UNIVERSITY OF EDUCATION, WINNEBA

ANTIOXIDANT AND INSECTICIDAL POTENCY OF ESSENTIAL OIL EXTRACT FROM LANTANA CAMARA GROWING IN CENTRAL REGION OF GHANA

GLADYS HAYFORD OWUSU

MASTER OF PHILOSOPHY

2022

UNIVERSITY OF EDUCATION, WINNEBA

ANTIOXIDANT AND INSECTICIDAL POTENCY OF ESSENTIAL OIL EXTRACT FROM *LANTANA CAMARA* GROWING IN CENTRAL REGION OF GHANA

GLADYS HAYFORD OWUSU
(200024952)

A thesis in the Department of Chemistry Education,
Faculty of Science Education, submitted to the
School of Graduate Studies in partial fulfillment

of the requirements for the award of the degree of
Master of Philosophy
(Chemistry Education)
in the University of Education, Winneba

AUGUST, 2022

DECLARATION

I, Gladys Hayford Owusu, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work and it has not been submitted, either in part or whole, for another degree elsewhere.

Signature :……

Date …….

Supervisors' Declaration

I hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of thesis as laid down by the University of Education, Winneba.

Supervisor's Name: Dr. E. K. Oppong

Signature :……

Date :…..……..

DEDICATION

This research is dedicated to my husband, Mr Elijah Asamoah Addai and my children Christabel Owusu Asantewaah and Lucy Dzigbodi Adwoa Deku.

ACKNOWLEDGEMENTS

I wish to express my heartfelt appreciation to my supervisor, Dr. E. K. Oppong, a Senior lecturer in the Department of Chemistry Education, University of Education, Winneba, for his technical guidance and encouragement throughout the supervisory work on the thesis. A special thanks to Dr. Justice Kwaku Addoh of University of Cape Coast for his guidance and moral support throughout the period of this research.

Another special thanks go to Mr. Elijah Asamoah Addai my husband, Christabel Owusu Asantewaah, Lucy Dzigbodi Adwoa Deku, Mr. Hayford Baah-Adomako, Rev. Fr. Flt. Lt. Jacob Ernest Quansah, Bernard Nana Kwame Danquah, for their technical input, inspiration and encouragement throughout the course of this research work. I also want to show appreciation to Rev. Owusu Achew Bediako, Pastor Eric Mensah, Pastor Precious Patrick Kofi Ahiamadia and Pastor Michael Abunyah for their continuous prayers and support.

Finally, I would like to say thank you to Felix Nelson Akompi and all my course mates.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Since ancient times, essential oils have been employed in cuisine as spices or herbs as well as in fragrance, cosmetics, and medicine. Although their primary usage in the nineteenth century was as additives for scent and flavor, their original application was in medicine. In this study, essential oil was extracted from fresh leaves of *Lantana camara* using hydro-distillation procedure. The yield of the oil obtained was 12.68g which represented a 2.54% yield. Gas Chromatography -Mass Spectrometry Analysis of the oil revealed that the essential oil from *Lantana camara* contains total of 76 compounds representing 99.40% of the oil with (E)- Caryophyllene as the predominant compound in the oil extract constituting 20.60%. The total flavonoid Content as well as the Total Antioxidant Capacity (TAC) of the essential oil extract from *Lantana camara* was determined. The essential oil extract was also examined using UV-Vis spectrophotometer according to the phosphomolybdenum assay. The free radical scavenging activity of the essential oil extract was also determined using the DPPH assay. A comparison of the total phenolic content (TPC) and the total antioxidant capacity (TAC) of the oil extract indicated a slight difference in activity of the TAC from the TPC, meaning there were more phenolic contents present in the oil extract, with a lower antioxidant capacity. Its (Lantana camara) widespread usage as a medicinal plant with the curative effect against a variety of diseases and conditions is supported by the presence of antioxidants in the essential oil.

CHAPTER ONE

INTRODUCTION

1.0 Overview

This chapter gives a general introduction to the study. It encompasses the background to the study, statement of the problem, the aim of the study, objectives of the study, as well as the significance of the study. This chapter also looks at the organisation of the report.

1.1 Background of Study

Plants have been recognized many years ago for their therapeutic properties. These plants have been a great source of medicine due to the presence of bioactive natural compounds. These bioactive natural compounds are widely used in both traditional and modern therapies for improving human health with relatively less or no side effects (Kuppusamy, Yusoff, Parine, & Govindan, 2015; Arumugam, Swamy & Sinniah, 2016). Globally, various medicinal plants have been well explored to discover novel drug molecules to combat the threat of ever-increasing human diseases (Arumugam et al., 2016; Swamy & Sinniah, 2016). There are many indigenous cultures around the world that have used and relied extensively on traditional herbal medicine to treat a myriad of maladies. By contrast, the rise of the modern pharmaceutical industry in the past century has been based on exploiting individual active compounds with precise modes of action (Li & Weng 2017). This surge has yielded highly effective drugs that are widely used in the clinic, including many plant natural products and analogues derived from these products has fallen short of delivering effective cures for complex human diseases with complicated causes, such as cancer, diabetes, autoimmune disorders and degenerative diseases. While the plant kingdom continues to serve as an important and rich source for bioactive compounds

supporting drug discovery and drug development, the rich traditions of herbal medicine developed by trial and error on human subjects over thousands of years contain invaluable biomedical information just waiting to be uncovered using modern scientific approaches (Li & Weng, 2017). An increase in the complexity and number of diseases has resulted in a dire need to develop alternate treatment sources with as little side contractions as possible. Chemically synthesized drugs have been used over the years in the treatment of many diseases and has led to many unwanted side effects. Due to such reasons, more research is being conducted on varieties of plants to optimize their medicinal abilities and other usefulness. Traditional medicine has been in use for a long time in many parts of the world, especially Asia and Africa. About 80% of the world's population still resort to traditional medicines for their basic healthcare needs (Owolabi, Omogbai & Obasuyi, 2007).

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plant (Sánchez, García & Heredia, 2010). The chemicals in essential oils are secondary metabolites, which play an important role in plant defense as they often possess antimicrobial properties (Hyldgaard, Mygind & Meyer, 2012). Essential oils have been used for centuries in medicine, perfumery, cosmetic, and have been added to foods as part of spices or herbs. Their initial application was in medicine, but in the nineteenth century their use as aroma and flavor ingredients increased and became their major function. Almost 3000 different essential oils are known, and 300 are used commercially in the flavor and fragrances market (Burt, 2004). The antibacterial properties of secondary metabolites were first evaluated using essential oil vapors by De la Croix in 1881 (Burt, 2004). Since then, essential oils or their components have been shown to not only possess broad-range antibacterial properties (Oussalah et al., 2007), but also anti-

parasitic (George et al., 2009), insecticidal (Lee & Ahn, 2003) and antioxidant (Brenes & Roura, 2010) properties. Furthermore, they also function as growth enhancers for animals (Ahmadifar, Falahatkar & Akrami, 2011). Although the food industry primarily uses essential oils as flavorings, they represent an interesting source of natural antimicrobials for food preservation.

Essential oils are also defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavour or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. Essential oils are found in glandular hairs or secretory cavities of plant-cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots and/or fruits in different plants. The aromatic characteristics of essential oils provide various functions for the plants including (i) attracting or repelling insects, (ii) protecting themselves from heat or cold; and (iii) utilizing chemical constituents in the oil as defence materials. Many of the essential oils have other uses as food additives, flavourings, and components of cosmetics, soaps, perfumes, plastics, and as resins. Plant essential oils are a complex natural mixture of volatile organic compounds. These essential oils are also made up of monoterpenes and sesquiterpenes. These are considered to be among the best alternatives for the control of disease vectors (Dias & Moraes, 2014). One of such plant endowed with essential oils of medicinal properties is *Lantana camara.*

Lantana camara is listed as one of the significant medicinal plants of the world (Saxena *et al.,* 2012). The plant Lantana camara, generally known as wild or red sage is the most widespread species of this genus and it is a woody straggling plant with various flower colors, red, pink, white, yellow and violet. It is an ever-green strong-smelling shrub, with stout recurred prickles, leaves opposite, ovate, acute or sub-

acute, crenate -serrate, scab ride on both sides. *Lantana camara* shrub belongs to the family Verbanaceae and is located in the tropical parts of the globe. *Lantana camara* is native to tropical American countries like Brazil, Colombia, and Mexico (Barreto et al., 2010). *Lantana camara* has been used in many traditional medicinal applications but there is inadequate scientific confirmation of these traditional applications. It is known to be used in the treatment of itches, cuts, catarrh, tumour, headache, ulcers, eczema, tetanus (Abou & Shier, 1990). In Ghana, infusions of the plant are used to treat bronchitis and the powdered root is added to milk and given to children to treat stomach ache (Prakash & Rao, 2018). Barreto *et al*., (2010) published that Lantana is used as an antiseptic and antitumoral agent and that, its roots have been used for the treatment of malaria. The leave extracts of Lantana have shown analgesic and antimicrobial properties in many experiments (Lingamaneni, Rao & Mishra, 2011). Lantana oil can be used in the treatment of skin itches and as antiseptic for wounds (Bengum et al., 2000). Lantana is easily accessible due to its evasive nature. Phytochemical screening has shown that the leaves, stem and roots of *Lantana camara* contain tannins, steroids, flavonoids, catechin, saponins, phenols and many other chemicals that have diverse biological activities (Roudier et al., 2011). Day in 2003 published that leaf extracts of *Lantana camara* exhibited antimicrobial, insecticidal and fungicidal activity. Methanol extracts of shoots of *Lantana camara* from India, showed strong antioxidant activities (Basu, 2006). The shrub *Lantana camara* for a very long time is reported to be used in traditional medicine system for the treatment of a number of health-related conditions like swellings, itches, cuts, eczema, ulcers, rheumatism, bilious fever and cataract (Saxena *et al.,* 2012). Different parts of the plants are used in the treatment of whooping cough, cold, headache, chicken pox, asthma, eye injuries, bronchitis and arterial hypertension. *Lantana*

camara has been studied scientifically for various therapeutic activities like antioxidant, insecticidal, antibacterial, antipyretic, larvicidal, antimicrobial, wound healing and anti-hyperglycemic (Saxena *et al.,* 2012).

Analysis of essential oil of dried leaves of *Lantana camara* showed appreciable scavenging effect in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay (Zénabou et al., 2018). Antioxidants are a group of substances that slows or prevents oxidative damage to a target molecule (Yamagashis & Matsui, 2011; Mohamed, 2019). These antioxidants scavenge free radicals such as hydro-peroxides to inhibit the mechanism or chain of reactions that lead to degenerative diseases (Zhu, et al., 2011). In the human body, cellular mechanisms lead to the generation of unstable compounds such as reactive oxygen species and free radicals, which can destroy living cells and may cause several clinical diseases. Oxidative stress is an important factor in the pathogenesis of degenerative diseases such as asthma, cancer, diabetes, tumour and arteriole sclerosis. These natural antioxidants are nutrients that neutralize various species of free radicals and decline the damage caused by oxidative stress in diabetic patients (Doustkhah et al., 2021). Incorporation of antioxidants into our foods will help prevent diseases resulting from oxidation of free fatty acids and other free radicals. Recent development of continuous insect populations and resistant to conventional synthetic insecticides, together with the high cost of these synthetic insecticides, and the growing concern of environmental and human health challenges brought about over the use of many synthetic insecticides (Hagstrum, & Subramanyam, 2016) have necessitated and stimulated interest in the development of alternative control strategies of natural product origin. Among these methods is the use of plant essential oils, powdered plant parts and their extracts and/or bioactive isolates. The use of locally available plant material extracts as traditional botanical

insects and pest control agents such as essential oils from the leaves and seeds of plants has recently attracted considerable research to establish the scientific basis for their continued use in terms of efficacy, active constituents and appropriate application technology (Belmain, et al., 2001). These alternative control strategies also have advantage of being less toxic to nontarget organisms and are biodegradable which makes them eco-friendly.

1.2 Statement of Problem

Oxidation of free radicals in the body contributes to the development of cancer and other chronic degenerative diseases. Increase in microbial resistance to most clinical antibiotics is reducing medical options in antibiotic selection, resulting in an increase in microbial infections in humans and other organisms. There is therefore the need to seek other natural options or other natural sources of the mentioned challenges.

A range of synthetic and natural antioxidants have proven to be effective in controlling the activities of free radicals (Swamy & Sinniah, 2015). However, synthetic antioxidants are sometimes known to cause adverse effects on the human body as compared to the natural antioxidants with little or no side effects. Currently conventional drugs for diabetes-mellitus have undesired side effects such as weight gain, hypoglycemia, hypersensitivity, gastrointestinal disturbances, liver and heart failure, nausea and diarrhea (Baaloudj et al., 2021). Natural product-based remedies are options we need to consider to solve the mentioned challenges. Also, Malaria and other vector-borne diseases which are transmitted by mosquitoes is a major threat to health delivery in Ghana and Africa as a whole and it is becoming increasingly difficult to control them by conventional methods where there is continuous use of chemical insecticides. This is due to the fact that, there is wide spread indication of resistance by the arthropod vector mosquitoes to the existing chemical insecticides.

The World Health Organization (WHO) recently released Global report on insecticide resistance in malaria vectors from the period 2010–2016 (WHO, 2018). The results showed a high prevalence of resistance to the following commonly used chemical insecticide classes – Pyrethroids, Organochlorines, Carbamates and Organophosphates. The report shows a significant widespread resistance in all major malaria vectors across the WHO regions of Africa, the Americas, South-East Asia, the Eastern Mediterranean and the Western Pacific (WHO, 2018). Hence, there is the need to develop an alternate class of insecticides of plant natural product origin.

1.3 Purpose of the Study

It has been reported that herbal medications, diet, alternative and complementary therapies improve the homeostasis of glucose metabolism in patients with diabetes (Okur et.al., 2018). Recent clinical studies have supported the efficacy of many plant extracts in glucose homeostasis (Singab et al., 2014). Most communities consider *Lantana camara* as a pest plant and hence cut down the plant to make way for food crops, this study seeks to assess the chemical composition, antioxidant capacity and insecticidal activity of essential oil extracted from the fresh leaves of *Lantana camara* using the Clevenger-assisted hydro distillation method.

1.4 Objectives of the study

The objectives of this study is to;

- extract essential oil from the leaves of *Lantana camara.*
- screen for phytochemical constituents of the essential oil extract from the leaves of *Lantana camara.*
- assess the antioxidant capacity of the extracted oil from *Lantana camara* using DPPH assay.

- assess the total phenolic compounds, Flavonoids and FRAP from the *Lantana camara*.

1.5 Significance of the study

It has been reported that herbal medications, diet, alternative and complementary therapies improve the homeostasis of glucose metabolism in patients with diabetes (Okur et.al., 2018). Herbal products with large amounts of some chemicals such as flavonoids, phenolic compounds, terpenoids, alkaloids, glycosides and coumarins have beneficial effects on the regulation of carbohydrates metabolism (Hussain, et al., 2017). Thus, the oil obtained from the hydro distillation of *Lantana camara* would be used in pharmaceutical products to help curb free radical activity and therefore prevent degenerative diseases. The plant based essential oil from *Lantana camara* would be used in preparations of products such as skin pomades and other dermatological products to be used as insecticides.

1.6 Organization of the Report

The thesis is organized under five chapters. Chapter one dealt with, the background, the statement of the problem, purpose, significance and organization of the study. The second chapter reviewed literature related to the study. Under this chapter, the opinions of other researchers who have studied and written on essential oils, extraction of essential oils, applications of essential oils, beauty care products, essential oil application in food preservation, pest repellant potency, *Lantana camara,* principle of hydro distillation, spectroscopic analysis among others.

The third chapter is the methodology. It includes sample collection and treatment, extraction of bioactive compounds by hydro distillation of the essential oils, GC-MS

analysis of extracts, antimicrobial and antioxidant activity tests of the essential oil extracts.

The fourth chapter took care of analysis and discussion of results obtained from the research. Finally, the fifth chapter gives summary, conclusions and recommendations of the study.

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

This chapter reviewed literature related to the study. Under this chapter, the opinions of other researchers who have studied and written on essential oils, extraction of essential oils, applications of essential oils, beauty care products, essential oil application in food preservation, pest repellant potency, *Lantana camara,* principle of hydro distillation, spectroscopic analysis among others.

2.1 Definition and concept of essential oils

Essential oils are highly volatile and aromatic yields obtained from plants. Due to their volatility, they can easily be extracted by the method of steam distillation from different natural sources (Hyldgaard, Mygind & Meyer, 2012). The oil secreting glands are located in fruits, flowers, seeds, wood, leaves, roots, barks and sometimes present throughout the body of the plant. The essential oils are named after their mother plant from where they are isolated and the odor also resembles the organ of the plant from where they are extracted. The aroma of these volatile yields is generally more concentrated in the essential oils. Different authors have defined essential oils, but a precise one is proposed by Schilcher, Hegnauer, and Cohn Richter and further concluded by Sonwa (Mekem, 2000). “Essential is a biochemical product or a combination of similar products, which are generated in cytoplasmic fluid and are located in intercellular space in the form of minute droplets. They are highly odorous and volatile in nature.” They can be comprised of combinations of aromatic compounds or combinations of aromatic and non-aromatic compounds where an aromatic compound is defined as a “compound which possesses chemical purity,

volatile in ambient environment and possess odor which can please the aesthetic sense of the society". A more specific definition is proposed by International Organization for standardization (ISO) which states "extract procured from raw material of natural origin that is, plant by steam distillation, by processes that involve mechanical extraction from the epicarp of citrus fruits or by physical extraction such as dry distillation following elution of the aqueous phase and may also have post extraction physical analysis provided that no changes in its composition takes place (Mekem, 2003; Turek & Stintzing, 2013). As mentioned earlier, essential oils are multiplex, comprising of various components, mostly of liquid state but may also comprise of solid particles. At an ambient temperature, these oils may appear colorless to light yellow in color when obtained fresh, aromatic and are readily absorbed through the skin epidermis. These essential oils are low density fractions except for cinnamon, Sassafras and clove oils which have high density than water but are soluble in solvents such as diethyl ether, ethanol and miscible in vegetable oils, waxes and fats. Physical attributes of essential oils include high rotary strength and refraction index.

