EFFECT OF SPENT LUBRICATING OIL ON THE TOTAL ANTIOXIDANT PROPERTY (TAP), TOTAL FLAVONOID (TFC) AND TOTAL PHENOLIC CONTENT (TPC) OF SOYBEAN *Glycine Max (L.) Merr* SEEDLINGS AFTER 14 DAYS OF GERMINATION

BY

Collins Onohuomhen EBIAGUANYE

BMS1600854

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

OCTOBER, 2023.

CERTIFICATION

We the undersigned hereby certify that Mr. Collins Onohuomhen Ebiaguanye (BMS1600584) carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the reward of Bachelors/Masters of Science Degree (B.Sc.) in Medical Biochemistry.

Signed:

………………………… …………………………
DR. B.N AGUEBOR-OGIE Date
Project Supervisor

………………………… …………………………
DR. F.E OLUMESE Date
Acting Head of Department

…………………………. ………………………..
External Examiner Date

DEDICATION

This project is dedicated to God and my entire family for the love, support and care they have shown.

To my beloved Parents Mr. and Mrs. Clement Ebiaguanye.O.

ACKNOWLEDGMENT

First of all, I am grateful to God for establishing me to complete this project and for His protections and provisions.

I wish to express my sincere thankyou to my humble and kindhearted supervisor, Dr Bobby N. Aguebor-Ogie a distinguished gentleman and a great mentor, for his unflinching support and unparalleled dedication and encouragement to this research. He is knowledgeable, accessible and caring and always inspiring. I am deeply appreciative of his supervision and encouragement all through the research period.

The molecular aspect of this research relied heavily on the support from my immediate past Head of Department Prof. A.A. Omonkhua who is also the Head of Trigass Laboratory and her team members Mr. Aisosa for giving me the opportunity to use their laboratory where they were carried out.

The encouragement and assistants from colleagues and friends cannot be over emphasized. My deep appreciation goes to Arerebo Anthonet, Abdullahi Yusuf, Cindy Anenih Isimeh, Efe-Oise, Onyin, Mary Chigoziem Kogwu.

Special thanks to DR. F.E OLUMESE (Acting Head of Department) for his love; encouragement and continuous, support all through the research period.

Special thanks also go to my department Lectures in persons of Prof. (Mrs.) Henrietta A. Oboh, Prof. Akere A. Omonkhua, Dr. Fidelis E. Olumense, Dr. (Mrs.) Stella O. Olubodun, Dr. J.C. Anionye, Dr. E.F. Omoruwa, Dr. Bobby N. Aguebor-Ogie, Dr. E.S. Oghagbon, Dr. Kelly Oriakhi, Dr. (Mrs.) N. Eluehike.

Special thanks to the department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City for helping for helping to sort out my specimens and also providing Plant name identification as well as Voucher number.

Nobody has been more important to me in the pursuit of my academic successes and feats than my parents, Mr. And Mrs. Clement Ebiaguanye and my siblings Clinton, Christabel, Celestine, Christian, and Confidence whose love, prayers and guidance were with me from the very beginning of my pursuit. I am forever indebted to their nurturing, care, encouragements and love showered on me.

I also place on record, my sense of gratitude to one and all who directly or indirectly have lent their helping hand in this venture.

My regards to my course Mates (MBC Class of ’22), I love you all.

TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION

CHAPTER TWO: LITERATURE REVIEW

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

ABSTRACT

Environmental pollution is a growing concern, with its detrimental effects on ecosystems, biodiversity, and human health. Spent lubricating oil (SLO) represents a pervasive environmental pollutant, often finding its way into soil and posing a significant threat to plant life. In this comprehensive study, we delve into the intricate dynamics between SLO and soybean (Glycine max) seedlings during the early germination phase, with a specific focus on evaluating the impact on Total Antioxidant Property (TAP), Total Flavonoid Content (TFC), and Total Phenolic Content (TPC) after 14 days of germination. The investigation entailed the examination of four distinct SLO fractions: the aqueous extract, the water-insoluble fraction extract, the water-soluble fraction extract, and the whole SLO extract. The determination of TAP was executed using gallic acid as a standard, revealing that the water-insoluble fraction extract exhibited the highest TAP (40.00 mgGAE/g), outperforming both the whole SLO extract (30.00 mgGAE/g) and the water-soluble fraction extract (16.00 mgGAE/g). This discrepancy in TAP among the various SLO fractions suggests that specific components within SLO have varying effects on soybean seedlings' antioxidant defense mechanisms. Furthermore, the assessment of TPC unveiled a nuanced picture, with the water-insoluble fraction extract (28.00 mgGAE/g) displaying a notably elevated concentration of phenolic compounds in comparison to the whole SLO extract (16.00 mgGAE/g) and the water-soluble fraction extract (24.00 mgGAE/g). The disparities in TPC emphasize the selective influence of different SLO components on the production of phenolic compounds, which are crucial for plant defense mechanisms against oxidative stress. Turning our attention to TFC, using quercetin as a standard, the whole SLO extract (32.00 mgQE/g) exhibited the highest flavonoid content, surpassing both the water-insoluble fraction extract (24.00 mgQE/g) and the water-soluble fraction extract (12.00 mgQE/g). This observation suggests that certain elements within SLO might trigger the biosynthesis of flavonoids, essential compounds for plants in mitigating the harmful effects of oxidative stress. In conclusion, the findings from this meticulous investigation underscore the intricate and multifaceted nature of plant-pollutant interactions. Each fraction of SLO exerts a unique and context-dependent influence on soybean seedlings' biochemical properties, with profound implications for antioxidant defenses, phenolic compounds, and flavonoids. This study highlights the critical importance of adopting a tailored and precise approach when evaluating the effects of pollutants on plant biochemistry.

CHAPTER ONE

1.0 INTRODUCTION

Various studies have reported the adverse effect of petroleum products on plants ranging from reduced germination of seeds, reduced survival of plants to reduced yield of plants (Akinola *et al*., 2004; Andrade *et al*., 2004). Most of the reports on the effects of petroleum products on plants have focused on crude oil, diesel and gasoline (Siddiqui and Adams, 2002; Inoni *et al*., 2006) which get to the environment through accidental spillage. However, through the activities of automobile, generator, other machines, and servicing engineers (mechanics) spent oil is discharged to the environment indiscriminately.

Spent lubricating oil here refers to used motor oil collected from mechanical/automobile, workshops, garages, and industry sources like hydraulics oil, turbine oils, process oil and metal working fluids (Olugboji and Ogunwole, 2008). Spent oil is produced when new mineral-based crankcase is subjected to high temperature high mechanical strain (ATSDR, 1997). Spent oil is a mixture of different chemicals (Wang *et al*., 2000) including petroleum hydrocarbons, chlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products and heavy metals that come from engine parts as they wear away (ATSDR, 1997). Spent oil contains polycyclic aromatic hydrocarbons (PAHs) and chemical additives like lead, zinc, Sulphur, phosphorus, magnesium, iron, vanadium, aluminum, nickel, calcium, barium, phenols, amines and benzenes (Meinz, 1999). The concentration of PAHs in spent oil increases with time of usage (Vwioko and Fashemi, 2005).

Spent oil is usually obtained after servicing and subsequent draining from automobile and generator engines (Sharifi *et al*., 2007). Spent oil is a common and toxic environmental contaminant not naturally found in the environment (Dominguez-Rosado and Pichtel, 2004). It gets to the environment due to discharge by motor and generator mechanics (Odjegba and Sadiq, 2002) and from the exhaust system during engine use and due to engine leaks (Anoliefo and Edegai, 2000; Osubor and Anoliefo, 2003). Also, the discharge of spent oil to the environment takes place when plants are at different stages of growth.

The disposal of spent oil into open vacant plots and farms, gutters and water drains is an environmental risk (Odjegba and Sadiq, 2002). Since spent oil is liquid, it easily migrates into the environment and eventually pollutes either water or soil (Olugboji and Ogunwole, 2008). Contamination of soils with spent oil leads to significant reduction of soil moisture (Akoachere et al.,2008). Spent oil significantly inhibits the activities of soil catalase and dehydrogenase (Achuba and Peretiemo-Clarke, 2007). Spent oil delays germination of seeds and causes reduction in the growth of plants (Adenipekun *et al*., 2008). The PAHs in spent oil have been shown to have indirect secondary effects like disruption of plant- water-air relationship (Renault *et al*., 2000) and effects on microorganisms like mycorrhizal fungi (Nicolotti and Egli, 1998).

The disposal of spent oil on the environment can take place when the crops grown on such land are at their different stages of growth. Plants are known to respond differently to their environment at their different stages of growth. It therefore became necessary to study what the effect of disposal of spent oil into the environment will have on the growth and performance of crops with time.

Soybean is one crop that its cultivation is expanding in Nigeria as a result of its nutritive and economic importance and diverse domestic usage, nevertheless, there is however, paucity of information on the response of soybean to spent engine oil contamination in the Ultisols of South-eastern Nigeria (Probst and Judd 1973).

Soybeans (*Glycine max*) play a pivotal role in human nutrition and animal agriculture, serving as a primary source of protein and oil. As a cornerstone of global food security, the health and productivity of soybean crops are of paramount importance. However, the presence of contaminants in agricultural soil, such as spent lubricating oil, poses a grave threat to crop growth and quality. Spent lubricating oil typically contains a mixture of hydrocarbons and heavy metals, which can exert detrimental effects on plant life (Burton, 1997).

Oil contamination in soil can disrupt essential physiological processes in plants, including nutrient uptake, water absorption, and photosynthesis. Consequently, this can lead to stunted growth and decreased crop yields. Among the key biochemical parameters in plants that are sensitive to environmental stressors are Total Antioxidant Capacity (TAC), Total Flavonoid Content (TFC), and Total Phenolic Content (TPC). These parameters are vital for the defense mechanisms of plants against oxidative stress and pathogenic attacks (Schlosberg *et al.*, 2001).

