TOTAL PHENOL AND FLAVINOID CONTENT OF DRIED SEEDS OF SOYA BEAN (*Glycine max*) AND COTTON SEEDS (*Gossypium spp*)

BY

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UNIVERSITY OF BENIN

NOVEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE, B.SC. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

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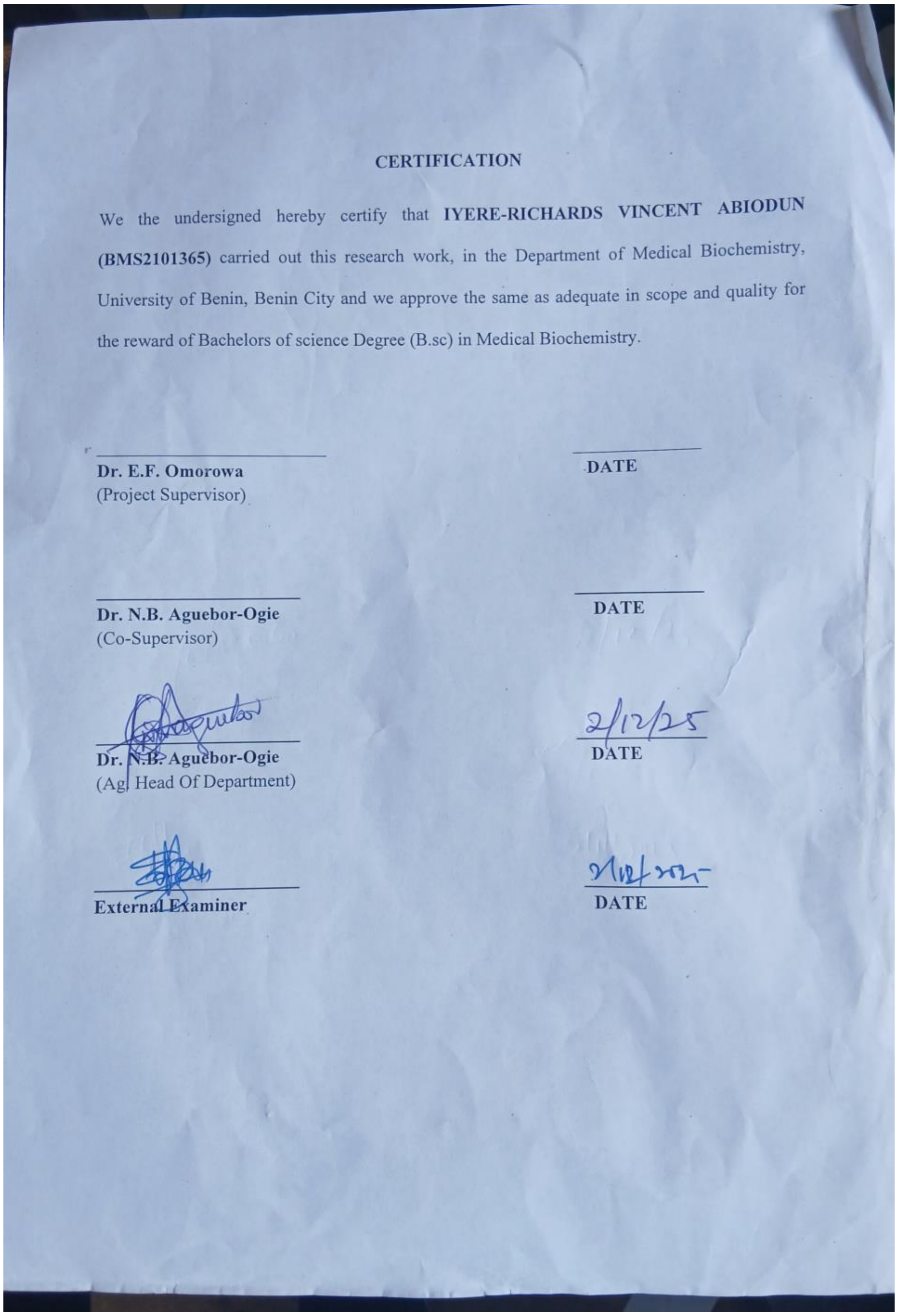
CERTIFICATION



CERTIFICATION

We the undersigned hereby certify that **IYERE-RICHARDS VINCENT ABIODUN (BMS2101365)** carried out this research work, in the Department of Medical Biochemistry, University of Benin, Benin City and we approve the same as adequate in scope and quality for the reward of Bachelors of science Degree (B.sc) in Medical Biochemistry.

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DEDICATION

This work is dedicated to the almighty God, and also to my parents, Mr. and Mrs. Iyere Ogbeiye.

ACKNOWLEDGEMENTS

It is with a grateful heart that I give all glory to God for the strength and grace through this academic journey. His guidance has helped me through my entire studies. I am grateful to him for existence, resilience, good health, protection and wisdom to scale through every obstacle.

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ABSTRACT

This study was carried out to investigate and compare the total phenolic and flavonoid content of dried seeds of Glycine max (soybean) and Gossypium spp. (cottonseed), recognising the growing interest in natural antioxidants and the limited comparative data available for these two commonly cultivated seeds. The aim of the study was to determine their antioxidant-related phytochemical composition and establish which seed possesses higher extractable phenolic and flavonoid levels. Quantitative data were generated from dried, ground seed samples extracted using methanol, and analysed using standard colorimetric procedures. Total phenolic content was determined using the Folin–Ciocalteu method while total flavonoid content was assessed with the Aluminium chloride assay, and absorbance readings were obtained spectrophotometrically. The resulting values were processed to obtain mean concentrations and standard error of mean for each parameter measured. The findings revealed that soybean contained notably higher total phenolic and flavonoid concentrations compared to cottonseed, indicating a stronger antioxidant potential and greater suitability for nutraceutical or functional food applications. Cottonseed, although lower in these constituents, still showed measurable levels, suggesting possible industrial utilisation after processing. In conclusion, the study successfully quantified and compared these bioactive compounds, demonstrating clear compositional differences and establishing a basis for further biochemical and application-focused research.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Wide range of chemical substances are produced by plants, which can be broadly categorized into main metabolites (such as proteins, carbohydrates, and lipids) and secondary (or specialized) metabolites. Because of their various functions in plant physiology, ecology, and human health, phenolic compounds and flavonoids are among the secondary metabolites of special interest (Dai *et al*., 2010; Zilic *et al*., 2013). Simple phenols, phenolic acids, tannins, lignins, stilbenes, and other more complex polyphenols are examples of phenolic compounds, often known as phenolics, which are characterized by the presence of one or more aromatic rings containing hydroxyl groups. Flavonoids, which include flavones, flavonols, flavanones, isoflavones, anthocyanins, and others, are a subclass of polyphenols distinguished structurally by a C6–C3–C6 backbone (Araujo and Leon-Bejarano, 2022).

These substances play important biological roles in plants, including protection against oxidative stress, pathogens, UV radiation, herbivory, and signaling molecules backbone (Araujo and Leon-Bejarano, 2022). They may affect dormancy, interactions with microbes, and the protection of the embryo in seeds. Phenolics and flavonoids are extensively researched for their antibacterial, anti-inflammatory, antioxidant, and other health-promoting qualities in both humans and animals. In fact, food science and nutraceutical studies frequently focus on phenolics obtained from plants backbone (Araujo and Leon-Bejarano, 2022; Prvulovic *et al*., 2016). A significant legume crop in the world, soybeans (Glycine max) are prized mostly for their oil and protein content. In addition to these macronutrients, soybean seeds are rich in flavonoids and phenolics, including isoflavones like genistein, glycitein, and daidzein (Malencic *et al*., 2013). As phytoestrogens and antioxidants, these isoflavones are gaining interest. Numerous studies have measured the total phenolic and flavonoid content of different soybean cultivars or processing methods and correlated them with antioxidant potential. Cotton (Gossypium spp.) is grown mostly for its fiber and seed oil, but its seeds are also utilized for bioactive component extraction, animal feed, and human consumption (in some situations). Although cottonseed residues, hulls, or extracts may include phenolic acids and flavonoid-like substances with antioxidant potential, the phenolic and flavonoid profiles of cottonseeds have received little attention (Malencic *et al*., 2013).

