DPPH SCAVENGING ACTIVITY OF *Gossypium hirsutum* (COTTON) SEED

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BMS2000027

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF MEDICAL SCIENCE

COLLEGE OF MEDICAL SCIENCE

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IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES DEGREE IN MEDICAL BIOCHEMISTRY

NOVEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY,
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REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS)
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CERTIFICATION

We the undersigned hereby certify that OBI FAVOUR EZICHUKWU (BMS2000027) 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

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DEDICATION

This project is dedicated to Almighty God, the giver of life who has made it possible to complete my Bachelor of Science Degree (B.Sc.) program in the Department of Medical Biochemistry and my entire family for their tender care and love for me.

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My gratitude goes for Almighty God for his grace in all my endeavors, unto him is all the glory. My sincere appreciation goes to my amiable supervisor Dr. N. B. Aguebor-Ogie who doubles as the head of department, alongside other lecturers in the department for their words of wisdom and encouragement.

ABSTRACT

Cotton (*Gossypium hirsutum*) seed, a major agricultural by-product, remains an under-explored source of natural antioxidants, which are increasingly sought after as alternatives to synthetic compounds. This study aimed to evaluate the *in vitro* antioxidant potential of an aqueous extract of cotton seed by assessing its DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. An aqueous extract was prepared from dried, powdered cotton seeds. Its free radical scavenging capacity was determined spectrophotometrically at concentrations ranging from 50 to 250 μg/mL and compared against ascorbic acid as a standard. The cotton seed extract exhibited dose-dependent activity, with inhibition ranging from 29.11% to 46.37%. Linear regression analysis was used to calculate the half-maximal inhibitory concentration (IC_{50}), which was found to be 288.88 μg/mL for the extract, compared to 86.12 μg/mL for the highly potent ascorbic acid standard. The findings demonstrate that aqueous cotton seed extract possesses moderate antioxidant properties, likely attributable to its inherent phytochemicals. This study validates the potential of cotton seed as a viable, low-cost source for natural antioxidants, supporting the valorization of this agricultural by-product for applications in the food and nutraceutical industries.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Oxidative stress is a physiological imbalance that occurs when the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) exceeds the body's antioxidant defense capacity (Jomova *et al.,* 2023). These reactive species, which include superoxide anion (O_2^-), hydroxyl radical ($OH\cdot$), and hydrogen peroxide (H_2O_2), are produced naturally during metabolic processes such as mitochondrial respiration (Forman and Zhang, 2021). While moderate levels of ROS play beneficial roles in cell signaling and immune defense, excessive production can lead to oxidative damage to biomolecules such as lipids, proteins, and DNA, contributing to various pathological conditions including cancer, diabetes, and neurodegenerative disorders (Gülçin *et al.,* 2022). Antioxidants are compounds capable of preventing or delaying the oxidation of biomolecules by neutralizing free radicals (Kedare and Singh, 2021). They function by donating electrons or hydrogen atoms to stabilize reactive species, thereby protecting cells and tissues from oxidative damage (Kiran and Pundir, 2023). Both enzymatic (e.g., superoxide dismutase, catalase) and non-enzymatic (e.g., vitamins C and E, flavonoids,) antioxidants play vital roles in maintaining redox homeostasis (Kothari *et al.,* 2022). Although synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used in food and pharmaceutical industries to retard oxidation, growing evidence has linked their excessive consumption to toxic and carcinogenic effects (Saini *et al.,* 2022). Consequently, there is increasing global interest in discovering natural antioxidants from plant sources that are effective and safe (Ungureanu *et al.,* 2024). Plants are rich reservoirs of bioactive secondary metabolites such as phenolics, flavonoids, tannins, terpenes, and alkaloids, which contribute significantly to their antioxidant potential (Qamar *et al.,* 2021). Among these, cotton, a member of the *Malvaceae* family, is an economically important crop primarily cultivated for its fiber and seed oil (Kumar *et al.,* 2022). Traditionally, various parts of the cotton plant have been used for medicinal purposes including antimicrobial, anti-inflammatory, anti-fertility, and antioxidant applications (Rehman *et al.,* 2022). However, compared to other oilseeds such as soybean and groundnut, the antioxidant properties of cotton seed remain under-explored, especially regarding its DPPH radical scavenging activity (Mohammed *et al.,* 2024). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is one of the most widely used methods for assessing antioxidant capacity in vitro (Baliyan *et al.,* 2022). Evaluating the DPPH scavenging activity of cotton seed extracts provides insight into their free radical neutralization potential and their possible applications as natural antioxidants in food, pharmaceutical, and nutraceutical industries (Canale *et al.,* 2023).

1.2 Aim of the Study

The aim of this study was to evaluate the DPPH free radical scavenging activity of cotton (*Gossypium* hirsutum) seed extracts.

1.3 Objective of the Study

The objective of the was to assess the DPPH free radical scavenging activity of cotton (*Gossypium* hirsutum) seed extracts in relation to their phytochemical composition.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Reactive Species (RS) and Oxidative Stress (OS)

Oxidation processes are essential for the survival of cells (Jomova *et al.,* 2023). Aerobic cellular respiration organisms provide energy from organic molecules such as glucose but also cause the formation of free radicals that cause cellular damage in metabolism (Forman and Zhang, 2021). A free radical contains an unpaired (free) electron with a quantum-mechanical property called spin (Gülçin *et al.,* 2022). Such an entity typically has high reactivity because of its open shell structure (Kedare and Singh, 2021). However, today there are many free radicals that are stable under laboratory conditions, that is, in the air and at room temperature (Kiran and Pundir, 2023). Free radicals are known to be mostly associated with oxidative stress (Kothari *et al.,* 2022). Oxidative stress is a comparatively new concept that has been commonly used in the medical sciences recently (Saini *et al.,* 2022). It occurs when there is an excess of reactive oxygen species (ROS) produced by a cellular mitochondrion (Ungureanu *et al.,* 2024). It is inevitable that free radicals, which are known to cause many degenerative diseases such as carcinogenesis, acute inflammation, high blood pressure, diabetes, preeclampsia, acute renal failure, atherosclerosis, Alzheimer's disease and Parkinson's disorders, mutagenesis, aging, and cardiovascular disorders, are produced in biological systems (Qamar *et al.,* 2021). There are many factors, including UV radiation and pollutants, that contribute to oxidative stress, which has a daily influence on human health (Kumar *et al.,* 2022). Cells metabolize oxygen, creating potentially harmful ROS (Chhikara *et al.,* 2022). Under normal conditions, the rate and amplitude of oxidant formation are balanced by the rate at which they are removed (Rehman *et al.,* 2022). However, disruption of the balance between antioxidants and pro-oxidants causes oxidative stress (Mohammed *et al.,* 2024). Recent extensive scientific research has classified reactive species (RS) and free radicals into three main categories: reactive nitrogen species (RNS), reactive oxygen species (ROS), and reactive sulfur species (RSS), composed of nitrogen, oxygen, and sulfur atoms, respectively (Baliyan *et al.,* 2022). Hydroxyl (HO·), superoxide anion (O2−), alkoxyl (RO·), nitric oxide (NO·), and peroxyl (ROO·) radicals are radicals (Krishnan *et al.,* 2024). Nitrogen monoxide (NO), singlet oxygen (1O2), hydrogen peroxide (H2O2), ozone (O3), nitrous acid (HNO2), nitrous oxide (N2O), lipid hydroperoxide (LOOH), and hypochlorous acid (HOCl) are non-radical reactive species (Canale *et al.,* 2023). RS also occur in living organisms as part of their defense systems (Jomova *et al.,* 2023). Phagocytes such as monocytes, macrophages, or neutrophils defend themselves against foreign organisms by synthesizing large amounts of O2·− or NO· as part of their killing or defense mechanisms (Forman and Zhang, 2021). Antioxidant molecules inhibit oxidative processes and reduce the hazardous effects of RS (Gülçin *et al.,* 2022). In this way, they are important in terms of health (Kedare and Singh, 2021). When free radicals occur excessively in the human body, they cause very serious negative effects in different tissues (Kiran and Pundir, 2023). One of the most important complications related to this is the formation of lipid peroxidation in the plasma membrane (Kothari *et al.,* 2022). This event promotes RNS and ROS formation (Saini *et al.,* 2022). Meanwhile, metals such as iron and copper enable Fenton and Haber–Weiss reactions and the formation of reactive species such as OH· (Ungureanu *et al.,* 2024). In the presence of metal ions and oxygen, H2O2 can easily form OH· by the Fenton reaction (Qamar *et al.,* 2021). In addition, the Haber–Weiss reaction produces OH· from O2•− and H2O2 catalyzed by iron ions (Kumar *et al.,* 2022). This impact was first suggested by Fritz Haber (Chhikara *et al.,* 2022). In later studies, it was known that both reactions constitute the main source of radicals and are the most important ones responsible for cellular damage (Rehman *et al.,* 2022).