1.1 Aim of Study

The aim of this study is to assess how exposure to spent lubricating oil influences the TAC, TFC, and TPC in soybean seedlings.

This study seeks to comprehensively examine the consequences of spent lubricating oil contamination on the TAP, TFC, and TPC of soybean seedlings following a 14-day germination period. The findings are expected to contribute significantly to our understanding of how environmental pollutants can disrupt the intricate biochemical processes within soybeans. Moreover, this research may provide insights into potential strategies for mitigating the adverse effects of industrial contaminants on agricultural ecosystems, food security, and the quality of soybean-based products. Ultimately, addressing these issues is crucial for ensuring the sustainable production of soybeans and safeguarding both environmental and human health.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Lubricating Oil (Engine Oil)

Motor oil, engine oil, or engine lubricant is any one of various substances used for the lubrication of internal combustion engines. They typically consist of base oils enhanced with various additives, particularly antiwear additives, detergents, dispersants, and, for multi-grade oils, viscosity index improvers. The main function of motor oil is to reduce friction and wear on moving parts and to clean the engine from sludge (one of the functions of dispersants) and varnish (detergents). It also neutralizes acids that originate from fuel and from oxidation of the lubricant (detergents), improves the sealing of piston rings, and cools the engine by carrying heat away from moving parts.(Klamman, Verlag Chemie, 1984)

In addition to the aforementioned basic constituents, almost all lubricating oils contain corrosion and oxidation inhibitors. Motor oil may be composed of only a lubricant base stock in the case of non-detergent oil, or a lubricant base stock plus additives to improve the oil's detergency, extreme pressure performance, and ability to inhibit corrosion of engine parts.

Motor oils (Fig. 2.1) are blended using base oils composed of petroleum-based hydrocarbons, polyalphaolefins (PAO), or their mixtures in various proportions, sometimes with up to 20% by weight of esters for better dissolution of additives. (Schlosberg *et al.*, 2001)

Figure 2.1. Using a funnel to assist with a motor oil refill

2.1.1 Uses of Lubricating Oil (Engine Oil)

Motor oil is a lubricant used in internal combustion engines, which power cars, motorcycles, lawnmowers, engine-generators, and many other machines. In engines, there are parts which move against each other, and the friction between the parts wastes otherwise useful power by converting kinetic energy into heat. It also wears away those parts, which could lead to lower efficiency and degradation of the engine. Proper lubrication decreases fuel consumption, decreases wasted power, and increases engine longevity.

Lubricating oil creates a separating film between surfaces of adjacent moving parts to minimize direct contact between them, decreasing frictional heat and reducing wear, thus protecting the engine. In use, motor oil transfers heat through conduction as it flows through the engine (Booser *et al*, 1994.) In an engine with a recirculating oil pump, this heat is transferred by means of airflow over the exterior surface of the oil pan, airflow through an oil cooler, and through oil gases evacuated by the positive crankcase ventilation (PCV) system. While modern recirculating pumps are typically provided in passenger cars and other engines of similar or larger in size, total-loss oiling is a design option that remains popular in small and miniature engines.

In petrol (gasoline) engines, the top piston ring can expose the motor oil to temperatures of 160 °C (320 °F). In diesel engines, the top ring can expose the oil to temperatures over 315 °C (600 °F). Motor oils with higher viscosity indices thin less at these higher temperatures. (Challen *et al.*, 1999.)

Coating metal parts with oil also keeps them from being exposed to oxygen, inhibiting oxidation at elevated operating temperatures preventing rust or corrosion. Corrosion inhibitors may also be added to the motor oil. Many motor oils also have detergents and dispersants added to help keep the engine clean and minimize oil sludge build-up. The oil is able to trap soot from combustion in itself, rather than leaving it deposited on the internal surfaces. It is a combination of this and some singeing that turns used oil black after some running.

Rubbing of metal engine parts inevitably produces some microscopic metallic particles from the wearing of the surfaces. Such particles could circulate in the oil and grind against moving parts, causing wear. Because particles accumulate in the oil, it is typically circulated through an oil filter to remove harmful particles. An oil pump, a vane or gear pump powered by the engine, pumps the oil throughout the engine, including the oil filter. Oil filters can be a *full flow* or *bypass* type.

In the crankcase of a vehicle engine, motor oil lubricates rotating or sliding surfaces between the crankshaft journal bearings (main bearings and big-end bearings) and rods connecting the pistons to the crankshaft. The oil collects in an oil pan, or sump, at the bottom of the crankcase. In some small engines such as lawn mower engines, dippers on the bottoms of connecting rods dip into the oil at the bottom and splash it around the crankcase as needed to lubricate parts inside. In modern vehicle engines, the oil pump takes oil from the oil pan and sends it through the oil filter into oil galleries, from which the oil lubricates the main bearings holding the crankshaft up at the main journals and camshaft bearings operating the valves. In typical modern vehicles, oil pressure-fed from the oil galleries to the main bearings enters holes in the main journals of the crankshaft.

From these holes in the main journals, the oil moves through passageways inside the crankshaft to exit holes in the rod journals to lubricate the rod bearings and connecting rods. Some simpler designs relied on these rapidly moving parts to splash and lubricate the contacting surfaces between the piston rings and interior surfaces of the cylinders. However, in modern designs, there are also passageways through the rods which carry oil from the rod bearings to the rod-piston connections and lubricate the contacting surfaces between the piston rings and interior surfaces of the cylinders. This oil film also serves as a seal between the piston rings and cylinder walls to separate the combustion chamber in the cylinder head from the crankcase. The oil then drips back down into the oil pan. (Challen *et al.,* 1999).

Motor oil may also serve as a cooling agent. In some engines oil is sprayed through a nozzle inside the crankcase onto the piston to provide cooling of specific parts that undergo high-temperature strain. On the other hand, the thermal capacity of the oil pool has to be filled, i.e. the oil has to reach its designed temperature range before it can protect the engine under high load. This typically takes longer than heating the main cooling agent – water or mixtures thereof – up to its operating temperature. In order to inform the driver about the oil temperature, some older and most high-performance or racing engines feature an oil thermometer.

Continued operation of an internal combustion engine without adequate engine oil can cause damage to the engine, first by wear and tear, and in extreme cases by "engine seizure" where the lack of lubrication and cooling causes the engine to cease operation suddenly. Engine seizure can cause extensive damage to the engine mechanisms. (Challen *et al*., 1999.)

2.1.2 Effects on Environments

Due to its chemical composition, worldwide dispersion and effects on the environment, used motor oil is considered a serious environmental problem. (*Vazquez-Duhalt* and *Rafael, 1989).)* Most current motor-oil lubricants contain petroleum base stocks, which are toxic to the environment and difficult to dispose of after use. Over 40% of the pollution in America's waterways is from used motor oil. Used oil is considered the largest source of oil pollution in the U.S. harbors and waterways, at 1,460 ML (385×10^6 US gal) per year, mostly from improper disposal. By far the greatest cause of motor-oil pollution in oceans comes from drains and urban street-runoff, much of it caused by improper disposal of engine oil. One US gallon (3.8 L) of used oil can generate a 32,000 m^2 (8 acres) slick on surface water, threatening fish, waterfowl and other aquatic life.

According to the U.S. EPA, films of oil on the surface of water prevent the replenishment of dissolved oxygen, impair photosynthetic processes, and block sunlight. Toxic effects of used oil on freshwater and marine organisms vary, but significant long-term effects have been found at concentrations of 310 ppm in several freshwater fish species and as low as 1 ppm in marine life forms. Motor oil can have an incredibly detrimental effect on the environment, particularly to plants that depend on healthy soil to grow. There are three main ways that motor oil affects plants:

- contaminating water supplies
- contaminating soil
- poisoning plants

Used motor-oil dumped on land reduces soil productivity. Improperly disposed used oil ends up in landfills, sewers, backyards, or storm drains where soil, groundwater and drinking water may become contaminated.

2.2 Soya Bean (*Glycine max* ***L. Merr***.)

Soybean (*Glycine max (L.) Merrill.)* occupies a premier position among agricultural crops, being the most important source of good quality concentrated proteins as well as vegetable oil. Seeds of soybean have been used in Asia and other parts of the world for many centuries to prepare a variety of fresh, fermented and dried foods (Probst and Judd 1973). Soy-based nutritious food products such as tofu, soy milk, soy sauce, miso, etc. have been developed for human consumption while oil extracted soy meal is used as a nutritious animal feed. Besides its use for domestic purposes, soy oil finds multifarious uses in industries related to production of pharmaceuticals, plastics, papers, inks, paints, varnishes, pesticides and cosmetics.

Recently, use of soy oil as biodiesel has opened up another possibility of renewable sources of energy for industrial uses. As a legume crop, soybean is capable of utilizing atmospheric nitrogen through biological nitrogen fixation and is therefore less dependent on synthetic nitrogen fertilizers. Keeping in view its vast utilities, there is ample justification for its significant involvement in major crop improvement programs throughout the world.

Soybean belongs to the family Leguminosae and subfamily Papilionaceous. The cultivated soybean (Fig. 2.2) has been proposed to be named correctly as *(Glycine max (L.) Merrill)* by Ricker and Morse in 1948 (Gazzoni 1994). The genus Glycine consists of two subgenera: Glycine (perennials) and Soja (annuals). The perennials consist of 22 recognized species and the annual two species, G. max L. Merrill. (cultigen) and G. soja (Sieb. and Zacc 2001). (Wild species and progenitor of G. max) (Hymowitz 2004). Natural cross-pollination is usually less than 1% in the highly self-pollinated annual *Glycine max* though it may sometimes reach up to 2–3%. The perennial species have been reported to have up to 60% out-crossing for Glycine argyria and Glycine clandestina (Brown et al. 1986). Both the cultivated and wild soybeans are paleopolyploid with $2n = 2x = 40$ and these are perfectly cross-compatible (Hymowitz 2004). Soybean has a relatively large genome (1.12 × 109 bp) (Arumuganathan and Earle 1991) and about 55% of its genome consists of highly repetitive sequences (Danesh *et al*. 1998).