Studies comparing the phenolic and flavonoid content of various seed varieties, or between well-studied seeds like soybean and understudied ones like cottonseed, can provide valuable information. Examining dried seeds in particular (as they are usually treated and kept) guarantees relevance to practical applications (food, feed, extractive industries). Therefore, determining the total phenol and total flavonoid content of dried soybean and cotton seeds is useful from a scientific standpoint. It can help determine the relative potential of these seeds as sources of antioxidants, direct breeding or selection for genotypes with high phenolic content, and guide the use of frequently underutilized seed resources like cottonseed (Guo *et al*., 2012; Araujo and Leon-Bejarano, 2022).

1.2 STATEMENT OF PROBLEM

There are still a number of important gaps in our understanding of phenolics and flavonoids in soybeans, despite extensive research. Comparative lack of cottonseed data: The literature on the phenolic and flavonoid composition of cottonseed is far less extensive than that on the secondary metabolite profile of soybeans. This indicates that cottonseed's potential as a source of antioxidants or nutraceuticals has not been fully investigated. Variability in drying/storage, circumstances, and cultivars: Total phenolic and flavonoid levels in soybeans are known to differ according on the cultivar, post-harvest treatment, growth conditions, maturity stage, and geographic region. However, rather than dried seeds in storage conditions, many previous research have concentrated on fresh or processed forms (fermented forms, sprouts). As a result, it's unclear how consistent or representative the phenolic/flavonoid levels are in dried seeds when they're eaten or stored over time.

Absence of direct comparisons or a uniform methodology Cross-study comparisons are frequently complicated by variations in extraction solvents, assay techniques, and expression units (e.g., gallic acid equivalents, quercetin equivalents). For both seeds, a carefully planned direct comparison research using regulated procedures is required. Cottonseed's underutilization in value addition Cottonseed may be valued beyond its conventional use if it is demonstrated to contain a high phenolic/flavonoid content. However, this chance is lost because its potential is not well documented.

Link to antioxidant activity and practical significance: Without an understanding of the functional meaning (such as antioxidant activity) and comparative relevance of phenolics and flavonoids, just measuring them is less helpful. Although there is a correlation between several phenolic chemicals and the ability to scavenge radicals, the intensity and pattern of this correlation might vary depending on the type of seed. In practice, it is impossible to determine whether soybean or cottonseed is a superior source of phenolic/flavonoids without data. Therefore, the issue is that the total phenol and flavonoid content of dried soybean and cottonseed is still poorly described, especially when using the same procedures, and it is uncertain how promising these seeds are as sources of phenolics and flavonoids.

1.3 JUSTIFICATION OF STUDY

This research is justified on several grounds — academic, practical, and economic:

This study would close a knowledge gap in plant secondary metabolite profiling by offering n trustworthy comparison data on phenolic and flavonoid levels in soybean versus cottonseed (dry). The results could lead to more research on the distribution, production, genetic regulation, and particular phenolic/flavonoid chemicals found. In breeding projects intended to produce "functional seeds" with improved health or antioxidant qualities, characteristics that produce higher phenolic/flavonoid content may be selected for. Given the function of phenolics in plant defense, such selection may also aid in research on disease or stress resistance. Cottonseed is frequently underutilized or assigned to low-value uses (such as seed cake or feed).

Industries could invest in the extraction of bioactive chemicals from cottonseed if it is demonstrated to be a viable source of phenolics or flavonoids. This would increase revenue streams and decrease waste. Marketing seed-derived extracts or enhanced goods could provide smallholder farmers or seed processors with an added source of revenue. Knowing the total phenolic and flavonoid content provides information on the antioxidant properties of meals or supplements produced from seeds. Food science, the development of functional foods, and perhaps public health recommendations might all benefit from this. The findings may support cultivar-matched processing designed to conserve phenolics and flavonoids in soybeans, which are already widely consumed. Repurposing underutilized seed materials, such as cottonseed or seed by-products, can help reduce waste and promote more circular agricultural uses if it is demonstrated that they contain beneficial bioactives.

1.4 AIM OF STUDY

Determining and comparing the total phenol and flavonoid levels of dried soybean and cotton seeds, as well as connecting those results to antioxidant potential or seed utility, is the main goal of this study. When these objectives are met, you will be able to draw conclusions about the relative phytochemical potential of cottonseed and soybeans based on data and offer recommendations for use, breeding, or additional phytochemical research.

1.5 RESEARCH QUESTIONS

Aligned with these objectives, the study will seek to answer the following research questions:

1. What is the total phenol content of dried soybean seeds under the extraction and assay protocols used?
2. What is the total flavonoid content of dried soybean seeds under the same extraction and assay conditions?
3. What is the total phenol content of dried cotton seeds under comparable conditions?
4. What is the total flavonoid content of dried cotton seeds under comparable conditions?
5. How do the phenolic and flavonoid levels of soybean seeds compare statistically with those of cotton seeds?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Plants: Glycine max and Gossypium spp

2.1.1 General Overview of Glycine max

The legume crop soybean (Glycine max) is a member of the Fabaceae family, which is a part of the Fabales plant order (Caproti *et al*., 2014). In terms of production and consumption, soybeans are the most significant economically utilized legume crop in the feed and food industry, particularly in Asian nations. In other regions, such as North and South America, cultivation and food processing are also on the rise, as are industrial products (Luthria *et al.*, 2018). The seeds have a significant nutritional value due to their high protein content as well as other healthy macromolecules including fiber, vitamins, minerals, unsaturated fatty acids, and biologically active substances. Low raw material prices, large-scale farming, and lower water usage than other crops are only a few benefits of soybeans.

Furthermore, in vegetarian diets, soybeans are the main substitute protein source. Soybeans account for around 70% of all protein meals consumed globally. A total of 380 million metric tons of soybeans are produced by the three biggest soybean-producing nations—the USA, Brazil, and Argentina—with $15 billion in sales in the USA alone. 209 distinct soybean proteins, encompassing all required amino acids for human nutrition in a balanced way, were identified using an LC-MS/MS proteomics technique, making soybeans a complete protein supply (Caproti *et al.,* 2014). Due to its great quantity and variety of high-quality proteins—up to 400 g/p;kg soybean, or 36–56% of the total soybean content—and oil content of roughly 200 g/kg soybean, soybeans have become a significant and popular ingredient in food (Preece, 2017).

2.1.2 General Information On SoyBean (*Glycine max*).

Scientific name: *Glycine max* (L.) Merr.

Common name(s): Soybean, soya bean, soya

Family: Fabaceae (Legume/pea family)

Plant type: Non woody, annual.

Origin: East Asia (particularly China)

Planting month: Late May and early July.

Availability: Widely cultivated worldwide in both temperate and tropical zones.

2.1.3 Description

Height: 3 to 5 feet

Spread: 1 to 2 feet

Plant Habit: Erect, bushy herbaceous annual

Growth rate: Rapid under favorable conditions

Texture: Thin leaves, herbaceous stem

Leaf arrangement: Alternate

Leaf type: Compound, typically Trifoliate

Leaf margin: Entire

Leaf shape: Elliptic or Ovate.

Leaf Venation: Pinnate

Leaf persistence: Deciduous

Leaf blade length: 6 to 14 centimeters

Leaf color: Green

Fall color: Annual, hence less prominent

Seed type: Legume seed

Seed cotyledons: Dicotyledonous

Seed color: varies from yellow, green, brown, and black. Commercial soybeans are yellow with a brown hilum.

Seed texture: smooth

Seed dormancy: low dormancy

2.1.4 Botanical Description of *Glycine max*

The soybean is an annual plant. It first grows taproots, then a lot of secondary roots. Through the development of root nodules, roots and the bacteria (Bradyrhizobium japonicum) form a symbiotic connection. Simple main leaves, pinnately trifoliolate leaves, the seed (initial pair of simple cotyledon leaves; epigeal germination), and prophylls (a pair of 1-mm-long simple leaves at the base of each lateral branch) are the four types of leaves found in soybeans (Carlson, 2004). Stem termination is known to be regulated by two loci (Dt1 and Dt2).

In contrast to the indeterminate stem type (Dt1), which continues stem elongation and node production after flowering to produce a longer, more tapered main stem and branches, the determinate stem type (dt1) typically experiences little growth in stem length after flowering with blunt stem termination and a terminal raceme (Lersen, 2004). Within each of these two types, stem growth varies greatly, and stem morphology is significantly influenced by the timing of flowering and maturity. After vegetative growth, soybean plants move into the reproductive stage. Axilary buds grow into groups of two to thirty-five blooms. Between 20 and 80 percent of the flowers are abscised. The blooms that are generated first and last tend to abort the most frequently.