Fe2++H2O2→Fe3++OH−+OH• (Fenton reaction).

O•−2+H2O2→O2+H2O+OH• (Haber–Weiss reaction).

2.2 Antioxidants: Mechanisms and Classification

Antioxidant molecules can be classified in different ways depending on their environment and the functions they perform (Krishnan *et al.,* 2024). An antioxidant is defined as a substance that can significantly delay or completely prevent the oxidation of substrate molecules, even at low concentrations (Canale *et al.,* 2023). They donate electrons to free radicals, rendering them harmless, and neutralize them by minimizing oxidative damage in biological processes (Jomova *et al.,* 2023). Antioxidants prevent free radical formation by interfering with the free radical-mediated oxidative process at any of its three main stages: initiation, propagation, and termination (Forman and Zhang, 2021). The effectiveness of an antioxidant compound depends on different parameters and factors (Gülçin *et al.,* 2022). The most important are the physical system state, temperature, structural properties, properties of the oxidation-sensitive substrate, concentration, synergistic effect, and presence of pro-oxidant compounds (Kedare and Singh, 2021). The chemical structure of an antioxidant molecule determines its intrinsic reactivity and antioxidant ability towards free radicals and other ROS (Kiran and Pundir, 2023). In addition, the effectiveness of the antioxidant also depends on its concentration in the system and localization, such as interface distribution (Kothari *et al.,* 2022). The reaction kinetics are another factor that plays an important role in the protective effect of the antioxidant in the long or short term (Saini *et al.,* 2022). This includes the thermodynamics of the reaction between an antioxidant and a different oxidant, the reaction rate, and the antioxidant's ability to react (Ungureanu *et al.,* 2024). All of these parameters must be considered when testing the effectiveness of a particular antioxidant substance (Qamar *et al.,* 2021). In this way, they maintain the balance between oxidants and antioxidants in metabolism (Kumar *et al.,* 2022). In addition, antioxidants delay lipid peroxidation formation during storage and processing of foods, prevent the deterioration of drugs and food products, and extend the shelf life of products (Chhikara *et al.,* 2022). For this purpose, a wide variety of synthetic or natural antioxidants are often used to prevent food spoilage (Rehman *et al.,* 2022). To address this, the pharmaceutical industry has mainly used synthetic antioxidants to block or reduce the intracellular amounts of reactive oxygen or nitrogen species (Mohammed *et al.,* 2024). Of these, synthetic antioxidants are widely used because they can be found in high purity, have low costs, and are highly reactive even at low concentrations (Baliyan *et al.,* 2022). However, some harmful effects have been reported (Krishnan *et al.,* 2024). Therefore, antioxidants of natural origin rather than synthetic antioxidants are preferred (Canale *et al.,* 2023). There has been a parallel increase in methods used to estimate the efficacy of antioxidants (Jomova *et al.,* 2023). The use of a free 1,1-diphenyl-2-picrylhydrazil radical (DPPH) is the most common method (Forman and Zhang, 2021). Butylated hydroxytoluene (BHT), propyl gallate (PG), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) are the synthetic antioxidants that are most preferred by manufacturers, and therefore consumers have to use them despite their known negative effects (Gülçin *et al.,* 2022). The chemical structures of the synthetic antioxidant molecules are given in Figure 2.1 (Kedare and Singh, 2021). These chemicals have been widely used as food additives for the prevention of oxidative deterioration in food and pharmaceutical products (Kiran and Pundir, 2023). However, new studies have raised concerns regarding the safety of these synthetic compounds owing to unexpected consequences, particularly their inhibitory ability against numerous enzymes (Kothari *et al.,* 2022). Due to the toxic effects of these synthetic additives, researchers are working hard to find new and alternative antioxidant substances with fewer side effects (Saini *et al.,* 2022). In this context, there is considerably increasing trend to replace synthetic antioxidants with natural antioxidants, which have lower toxicity, high biodegradability, and safer methods of action (Ungureanu *et al.,* 2024). In the case of long-term use of these synthetic antioxidants, it has been stated that they cause some health problems, including carcinogenesis, skin allergies, fatty liver, and gastrointestinal distress (Qamar *et al.,* 2021). Therefore, conscious consumers are concerned about the negative effects of synthetic antioxidants and prefer natural antioxidants (Kumar *et al.,* 2022). The main and most accessible sources of these natural and safer antioxidants are fruits, vegetables, herbs, and spices (Chhikara *et al.,* 2022). For this purpose, plants such as tea, linden, cinnamon, cloves, fennel, anise, and rosemary are used as sources of natural antioxidants due to their rich tannin, catechin, theine, phenolic, and flavonoid contents (Rehman *et al.,* 2022). Consumption of herbal products rich in phenolic content, which has an antioxidant effect, both reduces the risk of catching diseases and prevents the development of degenerative disorders (Mohammed *et al.,* 2024). However, the antioxidant capacity and quality of natural antioxidants and extracts depend not only on the natural source but also on the applied isolation and extraction processes (Baliyan *et al.,* 2022).

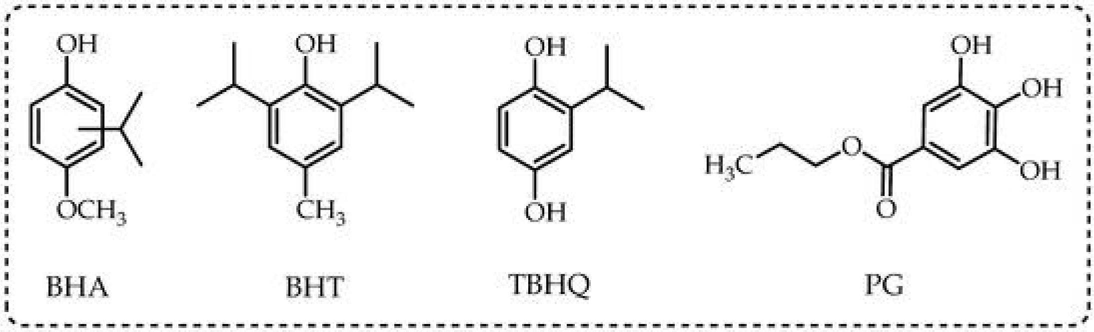

Figure 2.1. The chemical structures of the most putative and commonly used synthetic antioxidants (Krishnan *et al.,* 2024).

Antioxidants can be divided into several major categories, including water soluble and liposoluble, biological, and synthetic (Figure 2.2) (Canale *et al.,* 2023). Water-soluble antioxidants are found in vegetables and fruits and are best absorbed by the body (Jomova *et al.,* 2023). On the other hand, the body quickly removes them through urine (Forman and Zhang, 2021). Both vitamin C and polyphenols are examples of water-soluble antioxidants (Gülçin *et al.,* 2022). Antioxidants that are absorbed in the presence of lipids are known as liposoluble or fat-soluble antioxidants (Kedare and Singh, 2021). Therefore, without lipids, the body is unable to absorb and utilize these antioxidants (Kiran and Pundir, 2023). However, note that they are difficult to remove from cells and tissues and can build up over time, preventing you from getting adequate amounts (Kothari *et al.,* 2022). A prime example of a fat-soluble antioxidant is vitamin E (Saini *et al.,* 2022).

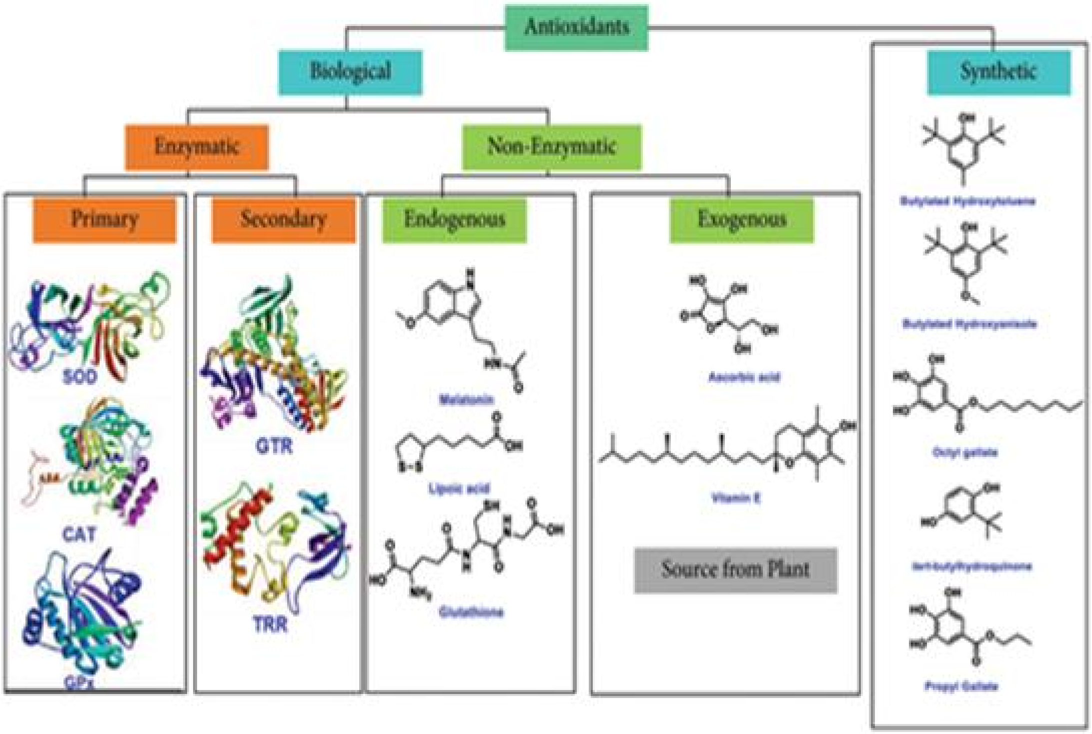

Figure 2.2: Schematic approaches for the classification of antioxidants (Ungureanu *et al.,* 2024).