(A) (B)

Figure 2.2 (A) Soya Beans Plant (B) Soya Beans Seeds

2.2.2 Biology and Breeding Behavior of Soya Beans

In (Fig 2.3) Soybean is a hairy annual with an extensive tap root system, most of it in the top 15 cm of the soil. The tap root may grow as deep as 2 m and adventitious roots grow from the hypocotyls (Chaturvedi *et al*. 2011).

Cultivated soybeans have an erect growth habit though procumbence is not uncommon in germplasm resources (Burton, 1997). The modern cultivars of soybean are mostly erect, bushy, 20–180 cm tall, usually with a few primary branches and no secondary branches. Exceptionally prostrate and freely branching forms are also found, particularly in those varieties which are meant for forage purposes. The leaves are trifoliate and alternate with long petioles and small stipules and stiples; the leaflets are ovate to lanceolate with mucronate tip.

In soybean, flowering and maturity are greatly influenced by photoperiod. The flowers are typical papilionaceous, white or pale purple, with a tubular calyx of five unequal sepal lobes and a five-member corolla that consists of a posterior standard petal, two lateral wing petals and two anterior keel petals (Guard, 1931).

The androecium is diadelphous with 9 + 1 arrangement. The single pistil is unicarpellate and has 1–4 campylotropous ovules (Palmer 2001). The style curves back toward the posterior stamen and is surrounded by a knob-like stigma (Carlson and Lersten 1987). Each flower is subtended by two bracteoles and has a hairy calyx of five pointed sepals united for about half of their length. The pods are short stalked and occur in groups of 3–15, 3–7 cm long and hairy, light brown at maturity and slightly constricted between the seeds. The seeds vary greatly in shape, size and color though these are mostly often round and yellowish, brown or black with epigeal germination.

Soybeans are mostly self-pollinated, though rates of natural cross-pollination have been observed to be between 0.03 and 1.14% in natural conditions (Culter 1934; Caviness 1966). The wild annual soybean, G. *soja* is predominantly self-pollinated, while the perennial wild relative, G. *argyrea* (Ting.) and its closely related species, G. *clandestine* (Wendl.), have both self-fertilized cleistogamous as well as chasmogamous flowers on the same plant (Brown *et al*. 1986; Schoen and Brown 1991; Palmer *et al*. 2001).

The chasmogamous flowers are frequently visited by insect pollinators leading to cross-pollination. Small insects such as thrips and honeybeas are mainly responsible for natural outcrossing in soybean, but other insects are also observed to be working on soybean flowers. Insect-mediated cross pollination in soybean has been discussed in detail by Palmer *et al*. (2001).

Self-pollinating soybean flowers have 3–4 ovules, which reach maturity prior to anthesis (Stelly and Palmer 1985). Flowers open and normally self-pollinate at anthesis. For planned controlled pollination, first the sepals and petals are carefully removed from the young unopened flowers. This is followed by removal of anthers by forceps or tweezers though removal of anther is not always necessary (Stelly and Palmer 1985). The remaining flowers on the inflorescence are also removed. Pollination is done the next morning. For this, the flowers which are about to open should be taken. In these, with the help of forceps, first sepals are removed, followed by removal of the standard petal, wing and keel petals to expose the anthers. These anthers are then gently brushed on the stigma till the pollen is clearly visible on it. A small pod is usually visible in 6–7 days.

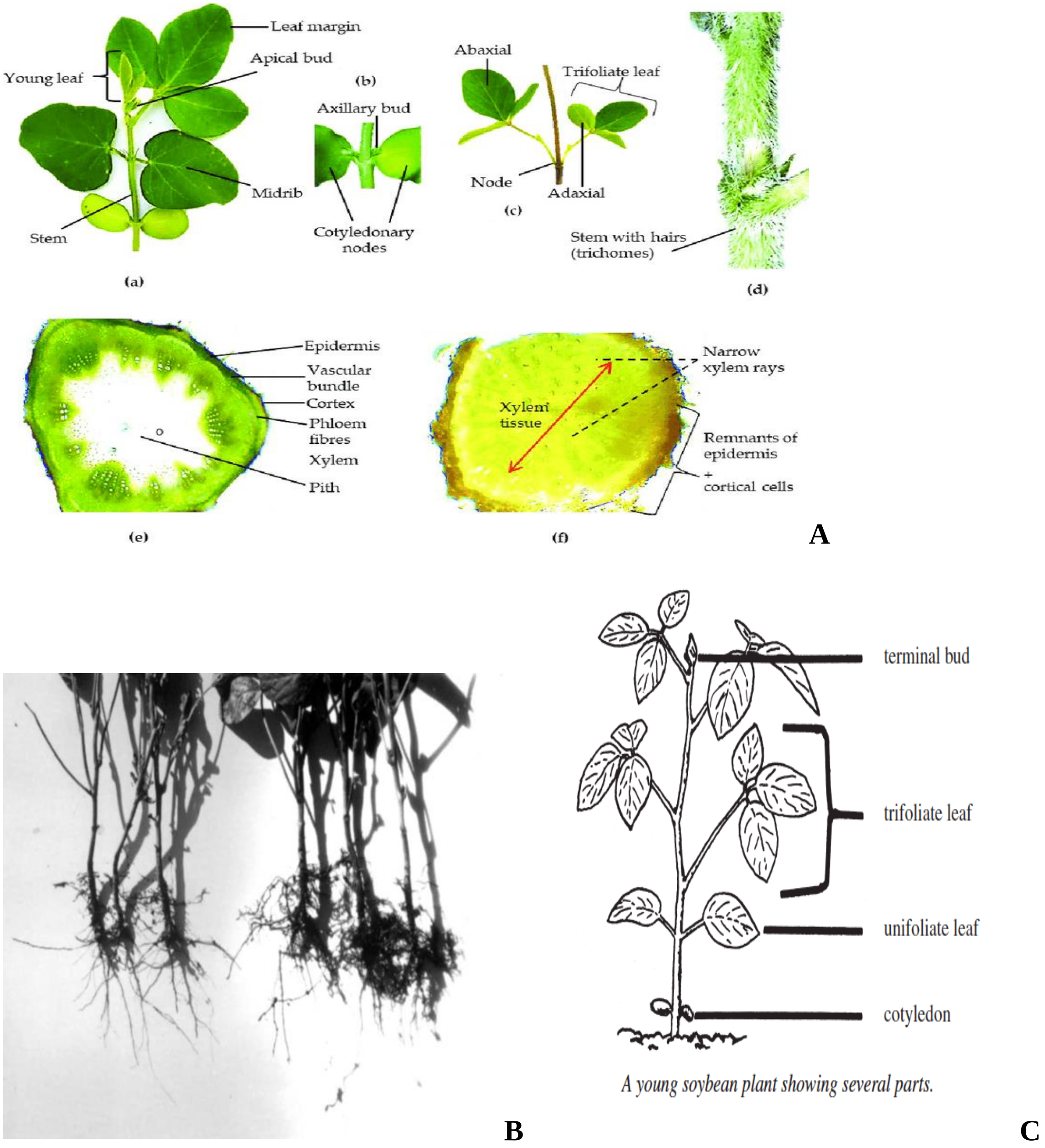

Figure 2.3 (A) Overview of soybean plant morphology and anatomy. (a) Vegetative first trifoliate (V1) stage. (b) Example of cotyledons and axillary buds at the axil. (c) Trifoliate leaves showing adaxial-abaxial leaf surfaces. (d) Example of soybean stem with epidermal hairs. (e) A micrograph of soybean stem cross-section. (f) A micrograph of soybean root cross-section. (B) SoyaBean Root (C) A young soybean plant

2.2.3 Polyploid Nature of Soybean

The genus Glycine is of an ancient polyploid origin and its genome has been reported to have gone through two major rounds of duplication events during speciation (Schlueter et al. 2004; Van et al. 2008). The haploid genome studies also suggested that soybean is a diploidized ancient tetraploid (Safari and Schlueter 2011). Studies showed that these duplication events occurred at ~14.5 and 45 MYA (Schlueter *et al*. 2004; Blanc and Wolfe 2004).

The genetic map of soybean revealed multiple nested duplications that appeared to reflect an even more ancient round of poly-ploidy at some point in the ancestry of the genus (Shoemaker et al. 2006). It has been suggested that the ancestral “diploid” genome donors of modern “allopolyploid” soybean were themselves stabilized paleopolyploid from an earlier round of genome duplication.

The soybean genome has been described as having both allo and autopolyploid origin. An allopolyploid soybean genome was first hypothesized based on cytogenetic (Singh and Hymowitz 1985) and molecular studies (Shoemaker *et al*. 1996), while the phylogenetic analysis of nuclear genes has hypothesized its origin to be of autopolyploid nature (Doyle et al. 2003; Straub *et al*. 2006).

A novel molecular cytogenetic tool, the fluorescence in situ hybridization (FISH) has clearly distinguished ten chromosome pairs in soybean suggesting that there are two distinct and co-resident genomes in its nucleus having two types of centromeres, which reflect divergence in its two diploid progenitors (Udall and Wendel 2006). Most of the molecular studies suggest that cultivated species, G. max, has close phylogenetic relationship with wild species, G. soja, which is known as a progenitor of this species. The North Asian subgenus soja has been suggested to be the probable wild progenitor of cultigen G. max (L.) Merrill. (Doyle et al. 2003).