Figure 2.1: Soya Bean (*Glycine max*) Plant

(Source: Blalock, 2024)

The typical papilionaceous flower of soybeans has a five-parted corolla and a tubular calyx of five unequal sepals. A standard (posterior banner) petal, two lateral wings, and two anterior keel petals that touch but are not fused make up the corolla. To ensure self-pollination, stamens are grouped around the stigma. The ovary, style, and stigma make up the gynoecium. The ovary can produce up to four ovules. There are two whorls of nine stamens, with five stamens in the outer whorl and four in the inner whorl. On a staminal tube, the two whorls of nine stamens align to form a single whorl. Around the growing gynoecium, the larger, older stamens alternate with the smaller, younger stamens in a sequential manner. The stigma is covered in pollen. Pollen tubes enter the filiform apparatus after passing through style. Two sperm nuclei are released when the pollen tube tip bursts. While the second sperm connects with the secondary nucleus to generate an endosperm, the first sperm nucleus fuses with the egg to form a zygote. After fertilization, mature seeds need 30 to 50 days to develop. They have two big fleshy cotyledons, a hypocotyl-radicle axis, a micropyle, a hilum with a central fissure, a raphe, a plumule with two well-developed main leaves enclosing one trifoliolate leaf primordium, and no endosperm (Carlson and Lersten, 2004). Each soybean plant node's inflorescence can develop into one to more than twenty pods; a plant can have up to 400 pods.

Figure 2.2: Seeds of Soya Bean (*Glycine max*)

(Source: Maria, 2025)

2.1.5 Taxonomical Classification

The genus Glycine is a member of the tribe Phaseolae and the Subfamily Papilionoideae of the family Leguminosae/Fabaceae. Linnaeus first used the term "glycine" in the first edition of his book Genera Plantarum. There are 22 perennial wild species in the subgenus Glycine. *Glycine max (L.) Merrill*, its wild annual cousin *Glycine soja Sieb*, and *Zucc* are all included in the subgenus Soja. Furthermore, *Glycine gracilis*, a variety with intermediate morphological traits, is included in the Subgenus Soja. In between *Glycine soja* and *Glycine max* (Mishra, 2021).

The taxonomy of the soybean is as follows:

Division: Magnoliophyta (Flowering plants / Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Subclass: Rosidae

Order: Fabales

Family: Fabaceae (Leguminosae / Pea family)

Subfamily: Papilionoideae (Faboideae)

Genus: Glycine

Species: Glycine max (L.) Merr.

2.2 Medicinal and Non-medicinal uses of Glycine max

2.2.1 Medicinal uses of Glycine max.

The ISOs (Isoflavones) (Khan et al., 2010), particularly genistein, have been credited with the numerous health benefits of a soy diet. The use of soybeans is linked to immune system regulation, antioxidation, carcinogenesis inhibition, and cholesterol reduction (Montales *et al*., 2012).

Soybean's capacity to function as a detoxifier, anti-inflammatory, and source of blood plasma profile improvement are among its most significant therapeutic qualities. Different soybean components such as proteins, phytosterols, fiber, and polysaccharides play their role in the reduction of diseases, but the soy ISOs are chiefly associated with control of chronic diseases because of their antioxidant activities (Takahashi *et al*.,2005).

a. Prebiotic Effect

The presence of oligosaccharides in soybeans is another advantage; these oligosaccharides function as a prebiotic for intestinal microorganisms and increase the number of probiotics in the intestine. When fermented soymilk is used, the entire ecosystem of GI bacteria is improved (Cheng *et al.,* 2005). This increase in probiotics will improve the GI tract's barrier function and lessen the likelihood of a malfunctioning GI tract, which is mostly caused by bacterial invasion (Chow *et al*., 2002).

b. Control of Obesity

It has also been suggested that soybean phytoestrogens contribute to the prevention of obesity. The soybean's genistein can shrink the fat pads that contribute to obesity, but daidazin also has anti-obesity effects in obese rats (Szkudelska *et al*., 2007). Additionally, it was shown that soybeans play a role in controlling obesity; as a result, they can be incorporated into diets that limit energy intake (Naaz *et al*., 2003).

c. Management of Postmenopausal Problems

According to reports, soybeans can help treat postmenopausal diseases and menopausal issues like hot flashes, mood swings, and sleep disruptions. Women who have gone through menopause are susceptible to coronary artery disease (Welty *et al*., 2007). There are numerous more, non-hormonal impacts of phytoesterogens. One of these is a decrease in free circulating endogenous hormones due to an increase in the concentration of globulins, which bind sex hormones. More than 300 distinct plants contain phytoesterogens, which are polyphenolic nonsteroidal compounds similar to dietary estrogen (Ribotta *et al*., 2004).

d. Prevention of Diabetes

The brush-border cell at the intestinal surface membrane contains glucosidases, which are enzymes involved in the digestion of carbohydrates. In terms of their function as therapeutic agents for some diseases, particularly degenerative disorders, glucosidase inhibitors are crucial (Hirsh, 1997). Genistein has demonstrated its ability to inhibit α-glucosidases. The enzyme α-glucosidase, which is crucial for the digestion of carbohydrates as well as the processing of glycoproteins and glycolipids, is specifically inhibited by genistein. Numerous metabolic diseases, including diabetes, viral attachment, and the development of cancer, are also caused by this enzyme.

e. Lowering of Cholesterol

According to He *et al*. (2005), soybeans have a positive effect on the risk factors for cardiovascular diseases, such as lowering blood or liver triglycerides, lowering low density lipoprotein (LDL) levels, raising high density lipoprotein (HDL) levels and the ratio of both Lipoproteins cholesterol, and lowering blood pressure. It has been claimed that soy protein lowers intestinal absorption of cholesterol and plasma cholesterol concentrations. In animal models, soybean proteins have demonstrated a higher capacity to lower cholesterol, particularly LDL, than casein. Blood cholesterol is lowered by some of the soy proteins' indigestible portions.

f. Reduction of Carcinogenesis

Because soybeans contain phytoestrogens like genistein and daidzein, they are crucial in reducing carcinogenesis, particularly hormone-dependent cancer. The most significant and possibly anticarcinogenetic substances found in dietary soybeans are isoflavonoids, which primarily contribute to the prevention of breast cancer (Jian, 2009). In addition, soy saponins have been linked to a lower risk of colorectal cancer, especially in women (Park, 2004). The two most major ISOs in soybeans are genistein and daidzein, both of which significantly lower the incidence of several malignancies. Although genistein is primarily responsible for the anticancer action of soybeans, daidzein is thought to be a more bioavailable molecule (Tin *et al.,* 2007)

2.2.2 Non-medicinal uses

In addition to being a nutritional and therapeutic crop, soybeans (*Glycine max*) have drawn a lot of attention from around the world for their numerous non-medical uses in the industrial, environmental, and technological domains. According to recent studies, soybean oil and its derivatives are essential feedstocks for the production of biodiesel because of their favorable fatty acid profile and high triglyceride content, which facilitate effective transesterification into fatty acid methyl esters (FAMEs) that satisfy international biodiesel standards (Belachew *et al*., 2023; Sarwar *et al*., 2024). According to studies, biodiesel made from soybean oil is a potential renewable energy substitute for petroleum diesel because of its high combustion efficiency, low sulfur emissions, and biodegradability (Mohammadi-Moghaddam *et al*., 2025). Beyond biofuels, soybean oil is a renewable raw material used in the production of bioplastics, lubricants, coatings, and surfactants, where chemical modification processes like epoxidation, polymerization, and hydroxylation are used to create environmentally friendly alternatives to petrochemical-based materials (Zhao *et al*., 2022; Wang *et al*., 2023). Soy protein isolates and concentrates are used as functional ingredients in meat analogs, bakery products, and emulsifiers. Because of its high protein digestibility and balanced amino acid profile, soybean meal—a by-product of oil extraction—represents almost 70% of the worldwide protein feed market and is essential to the nutrition of cattle, poultry, and aquaculture (Belachew *et al*., 2023; Olafimihan, 2025).