2.2 Variety in Essential Oils

Nearly 3000 diverse essential oils have been extracted. Out of the 3000, only about 300 are utilized monetarily in the seasoning and scents. In any case, the high diversity in the chemical composition of aromatic plants shows a possibly significant problem for the scent producing industry (Burt, 2004). Therefore, much research have been concentrated on the different factors adding to this assortment other than entirely genetic ones. For instance, researchers have identified particular races of similar species, for instance, *Melaleuca bracteata* is rich in chief constituents which produces an alternate essential oil with either methyl eugenol, methyl isoeugenol, and elemicin.

Other fragrant plants, such as sweet flag (*Acorus calamus*), wormwood (*Artemisia absinthium*), sweet basil (*Ocimumbasilicum*) camphorwood (*Cinnamomumcamphora*), lemon balm (*Melissa officinalis*), thyme (*Thymus vulgaris*), peppermint (*Menthapiperita*), or tansy (*Tanacetum vulgare*) have been broadly considered and distinctive chemotypes and their synthetic races have been identified (Evans, 2009). It has similarly been found that different variables can alter the chemical composition of essential oils, for example, atmosphere, precipitation, or geographic location of the plant.

2.3 Extraction of Essential Oils

Essential oils can be extracted by methods of steam distillation, steam and water distillation, or by steam distillation alone. These are the most conventional and regularly utilized strategies to isolate these aromatic essences. When the solubility of a specific essential oil in water is high, as in account of lavender, geranium or rose another procedure that is, cohobation can be utilized. In this method, the loss of hydro soluble compounds can be prevented by returning the condensed water from the separator back to the still. Other forms for acquiring essential oils incorporate enfleurage and maceration with the recent systems employing extraction with supercritical liquids or solvents. Maceration can be utilized when the yield from distillation is low, while enfleurage and solvent extraction is reasonable for sensitive, costly and thermally unstable materials (Chamorro, Morales, Sequeira, Velasco & Zambon, 2012). Generally, essential oils are acquired through water distillation or steam distillation from various organs of the plant, including the entire plant or simply the fruits, wood, leaves, roots, bark, or seeds (Hyldgaard, Mygind & Meyer, 2012).

The portions of plants collected for extraction purposes can be freshly obtained, partially dried or dehydrated but in the case of flowers they must be freshly picked. If the method of steam distillation is used to obtain these aromatic essences, the nature of extracted compounds will always be volatile. If solvents are utilized for isolation purposes, then the chemical assortment will differ from similar essential oil got through distillation. In addition, if the plant material is incubated with water at high temperatures prior to extraction phase, the composition of essential oil will also differ significantly. Whenever water and plant material are kept in different systems and the water vapor goes through the plant material, the contact is short and the oil can be gathered only a couple of minutes in the initial stages of the procedure (Chamorro et al., 2012). One other option to distillation that maintains a minor synthetic change in the extracted product is supercritical CO_2 extraction. This process tends to leave labile mixes and an extensive variety of different standards unaltered, for instance, when purification is done by using this technique, the extracted yield may contain flavonoids of nonvolatile nature. In similar manner, when essential oil is obtained by solvent extraction, including enfleurage, the extractive fluid may dissolve mixes for example, resinoids and other nonvolatile fractions. Diverse outcomes can be obtained with different procedures, for example, cold squeezing or crushing of the external peel of mainly citrus fruits in presses subsequently decanting or centrifuging the oils to isolate the plant material and the fluid remains. In every one of these cases, the chemical composition of the essential oils are not the same as that obtained through distillation (Chamorro et al., 2012).

2.4 Applications of Essential Oils

The utilization of essential oils is to a great degree assorted relying upon the source, quality, extraction strategy, and so on. Essential oils are largely used in the fabrication of fragrances, beautifying agents, soaps, shampoos or cleaning gels. Another fascinating part of these oils is their potential as medicines in aroma-based therapies or as carriers for drug delivery (Naeem et al., 2018). Another major utilization of essential oils is in the agro food business, both for creating refreshments and for enhancing sensorial properties of food items.

2.5 Beauty Care Products of Essential oils

The utilization of essential oils in the beauty care products, detergent, soap and perfume industries is of great concern from a financial point of view. The generation of essential oils for preparation of perfumes and scents has expanded enormously on a global level and simultaneously collection of these aromatic plants. Salvia, lavender and thyme species are highly consumed to produce these aromatic yields. Sufficient determination of the crude source material and method employed for the extraction are fundamental components for enhancing the nature of the volatile yields.

2.6 Pharmaceutic and Therapeutics of Essential oils

Essential oils are utilized as a part of pharmaceutics for their potential as therapeutic agents (Edris, 2007; Roberts et al., 2013). This is particularly the instance of the essential oils from peppermint (Menthapiperita), sage (*Salvia officinalis*), anise (*P. anisum*), eucalyptus (*E. globulus*), clove (*S. aromaticum*), and tea tree (*M. alternifolia*). These oils are utilized as an expectorant for treating bronchitis and cough (eucalyptus essential oil), as antibacterial agents (sage, clove and tea tree oil), as a decongestant of the respiratory tract (peppermint oil), and as a carminative (anise

oil). Moreover, clove oil is utilized as a part of dentistry for its antimicrobial and pain-relieving properties while tea tree oil is utilized in the field of dermatology (antiacne drug) as it possesses antimicrobial properties against Gram-positive microbes (Baser & Buchbauer, 2009). In pharmaceutics, essential oils are used to enhance sensory attributes of pharmaceutical drugs. The prime application of essential oils in pharmaceutics is aromatherapy.

Different strategies can be utilized to administer essential oils isolated from different plant sources. The term "aromatherapy" was coined by Gattefossé in the 1920s and was restored by Maury in the 1960s. Since the 1980s, its prominence has expanded relentlessly. In current times, it is genuinely entrenched in Germany, New Zealand, Australia, Canada, France, Switzerland, the United States, and United Kingdom (Koroch et al., 2007). The most well-known application strategy for essential oils is local application of these oils along with some carrier oils after being diluted to a concentration. Moreover, they can be used as balms, compresses and creams. In any case, oral utilization of essential oils through encapsulation or other customized discharge techniques has been presented as a successful strategy for getting the helpful impacts of these essences (Boehm, Bussing & Ostermann, 2012). They can also be used as extract infusions in the form of tea which is considered more exact dose while preventing unwanted impact. However, it is conceivable that the harmfulness of essential oils might be higher when taken by this method.

2.7 Agro food

Essential oils are utilized as a part of a wide range of food products, for example, confectionery sodas, and alcoholic drinks. Apart from being consumed as a seasoning material, they are also utilized as a part of agriculture and food industry for their

antimicrobial, antiviral, antifungal, insecticidal, nematocidal, and anticancer attributes (Adorjan, & Buchbauer, 2010; Silvestri et al, 2016).

Numerous essential oils have antibacterial as well as anti-oxidative properties (Lang & Buchbaue, 2012; Dandlen *et al*., 2010), yet their application as additives in food items requires a detailed learning of their properties, including the inhibition of the microorganisms on target, the particular method of activity, their antibacterial effectiveness, and the possible interaction impact on their antibacterial attributes with food components (Hyldgaard, Mygind & Meyer, 2012).

2.8 Essential Oil Application in Food Preservation

There is a leading interest for the utilization of new techniques for maintaining safety of food items and to have a native characteristic and 'Green' outlook. One of such plausibility is the utilization of Essential Oils (EOs) as food additives to preserve food quality and safety (Burt, 2004). Enhancing the food storage life, while guaranteeing its quality and safety, is a focal interest of the agro-food industry and government organizations. Storage life has been characterized as the time duration amid which the food item will stay unaltered; retain sensorial properties, physical, biochemical, microbiological, and functional attributes; and conform to any name affirmation of dietary information when stored under the conditions mentioned on the label. In this way, the definition of a preservative can be stated as a compound that maintain or elongate the storage life of a food product. Essential oils can be easily characterized as food preservatives due to their versatile biological activities.

2.9 Preservative Attributes of Essential Oils (EOs)

EOs are normally described by a strong scent and these blends comprise more than 200 constituents who can be characterized fundamentally into volatile and nonvolatile

components (Hanif et al., 2019). Around 90% to 95% of the EO is comprised of the volatile components and this comprises of sesquiterpene hydrocarbons, mono-terpenes and their oxygenated fractions alongside aliphatic aldehydes, esters and alcohols. The nonvolatile components, nonetheless, constitutes around 5% to 10% of the entire oil, which for the most part contains unsaturated fats, waxes, sterols, coumarins, hydrocarbons, carotenoids and flavonoids (Basile, Jimenez-Carmona & Clifford, 1998; De Castro, Jimenez-Carmona & Fernandez-Perez, 1999).

The phenolic constituents as indicated significantly contributes to the antibacterial properties of essential oils (Burt, 2004). These phenolic compounds contain at least one aromatic ring containing one or more hydroxyl groups and are produced as secondary metabolites by plants (Michalak, 2006). Phenolic compounds are mainly biosynthesized from aromatic amino acids by means of the shikimate pathway. The importance of this pathway is that under ordinary growth conditions, 20% of the carbon fixed by plants moves through this biosynthetic pathway. The aromatic amino acids Phenylalanine, and tryptophan, tyrosine is biosynthesized through this pathway and are later used for building up of proteins or converted by means of phenylpropanoid metabolic pathway to secondary metabolites, for example, phenolic compounds (Diaz, Bernal, Pomar & Merino, 2001). Phenolic compounds are subdivided on the basis of number of carbon atoms attached to the basic framework of phenolics (Michalak, 2006). Antibacterial activities of essential oils were observed by Burt (Burt, 2004). The antibacterial attributes of thyme EOs for example, have been accounted for to be for the most part contributed by carvacrol and thymol, which are the phenolic fractions of the oil. Carvacrol and thymol are structurally alike with a minor difference is the attachment of hydroxyl group to phenolic ring in thymol. The

two substances seem to alter the cell membrane integrity (Lambert, Skandamis, Coote & Nychas, 2001). Carvacrol and thymol can break down the outer envelope of Gram-negative microbes, leading in diffusion of lipopolysaccharides and alleviating the porousness of the cytoplasmic layer to Adenosine Triphosphate (ATP). This mechanism was shown to be unaltered by the presence of magnesium chloride ions (Helander *et al*., 1998). Minor fractions of EOs are involved in the synergistic activities with other fractions thereby playing an important role in the biological activities of EOs. Nevertheless, phenolic compounds are more potent to display antibacterial actions. These terpene phenols are joined to the amine and hydroxylamine groups of the proteins of the bacterial cell membrane, consequently altering their permeability consequently cell lysis (Juven, kanner, Schved & Weisslowicz, 1994). Olmedo et al., (2013) assessed the impact of oregano and rosemary EOs on the oxidative and fermentative qualities of seasoned cheddar with cream cheddar base. They concluded that oregano EO possessed anti lipid oxidation properties. Goni showed that Gram negative bacteria (*Escherichia coli*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*) and four Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Enterococcus faecalis*) can be inhibited by the combined vapors of rosemary and oregano (Goni, Lopez, Sanchez, Gomez-Lus, Becerril & Nerin, 2009). This study confirmed the inhibitory effect of EOs in vapor phase for the first time. Bounatirou et al., (2007) observed the biological activities of EOs isolated from aerial parts of Tunisian Thymus capitatus at different maturity stages and collected from different geographical locations. Higher antibacterial action was seen with the blossoming and the post-flowering stage EOs when the activities of these EOs were compared with synthetic antibiotics.

2.10 Pest Repellant Potency

Food items such as fruits, stored grains and other cellulose rich materials are highly contaminated by different vermin, for the most part arthropods. To prevent the food from being infested by these pests there is a dire need to preserve such commodities. The utilization of chemicals to control the pests raises a few human health issues. Different options being investigated is the utilization of compounds isolated from natural sources that have great adequacy and have no injurious effect on environment. EOs are among these chemicals to be used as repellants isolated from natural reserves and have a great deal of efficacy. In connection to human medical problems, the United States Environmental Protection Agency has affirmed the adequacy of a few EOs and therefore enlisted citrus EO, lemon EO and eucalyptus EOs as insect repellents to be used locally. Compounds derived from natural sources have shown to possess high potency, no toxic effects on humans and subsequently opted by consumers (Katz, Miller & Hebert, 2008). However, these decoctions may not always be a safer alternative as these compounds needed to be assessed by different assays which check their safety and efficacy. The broad investigation demonstrates that EOs from plant and their individual metabolites have shown ideal potential for repelling bugs, as well as different sorts of arthropods. The activity of these EOs is comparable to those exhibited by synthetic repellants when these EOs are mixed with fixative chemicals which overcome the problem of volatility of EOs and losing their function.

2.11 Use as Antibacterial Packaging

EOs have been affirmed to be of incredible significance in the control of microbial growth. As a rule, they are molded to target particular microorganisms so as to give higher bactericidal activity and therefore sustain quality and storage life of food

products. EOs have shown to be an active ingredient for food safety and packaging (Queen et al., 2002). The interest for the utilization of antibacterial packaging as an active packaging is on the expansion and due to the consumer demand for minimally processed foods are increasing these packaging materials can be of great use (Sadaka et al., 2014). EOs and particularly their bioactive properties such as antibacterial, antiviral, antifungal, insecticidal, and anti-oxidative attributes made them valuable to mankind (Kordali et al., 2005). The antibacterial potential of EOs in food systems is multi-dimensional factor and involves a variety of factors to contribute to the antibacterial efficacy.

Active component of food packaging material: Edible coatings are developments in the field of food safety and hygiene. These coatings are made up of complex carbohydrates, lipids and proteins. Despite the fact that protein and polysaccharide films possess better mechanical attributes, they have a poor conductivity to water molecules as they are hydrophilic in nature. The addition of Sodium Caseinate (SC) to these films can greatly improve their water barrier properties. SC is yielded as an end product of acid precipitation of casein which is soluble in water (Audic & Chaufer, 2005). These films have an advantage of having nutritional properties as well as they are well suited for food preservation due to their sensory attributes. In such manner, effectively enhanced the appropriateness of water vapor penetrability of the SC films for food items by incorporating oleic acid and beeswax (Fabra, Talens & Chiralt, 2008). These films can further be improved by the addition of EOs such as Ginger and cinnamon oil. Atarés et al., (2010) described SC-based films fused with cinnamon or ginger EO and confirmed that cinnamon oil remained homogeneously coordinated in the SC lattice, offering ascend to films with normal surfaces, yet extensively

influenced the optical properties of SC films (Preedy, 2015). Because of the collection of lipids amid drying, ginger oil caused a loss in smooth surface of films and lost gleam. The reason behind these undesirable properties is low oxygen permeability under low humid conditions (Aamer & Emara, 2016).

2.12 Lantana Camara

Lantana camara is a shrub that belongs to the family verbanacea of the kingdom plantae, and can be found in the tropical parts of the world in countries such as Mexico, Columbia, India, parts of America and sub-Saharan Africa (Saxena, Saxena & Khare, 2012). Mishra, (2014) published that Lantana is a low erect shrub that has stout prickles on the branches and stem, and has leaves that have strong smell of black currents, oval leaves that are serrated on both sides. It is a woody straggling plant with small flowers held in clusters (called umbels). The color usually is pink, white, yellow, violet, orange, and sometimes varying from white to red in various shades as shown in **Figure 1**. The leaves are arranged in opposite pairs and are broadly oval, rough with short hairs, with finely toothed edges along with a number of veins giving a wrinkled appearance. The leaves are 3 – 8 cm long and 3 – 6 cm wide, green in color. Leaves and stem are covered with rough hairs. *L. camara* is an important medicinal plant with several medicinal uses in the traditional medication system. It has been used to cure many health problems in different parts of the world (Bevilacqua *et al.,* 2011). Phytochemical screening of Lantana leaves has shown that the leaves consist of essential oils, phenols, flavonoid, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethananoid, oligosaccharides, quinine, saponins, steroids, triterpenes, sesquiterpenoides, tannin (Purkayastha *et a*l., 2012). In Ghana, lantana is locally called Abibriba by members of the Akan ethnic

group. The various components of the leaves are responsible for its medicinal abilities.

Figure 1: Images of Lantana camara

Different parts of *L. camara* are reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, quinine, saponins, steroids, triterpenes, and tannin as major phytochemical groups (Purkayastha *et al.,* 2012). These diversified phytochemicals attribute to multiple health potentials of plants and their extracts (Schmaal *et al*., 2017). Essential oil of lantana consists of 23 % caryophyllene oxide, 13 % spathulenol, 8 % humulen-1,2epoxide, 8 % β- caryophyllene, 7 % E- nerolidol and 5 % α- humulene (Sako *et al*., 2018). Chemical composition of oil is different for leaves of Lantana plants from different geographical locations.