A number of perennial diploid relatives of Glycine have been found throughout Australia and Papua New Guinea. Among these, there are reports of intercrossing among the diploid species which resulted in some allopolyploid taxa (Doyle et al. 2004). Doyle *et al*. (2004) has defined the tomentella and tabacina complexes, which have been described as allopolyploids found in the wild. These resulted from various combinations of diploid progenitors, which support that these polyploids have clearly arisen through multiple origins.

2.2.4 Economic Importance of Soya Bean

Soybean is a suitable food of great nutritional value. Soybean is probably the world's most valuable crop, used as food by billions of livestock, as "a source of dietary protein and oil by millions of people; and in the industry for manufacture of thousands of products. The crop is extremely rich in protein and oil, and is such a good source of energy, vitamins and minerals (Nwokolo, 1996). Comparing with other legumes and cereals, soybean (*Glycine max* (L) merill) has been observed to be the cheaper source of both quality proteins (Johnson, 1975; IITA 1994; Osodeke 2001). Osho and Dashiell (1998) reported that soybean which has less purchasing cost, has about 40% protein, 30% carbohydrate, 20% oil and 10% mineral. With a high-quality protein content, high quality oil, and good percent of vitamin C, that most legumes lack, soybean is a good raw material for animal production and industries (Peter, 1989; Lui, 1995).

Soybean has been found to be excellent for a number of different conditions such as high blood pressure, diabetes-related diseases and many others. It is very useful in improving the diet of malnourished children and revitalizing heart and breast cancer patients and has no cholesterol (Enwere 1998). Soybean can be used as a nutritional supplement for pregnant women, lactating mothers and children.

Agriculture is the cultivation of the land for the purpose of producing food for man, food for animals and fiber or raw materials for our industries. It also includes the processing and marketing of crops. Soybean is a grain legume that occupies greater position in world agriculture by virtue of its high protein content and its capacity for fixing atmospheric nitrogen. The crop is produced, processed and utilized today in many forms and formulations like paste, juice, cake, milk, chocolate, fiber, paint etc. These are used for human consumption, livestock feed, raw materials for food and pharmaceutical industries, and for cash. Potentials of soybean leads to greater improvement in agriculture by its remarkable contributions in meeting up with some demands of population on agriculture in any developing country like Nigeria. These demands include among others: Provision of food for the teeming population, Provision of raw materials for industries, Generation of income for farmers, Provision of employment for the working population, Improving fertility of the soil for maximum crop yield and Health care delivery or services.

A nation that depends on importation of food for her population will certainly be bankrupt. Soybean is a good source of protein and oil; used as both food and medication. Soymilk, with a high level of protein content, has been found to be the most economic substitute to imported animal milk which has become very costly. It serves as a good substitute to coast bean in preparation of 'dawadawa' (local maggi); and used in place of melon in soup. Strongly roasted
and grounded seeds are used as coffee substitute (Falliola 1990). Oil in the seed can be cooked and used as dressing salad, manufacture of margarine and vegetable oil with no cholesterol. Seeds can be grounded into flour and added as cereal flavour, roasted and eaten as peanut-like snacks, processed into paste and milk for infant nutrition.

Soybean seeds are useful materials for food industry for they are rich in oil and protein with good nutritive value. Industrial and domestic processing of soybean has given rise to numerous products utilized for both human and animal consumptions. Soybean products also serve as raw material in paint, pharmaceutical and confectionary industries. These products include:
Soybean meal: - Used as a supplement in poultry feeds and cattle feed.
Soybean oil: - Used as edible oil; refined to produce paints, varnishes, soap, lubricants, Sealants and in pharmaceuticals.
Lecithin: - used in oil and chocolate industries.
Soybean curd: - Used in vegetarian cooking.
The nutritional goodness of soybean is also fully utilized in other food processing operations for producing weaning foods. The market for weaning food is largely dominated by Nestle Food Plc, one of the multi-national companies operating in Nigeria. The company produces weaning foods from maize and soybean. The technology being used by this company allows for the use of the whole dehulled soybean. The quality of the product is standard and has been used by generations of Nigerian children.

The protein and oil content of soybean determine the economic worth of soybean seed. Soybean is commonly processed into commercial purposes. There are products like soybean oil, soybean milk, extrusion cooking, soy ogi, soy cake etc. These products command the most commercial attention and are sold for cash by farmers. Workers and labourers used for producing, processing and marketing of the products are paid their wages and salaries.

Through the export of agricultural products, a nation can earn foreign currency. The foreign exchange can in turn, be used to purchase goods necessary for improvement of agriculture for national growth and development. Nigerian soybean has been reported as one of the best quality soybean in the world. Its quality is said to compare favorably with 'yellow gold; the United States of America's variety (RMRDC 2004). According to RMRDC (2004) the major international buyers are the countries in the West African sub region: Niger Republic, Chad etc, whose climatic condition does not favor the cultivation of soybeans. Other consuming countries are Netherlands, United Kingdom, Turkey, France, Poland, Romania, Czech Republic and Saudi Arabia.

Agriculture and other agro-based industries provide employment opportunities for up to 60-70% of the population. Soybean production and industries engaged in the processing of soy products, provide employment opportunities for a good number of the population. The production and processing of soybean, developed into a commercial venture, will ensure ability of those employed under it to produce enough to feed the country and allow for export.

Soybean is generally acceptable to be capable of fixing nitrogen in association with *Rhizobium japonica*. The amount of fixed nitrogen remains in the nodules and is released to the soil, to increase nitrogen content of the soil.

Researchers have reported maximum increase in yield of cereals planted in soil where soybean is planted the previous season; which in part was as a result of increased nutrient content of the soil, which is one of the major determinants for increasing crop yield and yield components; thereby encouraging agriculture in Nigeria.

One of the determinants of growth and development of any nation is the reduction of diseases and illnesses which can hinder growth, productivity and development. A lot of soybean and soybean products can be utilized and consumed as drugs in the treatment of certain diseases and illnesses which may cause death. Duke (1983), Ayensi and Duke (1985), reported treatment of snake bite with bruised leaves of soybean; blindness and opacity of the cornea with flowers and immature seed pods; healthy functioning of bowels., heart, kidney, liver and stomach with the seeds as antidote.

Soybean constituents has been used for the cure of hypertension; reduce risk of heart diseases, developing coronary osteoporosis, and alleviation of menopause symptoms. Consuming soybean in diet has reduced cancer risk and in a limited number of study, found to have significant reductions in both diastolic and systolic blood pressure and diabetes. It also helps people to stay lean, and reduce obesity (Fabiyi 2006). Soybean has also been shown to promote serum insulin production (Fukushima, 2000). Soy protein helps to improve cholesterol profile. It is particularly important in post menopause years because it prevents hip fractures; reduce fat development, especially abdominal fat (Anderson 2003).

In areas of the world where soybeans are eaten regularly, rates of colon cancer as well as some other cancer including the breast cancer tend to be low. Soybean may be the most practical means of relief from kwashiorkor (protein calorie malnutrition) which is increasing in prevalence among children in many parts of a developing country.

2.3 Antioxidant Property

Antioxidants are compounds that inhibit oxidation (usually occurring as autoxidation), a chemical reaction that can produce free radicals. Autoxidation leads to degradation of organic compounds, including living matter.

Antioxidants are frequently added to industrial products, such as polymers, fuels, and lubricants, to extend their usable lifetimes.(Klemchuk, *et al*. 2000). Food are also treated with antioxidants to forestall spoilage, in particular the rancidification of oils and fats. In cells, antioxidants such as glutathione, mycothiol or bacillithiol, and enzyme systems like superoxide dismutase, can prevent damage from oxidative stress (Helberg, *et al*. 2021).

Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors (Kader AA and Kerbel EL 1989).

Consequently, packaging of fresh fruits and vegetables contains an ≈8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food (Zallen *et.al.*,1975). These preservatives include natural antioxidants such as ascorbic acid (AA, E300) and tocopherols (E306), as well as synthetic antioxidants such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321) (Iverson F 1995*)*. Unsaturated fats can be highly susceptible to oxidation, causing rancidification (Robards *et.al*., 1988*)*. Oxidized lipids are often discolored and can impart unpleasant tastes and flavors.

Thus, these foods are rarely preserved by drying; instead, they are preserved by smoking, salting, or fermenting. Even less fatty foods such as fruits are sprayed with sulfurous antioxidants prior to air drying. Metals catalyse oxidation. Some fatty foods such as olive oil are partially protected from oxidation by their natural content of antioxidants. Fatty foods are sensitive to photooxidation, (Del Carlo, *et. al* 2004) which forms hydroperoxides by oxidizing unsaturated fatty acids and ester (Frankel. *et.al* 2012). Exposure to ultraviolet (UV) radiation can cause direct photooxidation and decompose peroxides and carbonyl molecules. These molecules undergo free radical chain reactions, but antioxidants inhibit them by preventing the oxidation processes *(*Frankel, *et al* .,2012).

2.4 Flavonoid Content

Flavonoids are phenolic compounds commonly found in many plants, vegetables, and flowers. The flavonoid family comprises 15 classes of compounds, including the flavones, flavanols, flavanones, chalcones, and isoflavones. Although flavonoids are ubiquitous, isoflavones can be found only in legumes, particularly soybean (Boue *et al*., 2003). It is known that flavonoids are among the most potent plant antioxidants as well as the most widely distributed phenolic compounds in plant foods, and also the most studied ones (Bravo, 1998). Health-promoting effects of soybean have been attributed to its antioxidative effect, as well as to the estrogen-like activity of isoflavones, which has been reported to reduce the risk of breast cancer and osteoporosis (Messina and Messina, 2010).