2.3 Overview of the Plant

2.3.1 General Overview of Cotton (Gossypium spp)

The world's most significant natural textile fiber is derived from the fibrous seed coat trichomes of cotton (Gossypium spp.), a perennial shrub of the Malvaceae family that is grown as an annual crop all over the world. Of the more than fifty species in the genus Gossypium, four have been domesticated for the production of fiber and seeds: G. hirsutum, G. barbadense, G. arboreum, and G. herbaceum. While G. barbadense is valued for its long, fine fibers used in high-end textiles, G. hirsutum (upland cotton) dominates global output. According to contemporary genomic research, cotton is an allopolyploid plant that resulted from hybridization between ancestral A- and D-genome species. Cotton is thought to have been separately domesticated in both the Old and New Worlds about 5,000–6,000 years ago. According to botany, cotton plants have lobed leaves, yellowish blooms, and bolls, which are fiber-encased capsules that store seeds. Cotton is an essential industrial fiber and a renewable supply of biomaterials for biomedical and biotechnological applications because it is a single elongated epidermal cell that is mostly made of cellulose (85–90%), with trace amounts of proteins, waxes, and pectins. Beyond fiber, cottonseed is an important by-product that produces cottonseed oil, which is used extensively in foods, cosmetics, and pharmaceuticals, as well as cottonseed meal, a protein-rich feed component that was formerly restricted due to the presence of gossypol, a polyphenolic aldehyde that is harmful to both humans and animals. Gossypium, or cotton, is a tropical shrub that is a member of the Malvaceae family (Tariq *et al*., 2018). The cotton plant features big, eye-catching flowers with five petals, mostly white or cream purple, as well as upright branching stems and alternating leaves. With enough moisture and healthy soil, cotton may reach a depth of 60 cm thanks to its tap root system. The fruit has three to five leathery valves and resembles a capsule. Cotton seeds have an ovoid form and are coated in long hair-like threads or fibers. Cotton seeds can weigh up to 80 milligrams. It has cuticles covering its hard seed coat. Only four of the 50 species of cotton that have been identified are grown worldwide; the other species are found growing wild in tropical and subtropical regions (Gotmare *et al*., 2000). Four common cultivated cotton species are Gossypium hirsutum, G. herbaceum, G. barbadense, and G. arboreum. The most sought-after part of the plant is the seed cotton, which is utilized as a raw material in a variety of industries, including textiles, edible oil, paper, and animal feed. In addition to proteins (Hu *et al.,* 2011; Essien *et al*., 2011). 10 M. A. Ali et al. Pharmaceuticals (Aluri *et al.,* 2008; Ezuruike and Prieto, 2014; Hegde *et al*., 2004)

2.3.2 General Information

Scientific name*: Gossypium spp.* (L.)

Common name: Cotton, Seed cotton, Lint cotton

Family: Malvaceae

Plant type: Shrub or small perennial herb

Origin: Species dependent

Planting month: May to July

Growth requirement: Well drained loamy soil

Availability: Globally cultivated

2.3.3 Description

Height: 3 to 6 feet

Spread: 3 to 5 feet

Plant habit: Erect, shrubby, bushy perennial.

Plant density: Moderate

Growth rate: Moderate

Texture: pubescent

Leaf arrangement: Alternate

Leaf margin: Entire or slightly serated

Leaf shape: Palmate or heart shaped

Leaf venation: Palmate

Leaf type and persistence: Deciduous

Leaf blade length: 4 to 6 inches

Leaf color: Dark to medium green

Fall color: Yellow-green to pale-brown

Fall characteristics: Leaf wither and fall off

Seed type: Dicotyledonous

Seed shape: Ovoid to oblong

Seed coat: Covered with short fuzz and long lint fibers

Seed color: brown, grey or black

2.3.4 Botanical description of *Gossypium spp* (cotton seed)

About 50 species of perennial shrubs and small trees make up the genus Gossypium L. (family Malvaceae), which is primarily found in tropical and subtropical regions. Of these, four species—*Gossypium hirsutum*, *Gossypium barbadense*, *Gossypium arboreum*, and *Gossypium herbaceum*—are grown for their natural fiber and other industrial uses. In terms of botany, Gossypium species are distinguished by a deep taproot system, upright, woody stems that can grow to a height of one to three meters when grown, and alternating, palmately lobed leaves with three to five (and occasionally seven) lobes that are covered in tiny trichomes that help prevent water loss (Wendel *et al.,* 2020; Hu *et al*., 2022). The plants have axillary, single flowers with an eye-catching epicalyx of three enormous bracts (together referred to as the involucel) encasing the flowering bud (Wang *et al*., 2023). The blooms are transitory, staying open for about one day before fading, and are usually yellow to cream with a purple patch at the base of the petals. The pistil is surrounded by the staminal column, a characteristic of the Malvaceae family, which contains many monadelphous stamens that dehisce longitudinally to release pollen (Wang *et al*., 2023; Tyagi *et al*., 2024). The fruit is a loculicidal capsule (boll) that, when fully grown, dehisces to reveal the cotton fibers, which are long epidermal trichomes that act as dispersal mechanisms for the seeds (Hu *et al*., 2022). Due to their better fiber yield and adaptability, the tetraploid species (G. hirsutum and G. barbadense) dominate the world's cotton production (Tyagi *et al.,* 2024; Fang *et al.,* 2023). In terms of morphology, the genus shows significant variety in boll size, pubescence density, and leaf morphology, which reflects widespread domestication and ecological adaption.

Figure 2.3*: Gossypium spp*. (Cotton plant)

(Source: Osaka, 2019)

2.3.5 Taxonomical Classification

According to its classification, Gossypium (cotton) belongs to the family Malvaceae, order Malvales, and kingdom Plantae. With more than 50 species, the genus Gossypium is a diversified group that is further divided into many allotetraploid species and eight diploid genomic groups (A-G and K). Gossypium spp., or cotton plants, are categorized taxonomically from kingdom to species as follows:

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants / Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Subclass: Dilleniidae

Order: Malvales

Family: Malvaceae (Mallow family)

Genus: Gossypium

Species (examples):

Gossypium hirsutum L. — Upland cotton (most widely cultivated species)

Gossypium barbadense L. — Egyptian or Sea Island cotton

Gossypium arboreum L. — Tree cotton (Asian cotton)

Gossypium herbaceum L. — Levant cotton (African cotton)

Scientific Name (general): Gossypium spp.

Common Name: Cotton

2.4 Properties of *Gossypium spp*

2.4.1 Physical Properties of *Gossypium spp*

The post-harvest handling, storage, oil extraction, and processing efficiency of dried cotton seeds (*Gossypium spp*.) depend heavily on their physical characteristics. Recent research has shed light on the size, density, moisture content, and mechanical behavior of these seeds. The moisture level of dried cotton seeds typically ranges from 6% to 10% (wet basis), which has a substantial impact on their bulk density, flowability, and storability (Srinivasan *et a*l., 2022; Kyei-Boahen *et al.,* 2023). Different species have different average dried seed dimensions. For example, G. hirsutum

Figure 2.4: Image of Nigerian Cotton seed (*Gossypium spp*.)

(Source: Tridge, 2025)

and G. barbadense have length, breadth, and thickness values of 8–11 mm, 5–8 mm, and 4–6 mm, respectively (Nawaz *et al*., 2021). The sphericity and aspect ratio of the seed are determined by these factors, which are crucial for the design of oil expellers, dryers, and dehullers. Depending on compaction and fiber adhesion on the seed coat, the bulk density of dried cotton seeds is between 450 and 620 kg/m³, although the actual density typically falls between 1.0 and 1.2 g/cm³ (Onwualu *et al.,* 2020). Aeration and heat transmission during drying processes are impacted by the porosity, which averages between 40 and 50% and is dependent on seed packing and fiber coverage (Zhang *et al.,* 2022). Cotton seeds have a rough surface texture because of linters and fuzz, which raises the angle of repose (usually 35–40°) and affects the flow characteristics in processing machinery (Elbashir *et al*., 2021).