Lantana oil is used as a wound antiseptic and the roots are used in tooth ache treatment (Geethalakshmi et al., 2013). Over the years, there has been considerable research on antioxidants, as potential therapeutic agents in preventing damage to the

human body as a result of the action of free radicals in the body (Chandra *et al*., 2014). Antioxidants are substances that donate electrons to remove free radicals, thereby protecting the body from oxidative stress. Free radicals are produced in the body as byproducts of ATP production but can also be formed due to external substances such as cigarette smoke, ozone and pesticides (Huang et al., 2019). Free radicals in the body exists mainly as ROS, reactive oxygen species or RNS, reactive nitrogen species. In most living organisms, particularly humans, ROS are continuously produced during the normal physiological processes of the organism but when produced in excess, they cause damage to biomolecules such as nucleic acids, lipids, proteins and carbohydrates. ROS can also initiate the peroxidation of lipids in the cell membrane of animal cells (Gulcin, 2008). Antioxidants produced by the body (peroxidase, glutathione and Catalase) and some dietary antioxidants such as vitamin E, vitamin C and beta-carotene are used to remove free radicals and repair damaged molecules (Middleton et al., 2000). If ROS are not removed from the body, they may cause oxidative stress, which may lead to degenerative diseases such as, Alzheimer's disease, cancer, arteriolosclerosis, diabetes mellitus, Hypertension and human aging (Stevanovic *et al*., 2009). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) are often used as food additives but have been known to be unstable and associated with liver damage and carcinogenesis (Shahidi & Ambigaipalan, 2015). In recent years, consumption of fruits and vegetables as part of the average diet has been known to contribute to the prevention of degenerative diseases such as stroke, pulmonary heart disease and different types of cancer (Polidori, 2003). The benefits obtained from eating these fruits and vegetables are due to the presence of polyphenols, flavonoids and vitamins. Of these phytochemicals, polyphenols are highly regarded as anti-inflammatory, antimicrobial,

antiviral and antioxidant agents (Steinmetz & porter, 1996; Hsin & Manley, 2012). Phytochemicals like carotenoids, tocopherols and tocotrienols, ascorbates and phenols are strong antioxidants and have carved out an important role in the health care system. There is renewed interest in finding out new natural antioxidants from living system for application in food, pharmaceuticals and cosmetics (Kumar *et al*,. 2014). Natural antioxidants are better alternatives due to the variety of structures and many biological processes they can perform. Several research findings have proven that polyphenols such as flavonoids like quercetin are very powerful antioxidants with higher potency than Ascorbic Acid (Barroso *et al*., 2014). Flavonoids are water soluble phenolic compounds which are extremely common in plants as glycosides (Kumar *et al*., 2014). Several lines of evidence support the role of oxidative stress in artherogenesis.

Oxidative stress occurs when there is an imbalance of free radicals and antioxidants in the body. Epidemiological studies suggest that low levels of antioxidants in the body are associated with increased risks of cardiovascular diseases (Deveraj & Jialal, 2003). Higher levels of vitamin E plasma has been associated with the absence of atherosclerosis in octogenarians (Cherubini *et al*., 2001). Several scientific works have proven the potential therapeutic use of *Lantana camara* in both in-vivo and in-vitro experimental conditions. Oleanonic acid isolated from *Lantana camara*, showed anticancer activity against Murine tumour and three human cancer cell lines which are, malignant skin melanoma, lymphoma and laryngeal carcinoma. Oleanonic acid showed promising cytotoxicity against skin melanoma (Sarma & Baruah, 2010). In-vitro tests showed that methanolic extracts of Lantana leaves exhibited cytotoxicity and inhibited the growth of Vero cell lines (Pour, Latha & Sasidharan, 2011). In vivo

studies showed that ethanolic extracts of *Lantana camara* decreased the extent of lipid peroxidation in the kidneys of Urolithic rats due to its significant antioxidant activity. In-vitro studies by DPPH and Nitric oxide free radical scavenging assays showed a high antioxidant property for the ethanolic extracts of Lantana (Mayee & Thosar, 2011). Verbascoside isolated from the leaves of L. camara exhibited inhibition of protein kinase C and antitumor activity (Herbert *et al*., 1991). The different varieties of L. camara have been reported to contain different types and levels of lantadenes and other phytochemicals, and may contain different bioactive constituents among these varieties of this Lantana species (Sharma *et al*., 1991). Antioxidant analysis of methanol extracts of Lantana leaves has shown that the total phenolic content of Lantana leaves was greater than 100mg/g of extracts and this value was equivalent to about 230 AAE.mg/g of extracts (Ascorbic Acid Equivalent) and 130 GAE.mg/g of extracts (Gallic Acid equivalents), other parts of the Lantana plant, that is the roots, fruits, stem and flowers exhibited relatively lower phenolic contents. The DPPH scavenging effect of methanol extracts of Lantana leaves showed a percentage (%) scavenging effect equivalent to an IC_{50} value of 16.02 ug/ml (Mahdi-pour *et al*., 2012). IC_{50} value is the concentration of extracts that is able to scavenge 50 % of DPPH free radicals (Kumar *et a*l., 2014).

2.13 Principle of Hydro Distillation

Hydro distillation is a traditional method for removal of essential oils. One of the simplest and oldest methods is water distillation (Meyer-Warnod *et al*., 1984). Essential oils are extracted using this method. Hydro distillation is a method of extracting essential oils from aromatic and medicinal plants. Hydro distillation (HD) is a traditional method for extracting essential oils, in which the essential oils are

evaporated by heating a mixture of water or another solvent and plant materials, then the vapors are liquefied in a condenser (Aziz et al., 2018). A condenser and a decanter are also used in the setup to collect condensate and extract essential oils from water, respectively. The extraction principle is based on isotropic distillation. Water or other solvents, as well as oil molecules, are present under atmospheric pressure and during the extraction process (heating). The French invented hydro-distillation (HD), which is a type of steam distillation. Essential oil extraction from dried plants and quality control of essential oils in the lab are covered by pharmacopoeia. Water immersion, direct vapor injection, and water immersion and vapor injection are the three types of hydro distillation. It's a multilateral procedure that can be applied to both large and small businesses. The amount of time required for distillation is determined on the type of plant material being processed. Longer distillation yields a modest amount of essential oil but introduces undesirable high boiling point chemicals and oxidation products.

2.14 Solvent Extraction

Solvent extraction, also referred to as Liquid–liquid extraction or partitioning, may be define as a method use to separate compounds of a mixture supported the solubility of each component. This is often done using two liquids that do not mix, for a example, water and an organic solvent. Within the Solvent-Extraction method of Essential Oils recovery, an extracting unit is loaded with perforated trays of volatile oil material and repeatedly washed with the solvent. Perfumes, vegetable oils, and biodiesel are all processed using solvent extraction. On sensitive plants, solvent extraction is employed to provide more essential oils at a reduced cost (Chrissie *et al*., 1996). In material analysis, this can be the foremost commonly used sample preparation process.

Because the procedure is proscribed by the compound solubility within the specific solvent utilized, the sort of additional heat given determines the standard and amount of the extracted mixture. Although the method is simple and effective, it's variety of drawbacks, including a protracted extraction time, significant solvent consumption, and inconsistent repeatability (Dawidowicz *et al*., 2008).

2.15 Gas Chromatography – Mass Spectrophotometric Analysis

The GC-MS equipment separates chemical mixtures (GC component) and identifies the components at a molecular level (MS component). GC-MS is one of the foremost precise equipment for assessing environmental samples on the market. When a mixture is heated, it separates into different compounds, which is how the GC works. the new gases are sent via an inert gas-filled column (such as helium). The separated substances pour into the MS as they emerge from the column aperture. The mass of the analyte molecule is utilized in mass spectrometry to identify chemicals. A library of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is taken into consideration as the only definitive analytical detector. The GC generates a chromatogram, a graph during which each separated substance is represented by a peak. The number of peaks within the sample indicates the number of distinct chemicals. The retention time for each compound is indicated by the position of each peak. **Figure 2** gives a schematic representation of the basic operation of the GC-MS.

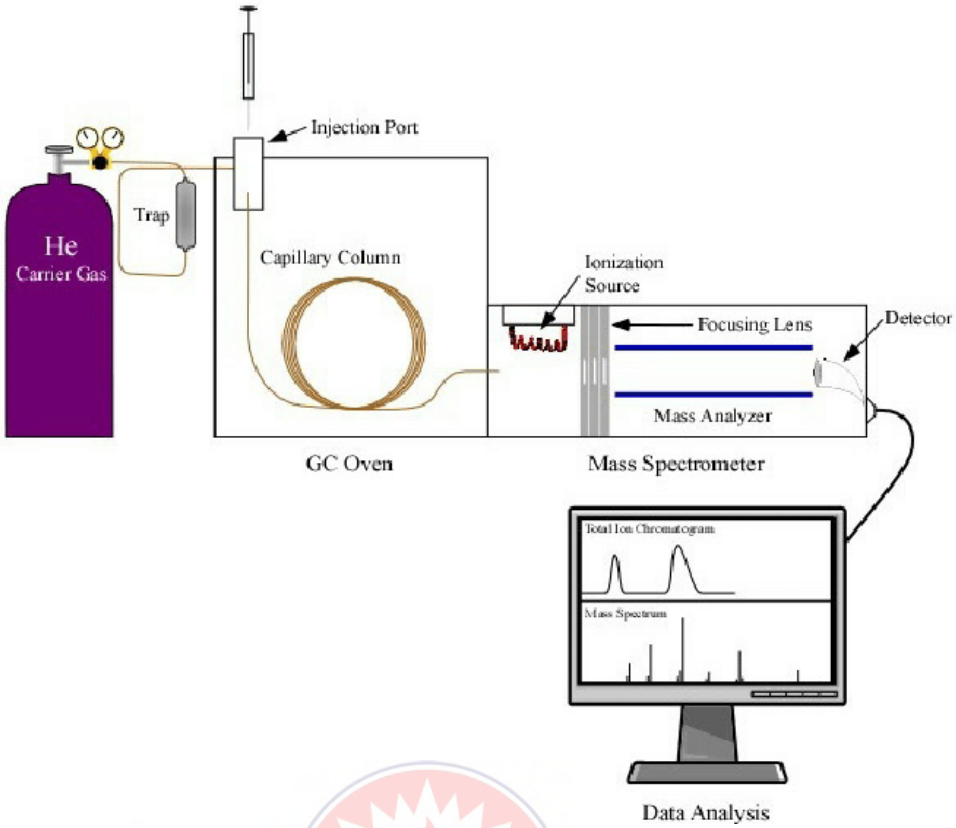

Figure 2: Schematic diagram of GC-MS. (Amirav et al., 2020)

2.16 Antioxidants in Plants

Plants are well known to produce a diverse array of secondary metabolites to engage with the world around them. The major classes of secondary metabolites are alkaloids, terpenes, and phenols. Phenols are typically classified as either low molecular weight simple phenols, or as high molecular weight polyphenols. The quantity and type of polyphenols created by plants vary considerably between species (El Gharras, 2009). Polyphenols became ubiquitous in the plant kingdom for protection of plants against UV radiation. Polyphenols further provide a repair mechanism for plants through oxidative polymerization by enzymes following mechanical damage. These compounds play an important role in plant growth and reproduction, providing an efficient protection against pathogens and predators, besides contributing to the

colour, sensory characteristics and nutritional properties of fruits and vegetables (Naczk & Shahidi, 2006).

Given the abundance of polyphenols in the typical human diet, and the potential bioactivities of phenols, their biological impact has been actively explored in the past decade. Some polyphenols are proposed as therapeutic agents for a variety of diseases or to promote general health (Škerget et al., 2005).

2.17 Roles of Antioxidants in Food and Human Health

In the past few decades, there has been growing evidence that oxidative stress and specific human diseases can be prevented by including in the diet plant foods that contain large amounts of antioxidants such as vitamins C, E or natural antioxidants such as flavonoids, tannins, coumarins, phenolics and terpenoids (Perumalla & Hettiarachchy, 2011). Dietary antioxidants can act as free radical scavengers, radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors (Karadag, Ozcelik, & Saner, 2009). Therefore, there is increasing interest in extending the range of antioxidants that can be used as food ingredients to prevent food oxidation. Furthermore, phenolic extracts prepared from plant materials (such as green tea, grape seed, aromatic herbs) are known to have antimicrobial effects against foodborne pathogens (Perumalla & Hettiarachchy, 2011). Antioxidants such as BHT, BHA and plant extracts have been widely used as additives, preservatives or supplements in foods (Zulueta et al., 2007). Ou et al., (2002) suggested that increasing the intake of dietary antioxidants may help to maintain the antioxidant status and normal physiological functions of the human body. Although antioxidants are recognized as important phytonutrients, presently, there is no recommended daily “total antioxidant” intake recommended due to the diversity and

complexity of antioxidants (Kaliora, Dedoussis, & Schmidt, 2006). Thus, *in vitro and in vivo* studies on the antioxidant properties and effects of foods such as fruit and vegetables are still required. Health diseases such as heart disease, macular degeneration, diabetes and cancer are all influenced by cellular oxidative damage. There has been increasing interest in the mechanism of action of antioxidants and whether they specifically intercept or remove free radicals from cells in the human body. Ames et al. (1993) as cited by Dröge, (2002) reported that antioxidants prevent injury to blood vessel membranes, optimize blood flow to the heart and brain, prevent cancer-causing DNA damage, and lower the risks from cardiovascular and Alzheimer's diseases. Jo et al. (2006) also indicated that antioxidants can prevent or slow the oxidative damage linked to various diseases such as carcinogenesis, atherogenesis and aging. Additionally, the flavan-3-ols from cocoa have been reported to have a vasodilatory effect which improved blood flow (Faridi et al., 2008). Chlorogenic acid, which is present in high amounts in coffee, is reported to have beneficial effect on cardiovascular disease (Bonita, Mandarano, Shuta, & Vinson, 2007) and to decrease the risk of type II diabetes (Bidel, Hu, & Tuomilehto, 2008). It is suggested that all these diseases might be retarded or prevented by protective compounds which have the ability to inhibit reactive oxygen species (ROS) formation, scavenge free radicals, or chelate metals (Panteleon et al., 2008). In the body, natural endogenous antioxidant systems have been developed to deal with the production of free radicals and have been divided into enzymatic and non-enzymatic groups. Examples of the enzymatic antioxidants are superoxide dismutase, gluthathione peroxidase and catalase (Rojas & Brewer, 2008) and non-enzymatic antioxidants are β-carotene, vitamin C, and vitamin E. There are also phytochemical

antioxidants, such a polyphenols, lycopene and lutein that can also protect the body from oxidation damage (Moon & Shibamoto, 2009).

Although there has been a focus on antioxidant effects of phytochemicals for many years, it is also recognized that non-antioxidant effects such as effects on cell signaling and gene expression are also important for health (Poulose et al., 2014).

To enhance the ability of cells to defend against these reactive species (free radicals), certain natural and chemical compounds called antioxidants are used. Antioxidant as relates to the biological system is any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays, prevents or inhibit oxidation of that substrate (Stocker & Keaney 2004). Such substrates include carbohydrates, fats and oil (lipids), proteins and deoxyribonucleic acid (DNA).

Biological antioxidants also include repair systems such as antioxidant enzymes, iron transport proteins (for example transferrin, albumin, ferritin and caeruloplasmin) and factors affecting vascular homeostasis (Frankel & Meyer, 2000). However, as it relates to food, antioxidant is a substance that in small quantities is capable of preventing, delaying or greatly retarding the oxidation of easily oxidizable materials leading to the development of rancidity or other flavour deterioration (Frankel & Meyer, 2000).

In a nutshell, the role of an antioxidant is to intercept a free radical before it can react with the substrate. Therefore, antioxidants of biological/therapeutic importance should have the ability to react/ trap the free-radical before it reacts with the susceptible substrate and initiate chain reaction.

2.18 Mechanisms of Antioxidants

Antioxidants may exert their effects by (i) inducing the biosynthesis of other antioxidants or defense enzymes (ii) suppressing the formation of active species (iii) reducing hydroperoxides (ROO.) and H_2O_2 (iv) sequestering metal ions (v) scavenging active free radicals (vi) repairing and/or cleaning damage (Valko *et al*., 2007). These mechanisms are illustrated below.

ROO. + AH ROOH + A.

RO. + AH ROH + A.

ROO. + A. ROOA

RO. + A. ROA

A. + A. AA

2.19 Characteristics of Antioxidants

Monohydroxy or polyhydroxy phenol compounds with various ring substitutions are the most current antioxidants utilized in foods. As they have low activation energy, the compounds can easily donate hydrogen and the resulting antioxidants radical is unable to initiate another free radical due to the stabilization of the delocalized radical electrons. The donation of hydrogen from the antioxidants, delay or minimize the initiation and propagation of free radicals' chain reaction. The resulting antioxidant free-radical is not subject to rapid oxidation due to its stability and can react with lipid free radicals to form a stable complex compound thus preventing them from being damaged.

2.20 Classification of Antioxidants

Antioxidants are categorized in two major groups namely natural and synthetic (David et al., 2016). These groups are in dietary antioxidants intakes and play major roles in maintaining the homeostasis of the oxidative balance. They are believed to protect humans from disease and aging. Sub-classification based on solubility contains oil-soluble antioxidants and water-soluble antioxidants while those based on the mechanisms of action are primary antioxidants (radical scavengers), secondary antioxidants (peroxide decomposers), and metal deactivators.