There are two categories of antioxidants, namely, biological antioxidants and synthetic antioxidants (Qamar *et al.,* 2021). Enzymatic and nonenzymatic biological antioxidants are further classified into two categories (Kumar *et al.,* 2022). Antioxidants-involved enzymes can break down ROS, thus protecting the body from free radical damage (Chhikara *et al.,* 2022). Superoxide dismutase, catalase, and glutathione peroxidase are the main classes of enzymatic antioxidants (Rehman *et al.,* 2022). On the other hand, glutathione reductase and thioredoxin reductase are the secondary enzymes of antioxidants (Mohammed *et al.,* 2024).

i. Primary Enzymatic Antioxidants

In human biological systems, superoxide dismutase (SOD) contains external body fluids and aerobic cells (Baliyan *et al.,* 2022). This enzyme accelerates the radical conversion of superoxide anions into hydrogen peroxide and oxygen (Krishnan *et al.,* 2024). There are three main types of SOD (all bound to metal cofactors) (Canale *et al.,* 2023). SOD bound to zinc or copper and SOD bound to manganese, iron, and nickel (Jomova *et al.,* 2023). In high altitude plants, copper- and zinc-bound SODs are commonly found on apoplasts, peroxisomes, chloroplast, and cytosols (Forman and Zhang, 2021). Manganese-bound SOD is also present in peroxisomes and mitochondria (Gülçin *et al.,* 2022). Iron-bound SOD is normally found in chloroplasts and peroxisomes (Kedare and Singh, 2021). The classification of SOD in humans can also be divided into zinc-bound SOD type 1 and manganese-bound SOD type 2 (Kiran and Pundir, 2023). Both enzymes are in the cytoplasm (Kothari *et al.,* 2022). The last is SOD type 3 that binds zinc extracellularly (Saini *et al.,* 2022). Almost all living organisms involved in oxygen processes in life contain a common catalase that is used to catalyze the breakdown of H2O2 into H2O and O2 (Ungureanu *et al.,* 2024). In the human biological system, there are primary or byproducts of metabolic activity that highly toxic H2O2 (Qamar *et al.,* 2021). This molecule must be converted into other compounds so as not to damage the cellular structure (Kumar *et al.,* 2022). These gaseous, less harmful water and oxygen molecules are produced by the rapid conversion of hydrogen peroxide in the body by the catalases (CAT) (Chhikara *et al.,* 2022). CAT have a Kcat value greater than 106/sec, and this allows to quickly change molecules (Rehman *et al.,* 2022). Although there has been significant debate regarding the function of CAT peroxynitrite, the current study revealed that CAT may be able to scavenge ONOO− (Mohammed *et al.,* 2024). Glutathione peroxidase (GPx) has four selenium cofactors that act as a catalyst for the breakdown of hydroperoxide radicals (Baliyan *et al.,* 2022). At least four different glutathione peroxidase isozymes exist in mammals (Krishnan *et al.,* 2024). GPx 1 is the most prevalent and highly efficient hydrogen peroxide scavenger, while GPx 4 is particularly active against lipid hydroperoxides (Canale *et al.,* 2023).

ii. Secondary Enzymatic Antioxidants

Classes of reductase enzymes such as glutathione reductase (GTR) and thioredoxin reductase (TRR) are enzymes that play a role in the secondary antioxidant system as they are responsible for the constant generation of NADPH to balance ROS production (Jomova *et al.,* 2023). NADPH can neutralize the effects of toxic molecules entering the human bod (Forman and Zhang, 2021). Another source of NADPH metabolism can also be through the pentose phosphate pathway with the help of enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Gülçin *et al.,* 2022).

iii. Non-enzymatic Antioxidants

Non-enzymatic antioxidants are compounds that can counteract free radicals without the intervention of enzymes (Kedare and Singh, 2021). Normally, this action does not occur catalytically and is found in low molecular weight chemicals (Kiran and Pundir, 2023). Therefore, non-enzymatic antioxidants consist of either endogenous (eukaryotic cells can produce them by themselves) or exogenous (antioxidants must be taken outside the body) (Kothari *et al.,* 2022). iv. Endogenous Non-enzymatic Antioxidants

Melatonin is a hormone derived from the amino acid tryptophan (Saini *et al.,* 2022). It can easily cross cell membranes and the blood-brain barrier, protecting cells from lipid peroxidation (Ungureanu *et al.,* 2024). Melatonin is called a terminal antioxidant because, once oxidized, it cannot return to its original state (Qamar *et al.,* 2021). Alpha-lipoic acid (α-lipoic acid) is a disulfide compound that participates in metal chelation and redox cycling (Kumar *et al.,* 2022). It helps scavenge reactive oxygen species (ROS), prevents Fenton reactions, and regenerates other antioxidants such as vitamins C and E (Chhikara *et al.,* 2022). Glutathione is a peptide made from amino acids that contains a thiol group, allowing it to be oxidized and reduced reversibly (Rehman *et al.,* 2022). It is a key cellular antioxidant that maintains the redox state of cells and directly reacts with oxidants (Mohammed *et al.,* 2024). Coenzyme Q10, also known as ubiquinone, is a lipid-soluble antioxidant synthesized in the body through the mevalonate pathway (Baliyan *et al.,* 2022). It protects cell membranes from lipid peroxidation, scavenges ROS, and regenerates other oxidized antioxidants (Krishnan *et al.,* 2024).

v. Exogenous Non-enzymatic Antioxidants

Exogenous antioxidants come from external sources, mainly plants and microorganisms, and must be obtained through the diet (Canale *et al.,* 2023). Vitamin C (ascorbic acid) is found in fruits such as citrus, tomatoes, and pineapples (Jomova *et al.,* 2023). It scavenges reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radicals (Forman and Zhang, 2021). Vitamin E (α-tocopherol) is a lipid-soluble antioxidant that protects cell membranes by stopping lipid peroxidation chain reactions and neutralizing free radicals (Gülçin *et al.,* 2022). Flavonoids are plant-based compounds that effectively reduce ROS through hydrogen atom transfer and can also chelate metals, preventing the formation of free radicals (Kedare and Singh, 2021). Quercetin, a type of flavonoid, stabilizes iron and blocks radical formation (Kiran and Pundir, 2023). Carotenoids, such as β-carotene found in carrots, pumpkins, and mangoes, are pigments that scavenge ROS and may have cancer-preventive properties (Kothari *et al.,* 2022).

vi. Synthetic Antioxidants

Synthetic antioxidants interact with free radical species through metal ion binding, oxygen deactivation, radical to nonradical species conversion, and UV radiation saturation (Saini *et al.,* 2022). Examples of synthetic antioxidants include propyl gallate (PG), octyl gallate (OG), tert-butylhydroquinone (TBHQ), butylhydroxytoluene (BHT), and butylhydroxyanisole (BHA) (Figure 3) (Ungureanu *et al.,* 2024). BHT is less effective than BHA due to the two sterically hindered tert-butyl groups (Qamar *et al.,* 2021). BHA is very effective in controlling the oxidation of short-chain lipids (Kumar *et al.,* 2022). BHA and BHT are less effective in inhibiting ROS activity when compared to TBHQ (Chhikara *et al.,* 2022). This is due to the two para-hydroxyl groups responsible for the antioxidation activity of TBHQ (Rehman *et al.,* 2022). PG is known as a safe antioxidant because it can preserve oils and foods from spoilage caused by peroxide formation (Mohammed *et al.,* 2024). In addition, PG stabilizes foods and cosmetics (Baliyan *et al.,* 2022). OG can be defined as a food preservative composed of gallic acid and 1-octanol ester (Krishnan *et al.,* 2024). BHT and BHA are very commonly used antioxidants in the diet industry (Canale *et al.,* 2023).