Recently, legumes are gaining interest because they are excellent sources of bioactive compounds and can be important sources of ingredients for uses in functional foods and other applications. The bioactive compound content of legumes is generally affected by genetic (genotype) and environmental (weather, soil type, geographical location) factors (Malenčić *et al.*, 2012). Regarding soybean grain composition, it can be treated as a functional food because of its innumerable desirable characteristics and because there is strong evidence that additive and synergistic interactions of natural antioxidants significantly strengthen the protective effects against oxidative damage (Prakash *et al.*, 2007).

2.5 Phenolic Content

Phenolic or phenol carboxylic acids (a type of phytochemical called a polyphenol) are one of the main classes of plant phenolic compounds. They are found in the variety of plant-based foods viz. seeds, skins of fruits and leaves of vegetables contain them in highest concentrations. Typically, they are present in bound from such as amides, esters, or glycosides and rarely in free form (Pereira *et* al., 2009). Phenolic acids possess much higher in vitro antioxidant activity than well-known antioxidant vitamins (Tsao and Deng, 2004). Phenolic acids are mainly divided into two sub-groups: hydroxybenzoic and hydroxycinnamic acid (Clifford, 1999). Hydroxycinnamic acids, derived from cinnamic acid, present in foods often as simple esters with quinic acid or glucose. The most abundant soluble bound hydroxycinnamic acid present is chlorogenic acid (a combined form of caffeic and quinic acids). On the other hand, hydroxybenzoic acids possess a common structure of C6-C1 and derived from benzoic acid. They are found in soluble form (conjugated with sugars or organic acids) and bound with cell wall fractions as lignin (Strack, 1997). As compared to hydroxycinnamic acids, hydroxybenzoic acids are generally found in low concentration in red fruits, onions and black radish etc. (Khoddami *et* al., 2013).

Polyphenols can also be found in cereals, dried legumes, and chocolate (Spencer *et* al., 2008). There has been a lot of focus on the potential health benefits of dietary plant polyphenols as antioxidants in the last decade (Beckman, 2000). According to epidemiological research and related meta-analysis, long-term consumption of diets high in plant polyphenols protects against cancer, cardiovascular disease, diabetes, osteoporosis, and neurological diseases (Arts and Hollman, 2005). Bitterness, astringency, color, flavor, odor, and oxidative stability are all things that polyphenols can help with in food. Plant polyphenols in the diet have a plethora of health benefits (Pandey and Rizvi, 2009).

(a) (b)

FIG 2.4. The two sub-groups of phenolic acid (a) hydroxycinnamic acid (b) hydroxybenzoic acid.

Phenols are a large class of secondary plant metabolites, showing a diversity of structures, from rather simple structures, e.g., phenolic acids, to phenols such as flavonoids that comprise of several groups, to polymeric compounds based on these different classes. Some phenolic compounds, such as isoflavones in soybeans, are extremely widespread, while others are restricted or particularly abundant in specific plants (Cheynier, 2012).

Along with their antioxidant properties, phenolic compounds play an important role in the metabolism of the plant itself. According to their carbon chain, they can be separated into 16 major classes (Dey and Harborne, 1989) and all of them are involved in many plant functions, such as pollination, structure, sensorial properties (color, aroma, taste and astringency), germination processes, resistance to pests and predators, growth, as well as development and reproduction (Colpas *et al*., 2003; Malenčić *et al.*, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials (General)

Atomic absorption Spectrophotometer, Oven, Refrigerator, Bucket centrifuge, Water Bath, Measuring Cylinder, Breakers, Test-tubes, , Test-tubes rack, Stirrers, Mortar and Pestle, Petri dish, Magnetic stirrer, Sample bottles, kitchen towel, Spatula, Micropipette, pipette tips, Cotton wool, Hand gloves, Filter, spent lubricating oil, Distill water, *Glycine max (L.) Merr* seeds, Sodium nitrite, Sodium hydroxide, Aluminum chloride, Quercetin, Folin-Ciocalteu reagent (FCR), Sodium carbonate, Gallic acid, Sodium phosphate, Ammonium molybdate, H2SO4, Citric ethanol, Normal Saline.

3.2 Methods

3.2.1 Sample collection and Processing

The seeds material, *Glycine max (L.) Merr* were obtained from a local market (Uselu market) in Benin City, Edo state, Nigeria and identification was done by a botanist in the Department of Plant Biology and Biotechnology, University of Benin and with the voucher number UBH-G628. A cup of *Glycine max (L.) Merr* seeds was soaked in a clean bowl with distill water for about 24 hours. Grains that floated were sieved out and others that submerged were planted in a cotton soil base, which was soaked with various fractions of the spent lubricating oil according to the groupings. After 14 days of planting, the stems and roots were harvested and homogenized. The mixture of the stems and roots homogenates were extracted into different sample bottles based on the groups by using 3ml of Normal saline for each sample. The samples are then centrifuged at 4000rpm for 5minutes and decant into a plain bottle for Total Antioxidant Properties, Total Phenolic Content and Total Flavonoid Content estimation

3.2.2 Total Antioxidant Properties (TAP) Estimation Total Antioxidant Properties (TAP) was determined by the procedure of Otitolaiye *et al.(*2023).

Principle

The antioxidants present in the extracts reduce the phosphomolybdate (VI) ion to a green phosphomolybdate (V) ion. This assay is also called phosphomolybdate (Sasikumar *et al*, 2014) and in this case, the reactions of sodium phosphate and ammonium molybdate in the presence of H2SO4 produce the free radical (phosphomolybdate ion). First, equal amounts of 4 mM ((NH4)2MoO4), 28 mM Na3PO4, and 0.6 M H2SO4 were added to produce the standard phosphomolybdate reagent solution. The standard used for this assay was (gallic acid) and was prepared in concentrations of 10, 25, 50, 100, 200, 300, 400, and 500µg/mL while 500µg/mL of extracts were utilized. To 1mL of extract or gallic acid reference was added 2 mL of the standard phosphomolybdate solution. Then, it was placed in a water bath at 95°C for 90 min. A subtle green colour developed after it was allowed to cool to ambient temperature. After determining the absorbance at 695 nm, the overall antioxidant strength was shown as the corresponding amount of gallic acid (mgGAE/g) in one gram of the extracts. Gallic acid (mgGAE/g) in one gram of the extracts.

Procedure

	Blank	Test	Standard
Standard (Gallic acid)	-	-	1ml
Distill water	1ml	-	-
Extract	-	1ml	-
Standard reagent solution (Na3PO4 + H2SO4 + (NH4)2MoO4)	2ml	2ml	2ml
Incubated in a water bath at 95°C for 90 min.			
Allowed to cool to 25°C			
Read absorbance at 695nm			

3.2.3 Total Flavonoid Content (TFC) Estimation

The flavonoid concentration in this assay was estimated using the aluminum chloride colorimetric method as described by Roy et al. (2018).

Principle

The standard quercetin (1mg/mL), was prepared in a range of concentrations, such as 10, 50, 75, 100 and 150 µg/mL to 1 mL of the extract, or quercetin was added 0.3 mL of sodium nitrite (5%). After covering, this solution was incubated for 5 min at ambient temperature. Thereafter, 10% aluminum chloride (0.3 mL) was introduced, stirred, and the solution was once more allowed to stand for 5 min at ambient temperature. The solution was then combined with 2mL of 1M NaOH and left to stand at room temperature for an additional 10 min. The flavonoid concentration was quantified as milligrams of quercetin equivalent per gram of extract (mgQE/g) after the absorbance was measured at 510 nm.

Procedure

	Blank	Test	Standard
Standard (Quercetin)	-	-	1ml
Distill water	1ml	-	-
Extract	-	1ml	-
5% Sodium Nitrite	0.3ml	0.3ml	0.3ml
Covered and incubated for 5mins at room temperature			
10% Aluminum chloride	0.3ml	0.3ml	0.3ml
Mixed and incubated for 5mins at room temperature			
1M NaoH	2ml	2ml	2ml
Incubated for 10mins @ room temperature			
Absorbance was read at 510nm			

3.2.4 Total Phenolic Content (TPC) Estimation

The phenolic content of the extracts was ascertained using the Folin-Ciocalteu reagent (FCR) method. As described by Roy et al. (2018).

Principle

The Folin-Ciocalteu reagent (FCR) assay's principle is that phenolic in the extracts reduce FCR, which causes the development of a blue colour that gets darker as phenolic concentration increases. One and a half (1.5) mL of 10% FCR reagent was put to a test tube already holding 200µL of extract/standard. The solution was covered and put in a dark cupboard at ambient temperature for 5 min. After that, 1.5mL of 5% Na2CO3 was introduced and thoroughly mixed. This was again covered and left to stand in a dark place for 2hr at room temperature. The absorbance was later read at 750nm. Gallic acid (1mg/mL) was prepared at different concentrations of 5, 10, 25, 50, 75, 100, 150, and 200µg/mL, to plot the standard curve. The equivalent amount of gallic acid, in milligrams (mgGAE/g), was used to determine the amount of phenol in the extracts.

Procedure

	Blank	Test	Standard
Standard (Gallic acid)	-	-	200µL
Distill water	200µL	-	-
Extract	-	200µL	-
10% Folin-Ciocalteu reagent	1.5ml	1.5ml	1.5ml
Allowed to stand in the dark at room temperature for 5min			
5% Na2CO3	1.5ml	1.5ml	1.5ml

3.3 Data Analysis

Each test was done three times and results were presented as mean ± SEM. The variations between the water insoluble fraction, water soluble fraction and whole spent lubricating oil fraction of root and stem extracts of *Glycine max (L.) Merr* for the quantitative Total Antioxidant Properties, Total

Flavonoid Content and Total Phenolic Content assays were analyzed by the Independent Samples t-test. $P<0.05$ was accepted as significant.

CHAPTER FOUR

4.0 RESULTS

The following below are the result on the effects of spent lubricating oil on the Total Antioxidant Properties, Total Phenolic Content and Total Flavonoid Content of *Glycine max (L.) Merr* seedlings after 14 days of germination. The result shows the various fractions of the spent lubricating oil according to the groupings.