2.21 Natural (Primary) Antioxidants

Natural antioxidants are compounds found in foods such as fruits, vegetables, nuts, grains, seeds, animal tissues etc. which can be consumed without much processing. They are the chain breaking chemical compounds that react with lipid radicals to convert them into more stable products (Hurrell, 2003). Natural antioxidants can be extracted from plants, microorganisms and animal tissues. They may possess several drawbacks including high usage levels, low antioxidant efficiency, undesirable flavour or odour, and possible loss during processing. These drawbacks occasionally call for their replacement by synthetic chemicals, which are cheaper, more easily available, consistent quality, and have greater antioxidant activity. Other sources of these antioxidants in plants are cereals, legumes, tea, coffee, wine, beer, herbs and spices (thyme, nutmeg, clove, black pepper, ginger, garlic, curcumin and derivatives) (Alok *et al*., 2014; Cies'lik *et al*., 2006). The animal sources include milk, fish lipids, eggs, glutathione (non-protein in animal tissue), carotenes and ubiquinone (coenzyme Q).

Examples of natural antioxidants include Minerals: Minerals are co-factors of antioxidants enzymes. Their absence affects metabolism of many macromolecules such as carbohydrates, proteins and lipids. Examples include copper, iron, manganese, selenium and zinc.

Flavonoids: Catechins (proanthocyanidins) - are flavanols, or flavan-3-ols. Major catechins are catechin, epicatechin, epicatechin gallate (ECG), and epigallocatechin-3-gallate (EGCG) that inhibit lipid oxidation in red meat, poultry and fish.

Carotenoids: Carotenoids are a group of isoprenoids synthesized by plants and microorganisms. Most of them have long carbon chain skeleton with series of conjugated carbon double bonds. Typical examples are lycopene, lutein, α –carotene and β –carotene.

Anthocyanins: These are natural colorants/pigments of fruits and vegetables. They are novel antioxidants and potent inhibitors of lipid peroxidation as compared to other classic antioxidants. The positively charged oxygen atom in the anthocyanin molecule makes it a more potent and distinct hydrogen donating antioxidant compared to oligomeric proanthocyanidins (OPCs) and other flavonoids.

Honey: It is a natural product formed from nectar by honeybees and has antioxidant properties. These properties are due to the presence of phenolic and flavonoid compounds such as benzoic acid, cinnamic acid, chlorogenic acid, caffeic acid, ferulic acids, ellagic acid, gallic acid, syringic acid, myricetin, hesperetin, coumaric acid, isoramnetin, chrysin, quercetin, galangin, luteolin, kaempferol and isoramnetin. In addition, it contains ascorbic acid, other organic acids and trace elements such as selenium.

Vitamins: Retinol (Vitamin A) - Vitamin A is obtained from the diet either as preformed vitamin A or as provitamin A carotenoids. Preformed vitamin A is ingested as long-chained fatty acids of retinol in foods such as butter, eggs, milk, liver and fortified cereals. Provitamin A carotenoids (α -carotene, β -carotene, and -cryptoxanthin) are found in vegetables such as carrots, spinach, collards, pumpkins, and squash.

Ascorbic acid (vitamin C) - widespread in plant tissues. It is gamma lactone of simple sugar. The antioxidant activity of ascorbic acid depends on its capacity to bind metal ions.

Tocopherols (vitamin E) - widely distributed in plant tissues, vegetables, animal fats, wheat germ oil and cottonseed oil. Antioxidant activity depends on concentration and temperature.

Phospholipids - Lecithin and cephalin are important commercial antioxidants

2.22 Physicochemical Properties of Some Natural Antioxidants

Physicochemical properties of Retinol (Vitamin A)

A white crystalline powder. Practically insoluble in water or glycerol. Soluble in absolute ethanol, methanol, chloroform, ether fats and oils. It has a melting point of 137-138ºC.

Physicochemical properties of Ascorbic acid (Vitamin C)

Vitamin C is γ-lactone of a C6 acid (hexose) having double bond between C2 and C3. The naturally occurring form (L-ascorbic acid) is a white, crystalline powder, odorless. It is a highly water-soluble substance. The stability is lost by heat and aerobic oxidation, particularly in alkaline solutions and cooking. It is a powerful

reducing agent that is stable below pH 6.8 at room temperature. Oxidation of ascorbic acid yields dehydro-ascorbic acid.

Physicochemical properties of Vitamin E

Chemically defined as (2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol. It is a clear viscous oil (slightly pale liquid) that is odorless and darkens upon exposure to air. It is insoluble in water, miscible with ether and freely soluble in ethanol. The density is 0.950 g/cm^3 while the refractive index [n] D/20 is 1,503 -1,507.

2.23 Synthetic (secondary) antioxidants

Synthetic antioxidants are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions. They are compounds produced artificially and added to processed or pre-packaged food to prevent rancidity, browning.

The most used synthetic antioxidants include butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary-butylhydroquinone (TBHQ), nordihydro guaretic acid (NDGA), propyl gallate (PG), octyl gallate, dodecyl gallate and metal chelating agents (ethylene diamine tetra acetic acid, polyphosphatases).

These synthetic antioxidants are easier to use, more reliable, and generally more cost-effective than natural antioxidants. They are also expected to be non-toxic, highly active at low concentrations (0.01–0.02%), concentrate on the surface of the fat or oil phase, relatively stable and able to penetrate the cells (Li & Weng, 2017). They are also used in vegetable oils, baking and confectionary products, cosmetics and margarine.

2.24 Physicochemical properties of synthetic antioxidants

Physicochemical properties of Butylated hydroxy anisole (BHA)

Consists of, a mixture of 3-tert-butyl 4-methoxyphenol and tert butyl-4 methoxyphenol. White waxy flakes, soluble in fats, oils and most organic solvents but insoluble in water. The stability of fat containing BHA decreases when they are heated to high temperature. Melting point is 50-52 ºC (Andre *et al*., 2010).

Physicochemical properties Butylated hydroxy toluene (BHT)

Chemically defined as 2,6-di-tert butyl-4-methylphenol. It is a white crystalline solid. The compound is insoluble in water but soluble in fats, oils and most organic solvents. Melting point is 68 - 70 ºC.

Physicochemical properties of Tertiary-butylhydroquinone (TBHQ)

It is a beige or white-to-tan crystalline powder. Solubility decreases in order of alcohol > fats > water. It is stable to heat and is most effective antioxidant in preventing oxidation of frying oils. Melting point is 126-128 ºC.

Physicochemical properties of Nordihydro guaretic acid (NDGA)

Chemically defined as 2,3-dimethyl-1,4-bis (3,4 dihydroxy phenyl) butane. It is a white, crystalline solid. It is soluble in dilute alkali and slightly soluble in water. Melting point is 184-185 ºC. It is a white crystalline powder, sparingly soluble in water. Soluble in alcohols. Melting point is 146-148 ºC.

Octyl gallate and dodecyl gallate (Synthetic water insoluble antioxidant).

Physicochemical properties Octyl gallate and dodecyl gallate are white to creamy crystalline powder, insoluble in water and have melting points of 91 - 92 ºC and 94 - 96 ºC respectively.

Table 1: Advantages and Disadvantages of Natural and synthetic antioxidants

Antioxidant	Advantages	Disadvantages
Natural	Wide range of antioxidant activity Common components of food are not subject to any legislative restriction Many chemical compounds can be found in human diet High aqueous solubility Pure, efficient, inexpensive Easily available	Low antioxidant efficiency High usage level Complex mixtures of many compounds of different activities therefore subject to Physical/chemical interactions. Expensive Assay could be time-consuming.
Synthetic	Harmless if added in concentration permitted by legislation Pharmacological effective against different diseases Shorter duration of assay	High degree of toxicity Low aqueous solubility Suspect of being chemicals Subject to radiosensitization

2.25 Antioxidants in Human Body System

An extensive range of antioxidant defenses, both endogenous and exogenous, are present in human body system to protect cellular components from free radical induced damage. They are grouped into three main types: antioxidant enzymes, chain breaking antioxidants, and transition metal binding proteins.

2.26 Enzymatic Antioxidants

Catalase: catalyses the conversion of hydrogen peroxide to water and oxygen. It consists of four protein subunits, each containing a haem group and a molecule of NADPH (Kirkman *et al*., 2007). The enzyme is largely located within cells in

peroxisomes, which also contain most of the enzymes capable of generating hydrogen peroxide.

$$\text{Catalase - } Fe^{3+} + H_2O_2 + O_2 + 2H_2O$$

Superoxide dismutase (SOD) –catalyzes the detoxification of superoxide radical (O_2.) to hydrogen peroxide (H_2O_2) and oxygen.

The hydrogen peroxide must then be removed by catalase or glutathione peroxidase as indicated in the equation above. There are three forms of superoxide dismutase in mammalian tissues. They include (a) Copper zinc superoxide dismutase (CuZnSOD) which is found in the cytoplasm and organelles of virtually all mammalian cells, (b) Manganese superoxide dismutase (MnSOD) which is found in the mitochondria of almost all cells, (c) extracellular superoxide dismutase (ECSOD) which is synthesized by only a few cell types, such as fibroblasts and endothelial cells. It is the major SOD detectable in extracellular fluids and is released into the circulation from the surface of vascular endothelium following heparin injection.

Glutathione peroxidase - catalyzes the oxidation of glutathione at the expense of a hydroperoxide, which might be hydrogen peroxide or another species such as a lipid hydroperoxide (Alia et al., 2005). Glutathione peroxidase requires selenium or any of these essential minerals such as copper manganese and zinc at the active site. The enzyme is widely distributed in almost all tissues; however, highest concentrations are found in liver. The activity of the enzyme is dependent on the constant availability of reduced glutathione.

2.27 Chain Breaking Antioxidants (Radical Scavengers)

These are compounds that stop chain propagation by blocking or reacting with free radicals generated in the initiation stage of oxidation. To block the radicals, the scavengers donate hydrogen atoms that react with alkyl or peroxy radicals, leading to the formation of quinones or quinine imines (Lu et al., 2014). Such radical scavengers include lipid phase types (tocopherols, ubiquinol, carotenoids, flavonoids and aqueous phase type (ascorbic acid, uric acid, glutathione and other thiols).

2.28 Transition Metal Binding Proteins (Metal Deactivators)

These are chelating agents that function by trapping metal ions in their structure in the form of stable complexes to reduce the catalytic oxidation activity of the metal ions. Many proteins bind copper and iron ions and -this can make the protein a target of attack by hydroxide radical (OH·). Therefore, it is important for organisms that free transition metals in biological fluids are restricted to very low levels. Such chelating proteins are ferritin, lactoferrin and transferring (Zadak *et al*., 2009)

2.29 Application of Antioxidants

Antioxidants as preservatives: The addition of antioxidants to foods as preservatives, are usually done during various stages of production. Antioxidants are mostly added to fats and oils used in food production, other food products like: (i) vegetables and their products (ii) cereals and their bakery products, (iii) fruits and their products (iv) milk and their products (v) meat and their products, (vi) fish and their products, (vii) dried foods like sugars, honey, and beverage. Antioxidants can also be added to packaging materials such as polyethylene plastics, paper boards to prevent oxidation of the materials or permitting the antioxidants to migrate into the

foods and prevent the degradation of foods during processing and storage (Morton *et al*., 2000).

Antioxidants as Prophylactic agents: Antioxidants may function as immune modulators and are emerging as potential prophylactic agents in many diseases. Supplements of exogenous antioxidants can act directly to stop the free radical reactions, prevent lipid peroxidation, boost the endogenous antioxidant system and hence deliver the prophylactic activity. A number of antioxidants namely; β-carotene, coenzyme Q10, ellagic acid, epigallocatechin-3-O-gallate, genistein, lycopene, indole-3-carbinol, quercetin, vitamin C and vitamin E have been found to be pharmacologically active as prophylactic agents against cancer, cardiovascular diseases, and others. Furthermore, cereals, legumes, spices, vegetables, fruits, crude palm oil, soybean oil, cod liver oil, sprouts, peppers, whole grain, honey, walnuts and black tea can prevent cardiovascular disease induced by oxidative stress, due to presence of unique dietary antioxidant components. Thus, targeting oxidative stress or boosting the endogenous levels of antioxidants by the use of antioxidants is likely to have beneficial outcome in the management of several disorders (Ratnam *et al*., 2006).

2.30 Antioxidants as therapeutic agents

Cardiovascular disease: Antioxidant vitamins by potentiating endothelial nitric oxide levels, increase in plasma antioxidant capacity, inhibit vascular inflammation, lipid peroxidation, platelet aggregation and oxidation of low-density lipoproteins (LDL) can be beneficial in the treatment of endothelial dysfunction. Other potential mechanism of actions include reduction in intracellular ROS generation, induction of

GSH, reduction in matrix metalloproteinase production, anti- inflammatory responses related to cardiovascular health (Lades *et al*., 2004; Khan *et al*., 2017).

Cancer: Antioxidant diet or consumption of antioxidants like vitamins E, C, and selenium may have potential role in enhancing the efficacy of cancer treatment (Dutta, 1959; Field, 2003). Vitamin E acts by increasing (a) humoral antibody production, (b) resistance to bacterial infections (c) cell-mediated immunity, (d) T lymphocyte response. It also inhibits mutagen formation, repairs membranes and DNA, blocks nitrosamine formation. Vitamin C displays its anti-carcinogenic activity by (a) blocking the formation of nitrosamines and fecal mutagens; (b) enhancing the immune response; (c) accelerating detoxification of liver enzymes. Antioxidants such as L-cysteine, N-acetyl cysteine (NAC), thiols, green tea polyphenols can block activation of NF-KB while β-carotene through its photo-protective property helps to protect against ultraviolet (UV)-light-induced cancer. Other antioxidants may act by (a) scavenging constitutive H_2O_2 including other free radicals or generate additional amounts of H_2O_2 to inhibit proliferation of cancer cells, (b) preserving normal cell cycle regulation, (c) inhibition of proliferation and inducing apoptosis, (d) inhibition of tumour invasion and angiogenesis (e) suppression of inflammation, and stimulation of phase II detoxification enzyme activity.

Diabetes: Antioxidant vitamins and phytochemicals with antioxidant activities are used to manage patients with diabetes and also prevent diabetic complications. Such phytochemicals include cinnamic acids, coumarins, diterpenes, flavonoids, lignans, monoterpenes, phenylpropanoids, tannins and triterpenes. They act by decreasing lipid peroxidation and oxidative stress markers in diabetic patients.

Rheumatoid Arthritis: Antioxidants like vitamins, β-cryptoxanthin, supplemental zinc, fruits and cruciferous vegetables are potential agents for therapeutic management of rheumatoid arthritis. Different studies have reported that the use of antioxidants as supplements with the conventional drugs yields even better results and the treatment of rheumatoid arthritis. They act by increasing total thiols, glutathione resulting in the lowering of oxidative stress and the resultant inflammatory damage.

Neurodegenerative diseases: Dietary intake or supplementary intake of natural antioxidants like vitamin C, vitamin E, β-carotene can ameliorate a variety of neurological disorders. Also, use of antioxidants like flavanoid, coenzyme Q10, α-lipoate, melatonin, phenyl-alpha-tert butyl nitrone and GSH-glycosid have shown beneficial effect in neurodegenerative diseases. These antioxidants act as therapeutic barrier to oxidative stress by preventing oxidation of proteins, lipid peroxidations and generation of reactive oxygen species (ROS).

Gastrointestinal diseases: Antioxidant is the most effective drug in the treatment of chronic inflammatory bowel disorders. Natural antioxidants, like vitamins A, C and E have been used in the treatment of gastrointestinal tract diseases. Synthetic antioxidants such as 5-amino-salicylic acid, N-acetyl cysteine, biotin, selenium, zinc, manganese, copper, magnesium, folic acid and coenzyme Q have also been used in GIT diseases such as liver disease and acute pancreatitis.

Renal disorders: Ascorbic acid (vitamin C) is most prominent antioxidant, exerting beneficial effects by an inhibition of lipid peroxidation and by reducing endothelial dysfunction. Other antioxidants employed in renal disorders are selenium and vitamin E.

Pulmonary disorders: Administration of vitamin C, vitamin E, or combination of both, selenium, zinc, and garlic oil have shown beneficial effects in pulmonary

disorders. Vitamin E acts by preventing the chain reaction involved in lipid peroxidation, while vitamin C acts to quench radicals inside the cell. Flavonoids as scavengers of nitric oxide have helped to reduce asthma inflammation by decreasing histamine release, arachidonic acid metabolism and cytokine productions (Heim *et al*., 2002). Treatment of chronic obstructive pulmonary disease (COPD) has been achieved using thiol antioxidants and mucolytic agents namely glutathione, N-acetyl-L-cysteine, erdosteine, fudosteine, carbocysteine and dietary polyphenols (curcumin, resveratrol, catechins/quercetin. Furthermore, increase in selenium administration may suppress asthma inflammation by saturating glutathione peroxidase and down-regulates the transcription factor NF-Kb.