2.3 Methods of Determining Antioxidant activity

Several studies have been performed recently on the oxidation process of free radicals and the general mechanism of action of antioxidants (Jomova *et al.,* 2023). This is because free radicals, although neutral, have a significant effect on the biological system (Forman and Zhang, 2021). In fact, some lipid derivative components, such as aldehyde, which can occur naturally during food processing and have adverse effects on human health, can easily occur as a result of the heat treatment of foods (Gülçin *et al.,* 2022). However, there are many antioxidant tests that directly measure the transfer of H atoms or electrons from antioxidants to free radicals (Kedare and Singh, 2021). The methods for measuring the activities of antioxidants have recently made remarkable progress (Kiran and Pundir, 2023). Early methods measure the effectiveness of antioxidants on the formation of certain types of oxidation products and therefore rely on measuring lipid peroxidation (Kothari *et al.,* 2022). So far, different chemical methods have been used for the evaluation of antioxidant activity by specific methods, combining highly automated and sensitive detection technologies, such as removal activity against several types of ROS or free radicals, reducing potency and metal chelation, and others (Saini *et al.,* 2022). The concept of antioxidant capacity first emerged as a chemical concept, and later it was adapted to fields such as medicine, biology, food, and epidemiology (Ungureanu *et al.,* 2024). It is very important to know the antioxidant profiles of these products in order to avoid loss of commercial and nutritional value during processing and preservation of foods and pharmaceutical products (Qamar *et al.,* 2021). Therefore, determining the potential antioxidant capacity of foods and pharmaceutical products requires the development of a fast and simple method (Kumar *et al.,* 2022). Today, many different antioxidant procedures have been developed and used effectively (Chhikara *et al.,* 2022). In this context, the most commonly used methods are inhibition of autoxidation of emulsions of linoleic acid, the β-carotene bleaching method, total radical-trapping antioxidant parameter (TRAP) and oxygen radical absorbance capacity (ORAC) analyses, ferric (Fe3+) and cupric (Cu2+) ions reduction assays, DPPH·, N,N-dimethyl-p-phenylenediamine radicals (DMPD·+), 2,2-azinobis 3-ethylbenzthiazoline-6-sulfonic acid radicals (ABTS·+), superoxide anion radicals (O2·−) removal experiments, and metal chelation tests (Rehman *et al.,* 2022). As is known, most of these methods use similar principles and techniques (Mohammed *et al.,* 2024). Measuring the ability of these antioxidant techniques is based on a suitable standard spectrophotometer measurement (Baliyan *et al.,* 2022). Antioxidant ability should not be tested with a single method; at least three different in vitro antioxidant methods must be performed together to determine antioxidant activity (Krishnan *et al.,* 2024). A pure-only method does not reflect antioxidant activity (Canale *et al.,* 2023). Given these, it is quite difficult to compare one method with another (Jomova *et al.,* 2023). Therefore, the methods to be used in analysis for research purposes should be carefully selected and applied (Forman and Zhang, 2021). Additionally, one of the most important objectives of this review is to detail the chemistry, mechanism, and application of the DPPH radical scavenging assay after giving some basic information about the antioxidant methods used to evaluate antioxidant properties (Gülçin *et al.,* 2022). In recent years, researchers have focused on the DPPH radical scavenging method (Kedare and Singh, 2021). Despite the antioxidant defense mechanisms found in living things, especially humans, cell damage accelerates the aging process and plays an important role in the development of diseases (Kiran and Pundir, 2023). Tissue damage may occur because of the oxidative modification of biological macromolecules such as lipids, proteins, and DNA (Kothari *et al.,* 2022). To understand and prevent these events, radical chain reactions in metabolism should be well understood (Saini *et al.,* 2022). Radical chain reactions are common mechanisms of lipid autoxidation and peroxidation (Ungureanu *et al.,* 2024). Radical scavenging agents can scavenge peroxide radicals to terminate radical chain reactions and improve the stability and quality of food products (Qamar *et al.,* 2021). The radical-scavenging properties of antioxidants are the most important lipid oxidation inhibition mechanism (Kumar *et al.,* 2022).

This method is an indispensable and standard test in antioxidant activity determination studies (Chhikara *et al.,* 2022). Radical scavenging-based methods such as DPPH·, DMPD+, ABTS+, and O2− are the most popular and putative spectrophotometric assays used for the determination of antioxidant activities of beverages, foods, and vegetable and fruit extracts (Rehman *et al.,* 2022). These chromogen radicals can react directly with antioxidant compounds (Mohammed *et al.,* 2024). These assays are also commonly used because they are sensitive, simple, fast, and reproducible (Baliyan *et al.,* 2022).

2.3.1 DPPH Radical Scavenging Assays

Radical chain reactions serve as a common mechanism for lipid peroxidation (Krishnan *et al.,* 2024). Radical scavengers increase the stability and quality of food products by ending peroxidation chain reactions (Canale *et al.,* 2023). For this purpose, radical scavenger molecules interact directly with peroxide radicals and scavenge them quickly (Jomova *et al.,* 2023). Free radical scavenging has a known mechanism where antioxidants directly inhibit lipid peroxidation (Forman and Zhang, 2021). This method is a standard, most widely used, and very fast and practical technique in antioxidant activity studies (Gülçin *et al.,* 2022). Radical removal activity has great importance due to the hazardous effects of free radicals in foods and pharmaceutical systems (Kedare and Singh, 2021). Many assays are used for the evaluation of the antioxidant activity of herbal extracts or phenolics (Kiran and Pundir, 2023). Different radicals and methods are used for antioxidant analyses and the determination of the final product of oxidation (Kothari *et al.,* 2022). ABTS+, DPPH·, DMPD+, or O2− radical removal methods are the most commonly used spectrophotometric methods for this purpose (Saini *et al.,* 2022). When antioxidants are added to these radicals, color removal occurs with a mechanism that reverses the formation of

DPPH·, ABTS+, and DMPD+ cations (Ungureanu *et al.,* 2024). $\mathrm{DPPH}\bullet+\mathrm{AH}\rightarrow\mathrm{DPPH2}+\mathrm{A}\bullet\mathrm{ABTS}\bullet{+}{+}\mathrm{AH}\rightarrow\mathrm{ABTS}{+}{+}\mathrm{A}\bullet\mathrm{DMPD}\bullet{+}{+}\mathrm{AH}\rightarrow\mathrm{DMPD}{+}{+}\mathrm{A}\bullet$

These three radical scavenging methods are extremely fast, requiring no expensive reagents or sophisticated instruments (Qamar *et al.,* 2021). Preparing and analyzing a sample takes half an hour and requires very little labor (Kumar *et al.,* 2022). These methods, which have high sensitivity, are very easy to use (Chhikara *et al.,* 2022). The analysis of antioxidant activity in many samples can be performed quickly and spontaneously (Rehman *et al.,* 2022). 2.3.2 Scope of DPPH Radical Scavenging Applications DPPH radical scavenging is a popular spectrophotometric method that has a wide application area and is used for determining the antioxidant capacity of beverages, pure substances, foods, and herbal extracts (Mohammed *et al.,* 2024). This method is simple, sensitive, fast, and reproducible, making it the most convenient and common radical removal method for evaluating the antioxidant capacity of compounds and herbal extracts (Baliyan *et al.,* 2022).

2.4 Cotton seeds

Figure 2.3: Cotton seeds (Krishnan *et al.,* 2024).

Cotton, the *Gossypium* genus in the tribe Gossypiae, in the family Malvaceae, can be generally divided into two types: cultivated and wild cotton (Canale *et al.,* 2023). Of 50 known species, only four (4) are cultivated, with the remaining 46 growing wild in the tropics and sub-tropics (Jomova *et al.,* 2023). The four common cultivated cotton species are *G. hirsutum*, *G. herbaceum*, *G. barbadense*, and *G. arboreum* (Forman and Zhang, 2021). These species vary in terms of fibre quality defined by length, maturity, strength, and micronaire (cell wall thickness) of the fibre (Gülçin *et al.,* 2022). The differences in fibre quality, yield, and adaptation to certain climatic conditions, has contributed to the preference of some cotton species over others (Kedare and Singh, 2021). All four cultivated cotton species are used for other purposes, including food production and medicinal application (Kiran and Pundir, 2023). *G. hirsutum*, sometimes referred to as "upland, American or Mexican cotton", is the most cultivated of all cotton species (Kothari *et al.,* 2022). *G. hirsutum*, is widely grown in its transgenic form because of its high yield and adaptability to different environmental conditions, although high temperatures can result in sterility and boll shedding (Saini *et al.,* 2022). The other species are predominant in parts of Asia and Africa and are seldom cultivated outside these regions, due to their inability to adapt to different climatic conditions and poor yields (Ungureanu *et al.,* 2024). *G. hirsutum* has been manipulated to improve yield and there has been some effort made toward enhancing the other cultivated species using transgenic approaches (Qamar *et al.,* 2021).