2.4.2 Chemical Properties of *Gossypium spp*

The commercial utility of dried cotton seeds (*Gossypium spp*.) for oil extraction, animal feed formulation, and the production of bio-based products is determined by their chemical characteristics; current research has provided comprehensive compositional insights. With slight differences owing to species, growing conditions, and processing techniques, dried cotton seeds normally contain 18–24% oil, 20–25% protein, 20–30% carbs, and 15–20% crude fiber (Singh *et al.,* 2023; Fang *et al*., 2023). The unsaturated fatty acids that make up the majority of the oil fraction—linoleic acid (50–55%), oleic acid (20–25%), palmitic acid (20%), and stearic acid (3–5%)—contribute to its oxidative stability and usefulness for both industrial and edible applications (Hu *et al.,* 2022; Zhao *et al.,* 2021). Tocopherols, sterols, and phospholipids—compounds that improve oxidative resistance but may be impacted by heat or extended storage—are abundant in dried cotton seed oil (Wang *et al*., 2023). Cottonseed meal has a high nutritional value due to its high protein content, which is mostly made up of globulins (60%) and albumins (25%). However, the presence of gossypol, a toxic polyphenolic aldehyde, limits its direct usage in feed (Singh *et al*., 2023). Depending on the species and processing, the free gossypol concentration of dried seeds usually varies from 0.3% to 1.2%; G. barbadense types often have lower gossypol levels than G. hirsutum (Hu *et al*., 2022; Zhang *et al*., 2022). To lower gossypol and increase protein bioavailability, detoxification techniques such heat treatment, solvent extraction, and microbial fermentation are frequently used Minerals including potassium, phosphorus, calcium, and magnesium, which are necessary for feed and fertilizer application, make up the majority of the ash content of dried seeds, which varies from 3% to 5% (Onwualu *et al.,* 2020).

2.5 Uses of the Plant (Medicinal and Non-medicinal Uses)

2.5.1 Medicinal Uses of *Gosssypium spp*

a. Wound healing

Vegetable oils' antioxidant and anti-inflammatory properties make them promising for wound healing. They repair the functions of the skin's lipid barrier, rebuild dermal tissues, and encourage the growth of healthy cells. CS-O's high vitamin E content acts as an antioxidant and offers numerous skin benefits, such as quicker wound healing. Additionally, psoriasis, skin ulcers, and other skin diseases and conditions have been successfully treated with vitamin E. Significant amounts of linoleic acid found in CS-O are thought to have an important role in the healing process of wounds, according to little data (El-Mallah *et al*., 2011; Isaac and Ekpa, 2013).

b. Therapeutic role in cardiovascular diseases

Due to greater public awareness of cholesterol and saturated fats, there is a noticeable decline in the consumption of animal fats like butter and lard and a rise in the consumption of vegetable oils (Senger *et al.,* 2017). The fact that CS-O is cholesterol-free, has a high linoleic acid content, and contains a significant amount of polyunsaturated fatty acids are significant characteristics. Saturated fat is thought to increase the risk of CVD via raising LDL-cholesterol (LDL-C) levels. Among the few seed oils that still have the ability to reduce consumption of saturated fat, CS-O is regarded as a very nutritious and healthful plant-based oil (Mahesar *et al*., 2017).

c. Anti-inflammatory properties

The word "inflammation" comes from a Latin syllable that describes a complicated biotic response of human tissues to a variety of damaging stimuli, including pathogenic invaders (viruses or bacteria), damaged cells, and different irritants, poisons, or dangerous insects. It is a defensive reaction involving blood vessels, immune system cells, or molecular mediators. The deadliest enemy of the human body, inflammation causes breakouts, redness, dandruff, and other pertinent issues. Terpenes, various phenols, and fatty acids—particularly linoleic acid—found in CS-O have anti-inflammatory properties and can prevent inflammation (Mueller, 2008).

d. Anti-oxidant potential

Reactive oxygen species (ROS) can be produced by a number of extrinsic (such as UV radiation) or mitochondrial respiration variables (Pillai *et al.,* 2005; Turrens, 2003). These radicals are known to contribute to a number of deadly health issues, such as cancer (Waris and Ahsan, 2006) and cardiovascular illnesses (Sugamura and Keaney, 2011). Therefore, the primary focus of many countries is finding novel antioxidant compounds that might potentially reduce the potentially fatal consequences of ROS, and CS is crucial in replacing this ROS (Gao *et al.,* 2010).

2.5.2 Non-medical Uses of *Gossypium spp*

Seed, a useful byproduct obtained during the manufacturing of fiber, makes up more than half of cotton (cotton seed). One ton of cotton seed can make up one-third of the seed coat, and after pressing, half of the seed can even be utilized as animal feed. Additionally, about 20% of the oil can be collected from the seed (Cotton Australia 2018). Half a billion people and billions of animals worldwide get their protein from cotton seeds. Cotton seed is most frequently used to make cooking oil and to feed cattle. Cotton seed is crushed or pressed to produce cotton seed oil, which has a variety of uses ranging from residential to commercial. Cleaning is the first step in the processing of cotton seeds, and this process continues until medicinal or industrially significant chemicals like Gossypol are extracted. Among the cleaning techniques to obtain fine cotton necessary for producing high-quality paper are the removal of dirt, inert matter, plant debris, and short fine fibers. In theory, paper made of both long and small staple fibers is more resilient to handling errors. Certain cotton and linen blends determine the value of even printing currency. For example, dollar notes made from a blend of ¼ linen and ¾ cotton cost 9.6 cents each (Kavilanz, 2011). Cotton seed is frequently fed to cattle and other animals and can be cooked as a meal. Seed oil can also be used in making of various industrial goods, i.e., margarine, soap, rubber, candles, emulsifiers, medications, cosmetics, waterproofing, etc. The fact that cotton seed oil is cholesterol-free, high in polyunsaturated fats, and rich in antioxidants like vitamin E prolongs the shelf life of pigs, rabbits, and chickens, among other animals.

2.6 Secondary Metabolites and Phytochemicals

Although they are not directly involved in main metabolic activities like growth or reproduction, secondary metabolites are a broad class of biologically active molecules produced by plants that are essential for defense, adaptation, and ecological interactions. They can be roughly classified into phenolic chemicals, alkaloids, terpenoids, flavonoids, saponins, tannins, and glycosides, each of which makes a distinct contribution to the biochemical defense mechanisms of the plant and the health advantages it offers to humans (Shahidi and Ambigaipalan, 2018; Wink, 2020). These metabolites' antioxidant, antibacterial, anti-inflammatory, and anticancer qualities make them essential to phytochemical study. Phenolics and flavonoids are examples of phytochemicals that chelate pro-oxidant metals, reduce reactive oxygen species (ROS), and alter redox-related enzymes including glutathione peroxidase and superoxide dismutase (Li *et al*., 2022). Secondary metabolites are essential in the field of medical biochemistry for comprehending the mechanisms underlying disease prevention as well as for creating nutraceuticals and medicinal substances made from plant-based sources.

Numerous kinds of secondary metabolites have been thoroughly studied in dried cotton seeds (Gossypium spp.) and soybean (Glycine max), highlighting their nutritional and medicinal potential. In addition to the polyphenolic compound gossypol, which has strong antioxidant and antimicrobial qualities but needs to be detoxified before being consumed, cotton seeds are especially rich in phenolic acids (gallic, caffeic, ferulic, and p-coumaric acids), flavonoids (quercetin, kaempferol, and catechins), and terpenoids (Hu *et al.,* 2022; Fang *et al*., 2023).

Cottonseed's antioxidant potential and resistance to oxidative stress during drying and storage are also enhanced by tannins and lignans.

2.7 Phenols and Flavonoids as major antioxidant

Among the most important antioxidant phytochemicals found in plants are phenols and flavonoids, which are essential for human disease prevention, redox control, and free radical scavenging. These substances, which are generally categorized as polyphenolic secondary metabolites, have hydroxyl groups that can donate hydrogen atoms or electrons to counteract reactive oxygen species (ROS) such hydroxyl radicals and superoxide anions (Shahidi and Ambigaipalan, 2018). In terms of structure, phenolics comprise complex derivatives like tannins, lignins, and flavonoids as well as simple phenolic acids like gallic, ferulic, and caffeic acids (Li *et al.*, 2022). Subclasses of flavonoids include flavones, flavonols, isoflavones, and anthocyanidins. These substances function biochemically by regulating oxidative enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), thereby preserving cellular redox balance. Through mechanisms involving lipid peroxidation inhibition, metal ion chelation, and DNA protection, numerous studies have connected increased consumption of phenolic and flavonoids to lower risks of chronic diseases, such as cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases (Wink, 2020; Ahmad *et al.*, 2023). Additionally, by modifying the NF-κB, Nrf2, and MAPK pathways, phenolics affect cellular signaling pathways, resulting in cytoprotective, anti-inflammatory, and anti-aging properties (Li *et al.*, 2022). Since oxidative stress is a major factor in the genesis of the majority of metabolic and degenerative disorders, these antioxidant processes have significant ramifications for medical biochemistry. A crucial biochemical method for evaluating the antioxidant potential of plant-derived food sources is the measurement of total phenolic content (TPC) and total flavonoid content (TFC) using Folin–Ciocalteu and aluminum chloride assays, respectively (Deng *et al.,* 2022).