2.31 Pharmacokinetics of Antioxidants

The pharmacokinetics of some major classes of antioxidants include maintaining the homeostasis of the oxidative balance (Rigotti, 2007). Common food carotenoids present in fresh vegetables/salads and in fruits, (bananas, mango and papaya) have been found to be bioavailable. The beta-carotene has blood serum of 0.28 - 0.52 micro mol/L while blood serum of 0.2 - 0.28 and 0.29 - 0.60 have been obtained for lutein and lycopene respectively. All-trans-carotenoids have a better bioavailability than the 9-cis-forms. Absorption is thought to occur by passive diffusion following release from the food matrix in the gastrointestinal tract. The degree of bioavailability depends on the type and original concentration of the carotenoid (s). Food additives, such as oil or co-administration of soy germ or testosterone have enhanced the bioavailability of carotenoid compounds. Frequent meals and higher dietary contents have also enhanced the bioavailability of many carotenoids. Electrolytes such as calcium and magnesium ions have shown significant inhibition effect on the

bioavailability of carotenoids. In general, the relative bioavailability of carotenoids varies from less than 10 % in raw, uncooked vegetables to 50 % in oils or commercial preparations.

Dietary carotenoids following distribution could accumulate in many tissues including the liver, adipose, serum, breast milk, adrenal, prostate, macula, kidney, lung, brain, and skin. Elimination of carotenoids takes several days with elimination half-life of 5 -7 and 2 - 3 days for beta-carotene and lycopene, respectively. Several oxidized metabolites of carotenoids are known.

Polyphenols: Polyphenols show poor bioavailability probably due to such factors as poor absorption, instability, excessive metabolism or intestinal microbial transformation. Different dietary polyphenols show different rates of absorption and bioavailability. For example, isoflavones are the best absorbed dietary flavonoids, flavanols, flavanones and flavonol glycosides are intermediate, whereas pro-anthocyanidins, flavanol gallates and anthocyanins are the least absorbed. Absorption into systemic circulation which is by passive diffusion may be influenced by the matrix in which they are consumed. Absorption is also affected by dosage, vehicle of administration, antecedent diet, sex differences, individual genetic properties and the microbial population of the colon. Despite low bioavailability many polyphenols still display therapeutic antioxidant activities.

Microemulsion, nanoemulsions and other nano-size formulations have been found to enhance the bioavailability of polyphenolic compounds. Distribution into tissues is rapid. Liver is the crucial organ responsible for various biotransformations of polyphenols. However, the intestinal mucosa, kidney and other tissues are also involved in the metabolism of polyphenols. The most abundant metabolic

biotransformation of polyphenols are oxidation, reduction, hydrolysis and conjugation with O-methylation, sulphate and glucuronate. Excretion is by bile recirculation or directly by the enterocyte back to small intestine reaching the colon with different chemical structures. Enhanced excretion in urine of easily recognizable conjugates, have been observed in foods with higher fat contents.

Vitamins: Vitamin A - The bioconversion of beta-carotene to retinal is dose-dependent, and ranges between 27% and 2% for a 6 and 126mg dose, respectively. Several oxidized metabolites of carotenoids are known. Flavonols such as quercetin glycosides and rutin are predominantly absorbed as aglycones, bound to plasma proteins and subsequently conjugated to glucuronide, sulfate, and methyl moieties. The elimination half-life ranges from 12 -19 hours.

Vitamin C - is readily absorbed from the small intestines, peritoneum and subcutaneous tissues following dietary intake. It passes through the portal vein into general circulation. The bioavailability of vitamin C is dose-dependent. Saturation of transport occurs with dosages of 200-400 mg/day. Adrenal cortex and medulla, pituitary, liver and corpus luteum store vitamin C up to an optimal saturation level. It is distributed throughout the body and not protein-bound. The elimination half-life is about 10 hours. Excess Vitamin C is excreted in urine.

Vitamin E (α-tocopherol) - is the most abundant tocopherol in human tissue. Its bioavailability is estimated to be 50%. It has elimination half-life of 81 hours. As a fat-soluble vitamin, intestinal absorption, hepatic metabolism and cellular uptake of vitamin E follows that of other lipophilic molecules. The metabolism is primarily located in the liver. Conjugation of the metabolites takes place during metabolism,

resulting predominantly in sulfated and glucuronidated metabolites. Glycine–glucuronide and taurine-modified metabolites of vitamin E have also been reported.

2.32 Total Phenolic Content (TPC)

The Folin–Ciocalteu reagent, which is made up of tungstates and molybdates, is used to study the oxidation–reduction reaction process (Wolf *et al*., 2003). The approach is based on the phenolic compound reducing the mixture of heteropolyphosphotungsates–molybdate, resulting in the synthesis of blue-colored chromogen. Only under basic conditions, such as those provided by a sodium carbonate solution, do phenolic substances react with the Folin–Ciocalteu reagent. Under basic circumstances, the phenolic compound dissociates to generate a phenolate anion, which reduces the Folin–Ciocalteu reagent, which is a combination of tungstates and molybdates, resulting in a blue-colored solution. The absorbance values from a spectrophotometer can be used to determine the color intensity of the generated blue chromogen.

2.33 Total Antioxidant Capacity (TAC)

In the total antioxidant capacity assay protocol, the Cu^{2+} ion is converted to Cu^{+} by both small molecule and protein antioxidants. The Protein Mask prevents Cu^{2+} reduction by proteins, enabling the analysis of only the small molecule antioxidants. The reduced Cu+ ion is chelated with a colorimetric probe giving a broad absorbance peak around 570 nm, proportional to the total antioxidant capacity.

2.34 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical method produces a violet solution in ethanol and is an antioxidant assay based on electron transfer. In the presence of an antioxidant molecule, this free radical, which is stable at room

temperature, is diminished, resulting in a colorless ethanol solution (Prior *et al.*, 2005). The DPPH test is a simple and quick technique to evaluate antioxidants using spectrophotometry and it may be used to evaluate multiple items at once.

2.35 UV-Vis Spectrophotometric Analysis

The principle behind the UV-Visible Spectrophotometry is based on chemical compounds absorption of ultraviolet or visible light, which results in the formation of different spectra. The interaction of light and matter is the basis of spectroscopy. Excitation and de-excitation occur as matter absorbs light, resulting in the formation of a spectrum.

When matter absorbs ultraviolet light, the electrons inside it become excited. This leads to leap from a ground state to an excited state (an energy state having a little amount of energy connected with it) (an energy state with a relatively large amount of energy associated with it). It is worth noting that the difference between the energies of the electron's ground and excited states is always equal to the quantity of ultraviolet or visible energy it absorbs.

The inside of the T70 UV-Vis spectrophotometer chamber was checked to assure that the appropriate sample holder was in place for liquid sample (the essential oil extract). The T70 UV-Vis spectrophotometer was turned on by pressing the power button in the front of the unit. The Carry WinU was opened to click on scan to start the program. The setup button was clicked to set the experimental parameters; Carry Tab to change the wavelength range setting, the Baseline Tab to select zero/ baseline corrections. The OK button was clicked after the parameters were set. The blank solution was transferred into the cuvette and placed in its holder with the transparent face facing the light source. The start button was pressed and the absorbance value

displayed on the monitor. This procedure was repeated for each sample analyzed. Standard calibration curves were then constructed from the standards and the concentrations of the essential oil extract determined from the equations obtained.

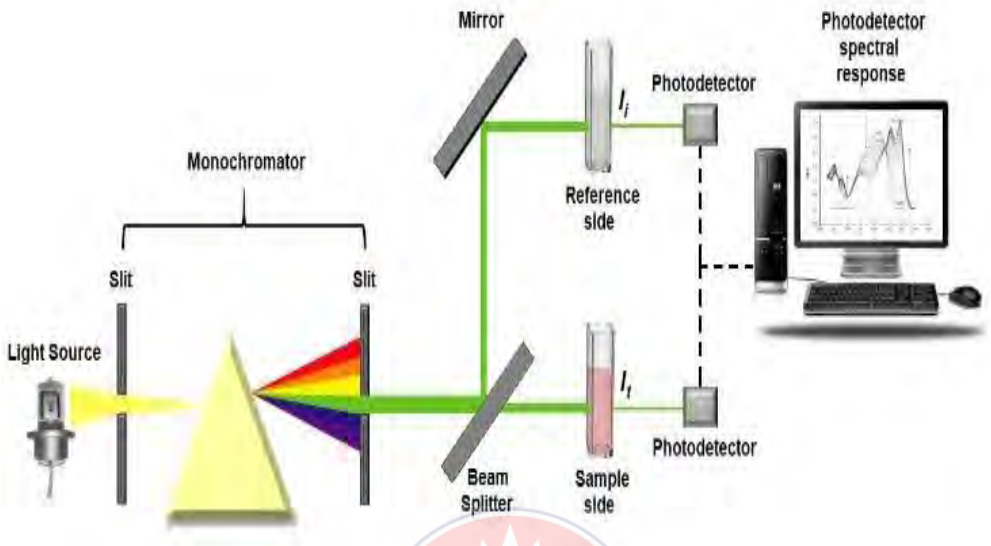

Figure 3: Schematic diagram of UV-Vis Spectrophotometer (Akash & Rehman, 2020).

2.36 Phytochemistry

The constituents of essential oil of Lantana camara are Sabiene (19. 6 - 21.5%), 1, 8 - Cineole (12.6 - 14.8%), ß-caryophyllene (12.7 -13.4%), α-humulene (5.8 - 6.3%), two rare sesquiterpenoids humulene epoxide-III and 8-hydroxy bicyclo germacrene (Kasali et.al.,2004), 1, 8-cineol (15.8%), sabinene (14.7%) and caryophylene (8.9%) (Sonibare & Effiong, 2008). Phytochemical screening revealed that leaf, stem and root of Lantana camara contained tannin, catachin, saponin, steroids, alkaloids, phenol, anthroquinone, protein, several tri-terpenoids, flavonoids, glycosides and reducing sugar (Mawal & Patil, 2019) which are mainly responsible for exerting diverse biological activities.

2.37 Pharmacological Activities

Lantana is basically used as an herbal medicine. All parts of this plant have been traditionally used for several ailments throughout the world. The plant extracts have been used in folk medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism and malaria. Further, it is used for the treatment of skin itches, as an antiseptic for wounds, and externally for leprosy and scabies have been documented. Beside this traditionally, Lantana is considered to be antiseptic, antispasmodic, carminative and diaphoretic agent (Barreto et al., 2010). The leaves of this plant have been used as an antitumoral, antibacterial, antihypertensive agent (Lingamaneni, Rao & Mishra, 2011) tonic and expectorant, while roots used for the treatment of malaria, rheumatism, and skin rashes (Sousa et al., 2015). Infusions of the leaves and other parts are used as an anti-inflammatory (Lingamaneni, Rao & Mishra, 2011) and added to baths as an anti-rheumatic agent. The methanolic extract of Lantana leaves shown healing potential against gastric ulcers and also prevents development of duodenal ulcers in rats (Kumadoh et al., 2021). The extracts from leaves have antipyretic and analgesic properties (Lingamaneni, Rao & Mishra, 2011). Extracts of lantana leaves have shown strong insecticidal and antimicrobial activity in numerous experiments (Mansoori et al., 2020); additionally leaves oil, stem and roots have sufficient bactericidal activity against pathogenic strains (Barreto et al., 2010). Lantana extract is powerful febrifuge (Abdollahzadeh et al., 2011) as the leaves and some other parts of lantana are poisonous, care must be taken when it is used medicinally. The lantana root extracts are the most toxic part and it has anticancer activity (Pour & Sasidharan, 2011). However, leaf and flower extracts obtained using different solvents shown to have

larvicidal activity (Chavan & Nikam, 1982; Kumar & Maneemegalai, 2008; Patel et al., 2011) flowers of the plant showed mosquitoes repellent activity (Dua et al., 2003) and thus flowers can be used as a mosquito control agent (Kumar & Maneemegalai, 2008). Additionally, storing potatoes with Lantana leaves nearly eliminates damage caused by Phthorimaea operculella Zeller, the potato tuber moth (Tsedaley, 2015).

CHAPTER THREE

METHODOLOGY

3.0 Overview

This chapter describes the methodology used in the study. It includes sample collection and treatment, extraction of bioactive compounds by hydro distillation of the essential oils, GC-MS analysis of extracts, Antimicrobial and antioxidant activity tests of the essential oil extracts.

3.1 Study Area

The fresh leaves of *Lantana camara* were harvested from Amamoma community, near the University of Cape Coast in Ghana. The plant was subsequently identified and authenticated at the herbarium unit of the School of Biological Sciences of the University of Cape Coast.

3.2 Research Design

Purposive sampling technique was used where only the fresh leaves of the plant *Lantana camara* was used for the extraction. The research was based on the analysis of the chemical composition, antioxidant capacity and insecticidal activity of essential oil extract from *Lantana camara* leaf obtained from Amamoma near the University of Cape Coast botanical gardens. The laboratory extraction process of the oil was carried out at the Natural Product Research laboratory of the Department of Chemistry, University of Cape Coast.

3.3 Materials and Equipment

3.3.1 Materials and chemicals used for the extraction of the essential oil.

Fresh leaves of *Lantana camara*

Quick-fit hydro distillation apparatus

Clevenger apparatus

Heating mantle

Distilled water

Filter paper

Analytical balance

Separating funnel

Retort stand and clamp

Diethyl ether

Anhydrous sodium sulfate

3.3.2 Materials and chemicals used for the antioxidant analysis

The chemicals used in this study include 2,2-ethylbenzothiazoline-6-picryhydrazyl(DPPH), butylated hydroxytoluene (BHT), ferrous chloride, potassium ferricyanide, catechin, ascorbic acid, quercetin, trichloroacetic acid (TCA), phosphate buffer, glacial acetic acid, folin-ciocalteu reagent, sodium carbonate, aluminium chloride, ascorbic acid and potassium acetate; analytical balance, test tubes, test tube rack, centrifuge, spectrophotometer, glassware, distilled water, micropipette with tips, were obtained from the department of Chemistry, University of Cape Coast. All other chemicals used, including the solvents, were of analytical grade without any further purification.

3.4 Sample Treatment

The leaves were sent to the Natural Product Research laboratory of the Department of Chemistry, University of Cape Coast for the essential oil extraction. The sampled leaves were weighed, washed and cut into smaller pieces to enhance its surface area for the oil extraction. This was then packed into a still compartment of the hydro distillation setup as shown in Figure 4.

3.5 Extraction of Essential Oil

About 500g of the treated leaves were packed into the still compartment (round-bottom flask) seated in the heating mantle. A volume of 300ml of distilled water was then added and the mixture distilled. As the temperature of the heating mantle was regulated, steam and hot air caused evaporation of the essential oil in the leaves. This caused the generated steam to have a mixture of both water and essential oil. The process was allowed to run for about 2 hours when the liquid portion of the mixture was fully distilled. The distillate was collected in a flat-bottom flask and allowed to cool to room temperature with aluminium foil seal to prevent volatilization of the essential oil. A 200 ml distillate was obtained after the hydro distillation procedure.

The essential oil in the distillate was extracted using separating funnel. The organic solvent, diethyl ether was used to extract the oil. For each 50 mL of the distillate in the separating funnel, 10 mL of the diethyl ether was added and shaken for several times with the release of pressure build up at time intervals. This was then mounted on a retort stand until there is clear separation of the organic layer and the aqueous phase. The organic phase (the essential oil) was transferred into a conical flask and covered with aluminum foil. The extraction with diethyl ether was repeated three times to ensure maximum extraction of the desired oils. The combined organic phase

extracts were dried using anhydrous sodium carbonate (Na_2CO_3) (covered with aluminum foil) for about 15 minutes to remove any traces of moisture. This was later filtered through a glass wool into a beaker to get rid of the drying agent. The diethyl ether solvent was removed through vacuo in a fume chamber leaving behind the desired essential oil. The essential oil extracted was then transferred into clean air-tight vials and kept in a desiccator for further analysis. The yield of the oil obtained was 12.68 g which represented a 2.54 % yield.

Figure 4: A Hydro-distillation setup

3.6 Gas Chromatography -Mass Spectrometry Analysis

100 mg of the sample was weighed and dissolved in dichloromethane (1 mL), vortexed for 10 s, sonicated for 1 hr., centrifuged at 14,000 rpm for 5 min and then dried by passing through anhydrous sodium sulphate before analysis (100 mg/µL) by

an Agilent Gas Chromatograph 7890A / 5975 C Mass Spectrometer in full scan mode. The extraction and analysis were carried out in triplicates.

An Agilent Gas Chromatograph 7890A / 5975 C Mass Spectrometer equipped with a Rtx-5mS fused HP-5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25 µm) (J&W, Folsom, CA, USA) and an FID detector were used for the quantitative determination of oil composition. Oven temperature was programmed as follows: 35 °C (5 min.) to 280 °C @10 °C/min (10.5 min) then to 285 °C @50 °C/min (29.9 min); run time 70 min. It was then ramp at 5 ºC/min rising to 280 °C. Injector temperature: 250 °C. Carrier gas: Helium with a flow rate of 1.25 ml/min, constant flow mode. Detector temperature: 250 °C, split ratio: 30:1. Diluted samples (1.0 µL, 1/100, v/v, in dichloromethane, DCM) were injected manually in the split mode. Identification of the oil components was based on their retention indices and mass spectra obtained from GC/MS analysis on an Agilent Gas Chromatograph 7890A / 5975 C Mass Spectrometer in full scan mode. The GC analysis parameters are listed above and the MS ones were obtained (full scan mode: scan time: 0.3 s, mass range was *m/z* 35 - 450) in the electron ionization mode at 72.4 KPa. All the data were the average of triplicate analyses. Detection and identification of the constituents of the oils was based on comparisons of the individual retention times and mass spectra with those obtained from using NIST'11, 08, 05, Adams and chemecol mass spectral databases and literature.