2.4.1 Cotton Industry and Processing

When fully matured, cotton bolls are picked and transported for processing, leaving the remaining plant as field trash (Kumar *et al.,* 2022). During the refining process or ginning of the harvested cotton, impurities are removed from the cotton fibres and are recovered as a processing by-product (CGT) (Chhikara *et al.,* 2022). Moreover, cotton seed (Figure 2.3) is also processed to recover cotton seed oils and cotton seed meals (Rehman *et al.,* 2022). Cotton production generates three categories of waste products: (i) field trash (stems, flowers, leaves, and stalks); (ii) CGT (leaves, fibre, flowers, immature seeds, sticks and soil) and cotton seed meal (from which oil has been extracted) (Figure 2.4) (Mohammed *et al.,* 2024).

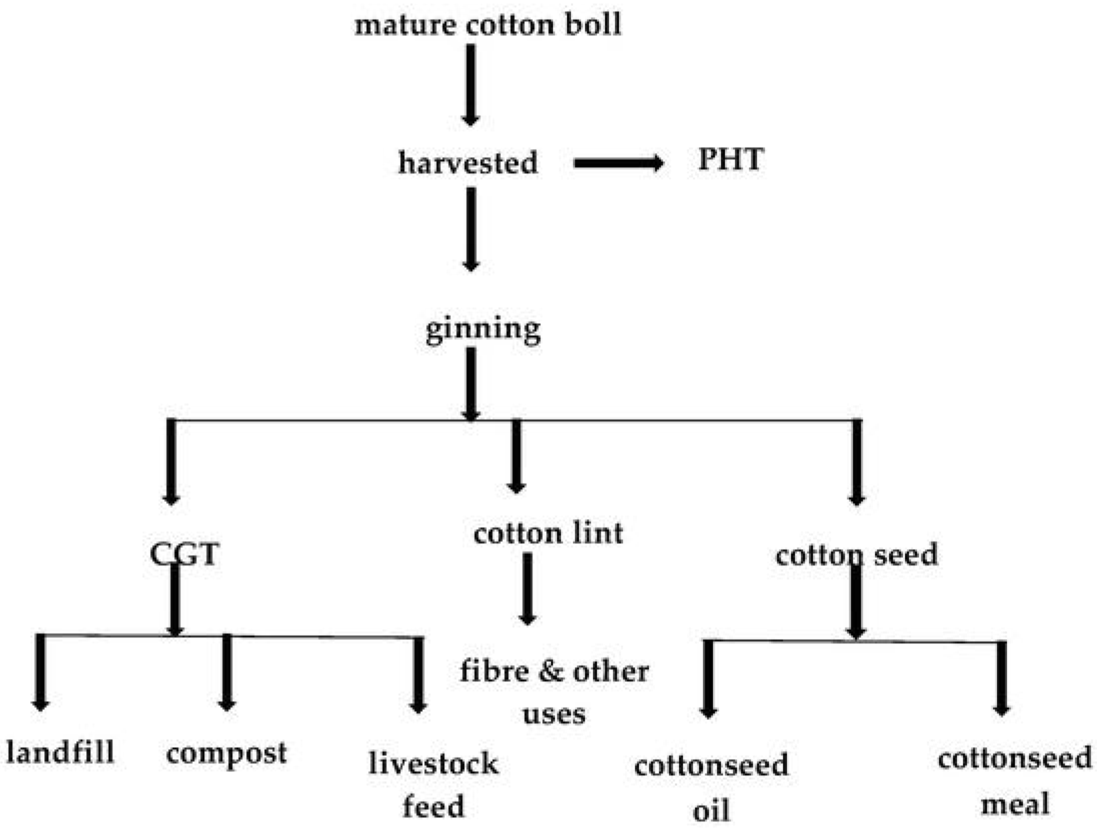

Figure 2.4: Flowchart of cotton processing from field to cotton gin products (Baliyan *et al.,* 2022).

2.4.2 Phytochemical Compounds in Cotton

Different compounds present in cotton play important roles during metabolism or interaction with the environment (Krishnan *et al.,* 2024). Naturally-occurring compounds in cotton include terpenes, phenols, proteins, carbohydrates, fatty acids, and lipids (Canale *et al.,* 2023). As with most plants, the distribution of these compounds vary between different parts of the cotton plant with some compounds concentrated in specific parts of the plant (Figure 2.5) (Jomova *et al.,* 2023). The distribution of these chemical compounds is related to their different properties and functionality in the plant (Forman and Zhang, 2021). The various compounds found in cotton plant will be discussed, highlighting the chemistry, as well as their distribution within the plant (Gülçin *et al.,* 2022).

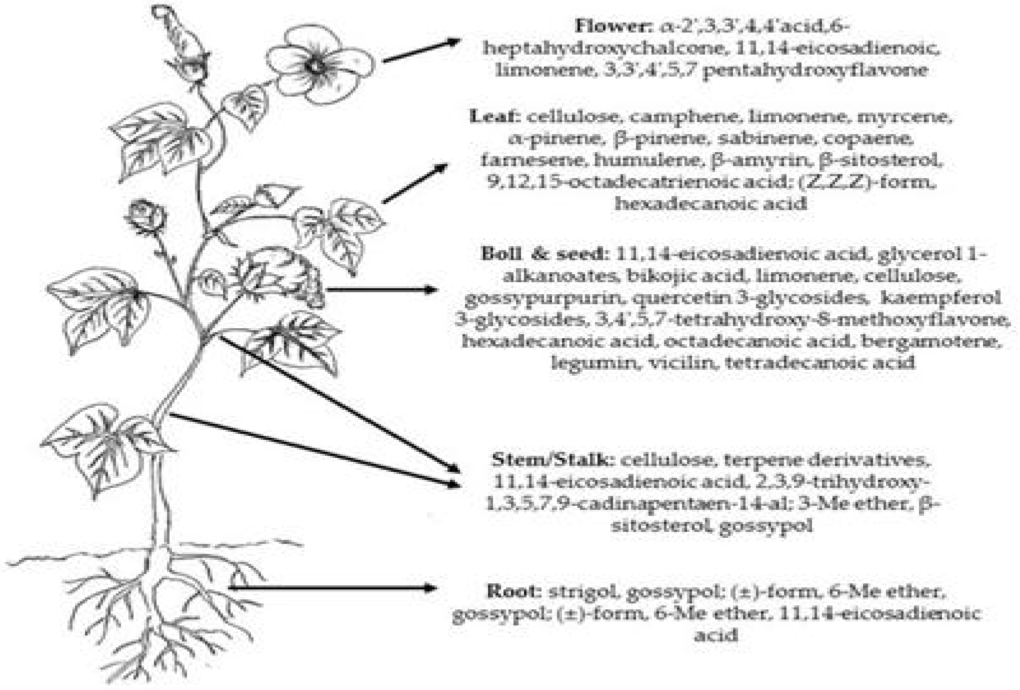

Figure 2.5: Distribution of common secondary metabolites in cotton plant (Kedare and Singh, 2021).

i. Terpenes

Like most plants, the cotton plant is susceptible to insect, herbivore, and pathogen attack (Kothari *et al.,* 2022). In a bid to ward off these predators, compounds are produced by the plant as a defense mechanism (Saini *et al.,* 2022). Terpenes are an important class of defense compounds synthesized in the cotton plant and are also the largest group of plant defense compounds (Ungureanu *et al.,* 2024). They are major constituents of essential oils found in most plants and, as such, have been applied in the food, chemical, and cosmetic industry (Qamar *et al.,* 2021). Terpenes are composed of units of a five-carbon compound, isoprene, linked together in a head to tail fashion, forming long chains or rings (Kumar *et al.,* 2022). They are classified into seven classes by the number of isoprene units they contain and include hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes (Chhikara *et al.,* 2022). Generally, hemiterpenes do not occur as free compounds but are bound to other non-terpene compounds, while terpenes modified by oxidation or a re-arrangement of the carbon skeleton are referred to as terpenoids (Rehman *et al.,* 2022).

