antioxidant activity. Medical Laboratory *Analytical Program,* 19(2): 1-6.

Prior, R.L., Wu, X., and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry,* 53: 4290-4302.

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: 3396-3402.

Rayman, M.P. (2000). The importance of selenium to human health. *The Lancet,* 356(9225): 233-41.

Roginsky, V., and Liss, E. A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry,* 92: 235–254.

Saliu, O. A., Akanji, A. M., Idowu, O. A., and Saliu, B. N. (2020). Free radical and reactive oxygen species scavenging potentials of *Luffa cylindrica* leaf extracts. *Journal of Cell Biology and Biochemistry Research,* 4(1): 13-19.

Sanchez-Moreno, C. (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International,* 8(3):121-137.

Sangh, P., Amit, K., Neeraj, K.S., and Jha, K.K. (2012). *Luffa cylindrica:* An important medicinal plant. *The Journal of Natural Product and Plant Resources,* 2 :127-134.

Sati, S.C., Sati, N., Rawat, U., and Sati, O.P. (2010). Medicinal plants as a source of antioxidants. *Research Journal for Phytochemistry,* 4: 23–224.

Sendra, J.M., Sentandreu, E., and Navarro, J.L. (2006). Reduction kinetics of the free stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) for determination of the antiradical activity of citrus juices. *European Food Research and Technology,* 223: 615-624.

Sharma, N. K., Sangh, P., Priyanka, P., Jha, K. K., Singh, H. K., and Shrivastava, A. K. (2012). Free radical scavenging activity of methanolic extract of *Luffa cylindrica* leaves. *International Journal of Green Pharmacy (IJGP),* 6(3).

Siddiqui, Masoom Raza, Alothman, Zeid, A., Rahman, and Nafisur (2013). "Analytical techniques in pharmaceutical analysis: A review". *Arabian Journal of Chemistry,* 10: 1409-1421.

Sies, H., (1997). Oxidative Stress: oxidants and Antioxidants. *Experimental Physiology,* 82(2): 81-295.

Souri, E., Amin, G., Farsam, H., Jalalizadeh, H., and Barezi, S. (2008). Screening of thirteen medicinal plant extracts for antioxidant activity. *Iranian Journal of Pharmaceutical Research,* 7(2): 149–154.

Sugamura, K., and Keaney Jr, J.F., (2011). Reactive oxygen species in cardiovascular disease. *Free radical biology and medicine,* 51(5): 978-992.

Traber, M.G., and Stevens, J.F., (2011). Vitamins C and E: beneficial effects from a mechanistic perspective. *Free radical biology and medicine,* 51(5): 1000-1013.

Tripathi, A., Tandon, M., Chandekar, A., Soni, N., and Upmanyu, N. (2016). In vitro antioxidant and anthelmintic activity on *Luffa cylindrica* leaf extract, *Journal of Herbs, Spices & Medicinal Plants,* 22(4): 348-355.

Tsai, I., Lin, Weng, Te, I., Tseng, Yufeng, J., Tan, Happy Sun, Hsiao-Ju, Kuo, and Ching-Hua (2013). "Screening and confirmation of 62 drugs of abuse and metabolites in urine by ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry". *Journal of Analytical Toxicology,* 37 (9): 642-651.

Tsai, P.J., Tsai, T.H., Yu, C.H., and Ho, S.C. (2007). "Evaluation of NO-suppressing activity of several Mediterranean culinary spices," *Food and Chemical Toxicology,* 45(3): 440-447.

United States Pharmacopoeia, 2004. 27th ed. The USP Convention Incorporation, Rockville, MD.

Valko, M., Jomova, K., Rhodes, C.J., Kuča, K., and Musilek, K. (2016). Redox-and non-redox metal-induced formation of free radicals and their role in human disease. *Archives of Toxicology,* 90: 1-37.

Valko, M., Jomova, K., Rhodes, C.J., Kuka, K., and Musilek, K., (2016). Redbox and non-redox metal-induced formation of free radicals and their role in human disease. *Archives of Toxicology,* 90: 1-37.

Veronika R. Meyer (2010). Practical High-Performance Liquid Chromatography, 5th Edition. ISBN: 9780470093771.

Vincken, J.P., Lynn Heng, Aede de Groot, and Harry Gruppen. Phytochemistry. *Journal for Phytochemistry,* 68(3): 275-297.

Wang, M., Li, K., Nie, Y., Wei, Y., and Li, X. (2012). Antirheumatoid Arthritis Activities and Chemical Compositions of Phenolic Compounds-Rich Fraction from Urtica atrichocaulis, an Endemic Plant to China. *Evidence-Based Complementary and Alternative Medicine,* 10: 155-182.

Weinmann, W., Renz, M., Vogt, S., and Pollak, S. (2000). "Automated solid-phase extraction and two-step derivatisation for simultaneous analysis of basic illicit drugs in serum by GC/MS". *International Journal of Legal Medicine,* 113 (4): 229-235.

Williamson, G., (2017). The role of polyphenols in modern nutrition. *Nutrition Bulletin,* 42(3): 226-235.

Yu, L.L. (2001). Free radical scavenging properties of conjugated linoleic acids. *Journal of Agricultural and Food Chemistry,* 49: 3452-3456.

Ziyatdinova, G., Nizamova, A., and Budnikov, H. (2011). Novel coulometric approach to evaluation of total free polyphenols in tea and coffee beverages in presence of milk proteins. *Food Analytical Methods,* 4: 334–340.

Ziyatdinova, G., Salikhova, I., and Budnikov, H. (2014). Coulometric titration with electrogenerated oxidants as a tool for evaluation of cognac and brandy antioxidant properties. *Food Chemistry,* 150(2014): 80–86.