Table 4.1. Effect of Spent Lubricating Oil (SLO) and its Fractions on TAP, TPC and TFC of *Glycine max (L.) Merr.*

Parameter	Aqueous extract (µg/mL)	Water insoluble fraction extract (µg/mL)	Water soluble fraction extract (µg/mL)	Whole spent lubricating oil extract (µg/mL
TAP (mgGAE/g)	24.00	40.00	16.00	30.00
TPC (mgGAE/g)	32.00	28.00	24.00	16.00
TFC (mgQE/g)	12.00	24.00	12.00	32.00

QE-Quercetin Equivalent; GAE-Gallic Acid Equivalent.

From the results in the table above (Table 4.1), I'm aiming to uncover the intricate effects of spent lubricating oil (SLO) on critical parameters of Glycine max (L.) Merr seedlings. Over a rigorous 14-day germination period, I was particularly interested in elucidating how various fractions of SLO influence key aspects of the plant's health, specifically focusing on Total Antioxidant Properties (TAP), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC). The comprehensive presentation of these findings is meticulously documented in Table 4.1.

In the above table, The Total Antioxidant Power (TAP) of the different SLO fractions. I used gallic acid as the reference standard. The most compelling revelation was that the Water Insoluble Fraction (WISF) extract stood out significantly, exhibiting an impressive TAP value of 40.00

mgGAE/g. In contrast, the Whole Spent Lubricating Oil (WSLO) extract showed a slightly lower TAP of 30.00 mgGAE/g, while the Water Soluble Fraction (WSF) extract displayed a notably lower TAP value of 16.00 mgGAE/g. This suggests that the WISF extract holds remarkable potential in enhancing the antioxidative capacity of Glycine max seedlings.

Moving on to the Total Phenolic Content (TPC) evaluation, I also found equally captivating results. The Water Insoluble Fraction (WISF) extract exhibited a substantial concentration of 28.00 mgGAE/g, surpassing both the Water Soluble Fraction (WSF) extract with a TPC of 24.00 mgGAE/g and the Whole Spent Lubricating Oil (WSLO) extract, which displayed a notably lower TPC value of 16.00 mgGAE/g. These findings highlight the distinctive phenolic composition of these fractions, and the pivotal role they play in the overall health of the seedlings.

Moreover, The Total Flavonoid Content (TFC), with quercetin serving as the standard reference. The results unveiled even more substantial differences. The Whole Spent Lubricating Oil (WSLO) extract stood out remarkably, presenting a remarkable TFC value of 32.00 mgQE/g. The Water Insoluble Fraction (WISF) extract demonstrated a substantial TFC of 24.00 mgQE/g, while the Water Soluble Fraction (WSF) extract lagged behind with a TFC of 12.00 mgQE/g. This indicates that the WSLO extract possesses a significantly higher flavonoid concentration compared to the other fractions, potentially contributing to the enhanced flavonoid content in Glycine max seedlings.

CHAPTER FIVE

5.1 DISCUSSION

The dawn of the 21st century has ushered in an era of heightened awareness about the perils of environmental pollution. In the agricultural sector, where the cultivation of crops is essential for global food security, the indiscriminate disposal of industrial waste, such as spent lubricating oil (SLO), raises alarm bells. This study represents a significant step forward in our quest to understand the impact of such pollutants on plant ecosystems, focusing on soybean (Glycine max) seedlings. The study meticulously investigates the variations in Total Antioxidant Property (TAP), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) following a 14-day germination period. By delving into the biochemical responses of soybean seedlings to SLO exposure, this research bridges the gap between environmental science and plant physiology (Adegbite *et.al*.,2018)

Soybeans are not merely agricultural commodities; they are integral to human nutrition. These legumes provide a significant source of protein and oil, making them a global dietary staple. However, the cultivation of soybeans faces mounting environmental challenges, including the insidious contamination by pollutants like SLO. Spent lubricating oil is a noxious blend of hydrocarbons, heavy metals, and other hazardous compounds. Its rampant presence in the environment, often due to improper disposal and machinery leaks, jeopardizes plant ecosystems and threatens food safety. This study represents a beacon of light in the murky landscape of environmental pollution, aiming to uncover the biochemical intricacies of how SLO affects soybean seedlings (Bellincontro *et.at*.,2017)

The ability of plants to thrive and endure in a hostile environment hinge upon their antioxidative potential. Measuring Total Antioxidant Property (TAP) is pivotal, as it reveals the plant's aptitude to counteract the menace of free radicals and protect its delicate cellular structures. Stressors, especially those of an environmental nature, disrupt this delicate balance. Here, TAP serves as an invaluable metric to gauge the influence of SLO on soybean seedlings' antioxidative capacity (Bertrand *et.al*.,2019)

The results are nothing short of revelatory, illustrating how different SLO fractions can modulate TAP in distinctive ways.

The results expose the pronounced variations in TAP among diverse SLO fractions. The water-insoluble fraction (WISF) emerges as a central protagonist, showcasing the highest TAP value. This revelation tantalizingly hints at the potential of this fraction to augment the antioxidative capacity of soybean seedlings. It prompts a cascade of questions about the specific compounds residing within the WISF that could fortify the plant's ability to fend off oxidative stress. Is this enhancement attributed to the presence of antioxidants within this fraction, or is it the plant's adaptative response to the unique stressors confined to this fraction (Hatami *et.al.*,2017)

TAP is not a mere abstract concept; it signifies the plant's fortitude against the encroachment of oxidative stress. In the context of environmental pollution, understanding the fluctuations in TAP reveals invaluable insights into the mechanisms that plants deploy in response to stressors. These insights form the bedrock upon which future strategies to ameliorate the negative effects of contaminants can be constructed. By bolstering plant health, these strategies hold the potential to enhance ecosystem well-being and human food security (Hancock *et.al.*,2019)

Another linchpin of plant health and adaptability lies in Total Phenolic Content (TPC). Phenolic compounds are celebrated for their potent antioxidant capabilities, protecting plant cellular structures from the scourge of oxidative damage. The research results underscore the palpable effect of SLO on the TPC of soybean seedlings. The conspicuous disparities in TPC across different SLO fractions are particularly thought-provoking (Hancock *et.al.*,2019)

The ascendancy of the water-insoluble fraction extract in the TPC arena, trumping the other fractions, accentuates the presence of specific phenolic compounds in this fraction. These compounds aren't passive spectators but are active participants in the plant's battle against oxidative stressors. Phenolic compounds are not just silent sentinels; they play a pivotal role in the complex interplay between plants and the environment.

Intricacies in the plant's responses to environmental stressors are underscored by the ebb and flow of Total Flavonoid Content (TFC). Flavonoids, renowned for their antioxidant potency, are the unsung heroes in the tales of plant-microbe interactions and environmental adaptation. The present study unveils a conspicuous augmentation in TFC within the whole spent lubricating oil (WSLO) extract. This underscores that SLO, while a pollutant, can also act as a catalyst for soybean seedlings to upregulate flavonoid production in response to oxidative stress (Alharbi *et.al.*,2018)

This surge in flavonoid production might be perceived as a defense strategy employed by the soybean seedlings to ward off the oxidative stress inflicted by SLO. However, the identity of these flavonoids and their potential applications in enhancing stress tolerance in crop plants remain shrouded in mystery. This is a realm ripe for dedicated exploration, as these compounds could hold the key to the development of strategies for sustainable agriculture and stress-resilient crops (Castano *et.al*.,2017)

5.2 CONCLUSION

In Conclusion, this study traverses the labyrinth of environmental pollution and plant resilience, illuminating the biochemical responses of soybean seedlings to SLO exposure. The intricate and varying reactions across different SLO fractions underscore the multilayered and context-dependent nature of the plant-pollutant relationship (Onuegbu, *et.al*.,2019)

These findings emphasize the need to differentiate between SLO fractions when assessing their impact on plant biochemistry. A tailored, precise approach to environmental research is imperative, as this nuanced understanding of pollution effects has ramifications far beyond the laboratory walls (Onuegbu, *et.al*.,2019)

The legacy of this study reaches beyond academia. It carries profound implications for the realms of sustainable agriculture and environmental conservation. In an era where environmental pollution casts a long shadow over our ecosystems and food security, comprehending how plants adapt and prosper in the face of adversity becomes not only a scientific endeavor but a cornerstone for resilience and sustainability (Murugaiyah, *et.al.,*2014)

The lessons gleaned from this study will reverberate through the corridors of science, agriculture, and environmental stewardship. They will inspire innovative solutions to protect our planet and its inhabitants, fostering a future where crops not only withstand environmental challenges but thrive amidst them. In the grand tapestry of environmental science, this research serves as a radiant thread, contributing to the grand narrative of a sustainable, resilient, and harmonious coexistence between nature and humanity (Yang *et.al*., 2017)

REFERENCES

Adegbite, A. A., and Alharbi, B. M. (2019). Phytotoxic effects of used automotive engine oil on germination, seedling growth, and biochemical characteristics of Zea mays L. Archives of Agronomy and Soil Science, 65(5), 653-665.

Agati, G., Azzarello, E., Pollastri, S., and Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. Plant Science, 196, 67-76.

Agu, R. U., and Onuegbu, N. C. (2019). Effects of spent engine oil contamination on soil physical properties, growth, and yield of Zea mays L. in southeastern Nigeria. Cogent Environmental Science, 5(1), 1650161.

Alam, M. N. , Bristi, N. J. , and Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21, 143–152. [PMC free article] [PubMed] [Google Scholar]

Almansoub, M. A. , Asmawi, M. Z. , and Murugaiyah, V. (2014). Effect of extraction solvents and plant parts used on the antihyperlipidemic and antioxidant effects of garcinia atroviridis: A comparative study. *Journal of the Science of Food & Agriculture*, 94(8), 1552 10.1002/jsfa.6456 [PubMed] [CrossRef] [Google Scholar]

Apel, K., and Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology, 55, 373-399.