3.7 Antioxidant Assays

3.7.1 Total Phenolic Capacity

The Total Phenolic Capacity of the essential oil extract was determined by UV-Vis spectrophotometer using the Folin-Ciocalteu's reagent, as described by Wolf *et al.*

(2003). Gallic acid, as a standard, was prepared by dissolving 0.01g of the Gallic acid in a 100 mL ethanol, hence a 0.01% (%w/v) Gallic acid was obtained. Also, a 7.5 g of sodium carbonate (Na_2CO_3) was diluted to 100mL dilute water to obtain 7.5 % (%w/v) Na_2CO_3. 10mL of Folin-Ciocalteu's phenol was prepared to 100mL by adding distilled water to obtain a concentration of 10% (%V/V). 500 µl of the essential oil was pipetted to 500 uL ethanol into a test tube. 2 mL of the 7.5 % anhydrous sodium carbonate (Na_2CO_3) and 2 ml of the 10% Folin-Ciocalteu's reagent were added to the content of the test tube. This was done for three different test tubes and labelled; C1, C2 and C3. The final mixture was then incubated for about 15 minutes at a temperature of 50 ^oC A blue-black colouration (chromophore) occurred, indicating the presence of phenolic compounds in the essential oil. A blank solution was also prepared – without the essential oil extract. Varying concentrations of different test tubes, labelled, A (100 µL GA + 900 mL H_2O + 2 mL Na_2CO_3), B (200 µL GA + 800 mL H_2O + 2 mL Na_2CO_3), C (300 µL GA + 700 mL H_2O + 2 mL Na_2CO_3), D (400 µL GA + 600 mL H_2O + 2 mL Na_2CO_3), D (400 µL GA + 6000 mL H_2O + 2 mL Na_2CO_3), E (50000 µL GA + 500 mL H_2O + 2 mL Na_2CO_3), F (600 µL GA + 400 mL H_2O + 2 mL Na_2CO_3), G (700 µL GA + 300 mL H_2O + 2 mL Na_2CO_3) and H (800 µL GA + 200 mL H_2O + 2 mL Na_2CO_3). These were also incubated for 15 minutes at 50^oC. Using the T70 UV-Vis spectrophotometer, the absorbance was obtained for the samples (C1, C2 and C3) and the standards (A to H) respectively at a wavelength of 765 nm. A standard calibration curve of absorbance against concentration was constructed from the absorbance values of the Gallic acid.

3.7.2 Estimation of Total Flavonoid Content

The total flavonoid was determined using the method of Ordonez et al. (2006). A volume of 0.5m of 2 % $AlCl_3$ ethanol solution was added to 0.5ml of the extract solution. The mixture was incubated for 1hr at room temperature for yellow colour appearance; the absorbance was measured at 530 nm. Plant extract were evaluated at a final concentration of 0.1 mg/ml. Total flavonoids content was calculated as quercetin(mg/g) using the equation obtained from the curve: $Y = 0.0004x$, $R^2 = 0.9532$, where x is the absorbance and Y is the quercetin equivalent.

3.7.3 Total Antioxidant Capacity (TAC)

The Total Antioxidant Capacity (TAC) of the essential oil extract of the *Lantana camara* was examined using UV-Vis spectrophotometer according to the phosphomolybdenum assay outlined by Wolfe *et. al*. (2003). A 1.20 g Na_2SO_4 was dissolved in a 250 mL volumetric flask of H_2SO_4 at initial concentration of 12.48 M. Also, a 1.25 g of ammonium molybdate was dissolved into the 250 ml H_2SO_4 volumetric flask. The mixture was swirled to ensure homogeneity. Using ascorbic acid as the standard, a mass of 0.01 g of ascorbic acid ($C_6H_8O_6$) was dissolved with distilled water to 100mL to obtain a concentration of 0.01% (% w/v). 500 µL of the essential was pipetted into three different test tubes; C1, C2 and C3. 3mL of the H_2SO_4 together with sodium phosphate and ammonium molybdate (phosphomolybdenum) was added to each of the three test tubes. The test tubes were incubated for 60 minutes in a water-bath at 95 ^{O}C and allowed to cool to ambient temperature. Serial dilutions of the standard ascorbic acid were also prepared in ten different test tubes, labelled S_1 to S_{10}, in varying concentrations of 10 µg/mL to 100 µg/mL respectively, with the addition of 3 mL phosphomolybdenum solution. These

test tubes were also incubated for 60 minutes at 95 ^{O}C. A blank solution was also prepared. Using the T70 UV-Vis spectrophotometer, the various absorbance were obtained for the standard, essential oil and the blank. A standard calibration curve of absorbance against concentration was constructed from the absorbance values of the ascorbic acid.

3.7.4 DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the essential oil extract was determined as described by Prior *et. al*. (2005), using the DPPH assay. About 0.001 g of the powdery DPPH was weighed and dissolved in a 100 ml volumetric flask using methanol. The flask was covered with aluminium foil as the DPPH is volatile. Ascorbic acid was used as the standard test. 0.01 g of $C_6H_8O_6$ was dissolved in 100mL distilled water in a volumetric flask. 1mL of the 0.001% DPPH was added to C1 (200 µl *L. camara* + 800 µL methanol + 1 mL $C_6H_8O_6$), C2 (400 µL *L. camara* + 600 µL methanol + 1ml $C_6H_8O_6$), C3 (600 µL *L. camara* + 400 µL methanol + 1 mL $C_6H_8O_6$) and C4 (800 µL *L. camara* + 200 µL methanol + 1 mL $C_6H_8O_6$). These were allowed to stand for 30 minutes. A color change from purple to pale yellow indicates the reduction of the oil and their absorbance values were determined using the T70 UV-Vis spectrophotometer at a wavelength of 517 nm. The same procedure was used for the standard ascorbic acid at varying concentrations of 200 µg/mL, 400µg/mL, 600 µg/mL and 800 µg/mL. A standard calibration curve of absorbance against concentration was then constructed from the absorbance values obtained. The equation from the curve was used to calculate the concentration of the essential oil per its scavenging activity.

3.8 Insecticidal Assay

3.8.1 Test Concentrations for the Biological assays

The essential oil extract was dissolved in dimethylsulphoxide (DMSO) to prepare dilute solutions for laboratory testing. About 100 ml of 1 % DMSO stock solution of the essential oil extract was prepared by dissolving 100 mg of it in 1ml of DMSO and 99ml of distilled water. These solutions were kept in a screw-cap vial, with aluminium foil over the mouth of the vial. The solutions were shaken vigorously to dissolve or disperse the materials in the solvent. The stock solution was serially diluted to prepare the following concentrations 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml. These test concentrations were obtained by the addition of the appropriate volume of the stock solution to 100ml chlorine-free or distilled water in 200 ml beakers.

3.8.2 Larvicidal Activity

Larvicidal activity was carried out as described by (WHO, 2005) with minor modifications as described by Wachira *et al.,* 2014 using second instar larvae of the *Culex spp.*

The different concentrations of the essential oil prepared 1.25 mg/ml, 2.5 mg/ml, 5.0mg/ml and 10.0mg/ml were tested to determine their larvicidal potency. The range of concentrations yielding mortality in 12 hours, 24 hours, 48 hours and 72 hours were used to determine the LC_{50} and LC_{90} values which was compared to the LC_{50} and LC_{90} values of other synthetic insecticides.

The DMSO used as a solvent system for the dissolution of the test compounds served as a negative control by preparing 1% DMSO solution. Batches of 20 second instar larvae were transferred by means of droppers to the 200 ml beakers, each containing

100 ml of water. As a precautionary measure, unhealthy or damaged larvae were removed and replaced. The appropriate volume of dilution of the test compounds were added to the 100ml water in the beakers to obtain the desired target dosage starting with the lowest concentration. Four replicates set up for each concentration item for the various plant test oil extracts and controls were as well set up simultaneously with tap water only and 1% DMSO solution. The test containers were held at 25-28ºC and preferably a photoperiod of 12 - hour light followed 12 - hour dark (12L: 12D). Larval mortality was recorded, whereby the number of dead larvae in each test is counted and removed after 12 h, 24 h, 48 h and 72 h of exposure time. Moribund larvae were counted and added to dead larvae for calculating the percentage mortality.

3.8.3 Adulticidal Activity

Same concentrations for the larvicidal activity 1.25 mg/mL, 2.5 mg/mL, 5.0 mg/mL and 10.0 mg/ml were prepared and tested on the adult mosquitoes by the feeding assay method where an appropriate volume of the diluted stock solution was added to 20 ml 10 % glucose in a 20 mL vial.

Mosquitoes used for the experiments were obtained from the established laboratory-reared colonies of sample larvae initially collected from the three communities around the Cape Coast municipality (Amamoma, Akoktokyir and Ola communities). The larvae were fed daily on (3 mg/larvae/day) Tetramin® fish food (Tetra, Germany). The adult mosquitoes when emerged were kept in cubic cages (20 ×20 × 20 cm) in a separate room maintained at 26 ± 2°C, 70 – 80 % R.H. with a photoperiod of LD 12:12 h, the light being provided by a fluorescent lamp (40 watt). Both male and female mosquitoes were kept together after emerging and then separated during the

assay. Mosquitoes were fed on 10% glucose solution *ad libitum* after emergence. The conditions in the bioassay rooms were the same as those of the rearing room.

Adulticidal tests were carried out using the feeding assay method as described by Wachira *et al.,* 2014 with minor modification. The female adult mosquitoes (3 – 5 days old) previously starved for 12 h were released into the experimental cages (20 × 20 × 20 cm) and left to acclimatize for 1 hour. Twenty (20) adult mosquitoes were placed in a single cage. The prepared concentrations of the essential oil extract and controls were then introduced into the center of their respective cages and the mosquitoes were made to feed on the test and control solutions through an immersed rolled up filter paper (Whatman No. 1) with 5 cm of it exposed above the top of the 20 mL vial. The female mosquitoes fed on the essential oil extract dissolved in dimethyl sulfoxide (DMSO) with the various concentrations to be prepared in 10 % glucose solution contained in a 20 mL vial, while the control groups fed on a similar essential oil-free solution. The four different concentrations; 1.25, 2.5, 5.0 and 10.0 mg/ml of the essential oil were tested in three replicates per dose. The control solutions were 10 % glucose solution with water; 10 % glucose solution with 1 % DMSO. Again, to find out whether the mosquitoes were feed on the glucose solutions with the essential oil, two other groups of cages were prepared with 10% glucose solution without water and another without food (10 % sugar solution) and water. Daily mortality was recorded in all the mosquito groups for seven consecutive days.

3.9 Data Analysis

The data for the antioxidant assays were structured in tables, and the results were displayed in graphs. The data were analyzed using MS Excel version 2016 as the statistical package. The data were analyzed utilizing Excel's descriptive statistics tool

and Graphpad prism version 5.0. The oil extract's percent radical scavenging activity was calculated and compared to that of standard ascorbic acid for the DPPH. The TPC and TAC of the oil extract were calculated using the Gallic acid and Ascorbic acid standard calibration curves, respectively. The calibration curves were created by graphing absorbance against concentration in MS Excel and Graphpad. The oil extract's total phenolic content and total antioxidant capability were compared to each other.

Data from all replicates from the insecticidal assay were pulled together for analysis. The mortality data was subjected to probit analysis in calculating the lethal concentration values (LC_{50} and LC_{90}) and lower and upper 95% fiducial limits. The LC_{50}, LC_{90} and Chi-Square values were computed using the EPA (U.S Environmental Protection Agency) computer probit analysis program (version 1.5). The potency of the essential oil against the larvae of a particular arthropod vector and strain and the adult female Culex spp can then be compared with the LC_{50} or LC_{90} values of other insecticides.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Overview

In this chapter we discuss the results obtained from extraction and percentage yield of the oil, GC-MS analysis and bioactivity tests conducted as well as discussions on them.

4.1 Extraction and Percentage Yield of the Oil Extract

Hydro-distillation is a traditional method for extracting essential oils from plant tissues in which a suitable amount of water is poured into the compartment containing the materials, which is then brought to a boil (Azmir *et al*., 2013). This method was used to extract the essential oil from 314.0 g of fresh lantana camara leaves, with diethyl ether as the extracting solvent of choice due to its low water solubility (Guardiola-Albert et al., 2020). The oil extract produced had a mass of 42.0 g and a volume of 20.00 mL. This resulted in a percentage yield of 13.38 %, which is significantly greater than the 12.7 % reported by Ali-Emmanuel *et al*. (2003).

The essential oils extracted by Conventional hydro-distillation method was evaluated based on chemical composition using gas chromatography-mass spectroscopy (GC-MS). A total of 76 compounds were identified representing 99.40 % of the oil with (E)- Caryophyllene as the predominant compound in the oil extract constituting 20.6 %.

4.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Oil Constituent

The GC-MS results of the oil extract shows that oil extract contains mixtures of various groups of compounds. A split ration of fifty (50) was used so as to obtain

much clearer peaks. The following are table of the GC-MS results and chromatogram of the oil extract.

Table 2: GC-MS result of the oil extract

RT	Library/ID	Peak Area_1	Peak Area_2	Peak Area_3	Mean Area	Abundance(%)
3.48	2-Pentanone	368884	405772	331995	368884	0.3
4.29	2,3-dimethyl-2-butanol	638409	702249	574568	638409	0.5
4.38	2-ethoxy-2-methylpropane	3532653	3885918	3179387	3532653	2.8
4.60	4-methyl-1-hexene	287393	316132	258653	287393	0.2
4.67	Methyl Isobutyl Ketone	354603	390063	319142	354603	0.3
4.95	3-methyl-3-pentanol	981055	1079160	882949	981055	0.8
5.23	4-methyl-2-pentanol	184856	203341	166370	184856	0.1
5.37	4-methylheptane	325097	357606	292587	325097	0.3
5.60	2-methyl-3-pentanol	125631	138194	113067	125631	0.1
7.02	2,4-dimethylheptane	384267	422693	345840	384267	0.3
7.57	2,4-Dimethyl-1-heptene	243982	268380	219583	243982	0.2
7.94	2E-Hexenal	306006	336606	275405	306006	0.2
8.00	3Z-Hexenol	6698962	7368858	6029065	6698962	5.4
8.15	4-methyl-octane	388874	427761	349986	388874	0.3
8.32	2E-Hexen-1-ol	131700	144870	118530	131700	0.1
8.36	1-Hexanol	728995	801894	656095	728995	0.6
9.78	α-pinene	499798	549777	449818	499798	0.4
10.63	β-pinene	1310637	1441700	1179573	1310637	1.0
10.69	Limonene	650071	715078	585063	650071	0.5
10.78	1-Octen-3-ol	2466746	2713420	2220071	2466746	2.0
11.00	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	289537	318490	260583	289537	0.2
11.10	3-Octanol	224100	246510	201690	224100	0.2
11.53	2,2,3,3-tetramethylhexane	139476	153423	125528	139476	0.1
11.59	4-methyldecane	423667	466033	381300	423667	0.3
11.64	Benzene, 1-ethyl-2,4-dimethyl-	244307	268737	219876	244307	0.2

11.71	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-	252120	277332	226908	252120	0.2
11.76	Eucalyptol	279391	307330	251451	279391	0.2
12.32	Nonane, 5-methyl-5-propyl-	428925	471817	386032	428925	0.3
12.43	Sabinene hydrate<trans->(IPP vs OH)	662909	729199	596618	662909	0.5
12.99	Linalool	3212801	3534081	2891520	3212801	2.6
13.43	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	279159	307074	251243	279159	0.2
13.75	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	464306	510736	417875	464306	0.4
14.09	endo-Borneol	175186	192704	157667	175186	0.1
14.28	Terpinen-4-ol	1551363	1706499	1396226	1551363	1.2
14.79	Verbenone	1218253	1340078	1096427	1218253	1.0
14.88	4,8-dimethyltridecane	786100	864710	707490	786100	0.6
15.28	5-Ethyl-5-methylnonadecane	175269	192795	157742	175269	0.1
15.37	4,6-dimethyldodecane	522073	574280	469865	522073	0.4
16.61	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	321992	354191	289792	321992	0.3
17.16	α-Copaene	867247	953971	780522	867247	0.7
17.30	β-Bourbonene	493989	543387	444590	493989	0.4
17.34	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	3390201	3729221	3051180	3390201	2.7
17.54	Tetradecane, 4-ethyl-	453627	498989	408264	453627	0.4
17.58	α-Selinene	665609	732169	599048	665609	0.5
17.63	Nonadecane	346868	381554	312181	346868	0.3
17.76	(*E*)-Caryophyllene	25704219	28274640	23133797	25704219	20.6
17.87	β-Copaene	2193445	2412789	1974100	2193445	1.8
17.96	α-Guaiene	305053	335558	274547	305053	0.2
18.13	(*Z*)-β-Farnesene	1298872	1428759	1168984	1298872	1.0
18.20	α-Humulene	16887538	18576291	15198784	16887538	13.5
18.30	α-Muurolene	876525	964177	788872	876525	0.7