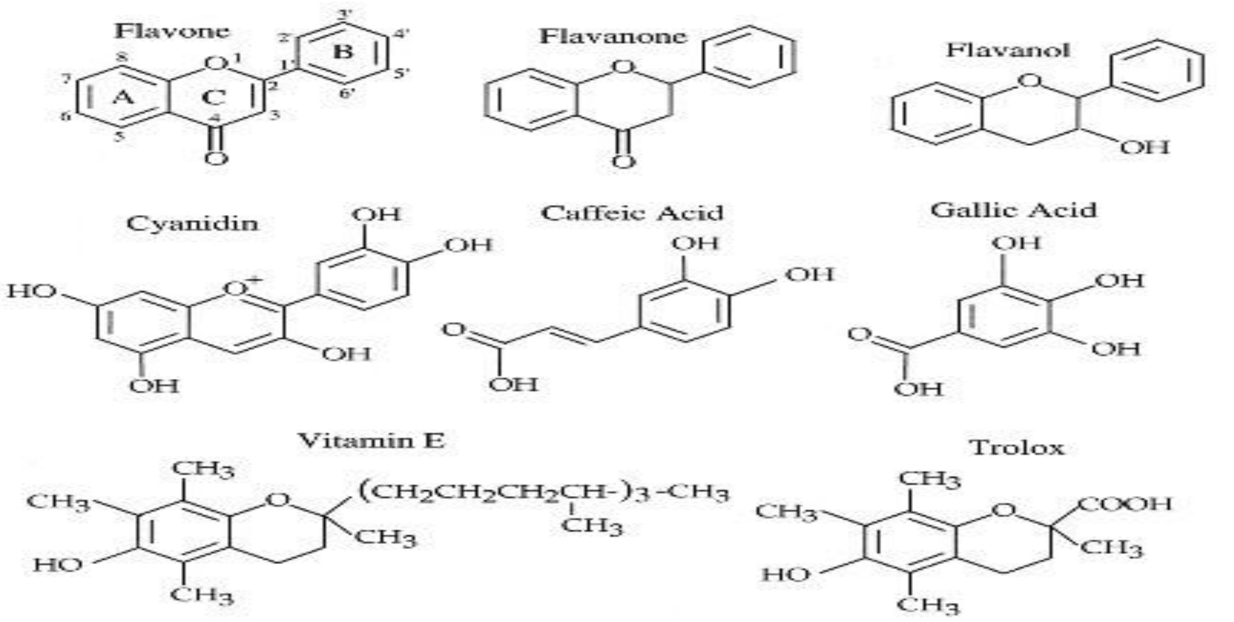

Figure 2.5: Structure of common phenol and flavonoids found in *Glycine max* and *Gossypium spp*.

(Source: Aaron, 2017)

Phenols and flavonoids play a significant role in the antioxidant capacity and health-promoting qualities of dried cotton seeds (Gossypium spp.) and soybean (Glycine max). Particularly when dried, cottonseed extracts' phenolic acids (gallic, ferulic, and p-coumaric) and flavonoids (quercetin, kaempferol, and catechin) work in concert to scavenge free radicals and improve oxidative stability (Hu *et al*., 2022; Singh *et al.,* 2023). By avoiding rancidity and peroxidation, these substances also aid in lipid protection in seeds that are stored, preserving the biological integrity of seed oils. Additionally, gossypol, a polyphenolic aldehyde exclusive to cottonseed, has strong antibacterial and antioxidant qualities, but its concentration needs to be regulated because of its slight cytotoxicity. (Fang *et al.,* 2023). The main flavonoids found in soybeans are isoflavones like genistein, daidzein, and glycitein. The phenolic and flavonoid content of both seeds is highly correlated with their high overall antioxidant potential. The retention of these compounds is greatly impacted by processing procedures, especially drying and solvent extraction; regulated low-temperature drying preserves more antioxidant components than high-temperature or sun-drying processes (Kyei-Boahen *et al*., 2023). Therefore, phenols and flavonoids from Gossypium spp. and Glycine max are of significant biomedical and nutraceutical importance because they not only improve the nutritional value of these seeds but also play essential roles in oxidative stress regulation, chronic disease prevention, and functional food formulation (Shahidi and Ambigaipalan, 2018; Li *et al*., 2022).

2.7.1 Methods for Detecting Total Phenol and Flavinoid in Dried Cotton Seeds (Gossypium spp) and Soya Bean (Glycine max)

In order to evaluate the antioxidant and nutraceutical potential of plant matrices such dried cotton seeds (*Gossypium spp*.) and soybeans (*Glycine max*), it is essential to detect and quantify the total phenol and flavonoid content. The Folin–Ciocalteu colorimetric assay, which relies on phenolic compounds reducing the phosphomolybdic-phosphotungstic reagent under alkaline conditions to produce a blue chromophore measurable spectrophotometrically at 760–765 nm, is the most popular technique for determining total phenolic content (TPC) (Shahidi and Ambigaipalan, 2018). Gallic acid equivalents (GAE) in milligrams per gram of material are commonly used to express the results. For effective extraction, certain solvent systems (ethanol-water or methanol-acetone combinations) are needed for phenolics like gossypol, gallic acid, and catechol derivatives in Gossypium spp. (Fang *et al*., 2023). Similarly, 80% aqueous methanol extraction improves Glycine max phenolic quantitation and produces high recovery rates of isoflavones (genistein, daidzein, and glycitein) (Li *et al*., 2022). Because it is selective for flavonoid hydroxyl groups, the aluminum chloride ($AlCl_3$) colorimetric method is still the gold standard for total flavonoid content (TFC). Flavonoids and $AlCl_3$ form stable acid complexes in this experiment, resulting in a yellow hue detected at 510–420 nm. The results are given as milligrams of quercetin or catechin equivalents (QE or CE) per gram (Li *et al.,* 20 22; Deng *et al.,* 2022). More sophisticated versions of this method use reaction kinetics modeling for increased sensitivity and microplate readers for high-throughput screening. Particle size, solvent polarity, temperature, and pH are extraction parameters that have a substantial impact on TFC findings in both Gossypium spp. and Glycine max (Singh *et al*., 2023).

Researchers have discovered that methanol and ethanol mixtures (60–80%) offer the best extraction of flavonoid glycosides such as rutin, kaempferol, and quercetin for dried cottonseed (Hu *et al*., 2022), whereas acidified ethanol extraction is recommended for effective isoflavone recovery in soybeans (Zhao *et al*., 2022). When combined, these techniques not only guarantee the accurate measurement of antioxidant phytochemicals in dried Gossypium and Glycine species, but they also lay the groundwork for establishing a connection between chemical composition and biological activity—a crucial aspect of functional food research and medical biochemistry.

2.7.2 Biochemical Relevance of Phenolic and Flavonoid Compounds

Both plant physiology and human health depend heavily on phenolic and flavonoid molecules, which are biochemically relevant secondary metabolites. They serve as UV protectors, antioxidants, antibacterial agents, and signaling molecules that improve stress tolerance in plants (Shahidi and Ambigaipalan, 2018). Their redox-modulating ability, which enables them to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) and preserve oxidative equilibrium at the cellular level, is their main biochemical significance in humans (Ahmad *et al*., 2023). Phenolic compounds have a high electron-donating capacity and are effective free radical quenchers due to the structural presence of one or more hydroxyl groups linked to aromatic rings. This antioxidant effect helps prevent diseases like atherosclerosis, cancer, diabetes, and neurological disorders that are linked to oxidative stress (Li *et al*., 2022).

A significant subgroup of polyphenols, flavonoids also show enzyme modulation activity, affecting the activity of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (Wink, 2020). These substances have the ability to affect important cellular pathways at the molecular level, such as NF-κB, Nrf2, and MAPK, which controls oxidative defense-related gene expression, inflammation, and apoptosis (Zhao *et al.,* 2022). Beyond their antioxidant properties, phenolic and flavonoid molecules have biochemical significance in hormone regulation, metabolic control, and cardiovascular protection. For example, isoflavones like genistein and daidzein, which are plentiful in soybeans, function as phytoestrogens by binding to estrogen receptors and providing protection against postmenopausal problems and hormone-dependent malignancies (Li *et al*., 2022; Deng *et al*., 2022).