ii. Phenols

Phenolic compounds are secondary metabolites found in most plants and normally comprise of one or more hydroxyl groups directly attached to one or more aromatic hydrocarbons (Mohammed *et al.,* 2024). Phenols occur in many lower and higher plants, medicinal plants/herbs, and dietary herbs, and their distribution is mainly governed by the physiological roles they play within the plant (Baliyan *et al.,* 2022). There are up to nine (9) groups of compounds classified as phenols, including phenolic acids, phenolic acid analogs, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, and quinones (Krishnan *et al.,* 2024). Despite the wide occurrence of phenols in higher plants, only phenolic acids, phenolic acid analogs, flavonoids, tannins, and coumarins have been reported to occur in cotton seeds (41 ppm), bracts (22.6 ppm), leaves (21.6 ppm), and roots (Canale *et al.,* 2023). Phenolic compounds are synthesized within the chloroplast of plant cells through a series of reactions which are preceded by the synthesis of aromatic amino acids tyrosine and phenylalanine via the shikimate-chorismate pathway (Jomova *et al.,* 2023). This pathway involves reactions between phosphoenol pyruvate (a by-product of glycolysis) and erythrose 4-phosphate (a by-product of the oxidative pentose phosphate pathway) (Forman and Zhang, 2021). These two aromatic amino acids, regarded as the major precursors in the synthesis of phenolic compounds, undergo a series of reactions via the phenylpropanoid pathway resulting in different classes of phenolic compounds (Gülçin *et al.,* 2022). Several other key enzymes are implicated in the synthesis of phenols from one class to another (Kedare and Singh, 2021).

iii. Flavonoids

Flavonoids are the most abundant class of phenolic compounds (Kiran and Pundir, 2023). Huang, Cai and Zhang reported that over 4000 flavonoids occur in nature while Cheynier suggested that the number is closer to 8000 (Kothari *et al.,* 2022). Flavonoids derive their name from the latin word “flavus” which means “yellow”, because of the prevalent yellow colour and are largely responsible for the colours of flowers, leaves, barks, fruits, and seeds of most plant species (Saini *et al.,* 2022). Flavonoids have a basic skeletal structure of phenyl benzopyrone (C6-C3-C6) comprised of two aromatic rings linked by three carbon atoms (Ungureanu *et al.,* 2024). Flavonoids occur as free compounds e.g., quercetin or as glycosides combined with different sugars e.g., kaempferol 3-glycosides, and quercetin 3-glycosides (Qamar *et al.,* 2021). There are several different classes of flavonoids such as the flavones, flavonols, isoflavones, aurones, anthocyanins, biflavonoids, flavanols, and flavanones (Kumar *et al.,* 2022). These flavonoids differ slightly in their chemical structures (Chhikara *et al.,* 2022). The flavonols possess hydroxyl side groups, which distinguishes them from the flavones (Rehman *et al.,* 2022). Isoflavones differ from flavones by the location of the phenyl group, whereas the anthocyanins differ from other flavonoids by possessing a positive charge (Mohammed *et al.,* 2024). Biflavonoids have a general formula of (C6-C3-C6)2 and aurones possess a chalcone-like group instead of the six-membered ring typical of flavonoids (Baliyan *et al.,* 2022). Several of these flavonoids have been identified in cotton including flavones, and flavonols which mostly occur as glycosides located in flowers, leaves, and seeds (Krishnan *et al.,* 2024). The most common flavonoids in cotton are glycosides of kaempferol, quercetin, and herbacetin (Canale *et al.,* 2023). Flavonoid glycosides are water- and ethanol-soluble, while free flavonoids are only soluble in organic solvents (Jomova *et al.,* 2023).

iv. Tannins and Coumarins

Tannins are a large class of poly phenolic water-soluble compounds which have molecular weights in the range of 500–4000 g/mol (Forman and Zhang, 2021). Plant tannins are divided into two classes, the hydrolysable tannins which derive their base unit from gallic acid, and condensed tannins, which arise from proanthocyanidins (condensed flavonols), as well as flavonoid and non-hydrolyzable tannins (Gülçin *et al.,* 2022). Condensed tannins are normally found in combination with alkaloids, polysaccharides, or proteins (Kedare and Singh, 2021). These are the class of tannins reported to occur in cotton and act as pesticides, protecting the cotton plant against predators (Kiran and Pundir, 2023). The coumarins are another group of phenolic acids isolated from cotton (Kothari *et al.,* 2022). Scopoletin, a coumarin derivative and its glycoside, scopolin presented in have been identified in cotton plant tissue confirming the report that coumarins occur in the free form and as glycosides in cotton, as well as other plants (Saini *et al.,* 2022).

v. Fatty Acids, Carbohydrates and Proteins

Fatty acids are carboxylic acids with long aliphatic chains that are synthesized in the cytosol of plant cells from malonyl-CoA, which in turn is derived from acetyl-CoA (Ungureanu *et al.,* 2024). Palmitic acid is a base fatty acid from which other fatty acids are formed by 2-carbon increments or reduction (Qamar *et al.,* 2021). The synthesis of palmitic acid from the precursor malonyl-CoA follows a five-step repeating cycle of acylation, condensation, reduction, dehydration, and reduction, which is catalyzed by the fatty acid synthase complex (Kumar *et al.,* 2022).

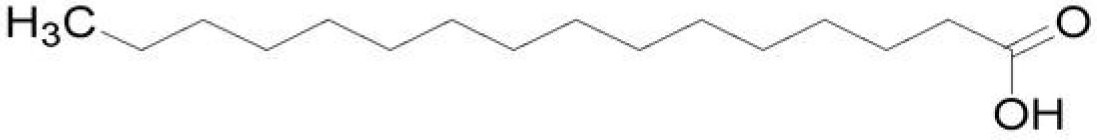

Figure 2.6: Palmitic acid, a base fatty acid from which other fatty acids are formed (Chhikara *et al.,* 2022).

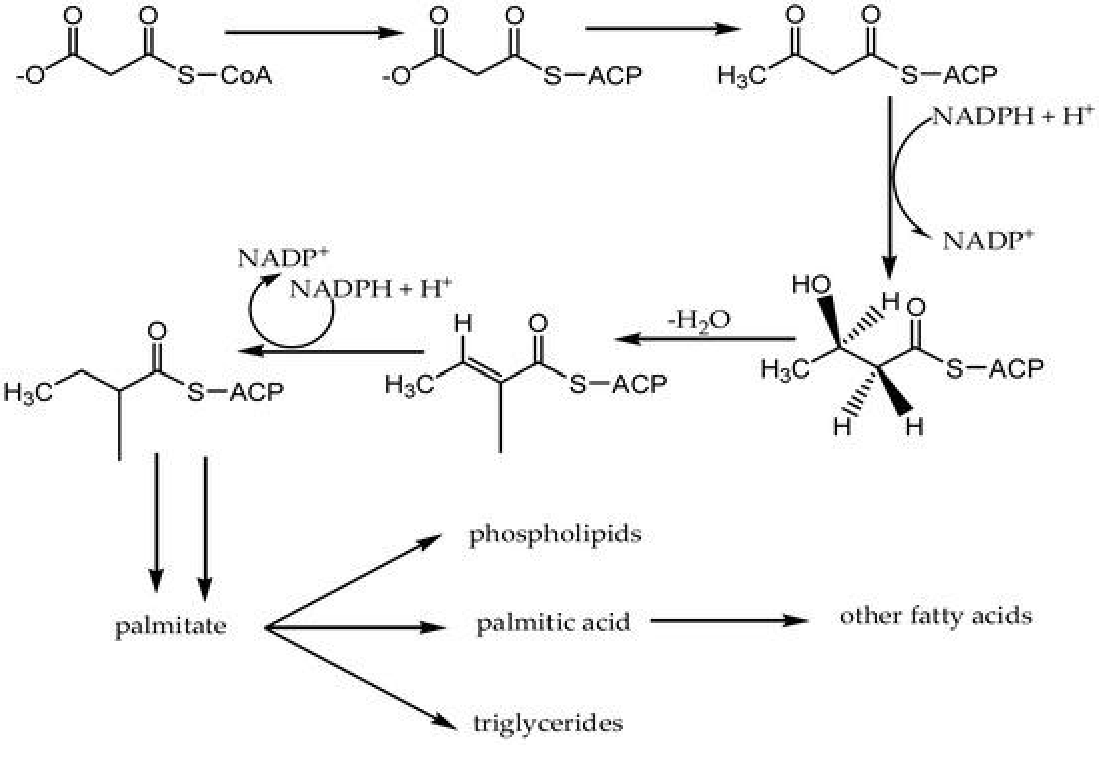

Figure 2.7: Fatty acid biosynthetic pathway in plants (Rehman *et al.,* 2022).