Baenas, N., and Moreno, D. A. (2014). Plant compounds for food, pharmaceutical, and cosmetics use. In G. F. Barbosa (Ed.), Dietary Phytochemicals and Microbes (pp. 1-21). Springer.

Bajpai, V. K. , Baek, K. H. , and Kang, S. C. (2017). Antioxidant and free radical scavenging activities of taxoquinone, a diterpenoid isolated from metasequoia glyptostroboides. *South African Journal of Botany*, 111, 93–98. 10.1016/j.sajb.2017.03.004 [CrossRef] [Google Scholar]

Baker, L. R., Parfitt, R. L., Collins, H. P., and Moir, J. L. (2007). Do contributions of roots and root exudates to soil carbon and nitrogen dynamics exceed the inputs of plant residues? Functional Plant Biology, 34(7), 659-673.

Bau, H. , Villaume, C. , Nicolas, J. , and Méjean, L. (2015). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*glycine max*) seeds. *Journal of the Science of Food & Agriculture*, 73(1), 1–9. [Google Scholar]

Bellincontro, A., Nicoletti, I., and De Rossi, A. (2017). Effects of abiotic stress in plants: a review. Journal of Berry Research, 7(3), 221-227.

Bellincontro, A., Nicoletti, I., and De Rossi, A. (2017). Effects of abiotic stress in plants: a review. Journal of Berry Research, 7(3), 221-227.

Bertrand, M., Castaño-Díaz, M., and Bouchard, M. (2019). Antioxidant properties of polyphenols: An emphasis on the effective factors. Food Science and Human Wellness, 8(3), 217-233.

Bertrand, M., Castaño-Díaz, M., and Bouchard, M. (2019). Antioxidant properties of polyphenols: An emphasis on the effective factors. Food Science and Human Wellness, 8(3), 217-233.

Bhattacharjee, S. (2016). Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. Current Science, 110(3), 424-431.

Buer, C. S., and Imin, N. (2010). A fine balancing act: A delicate interplay between defense and growth. Plant Science, 179(5), 414-425.

Chen, Y. , and Chang, S. K. C. (2015). Macronutrients, phytochemicals, and antioxidant activity of soybean sprout germinated with or without light exposure. *Journal of Food Science*, 80(6), 1391–1398. 10.1111/1750-3841.12868 [PubMed] [CrossRef] [Google Scholar]

Chen, Z. , Wang, P. , Weng, Y. , Ma, Y. , Gu, Z. , and Yang, R. (2017). Comparison of phenolic profiles, antioxidant capacity and relevant enzyme activity of different chinese wheat varieties during germination. *Food Bioscience*, 20, 159–167. 10.1016/j.fbio.2017.10.004 [CrossRef] [Google Scholar]

Félix, A. P. , Rivera, N. L. M. , Sabchuk, T. T. , Lima, D. C. , Oliveira, S. G. , and Maiorka, A. (2013). Effect of soy oligosaccharide extraction on diet digestibility, faecal characteristics, and intestinal gas production in dogs. *Animal Feed Science & Technology*, 184(1–4), 86–93. 10.1016/j.anifeedsci.2013.05.013 [CrossRef] [Google Scholar]

Ghorbanpour, M., and Hatami, A. (2017). Influence of lubricating oil on germination of Oryza sativa L. seeds and its subsequent seedling growth. Environmental Science and Pollution Research, 24(33), 26187-26194.

Ghorbanpour, M., and Hatami, A. (2017). Influence of lubricating oil on germination of Oryza sativa L. seeds and its subsequent seedling growth. Environmental Science and Pollution Research, 24(33), 26187-26194.

Hancock, J. T., and Neill, S. J. (2019). Nitric oxide: its generation and interactions with other reactive signaling compounds. Plant and Cell Physiology, 60(3), 19-28.

Harini, T. , Basetty, S. , Jasti, L. S. , Lavanya, K. , and Fadnavis, N. W. (2017). Recovery of functionally active trypsin inhibitor (SBTI) and lipoxygenase (LOX) from soymilk via multimodal technique. *Reactive & Functional Polymers*, 121, 45–50. 10.1016/j.reactfunctpolym.2017.10.009 [CrossRef] [Google Scholar]

Hasan, S., Singh, M. B., and Bhalla, R. (2020). Spent engine oil: A source of contamination in the environment and its adverse effects on human health. Environmental Science and Pollution Research, 27(3), 2053-2066.

Hasan, S., Singh, M. B., and Bhalla, R. (2020). Spent engine oil: A source of contamination in the environment and its adverse effects on human health. Environmental Science and Pollution Research, 27(3), 2053-2066.

Hasan, S., Singh, M. B., and Bhalla, R. (2020). Spent engine oil: A source of contamination in the environment and its adverse effects on human health. Environmental Science and Pollution Research, 27(3), 2053-2066.

Houben, D., and Sonnet, P. (2004). Impact of used motor oil on the biodegradation of polycyclic aromatic hydrocarbons (PAH) and loss of PAH diversity. Biodegradation, 15(5), 309-319.

Huang, X. , Cai, W. , and Xu, B. (2014). Kinetic changes of nutrients and antioxidant capacities of germinated soybean (glycine max L.) and mung bean (*vigna radiata* L.) with germination time. *Food Chemistry*, 143(2), 268 10.1016/j.foodchem.2013.07.080 [PubMed] [CrossRef] [Google Scholar]

Jahan, M. S., and Faridatun, A. (2018). Impact of oil spill in the aquatic ecosystem: A review. Environment and Natural Resources Journal, 16(1), 59-69.

Jahan, M. S., and Faridatun, A. (2018). Impact of oil spill in the aquatic ecosystem: A review. Environment and Natural Resources Journal, 16(1), 59-69.

Jia, D. , Huang, Q. , and Xiong, S. (2016). Chemical interactions and gel properties of black carp actomyosin affected by MTGase and their relationships. *Food Chemistry*, 196, 1180–1187. 10.1016/j.foodchem.2015.10.030 [PubMed] [CrossRef] [Google Scholar]

Kasala, E. R. , Bodduluru, L. N. , Barua, C. C. , Madhana, R. M. , Dahiya, V. , Budhani, M. K. , and Gogoi, R. (2016). Chemopreventive effect of chrysin, a dietary flavone against benzo(a)pyrene induced lung carcinogenesis in swiss albino mice. *Pharmacological Reports*, 68(2), 310 10.1016/j.pharep.2015.08.014 [PubMed] [CrossRef] [Google Scholar]

Kaushik, G. , Satya, S. , and Naik, S. N. (2010). Effect of domestic processing techniques on the nutritional quality of the soybean. *Mediterranean Journal of Nutrition & Metabolism*, 3(1), 39–46. 10.1007/s12349-009-0079-7 [CrossRef] [Google Scholar]

Kim, Y. M., Reed, D. W., Ha, J., Nijhawan, A., Cheng, Z., and Molnar, I. (2010). Effects of spent lubricating oil on the germination and growth of terrestrial plants. Water, Air, & Soil Pollution, 211(1-4), 355-365.

Kim, Y. M., Reed, D. W., Ha, J., Nijhawan, A., Cheng, Z., and Molnar, I. (2010). Effects of spent lubricating oil on the germination and growth of terrestrial plants. Water, Air, & Soil Pollution, 211(1-4), 355-365.

Krishnan, H. B. , Kim, W. S. , Oehrle, N. W. , Alaswad, A. A. , Baxter, I. , Wiebold, W. J. , and Nelson, R. L. (2015). Introgression of leginsulin, a cysteine-rich protein, and high-protein trait from an asian soybean plant introduction genotype into a north american experimental soybean line. *Journal of Agricultural & Food Chemistry*, 63(11), 2862 10.1021/jf505202z [PubMed] [CrossRef] [Google Scholar]

Kumar, V., Jain, M., Kumar, A., and Agarwal, R. (2019). Influence of oil spill on soil health and agricultural sustainability: A review. Catena, 183, 104210.

Kumar, V., Jain, M., Kumar, A., and Agarwal, R. (2019). Influence of oil spill on soil health and agricultural sustainability: A review. Catena, 183, 104210.

Lee, J. D. , Shannon, J. G. , Jeong, Y. S. , Lee, J. M. , and Hwang, Y. H. (2007). A simple method for evaluation of sprout characters in soybean. *Euphytica*, 153(1–2), 171–180. [Google Scholar]

Lee, W. H. , Wu, H. M. , Lee, C. G. , Sung, D. I. , Song, H. J. , Matsui, T. , … and Kim, S. G. (2014). Specific oligopeptides in fermented soybean extract inhibit nf‐κb‐dependent inos and cytokine induction by toll‐like receptor ligands. *Journal of Medicinal Food*, 17(11), 1239–1246. 10.1089/jmf.2013.3070 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Martinez‐Villaluenga, C. , Peñas, E. , Ciska, E. , Piskula, M. K. , Kozlowska, H. , Vidalvalverde, C. , and Frias, J. (2010). Time dependence of bioactive compounds and antioxidant capacity during germination of different cultivars of broccoli and radish seeds. *Food Chemistry*, 120(3), 710–716. 10.1016/j.foodchem.2009.10.067 [CrossRef] [Google Scholar]

Mehri, A., & Moaveni, P. (2017). Protective effects of exogenous salicylic acid on growth and some physiological characteristics of Safflower (Carthamus tinctorius L.) under spent lubricating oil pollution stress. Journal of Plant Interactions, 12(1), 142-151.

Mehri, A., and Moaveni, P. (2017). Protective effects of exogenous salicylic acid on growth and some physiological characteristics of Safflower (Carthamus tinctorius L.) under spent lubricating oil pollution stress. Journal of Plant Interactions, 12(1), 142-151.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science, 7(9), 405-410.