2.7.3 Influence of Drying and Processing on Phytochemical Retention

The impact of processing and drying on phytochemical retention has been extensively studied in recent years, especially in light of the increasing interest in preserving the therapeutic and nutritional value of foods and medicines produced from plants. Phenolics, flavonoids, alkaloids, and carotenoids are examples of thermolabile phytochemicals whose stability is greatly impacted by processing variables such temperature, drying time, and drying technique (Ahmed *et al*., 2022). According to studies, heat-sensitive chemicals can be severely broken down by thermal processing techniques as oven drying and autoclaving by oxidation, enzyme deactivation, and polymerization (Falade and Igbeka, 2021). For instance, the high temperatures involved in sun or hot-air drying often lead to losses in total phenolic content (TPC) and flavonoid concentration due to the breakdown of conjugated structures and the release of bound phenolics that are subsequently oxidized (Larrauri *et al.,* 2020). Conversely, low-temperature drying techniques such as freeze-drying (lyophilization) and vacuum drying tend to preserve higher levels of phytochemicals by minimizing oxidative stress and enzymatic browning, thus maintaining antioxidant potential (Huang *et al*., 2021). Moreover, mechanical and pre-processing factors—such as particle size reduction, blanching, and solvent exposure—affect the diffusion and retention of phytoconstituents, underscoring the need for optimized processing conditions that balance microbial safety and phytochemical stability (Kaur and Kapoor, 2023). New technologies that have the potential to improve drying efficiency while preserving bioactive chemicals include microwave-assisted drying, infrared drying, and pulsed electric field processing (Moses *et al*., 2022). For high-value phytochemical-rich materials like soybeans, cotton seeds, and medicinal plants, these non-thermal or minimally heat methods minimize oxidative degradation and shorten processing times (Nair *et al*., 2022).

2.8 Empirical Review

Recent empirical research has highlighted the biochemical and antioxidant significance of the total phenolic and flavonoid content in dried cotton seeds (Gossypium spp.) and soybean (Glycine max). According to research by Singh *et al*., (2023), dried Gossypium hirsutum seeds have substantial antioxidant capacity due to the presence of phenolic acids including gallic, caffeic, and ferulic acids as well as prominent flavonoids like quercetin and kaempferol. According to Li *et al*., (2022), Glycine max has high total phenol content (TPC) values ranging from 3.5 to 7.8 mg GAE/g, depending on cultivar and drying conditions, while total flavonoid content (TFC) varied between 2.1 and 5.3 mg QE/g, demonstrating soybean's potent capacity to scavenge radicals. Because excessive heat degrades thermolabile phenolics and flavonoids, comparative analyses reveal that freeze-dried samples typically retain higher TPC and TFC than their oven- or sun-dried counterparts (Huang *et al.,* 2021). Additionally, there is a substantial correlation between the content of these phytochemicals in dried seeds and antioxidant activities such FRAP and DPPH tests, indicating that they play a crucial role in oxidative stability and nutraceutical quality (Zhao *et al*., 2022). Empirical studies also show that phytochemical accumulation is influenced by the processing and varietal differences between soybean and cottonseed. According to Anokwuru *et al.* (2022), recovery rates are greatly impacted by solvent extraction techniques (ethanol vs. methanol), with methanol extracts exhibiting larger quantities of phenolic and flavonoids.

The phytochemical profiles of Gossypium spp. seed coats and kernels differ, with the seed coat having more phenolics because of its lignin-associated chemicals (Moses *et al*., 2022). In the meantime, Deng *et al.,* (2022) and Tian *et al.,* (2022) demonstrated that soybeans high in isoflavones, particularly genistein, daidzein, and glycitein, have potent DNA-protective and lipid peroxidation-inhibiting properties. Furthermore, various drying processes, correlations between total phenolic content and antioxidant index ($R^2 > 0.85$) have been found, highlighting the quantitative link between phytochemical retention and antioxidant capability (Li *et al*., 2023). Together, these empirical results demonstrate that Gossypium spp. and Glycine max are rich sources of bioactive compounds whose concentration and functionality are significantly impacted by post-harvest drying and extraction techniques, offering important information for pharmacognostic, biochemical, and nutraceutical applications.

2.9 Research Gap

Although phenolic and flavonoid compounds have been widely reported in Glycine max and Gossypium spp., comparative datasets focusing on dried seed biomass remain limited (Nguyen *et al*., 2022). Most existing studies emphasise fresh seeds, processed food derivatives, or oil extractions, overlooking how drying and single-solvent extraction influence measurable antioxidant metabolites. Furthermore, few studies have simultaneously quantified total phenols and total flavonoids in both soybean and cottonseed under uniform analytical conditions using Folin–Ciocalteu and aluminium chloride assays. Comparative investigations that evaluate antioxidant-linked phytochemicals in these two agriculturally significant seeds using identical preparation and spectrophotometric procedures are particularly scarce. This research addresses that gap by providing a controlled, side-by-side quantitative assessment of total phenolic and flavonoid content in dried Glycine max and Gossypium spp. Seeds. The study enhances understanding of how drying and methanolic extraction affect antioxidant-related phytochemical yield, clarifies compositional differences between the two seeds, and establishes baseline data relevant for nutraceutical, food formulation, and industrial valorisation. The findings also support further research into compound-specific profiling and functional antioxidant assays to optimise utilisation of both seed types.

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, UK), 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, Ferric chloride (Fecl3), Ethanol, Sodium acetate, Aluminum chloride, Sodium hydroxide, Methanol, Quercetin, Chloroform, Acetic acid, Hydrochloric acid, Distilled water.

3.2 Methods

3.2.1 Sample Collection and Preparation

The dried soybean (*Glycine max*) and cotton (*Gossypium spp*) used for this study were obtained from a local market in Benin metropolis. The seeds were thoroughly inspected to ensure they were free from foreign materials, debris, and mold contamination and were allowed to dry, the cotton seeds were soaked in boiled water and manually peeled to remove the hard shells and obtain the kernel. The soybean seed was dried and weighed till a constant mass was obtained, it was then crushed and blown to remove the shaft and obtain the inner kernel. The clean dried cotton and soybean seeds were then crushed and ground into a fine powder using a mortar and pestle to increase the surface area for efficient solvent extraction. The powdered sample was stored

in dried containers, properly labeled, and kept in a cool, dry place until required for extraction and analysis.

3.2.2 Extraction

The powdered soybean and cotton seed samples were extracted using standard phytochemical procedures with minor modifications. Each extraction was performed in triplicate to ensure accuracy and reproducibility. Solvents of different polarities were employed to achieve a wide range of phytochemical recovery. Polar solvents such as 70-80% methanol or ethanol were used to extract phenols and flavonoids.

Extraction was carried out through maceration, where the powdered samples were soaked in the chosen solvent at room temperature for several days with occasional shaking to enhance solvent penetration and compound dissolution. After soaking, the mixtures were filtered

3.2.3a. Detection of Phenols

This was done by treating 1.0 mL of the plant extract with four (4) drops of ferric chloride solution. The formation of a bluish-black colour indicated the presence of phenolic compounds.

3.2.3b. Detection of Flavonoids

This was done using the alkaline reagent test and the lead acetate test.

Alkaline reagent test: The extract was treated with a few drops of 2 mol dm3 sodium hydroxide solution. The formation of an intense yellow colour, which became colourless upon addition of dilute hydrochloric acid (2 mol dm3), indicated the presence of flavonoids. Lead acetate test: The extract was treated with a few drops of lead acetate solution, and the formation of a yellow-coloured precipitate confirmed the presence of flavonoids.

3.2.4 Quantitative Phytochemical Determination

The quantitative assessment of key phytochemical constituents in the dried soybean and cotton seed extract was conducted using standard spectrophotometric and gravimetric techniques, following the procedures of Singleton and Rossi (1965), Harborne (1973), Makkar *et al*., (2007)) with minor adjustments. All analyses were performed in triplicate, and the outcomes were expressed as mean values.

3.2.4a. Determination of Total Phenolic Content

The total phenolic content (TPC) of the extract was measured using the Folin-Ciocalteu method as outlined by Singleton and Rossi (1965), with slight modifications. Tannic acid served as the calibration standard.