Saturated fatty acids which occur in the cotton plant include myristic acid (tetradecanoic acid), melissic acid (triacontanoic acid), palmitic acid (hexadecanoic acid), stearic acid (octadecanoic acid), and palmitoleic acid (9-hexadecanoic acid) (Mohammed *et al.,* 2024). Unsaturated fatty acids identified in cotton include eicosadienoic acid, linoleic acid (octadecadienoic acid), linolenic (octadecatrienoic acid), and elaidic acid (octadecenoic acid) (Baliyan *et al.,* 2022). Most fatty acids identified in cotton are free fatty acids (not linked to any molecules) and play functional roles as a source of energy for plant growth (Krishnan *et al.,* 2024). Cotton, like all plants, is comprised of cellulose and hemicelluloses, proportions of which vary between different parts of the plant (Canale *et al.,* 2023). Cotton fibre itself is comprised mainly of cellulose at levels greater than 94% by weight (Jomova *et al.,* 2023). Raffinose is a unique minor sugar found in cotton plants predominantly in the seed (Forman and Zhang, 2021). Alkali and water-soluble proteins are also found in cotton, including water soluble globular proteins vicilin and legumin present in the seeds of cotton (Gülçin *et al.,* 2022). Proline-rich protein H6 is involved in the development of the cell wall structure of cotton fibre (Kedare and Singh, 2021).

2.4.2 Pharmacological Properties of Compounds

In Cotton Several studies have emphasized the importance of plants to the pharmaceutical and medical industry (Kiran and Pundir, 2023). Cotton is described as a medicinal plant because of the chemical compounds that have been isolated from it (Kothari *et al.,* 2022). Several compounds found in cotton play pharmacological roles in nature including anti-microbial, anti-inflammatory, cytotoxic, anti-cancer, and contraceptive roles in both humans and animals (Saini *et al.,* 2022). Monoterpenes such as myrcene, pinene, camphene, limonene, and sabinene isolated from cotton possess anti- microbial, anti-inflammatory, anti-cancer, anti-oxidant, and gastro-protective properties (Ungureanu *et al.,* 2024).

i. Anti-Microbial Properties

In vitro and in vivo studies with compounds derived from cotton have found they elicit various effects in most experimental cells and animals (Qamar *et al.,* 2021). Monoterpenes such as pinene present in the leaves of cotton possess anti-microbial activity against fungi and bacteria (Kumar *et al.,* 2022). Concentrations as low as 5 µg/mL and 117 µg/mL were reported to have anti-microbial activity towards bacteria and fungi, respectively (Chhikara *et al.,* 2022). Only positive enantiomers of the compound induced this effect (Rehman *et al.,* 2022). The phenolic acid 4-hydroxybenzoic acid which has anti-microbial properties against gram positive and gram-negative bacteria at IC50 value of 160 µg/mL is another compound present in the leaves of cotton (Mohammed *et al.,* 2024). The degree of anti-microbial activity of these compounds varies across micro-organisms (Baliyan *et al.,* 2022). This was observed in fungal toxicity assays with 4-hydetroxybenzoic acid on Ganoderma boninense at concentrations as low as 0.5–2.5 µg/mL (Krishnan *et al.,* 2024).

ii. Anti-Inflammatory and Anti-Oxidant Properties

Chemical compounds, such as trans-caryophyllene, caryophyllene oxide, α-humulene, and β-amyrin, are compounds which exert different anti-inflammatory properties (Canale *et al.,* 2023). α-Humulene and trans-caryophyllene are reported to prevent chemical-induced paw oedema in rats with 50 mg/kg of both compounds inducing the same anti-inflammatory effects as 0.5 mg/kg of dexamethasone (a steroid anti-inflammatory medication) (Jomova *et al.,* 2023). At doses of 12 mg/kg and 25 mg/kg body weight of experimental mice, caryophyllene oxide induced anti-inflammatory and analgesic properties almost equivalent to that of an aspirin at a dose of 100 mg/kg body weight of the experimental animals (Forman and Zhang, 2021). In humans, studies using peripheral blood mononuclear cells (PMBCs), 1, 2, and 5 µg/mL of β-amyrin promoted the secretion of IL-6 cytokine which is actively involved in pro-inflammatory and anti-inflammatory immune responses (Gülçin *et al.,* 2022). Anti-oxidant properties of β-amyrin and farnesene from "in vitro" studies using human blood cells showed that doses as low as 1 µg/mL and 100 µg/mL, respectively, induced anti-oxidant activities in a time-dependent manner (Kedare and Singh, 2021).

iii. Cytotoxic and Contraceptive Properties

Cytotoxic activities associated with compounds isolated from cotton are mostly reported in relation to cancer cell lines (Kiran and Pundir, 2023). α-Bisabolol, a common compound present in cotton possesses the ability to induce apoptosis in malignant carcinoma cell lines without affecting the viability of healthy cells (Kothari *et al.,* 2022). A dose of 2 µM of α-bisabolol is reported to be effective against cancer cell lines, but an increase in dosage from 50 to 250 µM can induce cytotoxicity in normal cells (Saini *et al.,* 2022). Another sesquiterpene, caryophyllene oxide, also exhibits cytotoxic properties against cancer cell lines with a minimum dose of 3.125 µM resulting in reduction in viabilities of the target cells, with this effect more pronounced as the dosage increased (Ungureanu *et al.,* 2024). Gossypol is a major compound present in cottonseed oil and other parts of the cotton plant and has been found to have contraceptive properties in mammals (Qamar *et al.,* 2021). In human males, a concentration of 0.3 mg/kg of body weight can induce azoospermia in a time-dependent manner, whereas in male rats, a concentration of 30 mg/kg will induce the equivalent effect (Kumar *et al.,* 2022). The contraceptive property of gossypol is not restricted to males alone as a study by Randel, Chase, and Wyse indicated that this compound, if administered at a dose of 40 mg/kg body weight of female mammals, induces abnormal oestrous cycles and reduced pregnancy rates (Chhikara *et al.,* 2022).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus and Equipments

The apparatus or equipment used for this study were gotten from the Chemistry laboratory at the University of Benin, and were confirmed to be in good working condition before use. They include: Beakers, spectrophotometer (Jenway 6100, Dunmow, Essex, U.K), water bath(37°C), oven, analytical balance, filter paper (whatman No.1), mortar and pestle.

3.1.2 Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical grade. They include:

Foli-ciocalteu reagent, Sodium carbonate, Vanillian reagent, Ethanol, Sulphuric acid, Aluminum chloride, Sodium acetate, Methanol, Quercetin, Folin-Denis reagent, Tannic acid solution, Acetic acid, Ammonium hydroxide (conc), Ammonium solution.

3.2 Methods

3.2 Sample Collection and Preparation

3.2.1 Sample Collection

Cotton seeds (*Gossypium spp.*) were procured from a local agricultural supplier at New Benin Market, Benin City, Edo State.

3.2.2 Preparation of Cotton Seed Aqueous Extract

The dried cotton seeds were ground into a fine powder using a mortar and pestle. A 1.0 g sample of the powdered seed was weighed using an analytical balance and dissolved in 50 mL of cool, boiled-out distilled water in a 100 mL beaker. The mixture was then quantitatively transferred to a 100 mL volumetric flask. The beaker was rinsed three times with approximately 10 mL of the same distilled water, with the rinsings added to the volumetric flask to ensure complete transfer. The solution was then made up to the 100 mL mark with distilled water. The flask was stoppered and inverted several times for thorough mixing. This produced a stock aqueous extract with a final concentration of 10,000 µg/mL (10 mg/mL), which was set aside for the antioxidant assay.

3.3 Determination of DPPH Radical Scavenging Activity

The ability of the cotton seed extract to scavenge free radicals was determined using the stable DPPH radical method as described by Siripongvutikorn et al. (2024) and Musa et al. (2016), with slight modification.

3.3.1 Preparation of Sample Concentrations

From the 10,000 µg/mL aqueous stock extract, serial dilutions were made using distilled water to obtain various working concentrations for the assay (e.g., 250, 200, 150, 100, and 50 µg/mL). A similar set of concentrations was prepared for the standard antioxidant, ascorbic acid, by dissolving it in distilled water.

3.3.2 Assay Procedure

1. Into a set of clean, dry test tubes, 2.0 mL of each diluted sample concentration was pipetted.

2. A control tube was prepared containing 2.0 mL of distilled water instead of the plant extract.
3. To each test tube, 2.0 mL of a freshly prepared methanolic solution of DPPH was added.
4. The tubes were agitated gently to ensure proper mixing of the contents.
5. All test tubes were then incubated in the dark at room temperature for 30 minutes to allow the reaction between the antioxidants and the DPPH radical to proceed.
6. After the incubation period, the absorbance of each solution was measured at a wavelength of 518 nm using a UV-Vis Spectrophotometer against a methanol blank. The reduction in absorbance, indicated by a color change from deep violet to yellow, signifies the radical scavenging potential of the extract.