Nielsen, P. M. , Petersen, D. , and Dambmann, C. (2010). Improved method for determining food protein degree of hydrolysis. *Journal of Food Science*, 66(5), 642–646. [Google Scholar]

Omidian, K. , Rafiei, H. , and Bandy, B. (2017). Polyphenol inhibition of benzo[a]pyrene‐induced oxidative stress and neoplastic transformation in an in vitro model of carcinogenesis. *Food & Chemical Toxicology*, 106, 165–174. 10.1016/j.fct.2017.05.037 [PubMed] [CrossRef] [Google Scholar]

Pandey, K. B., and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2(5), 270-278.

Pearce, K. N. , and Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural & Food Chemistry*, 26(3), 716–723. 10.1021/jf60217a041 [CrossRef] [Google Scholar]

Pietta, P. G. (2000). Flavonoids as antioxidants. Journal of Natural Products, 63(7), 1035-1042.

Plant Physiology and Responses to Environmental Stress:

Re, R. , Pellegrini, N. , Proteggente, A. , Pannala, A. , Yang, M. , and Rice-Evans, C. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology & Medicine*, 26(9–10), 1231 10.1016/S0891-5849(98)00315-3 [PubMed] [CrossRef] [Google Scholar]

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

Richert, S. H. (1979). Physical-chemical properties of whey protein foams. *Journal of Agricultural & Food Chemistry*, 27(4), 1804–1810. [Google Scholar]

Ritzoulis, C. , Marini, E. , Aslanidou, A. , Georgiadis, N. , Karayannakidis, P. D. , Koukiotis, C. , and Tzimpilis, E. (2014). Hydrocolloids from quince seed: Extraction, characterization, and study of their emulsifying/stabilizing capacity. *Food Hydrocolloids*, 42, 178–186. 10.1016/j.foodhyd.2014.03.031

Robles-Ramírez, M. C. , Ramón-Gallegos, E. , Reyes-Duarte, F. J. , and Mora-Escobedo, R. (2012). Effect of germinated soy protein on the growth of HeLa cervical cancer cells in female athymic mice. *Nutrition Cancer*, 64(8), 1261–1268. 10.1080/01635581.2012.717681 [PubMed] [CrossRef] [Google Scholar]

Sairam, R. K., Srivastava, G. C., and Saxena, D. C. (2000). Increased antioxidant activity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. Biologia Plantarum, 43(2), 245-251.

Sairam, R. K., Srivastava, G. C., and Saxena, D. C. (2000). Increased antioxidant activity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. Biologia Plantarum, 43(2), 245-251.

Shim, S. M. , Yoo, S. H. , Ra, C. S. , Kim, Y. K. , Chung, J. O. , and Lee, S. J. (2012). Digestive stability and absorption of green tea polyphenols: Influence of acid and xylitol addition. *Food Research International*, 45(1), 204–210. 10.1016/j.foodres.2011.10.016 [CrossRef] [Google Scholar]

Simirgiotis, M. J., Schmeda-Hirschmann, G., Bórquez, J., Kennelly, E. J., and Giordano, A. (2013). Antioxidant properties, quantification and phytochemical profile of Maqui (Aristotelia chilensis) berries from different Chilean geographical origins. Food Chemistry, 162, 108-115.

Simirgiotis, M. J., Schmeda-Hirschmann, G., Bórquez, J., Kennelly, E. J., and Giordano, A. (2013). Antioxidant properties, quantification and phytochemical profile of Maqui (Aristotelia chilensis) berries from different Chilean geographical origins. Food Chemistry, 162, 108-115.

Sreelatha, S. , Jeyachitra, A. , and Padma, P. R. (2011). Antiproliferation and induction of apoptosis by moringa oleifera leaf extract on human cancer cells. *Food & Chemical Toxicology*, 49(6), 1270–1275. 10.1016/j.fct.2011.03.006 [PubMed] [CrossRef] [Google Scholar]

Sritongtae, B. , Sangsukiam, T. , Morgan, M. R. , and Duangmal, K. (2017). Effect of acid pretreatment and the germination period on the composition and antioxidant activity of rice bean (*vigna umbellata*). *Food Chemistry*, 227, 280–288. 10.1016/j.foodchem.2017.01.103 [PubMed] [CrossRef] [Google Scholar]

Srivastava, P. (2002). Impact of lubricating oil on seed germination and seedling growth in Brassica juncea. Environmental Monitoring and Assessment, 77(3), 285-292.

Srivastava, P. (2002). Impact of lubricating oil on seed germination and seedling growth in Brassica juncea. Environmental Monitoring and Assessment, 77(3), 285-292.

Stamenic, M. , Vulic, J. , Djilas, S. , Misic, D. , Tadic, V. , Petrovic, S. , and Zizovic, I. (2014). Free-radical scavenging activity and antibacterial impact of greek oregano isolates obtained by SFE. *Food Chemistry*, 165(20), 307–315. 10.1016/j.foodchem.2014.05.091 [PubMed] [CrossRef] [Google Scholar]

Szőllősi, R. , Varga, I. S. , Erdei, L. , and Mihalik, E. (2009). Cadmium-induced oxidative stress and antioxidative mechanisms in germinating indian mustard (*brassica juncea* l.) seeds. *Ecotoxicology & Environmental Safety*, 72(5), 1337–1342. [PubMed] [Google Scholar]

Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., Agati, G., ... and Traversi, M. L. (2004). Differential accumulation of flavonoids and hydroxycinnamates in leaves of Ligustrum vulgare under excess light and drought stress. The New Phytologist, 163(3), 547-561.

Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., Agati, G., ... and Traversi, M. L. (2004). Differential accumulation of flavonoids and hydroxycinnamates in leaves of Ligustrum vulgare under excess light and drought stress. The New Phytologist, 163(3), 547-561.

Teixeira da Silva, J. A. (2003). Ecology, distribution, and management of invasive Hedychium gardnerianum in the Azores. Weed Technology, 17(4), 841-845.

Teixeira da Silva, J. A. (2003). Ecology, distribution, and management of invasive Hedychium gardnerianum in the Azores. Weed Technology, 17(4), 841-845.

Vadde, R. , Pochana, J. R. , and Pillatla, R. R. (2010). Nutritional quality of storage proteins during germination of indian bean (*dolichos lablab*. var. lignosus) seeds. *International Journal of Food Science & Technology*, 43(5), 944–949. [Google Scholar]

Verma, S., and Dubey, R. S. (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science, 164(4), 645-655.

Verma, S., and Dubey, R. S. (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science, 164(4), 645-655.

Vernaza, M. G. , Dia, V. P. , and Mejiab, E. G. D. (2012). Antioxidant and antiinflammatory properties of germinated and hydrolysed Brazilian soybean flours. *Food Chemistry*, 134(4), 2217 10.1016/j.foodchem.2012.04.037 [PubMed] [CrossRef] [Google Scholar]

Voesenek, L. A., and Bailey-Serres, J. (2013). Flooding tolerance: O_2 sensing and survival strategies. Current Opinion in Plant Biology, 16(3), 647-653.

Yoon, J., Cao, X., and Zhou, Q. (2006). Maize response to lubricating oil contamination in soils. Journal of Environmental Quality, 35(6), 2351-2357.

Yoon, J., Cao, X., and Zhou, Q. (2006). Maize response to lubricating oil contamination in soils. Journal of Environmental Quality, 35(6), 2351-2357.

Yuan, M. , Jia, X. , Ding, C. , Zeng, H. , Du, L. , Yuan, S. , … Liu, J. (2015). Effect of fluorescence light on phenolic compounds and antioxidant activities of soybeans (*glycine max*, L. merrill) during germination. *Food Science & Biotechnology*, 24(5), 1859–1865. 10.1007/s10068-015-0243-4 [CrossRef] [Google Scholar]

Zanatta, M. S., Vizzotto, M., Porciuncula, L. de O., and Silva, L. B. (2018). Plants' resilience to environmental stress: A review of some examples. Ciência Rural, 48(8), e20180093.

Zanatta, M. S., Vizzotto, M., Porciuncula, L. de O., and Silva, L. B. (2018). Plants' resilience to environmental stress: A review. Ciência Rural, 48(8), e20180093.

Zhang, H. J. , Zhang, H. , Wang, L. , and Guo, X. N. (2012). Preparation and functional properties of rice bran proteins from heat-stabilized defatted rice bran. *Food Research International*, 47(2), 359–363. 10.1016/j.foodres.2011.08.014 [CrossRef] [Google Scholar]

Zhang, X., Zhao, F., Wei, H., and Li, S. (2019). Effect of spent lubricating oil on the germination and growth of Kentucky bluegrass (Poa pratensis L.). Environmental Science and Pollution Research, 26(28), 29023-29031.

Zhang, X., Zhao, F., Wei, H., and Li, S. (2019). Effect of spent lubricating oil on the germination and growth of Kentucky bluegrass (Poa pratensis L.). Environmental Science and Pollution Research, 26(28), 29023-29031.

Zhang, X., Zhao, F., Wei, H., and Li, S. (2019). Effect of spent lubricating oil on the germination and growth of Kentucky bluegrass (Poa pratensis L.). Environmental Science and Pollution Research, 26(28), 29023-29031.

Zhang, Z. , Yang, Y. , Tang, X. , Chen, Y. , and You, Y. (2015). Chemical forces and water holding capacity study of heat-induced myofibrillar protein gel as affected by high pressure. *Food Chemistry*, 188, 111–118. 10.1016/j.foodchem.2015.04.129 [PubMed] [CrossRef] [Google Scholar]

Zhu, J. K. (2001). Plant salt tolerance. Trends in Plant Science, 6(2), 66-71.

Zhu, J. K. (2001). Plant salt tolerance. Trends in Plant Science, 6(2), 66-71.