18.45	γ-Muurolene	1125100	1237610	1012590	1125100	0.9
18.49	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	669011	735912	602109	669011	0.5
18.62	β-Selinene	1057188	1162906	951469	1057188	0.8
18.70	4-epi-cubedol	1862459	2048704	1676213	1862459	1.5
18.73	(-)-Aristolene	2856078	3141685	2570470	2856078	2.3
18.81	Phenol, 2,4-bis(1,1-dimethylethyl)-	2241693	2465862	2017523	2241693	1.8
18.96	Cubedol	4578873	5036760	4120985	4578873	3.7
19.02	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-	1802365	1982601	1622128	1802365	1.4
19.34	Guaia-1(10),11-diene	522893	575182	470603	522893	0.4
19.44	1,Z-5,E-7-Dodecatriene	549712	604683	494740	549712	0.4
19.50	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	379053	416958	341147	379053	0.3
19.62	4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-	148571	163428	133713	148571	0.1
19.74	Spathulenol	1486019	1634620	1337417	1486019	1.2
19.82	Caryophyllene oxide	6194854	6814339	5575368	6194854	5.0
19.92	Globulol	1175725	1293297	1058152	1175725	0.9
19.99	1,2-Dihydropyridine, 1-(1-oxobutyl)-	516360	567996	464724	516360	0.4
20.12	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	4651637	5116800	4186473	4651637	3.7
20.30	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1030000	1133000	927000	1030000	0.8
20.43	Epizonarene	2052237	2257460	1847013	2052237	1.6
20.50	α-Cubebene	1943027	2137329	1748724	1943027	1.6
21.35	Aromadendrene	713340	784674	642006	713340	0.6
22.17	Cycloisolongifolene, 8,9-dehydro-	224530	246983	202077	224530	0.2
24.58	Eicosane	122456	134701	110210	122456	0.1
25.13	Phytol	1055072	1160579	949564	1055072	0.8
38.91	Stigmastanol	168780	185658	151902	168780	0.1

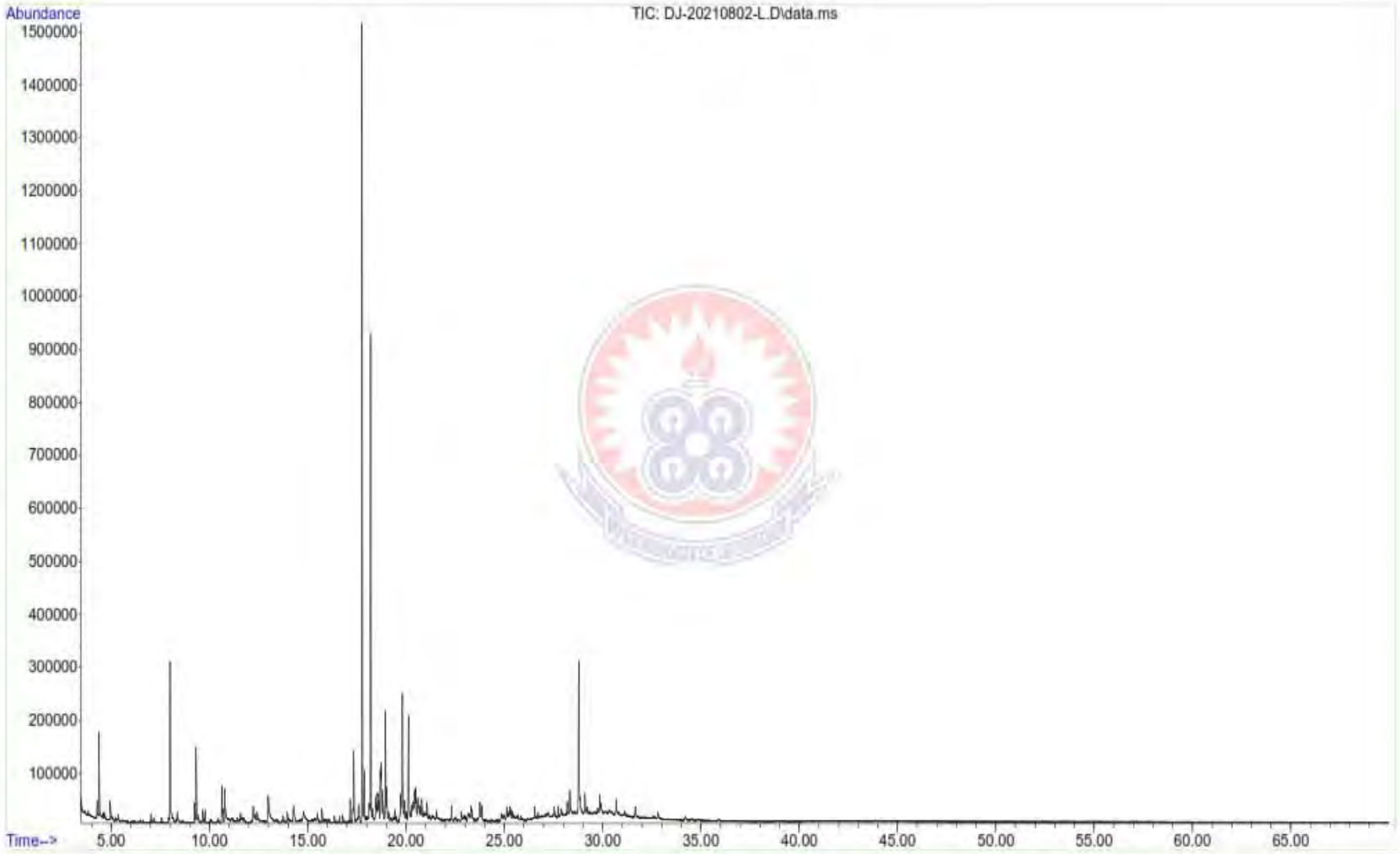

Figure 5: Non expanded total ion chromatogram without retention time annotation

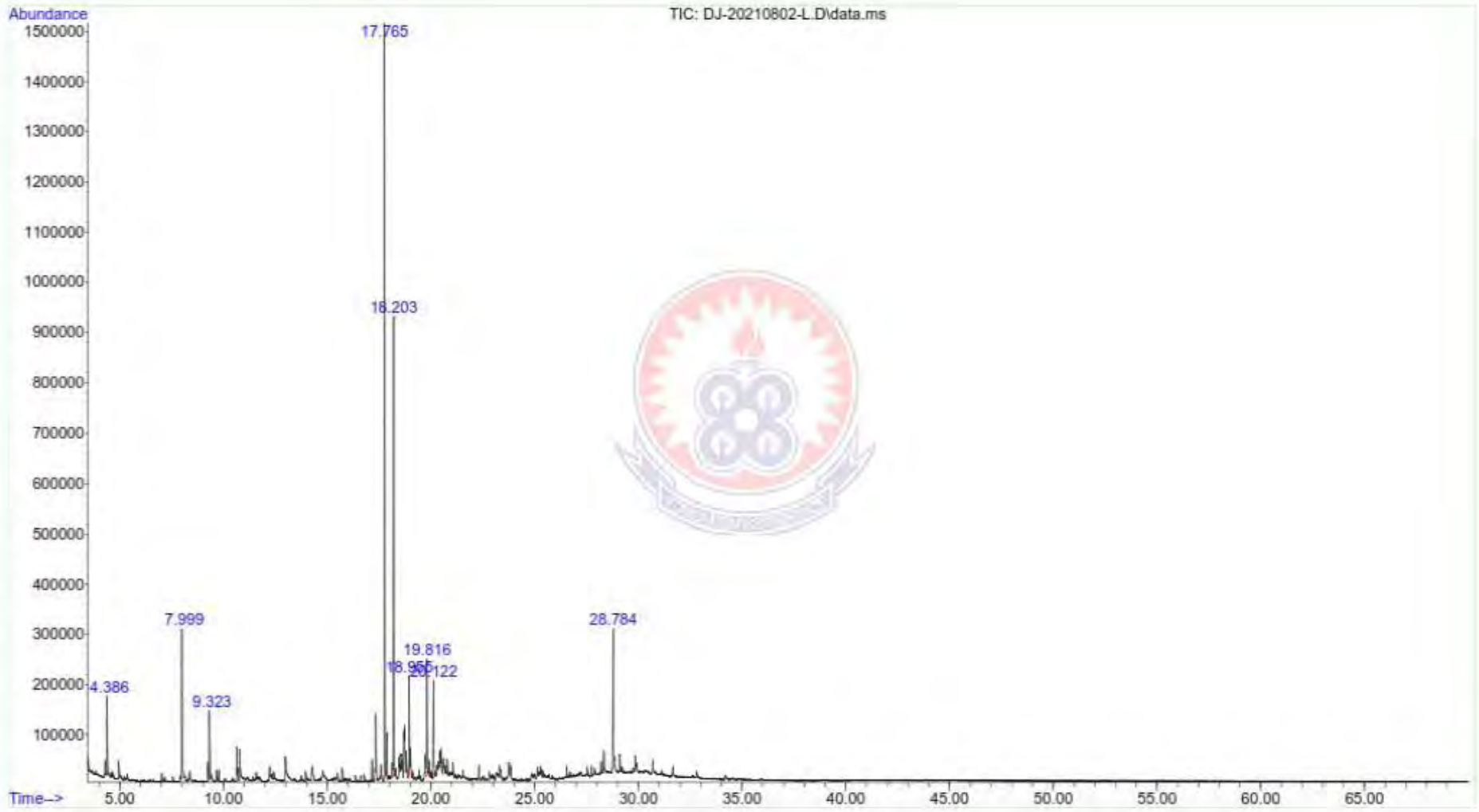

Figure 6: Non expanded total ion chromatogram with retention time annotation

The chromatograms from the GC-MS analysis as shown in **figure 5 and figure 6** represent the chemical composition of the essential oil extract from the fresh leaves of *Lantana camara* oil. The respective retention indices and relative peak area, along with their percentage abundance (%) are indicated on the chromatograms. The percentage peak areas were taken to represent the proportion of each compound relative to the total. The analysis revealed the identification of seventy-six (76) unique constituents in the oil extract. The oil is consisted of a complex mixture of numerous compounds, many of which were present in minute quantities. The identified compounds comprise of 99.4 % of the total extracted oil. The results could be compared to those reported by Limberger et al. (2001), obtained through hydro-distillation method of oil extraction and GC-MS technique of *L. camara.* Limberger et al., (2001) also reported concentration of constituents of Lantana similar to that recorded for this analysis.

The 76 chemical components analysed by GC-MS can be found in **table 2**. The predominant constituent compounds of the oil extract are (*E*)-Caryophyllene and α-Humulene with percentage abundance of 20.6 % and 13.5 % respectively. Other major chemical components analyzed are 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl) (2.7 %), 3Z-Hexenol (5.4 %), Caryophyllene oxide (5 %), (-)-Aristolene (2.3 %), 12-Oxabicyclododeca-3,7-diene, 1,5,5,8-tetramethyl (3.7 %), 1,6-Cyclodecadiene, cubedol (3.7 %), Linalool (2.6 %) and 2-ethoxy-2-methylpropane (2.8 %).

The chemical composition of the essential oils extracted from the *L. camara* leaves was consistent with previously works reported by Passos et.al., (2012) with some differences within the relative quantities of the volatile compounds. The observed differences in composition of the extracted oil and other studies could be due to climate differences in the harvesting of the Lantana camara leaves topographical location difference as well as the season. Most of the isolated compounds, namely a 12-Oxabicyclododeca-3,7-diene, 1,5,5,8-tetramethyl, 1,6-

Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl, and α-Muurolene are known for pharmaceutical applications as was reported by Abirami and Rajendran, 2011. Thus, α-Muurolene, has been reported as having pain-relieving and anti-inflammatory properties, and it exhibits antifungal activity against dermatophytes. Oxabicyclododeca oil, which is rich in dodecene (> 73 %), has been reported to show antioxidant, antibacterial, and insecticidal activities (Hemalatha *et al*., 2014). Apart from the medicinal value of *L. camara* oil, the sustained demand for synthetic flavorings and fragrances to be used within the pharmaceutical, food, and cosmetic industries makes this essential oil valuable for exploitation in these industries additionally. Again, the method of extraction of these essential oils can also bring about differences in the chemical composition of the recovered oil. Some researchers had used methods such as Conventional hydro-distillation, Clevenger-assisted hydro-distillation, Steam distillation, Microwave-assisted distillation etc. These had led to differences in the constituent of the oil extract in their concentrations and some cases differences in the chemical constituents of the oil.

4.3 Determination of Total Phenolic Content (TPC)

The total phenolic content of the oil (TPC) is the antioxidant activity that tells the amount of phenolic content or phenols in the oil samples and their contribution to the antioxidant activity of the oil. The Standard calibration curve of Gallic acid as well as the Absorbance and Concentration values of TPC of the oil extracts are represented in Fig. 7 and **Table** 3 respectively. The total phenolic content was calculated using the graph in Fig. 7, and the standard curve equation is $y = 0.0402x + 0.1733$, with $R^2 = 0.988$, implying a 99 % correlation between the data sets. The concentrations of essential oil extract were calculated using this equation, and the average is 14.966 ± 4.43 µg/mL. Thus, the oil extract as calculated shows a total phenolic content (Gallic acid equivalent, mg/g) of 14.966 ± 4.43 mg GAE/g.

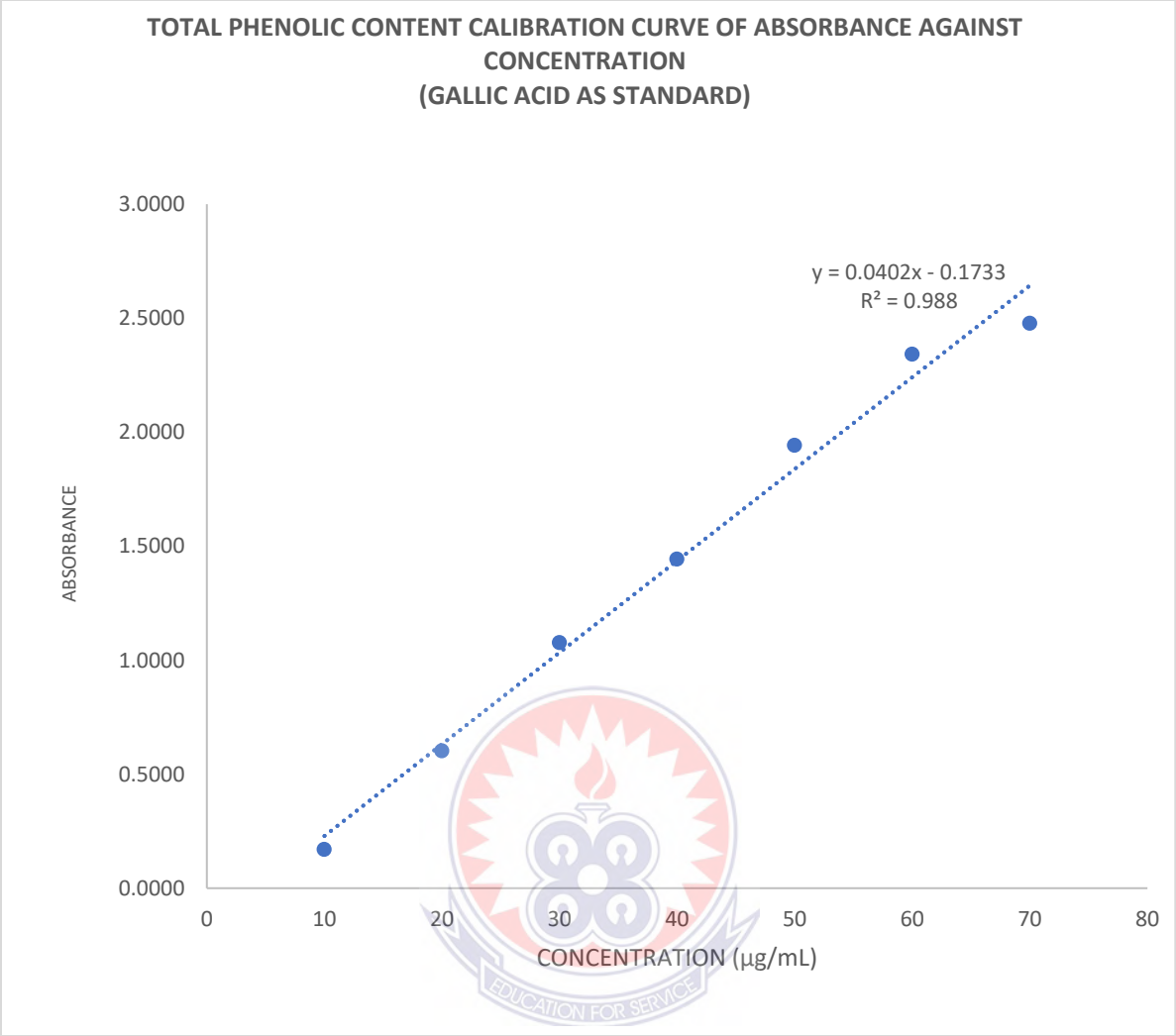

Figure 7: Standard calibration curve of Gallic acid

Table 3: Absorbance and Concentration values of TPC

SAMPLE	**ABSORBANCE**	**CONCENTRATION (µg/mL)**
C1	0.633	20.057
C2	0.343	12.843
C3	0.309	11.998
AVERAGE		**14.966 ± 4.43**

A comparison of the total phenolic content (TPC) and the total antioxidant capacity (TAC) of the oil extract as shown in Fig. 8 below, indicate a slight difference in activity of the TAC than the TPC. The R^2= 0.9033 (90 %) indicates a stronger correlation between the two data set. The P-value = 0.0036, therefore P<0.05, meaning there is a statistical significance difference their mean value. This means there are more phenolic contents present in the oil extract, with a lower antioxidant capacity.