3.3.3 Calculation of Scavenging Activity

The percentage of DPPH radical scavenging activity for each concentration of the cotton seed extract and ascorbic acid was calculated using the following formula:

$$\boldsymbol{Scavenging\ activity}\ (\%) = \frac{(\boldsymbol{A_0 - A_1})}{\boldsymbol{A_0}} \times \mathbf{100}$$

Where:

- **A_0** is the absorbance of the control (containing distilled water and DPPH).
- **A_1** is the absorbance of the sample (containing the cotton seed extract or ascorbic acid and DPPH).

CHAPTER FOUR

4.0 RESULTS

4.1 DPPH Radical Scavenging Activity

The antioxidant potential of the cotton seed extract was quantified by its ability to neutralize the DPPH free radical. The results, showing the percentage of inhibition at various concentrations for both the extract and the ascorbic acid standard, are presented in Table 4.1.

Table 4.1: Comparative DPPH Radical Scavenging Activity of Cotton Seed Extract and Ascorbic Acid

Concentration (µg/mL)	Scavenging Activity of Cotton Seed Extract (%)	Scavenging Activity of Ascorbic Acid (%)
50	29.113	40.87
100	33.129	56.56
150	39.583	64.11
200	45.766	70.63
250	46.371	88.71

Table 4.1 displays the percentage of DPPH radical inhibition for the aqueous cotton seed extract and the ascorbic acid standard at concentrations ranging from 50 to 250 µg/mL. The values indicate the dose-dependent scavenging potential of each sample.

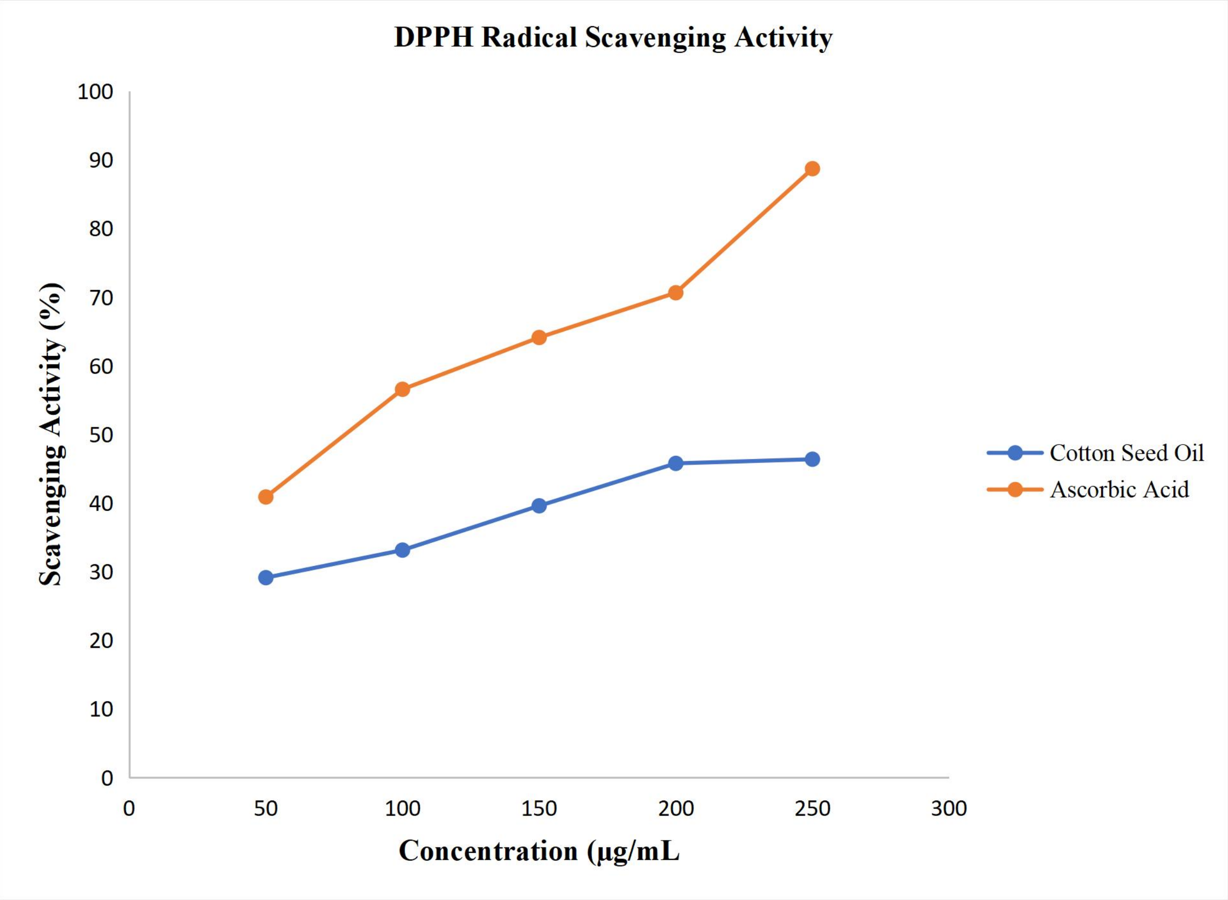

Figure 4.1 shows the comparative DPPH radical scavenging activities of the aqueous cotton seed extract and the ascorbic acid standard. Both samples exhibited a dose-dependent increase in activity, with ascorbic acid showing significantly greater potency across all concentrations.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The results of this study successfully demonstrate that the aqueous extract of cotton seed possesses notable antioxidant properties. The data presented in Table 4.1 and Figure 4.1 clearly show a dose-dependent relationship, where an increase in the extract's concentration from 50 µg/mL to 250 µg/mL resulted in a corresponding increase in DPPH radical inhibition from 29.11% to 46.37%. This trend is a fundamental characteristic of antioxidant activity and indicates the presence of bioactive compounds capable of donating hydrogen atoms or electrons to neutralize the DPPH free radical (Kedare and Singh, 2021).

The observed activity can be directly attributed to the phytochemicals present in cotton seeds. The literature review highlights that cotton seeds contain various secondary metabolites, including phenolic acids, flavonoids, and tannins (Canale *et al.,* 2023; Qamar *et al.,* 2021). These compounds, particularly those with hydroxyl groups on their aromatic rings, are well-known for their potent radical scavenging capabilities (Gülçin *et al.,* 2022). The quantitative measure of this activity, the IC_{50} value, was calculated from the linear regression analysis to be 288.88 µg/mL. This value serves as a benchmark for the extract's overall antioxidant potency.

To contextualize the potency of the cotton seed extract, its performance was compared against ascorbic acid, a universally recognized antioxidant standard. The results unequivocally show that ascorbic acid is a significantly more powerful scavenger of the DPPH radical. At every tested concentration, ascorbic acid exhibited much higher inhibition, culminating in 88.71% inhibition at 250 µg/mL.

This is further quantified by comparing their IC_{50} values. The IC_{50} of ascorbic acid was 86.12 µg/mL, which is more than three times lower than that of the cotton seed extract (288.88 µg/mL). A lower IC_{50} value signifies higher potency. This disparity is expected; ascorbic acid is a pure, highly efficient antioxidant molecule, whereas the cotton seed extract is a crude, unpurified mixture. The bulk of the extract's mass consists of proteins, carbohydrates, and lipids (Canale *et al.,* 2023), which do not contribute to radical scavenging. The antioxidant activity originates from a small fraction of bioactive phytochemicals. Therefore, the results should be interpreted not as the extract being weak, but as a demonstration of moderate antioxidant potential from a natural, complex source. This finding aligns with the aim of discovering effective natural antioxidants from plant sources to replace synthetic ones (Ungureanu *et al.,* 2024).

This study provides valuable insight into the under-explored antioxidant properties of cotton seed, a major by-product of the cotton industry. The findings scientifically validate the traditional medicinal applications of the cotton plant, which include antioxidant effects (Rehman *et al.,* 2022). By quantifying its DPPH scavenging ability, this research provides a basis for considering cotton seed extract as a potential source of natural antioxidants for the food, pharmaceutical, and nutraceutical industries (Canale *et al.,* 2023).

The valorization of agricultural by-products like cotton seed is of growing economic and environmental importance. Rather than being treated as low-value animal feed, the bioactive compounds within the seeds could be extracted and utilized as natural preservatives or healthpromoting supplements. This contributes to a more sustainable and circular economy for this globally significant crop.

5.2 CONCLUSION

This study was undertaken to evaluate the DPPH free radical scavenging activity of an aqueous extract of cotton (*Gossypium hirsutum*) seed. The research successfully demonstrated that the extract possesses moderate, dose-dependent antioxidant properties, with a calculated IC_{50} value of 288.88 µg/mL. While the extract was found to be less potent than the ascorbic acid standard (IC_{50} = 86.12 µg/mL), the findings confirm that cotton seed is a viable and under-explored source of natural antioxidant compounds. This provides a scientific foundation for its potential use in various health-related applications and for the value-added utilization of this important agricultural by-product.

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