In summary, 1.0 ml. of extract solution (250 µg/mL) was transferred into a test tube and mixed with 1.0 mL. of Folin-Ciocalteu reagent. After a 5-minute reaction period, 15.0 mL of 20% sodium carbonate (Na2CO3) solution was added. The mixture was then left to stand for 2 hours at room temperature, after which the absorbance was recorded at 760 nm using a UV-Visible spectrophotometer (Jenway 6100, Dunmow, Essex, UK).

The total phenolic concentration was calculated from a standard curve generated using tannic acid and expressed as micrograms of tannic acid equivalent (ug TAE) per gram of extract.

3.2.4b. Determination of Flavonoid Content

Flavonoid concentration was determined based on the colorimetric method of Lahouar *et al*. (2011) with minor adjustments.

A 30 µL aliquot of the methanolic extract was combined with 90 µL of methanol, 6 µL of 10% aluminum chloride (AICI), 6 µL of 1 M sodium acetate (CH,CO,Na), and 170 µL of methanol. The resulting mixture was incubated for 30 minutes, and absorbance was recorded at 415 nm using a UV-Visible spectrophotometer.

Quercetin was used as the reference standard, and the total flavonoid content was expressed as milligrams of quercetin equivalent (mg QE) per kilogram of extract.

3.2.5 Statistical Analysis

Data obtained from the spectrophotometric and gravimetric analyses were processed using IBM SPSS Statistics software (Version 21.0.0). Each test was performed in triplicate (n = 3), and the outcomes were reported as mean ± standard mean error (SEM) to enhance precision and reliability. Descriptive statistical methods were applied to interpret the data, and the results were clearly presented in tables for easy understanding.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 showing the Qualitative Analysis of dried seeds of soybean (*Glycine max*) and cotton (*Gossypium spp*)

Seeds	Phytochemicals	Indications
Soybean Seeds	Total Phenol	(+)
Cotton Seeds	Total Flavonoid	(+)
Soybean Seeds	Total Phenol	(+)
Cotton Seeds	Total Flavonoid	(+)

Table 4.2 showing the Total Phenolic and Flavonoid content of seeds (Mean ± SEM)

Parameter	Soya Bean	Cotton seed
Total Phenolic Content (gTAE/kg)	**98.42 ± 1.55**	**82.35 ± 0.37**
Flavonoid Content (g QE/kg)	**17.72 ± 5.04**	**9.15 ± 1.22**

The quantitative assessment of the dried seed extracts demonstrated clear differences in phytochemical composition between soya bean and cotton seed. The total phenolic content was higher in soya bean (98.42 ± 1.55 g TAE/kg) compared to cotton seed (82.35 ± 0.37 g TAE/kg), indicating a greater concentration of phenolic constituents in the soybean extract. Similarly, flavonoid levels were markedly greater in soya bean (17.72 ± 5.04 g QE/kg) than in cotton seed (9.15 ± 1.22 g QE/kg), suggesting a superior flavonoid profile despite slightly higher variability, as reflected by the SEM. The elevated phenolic and flavonoid contents in soya bean imply stronger antioxidant potential relative to cotton seed, while the lower yet more consistent values in cotton seed indicate a less potent but more uniform phytochemical presence. Overall, the results highlight soya bean as the richer source of bioactive antioxidant compounds between the two seed types.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Soybean (Glycine max) and cottonseed (Gossypium spp.) are recognized sources of plant-derived phytochemicals, particularly phenolic compounds and flavonoids, which contribute to antioxidant activity and biological protection against oxidative stress. Antioxidants from plant seeds are increasingly utilized in food preservation, dietary supplementation, cosmetics, and pharmacological formulations due to their ability to neutralise reactive oxygen species and prevent cellular damage. Soybean has long been acknowledged for its rich phenolic profile and nutraceutical relevance, while cottonseed, though historically viewed as a secondary agricultural by-product, has recently gained attention for its bioactive potential. Therefore, evaluating the antioxidant-related metabolites in these seeds is important for both nutritional science and industrial application. The general analysis of results obtained in this study showed a clear distinction between the two seed types. Soybean demonstrated significantly higher levels of both total phenolic and total flavonoid contents when compared to cottonseed. These findings indicate a stronger antioxidant potential in soybean extracts and support the hypothesis that its biochemical composition provides superior free-radical scavenging capacity. Conversely, cottonseed, although lower in both phenolic and flavonoid concentrations, still exhibited measurable levels, suggesting its possible utility where moderate antioxidant activity is adequate. These comparative results reflect inherent biochemical differences in seed metabolism, biosynthetic pathways, and adaptive defence mechanisms between legumes and malvaceous crop species. The total phenolic results for soybean confirmed a high concentration of phenolic compounds, indicating strong reducing capacity and antioxidant potential. This aligns with the known presence of bioactive compounds such as genistein, daidzein, and other isoflavones associated with oxidative protection in soybean. The high phenolic content also supports functional benefits linked to disease-prevention and cellular protection, demonstrating the significance of soybean as a dietary source of antioxidant molecules. These findings support earlier reports indicating high phenolic levels in soybean seeds and extracts (Malenčić and Popović, 2007; Li *et al*., 2022). In contrast, the phenolic results for cottonseed, while lower, were still significant, demonstrating that Gossypium spp. Contains extractable phenolic constituents capable of contributing to antioxidant mechanisms. Cottonseed phenolics are associated with compounds such as gossypol, catechin, and gallic acid, which possess measurable antioxidant and antimicrobial properties. The moderate phenolic level found in this research suggests that cottonseed could be exploited for industrial antioxidant extraction, particularly after detoxification processes. These findings correspond with research reporting moderate phenolic content in cottonseed, affected by variety, extraction conditions, and gossypol reduction (Singh *et al*., 2023; Hu *et al.,* 2022).

Flavonoid results for soybean also showed a distinctly higher concentration compared to cottonseed. Soybean flavonoids, particularly isoflavones, contribute to antioxidant activity through radical scavenging, metal chelation, and enzymatic modulation. The elevated flavonoid content supports the potential of soybean in nutraceutical development and functional food formulation. These findings align with previous studies reporting high flavonoid levels in soybean, especially when methanolic extraction methods are employed (Nguyen *et al*., 2022; Shahidi and Ambigaipalan, 2018). The flavonoid results for cottonseed showed considerably lower concentrations. This suggests that its antioxidant contribution may depend more heavily on phenolic acids rather than flavonoid subgroups. The lower flavonoid content may also be attributed to inherent plant biosynthetic differences, since cottonseed directs more metabolic carbon toward lignin development and fibre structural polymers rather than toward flavonoid biosynthesis. Similar observations were reported in literature showing lower flavonoid representation in cottonseed extracts compared to legumes (Fang et al., 2023; Moses *et al*., 2022).

When compared with previous studies, the findings from this research generally align with established literature. Studies on soybean routinely report higher phenolic and flavonoid concentrations, correlating strongly with antioxidant capacity (Malenčić *et al.,* 2007; Deng *et al*., 2022). Research comparing soybean cultivars demonstrates that phenolic variability is genetically influenced, yet consistently higher than in many non-legume seeds (Li *et al*., 2023). Conversely, the lower phenolic and flavonoid levels in cottonseed are consistent with studies indicating that refining, heat treatment, and gossypol removal decrease measurable antioxidant compounds (Hu *et al*., 2022). However, some contrast exists, as certain studies report higher flavonoid content in cottonseed hulls than in kernels, suggesting that sample preparation affects measured values (Singh *et al*., 2023). Extraction solvent selection has also been shown to influence antioxidant yields, which may explain slight differences between this study and previously published values (Falade and Igbeka, 2021). Therefore, while the overall trends align, minor variation reflects methodological and biological differences. This study has limitations. Not only total phenolic and total flavonoid levels were quantified, without identifying specific compounds. Antioxidant capacity assays such as DPPH, FRAP, and other phytochemical assays were performed, meaning functional antioxidant activity was inferred rather than directly measured. Extraction was conducted with a single solvent

5.2 CONCLUSION

At the end of this study, the total phenolic content and total flavonoid content of dried soybean (Glycine max) and cottonseed (Gossypium spp.) were successfully determined and compared. The results showed that soybean exhibited significantly higher levels of both phenolic and flavonoid compounds, indicating a stronger antioxidant potential. Cottonseed, although lower in both categories, still demonstrated measurable antioxidant-related constituents, confirming its underutilized value. These findings fulfil the aim of the study and provide a basis for further biochemical, industrial, and nutraceutical exploration.

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