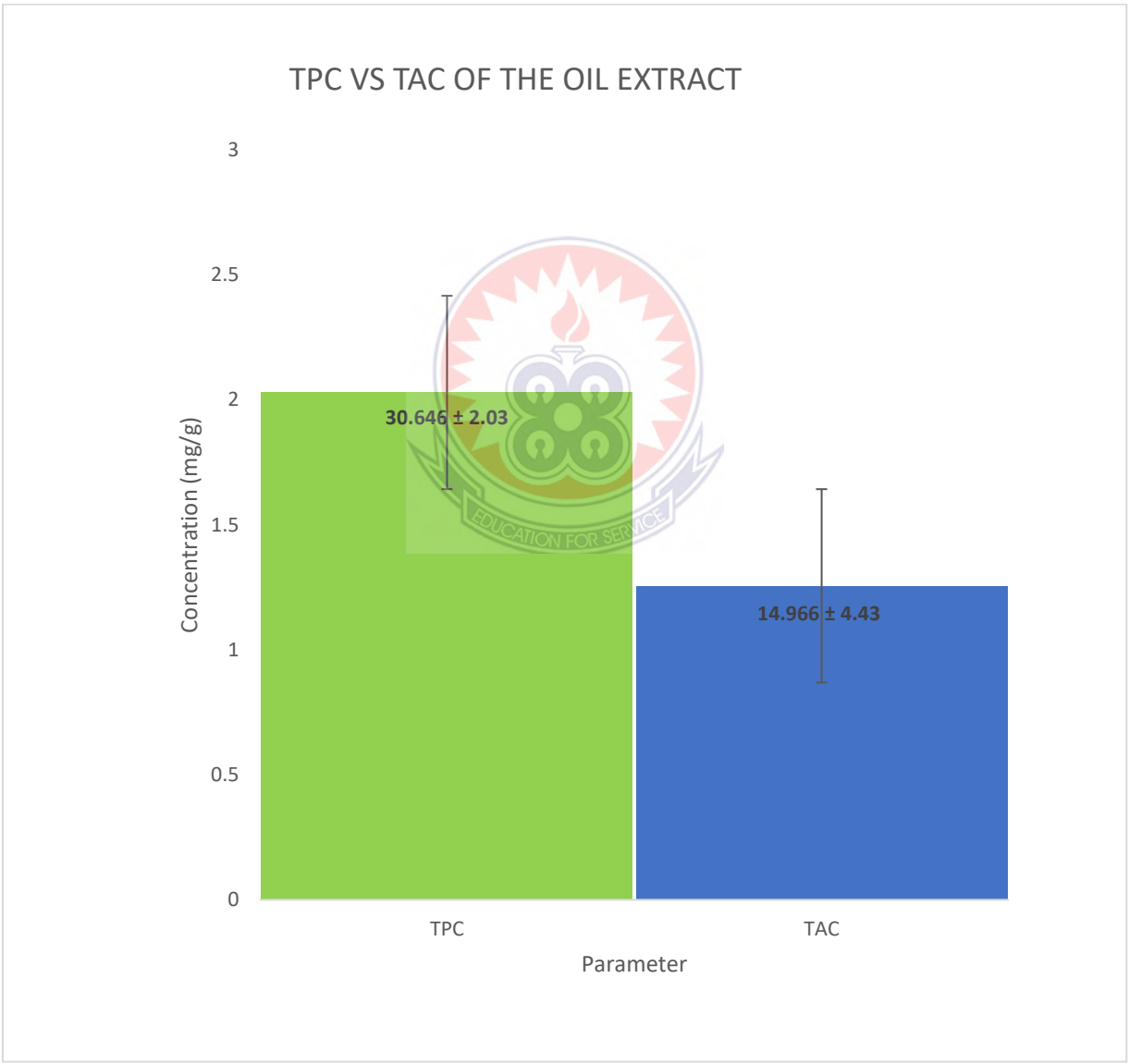

Figure 8: Comparison of TAC and TPC analysis of the oil extract

A phenol loses an H^+ ion to generate a phenolate ion, which decreases the Folic-Ciocalteu reagent in the basic reaction conditions (Fernandes et al., 2012). The change is measured using spectrophotometry. Because phenolics (including many flavonoids) include polar phenolic hydroxyl group(s), Gallic acid's greater TPC is understandable. Similarly, the oil extract's lower TAC could be explained in the same way.

4.4 Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) is a commonly used analyte for determining the antioxidant status of biological samples and can be used to assess the antioxidant response to free radicals produced in a given disease (Kohen & Nyska, 2002). Trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP), and cupric reducing antioxidant capacity (CUPRAC) are three different assays used to calculate a sample's TAC. This study reports the TAC values in mg AAE/g, where AAE is ascorbic acid equivalent. The assay is based on the fact that molybdenum (VI) is reduced to molybdenum(V) in the presence of a reducing agent (antioxidant), forming a green phosphomolybdate(V) complex, which can be evaluated spectrophotometrically at 765 nm (Prieto, Pineda & Aguilar, 1999; Alam et al., 2013). The assay involves an electron transfer (ET) mechanism. Many natural products, including phenols and flavonoids, can cause this reduction.

The **Figure 9** shows a plot of concentration against absorbance of the standard ascorbic acid, which gave a linear regression graph of $y = 0.0033x - 0.0078$, with $R^2 = 0.9462$, showing approximately a 95 % regression of how well the data set fit the model.

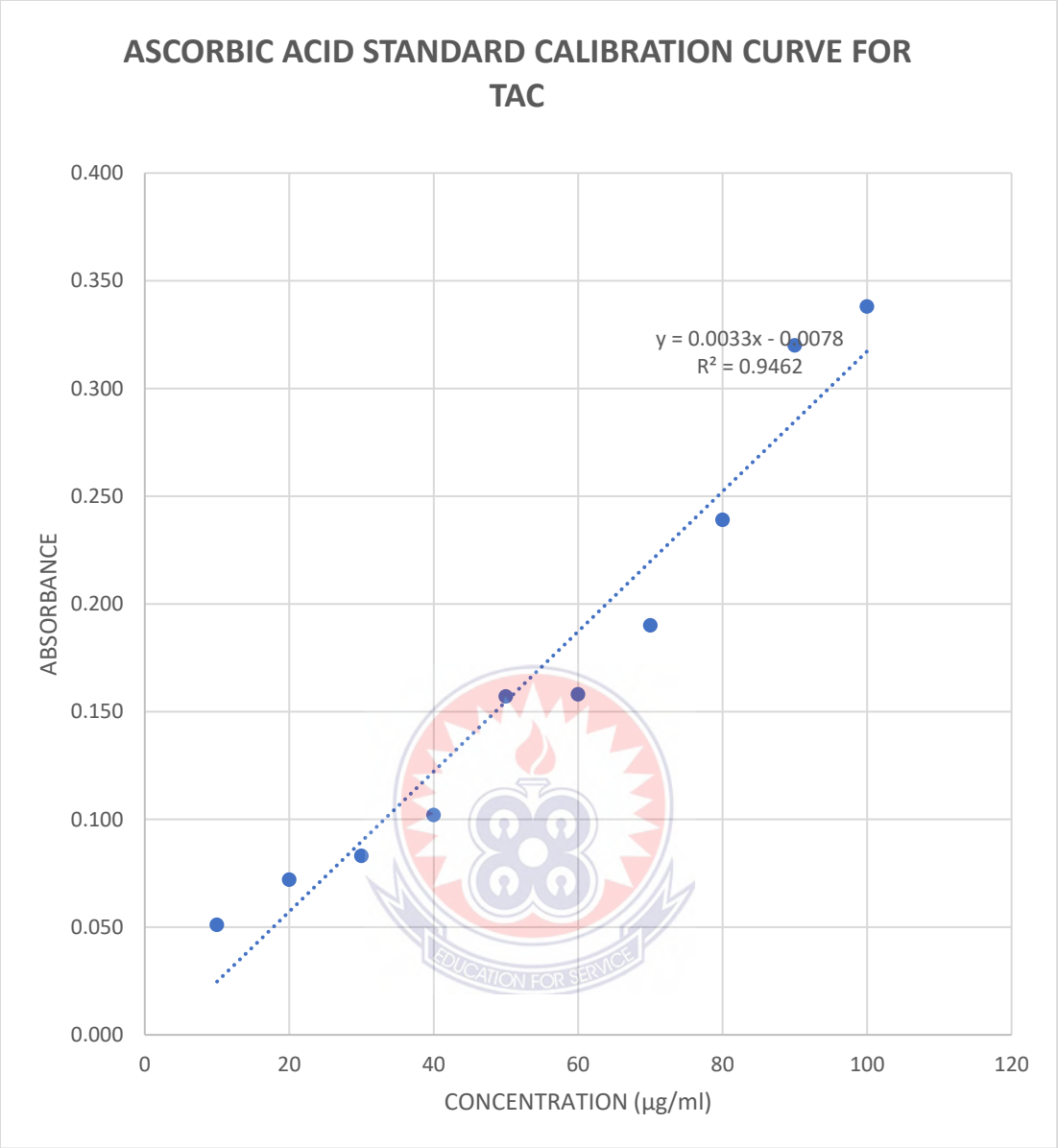

Figure 9: Standard calibration curve of Ascorbic acid

The Absorbance and Concentration values for TAC of the oil extract is shown in **Table 4** below.

Table 4: Absorbance and Concentration values for TAC

SAMPLE	ABSORBANCE	CONCENTRATION (µg/mL)
C1	0.095	31.152
C2	0.086	28.424
C3	0.099	32.364
AVERAGE		**30.646 ± 2.03**

The concentration of the *Lantana camara* oil extract determined to be 30.646 ± 2.03 µg/ml. Thus, the total antioxidant capacity (Ascorbic acid equivalents, mg/g) in the oil extract was reported as 30.646 ± 2.03 mg AAE/g, indicating the TAC of the essential oil extract in the 20 ml. This TAC value of the extract could have been quite higher if it were extracted with hexane or methanol, or even a different extraction method like Soxhlet extraction or Clevenger-assisted hydro-distillation etc. Thus, this work also explains why different extraction method or type of solvent used in the extraction process could lead to differences in the chemical constituent of the extracted oil and consequently differences in the activity as shown by the antioxidant assays. Nevertheless, the presence of antioxidant properties in the essential oil provides the basis for its (*Lantana camara*) wide use as a medicinal plant with the curative effect against numerous diseases and conditions. Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are part of these antioxidant systems, as are macromolecules such as albumin, ceruloplasmin, and ferritin, and a variety of small molecules such as ascorbic acid, -tocopherol, -carotene, reduced glutathione, uric acid, and bilirubin (Koracevic *et al.,* 2001). Cooperation among antioxidants provides greater protection against reactive oxygen or nitrogen species than any single compound alone. Thus, the overall antioxidant capacity may provide more relevant biological information than individual component measurements because it takes into account the cumulative effect of all antioxidants present in the oil extract (Koracevic *et al*., 2001).

4.5 Determination of DPPH Radical Scavenging Activity

The DPPH assay relies on the aptitude of an antioxidant to donate a hydrogen radical or an electron to DPPH radical, which is stable radical with deep violet color. When an odd electron becomes paired within the presence of atom scavenger of antioxidant agent, DPPH radicals get reduced to corresponding hydrazine, DPPH-H form (Paixao *et al.,* 2007) and therefore the solution gets decolorized from its initial deep violet to light yellow colour. The degree of fall within the absorbance measured is proportional to the concentration of the antioxidant. The measured absorbance at various concentrations is used to calculate the percentage radical scavenging activities of both the oil extract and standard ascorbic acid, obtaining a regression line of $y = 0.1928x + 15.107$ and coefficient of correlation, $R^2 = 0.8607$, which showed a comparatively good correlation (86 %) between the % RSAs with a mean of 24.75 ± 5.37. This could be observed from **fig. 10.** Also, the % RSA of the standard ascorbic acid from **fig. 10** below gave a linear equation of $y = 0.0947x + 52.492$ and coefficient of correlation, $R^2 = 0.9296$, which showed much higher correlation (93 %) than that of the oil extract, averaging 57.22 ± 2.54. These % RSA averages of the samples indicated that the radical scavenging activity of the standard ascorbic acid had a higher capability against the free DPPH compared to the *Lantana camara* oil extract.

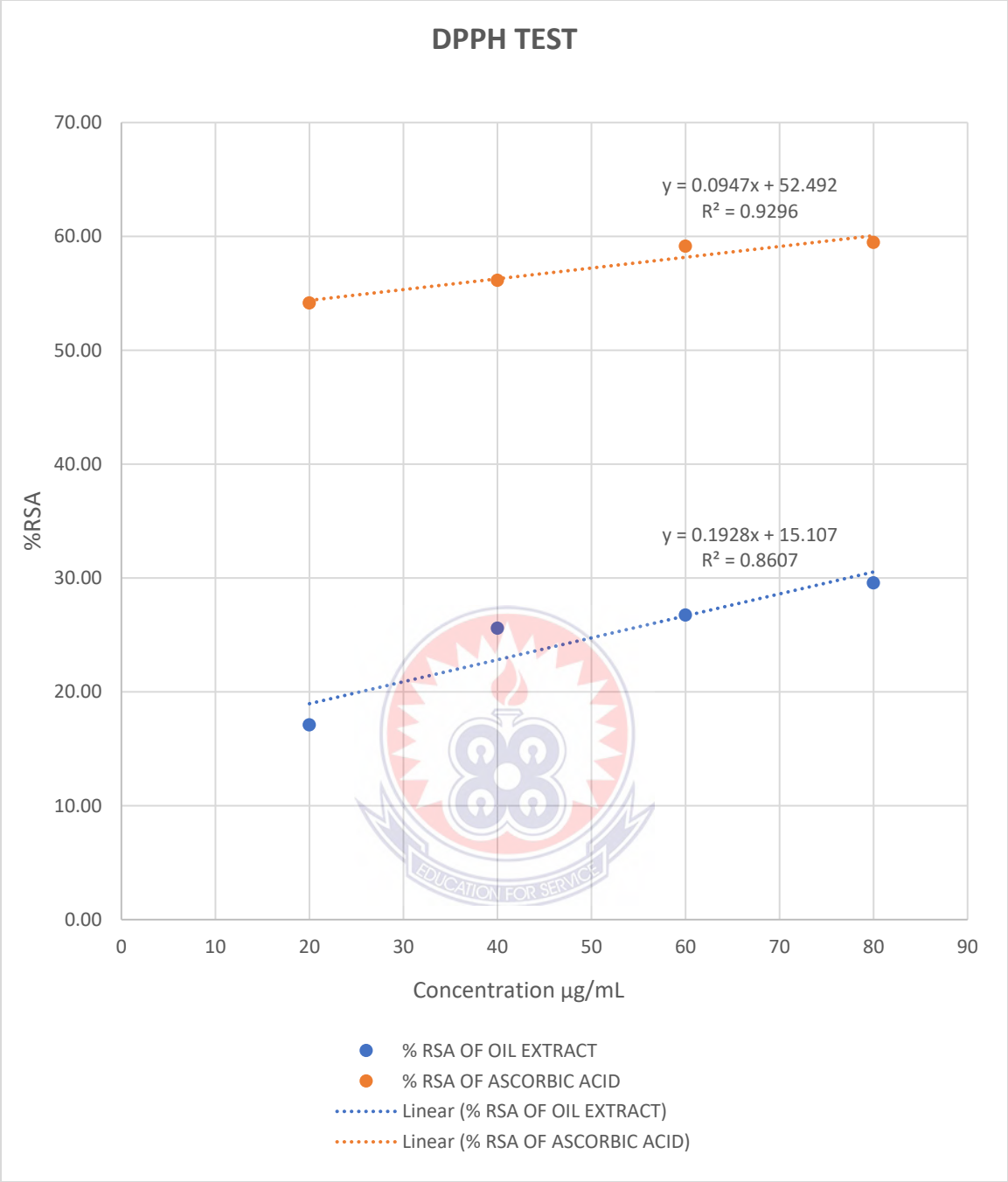

Figure 10: %RSA of both Lantana camara oil extract and standard ascorbic acid.

The DPPH Radical Scavenging Activity of oil extract and standard ascorbic acid at modified concentrations is presented in **table 5** below.

Table 5: DPPH Radical Scavenging Activity of oil extract and standard ascorbic acid at modified concentrations.

CONCENTRATION (µg/mL)	% RSA OIL EXTRACT	% RSA ASCORBIC ACID
20	17.10	54.15
40	25.58	56.15
60	26.74	59.14
80	29.57	59.47
AVERAGE	**24.748 ± 5.37**	**57.228 ± 2.54**

The free radical scavenging property of essential oil extracts from the Conventional hydro-distillation was dose dependent. The data obtained for the radical scavenging activity of the oil extract was compared with standard anti-oxidant drugs ascorbic acid and gallic acid with the different concentrations ranging from 20, 40, 60 and 80 µg/ml. In **table 5**, the DPPH free radical scavenging activity of the oil extract from the fresh leaves of *Lantana camara is indicated whereby ascorbic acid was employed as a comparative* standard antioxidant drug in the analysis. The percentage of inhibition of DPPH free radical for the oil extract showed dose dependent trend with these observed values 17.10, 25.58, 26.74, 29.57 (mean 24.75 ± 5.37) for oil obtained from the Conventional hydro-distillation compared to that of the standard ascorbic acid with values 54.15, 56.15, 59.14, 59.47 (mean 57.22 ± 2.54) with p-value 0.200. This is an indication of the superior antioxidant property of the standard drug ascorbic acid due to the higher DPPH free radical percentage inhibition compared to the value found for the oil extract. The DPPH free radical scavenging activity of the oil extract could be attributed to the constituents and the concentrations of the individual components of the oil extract as shown by the GC-MS data in **table 2.**

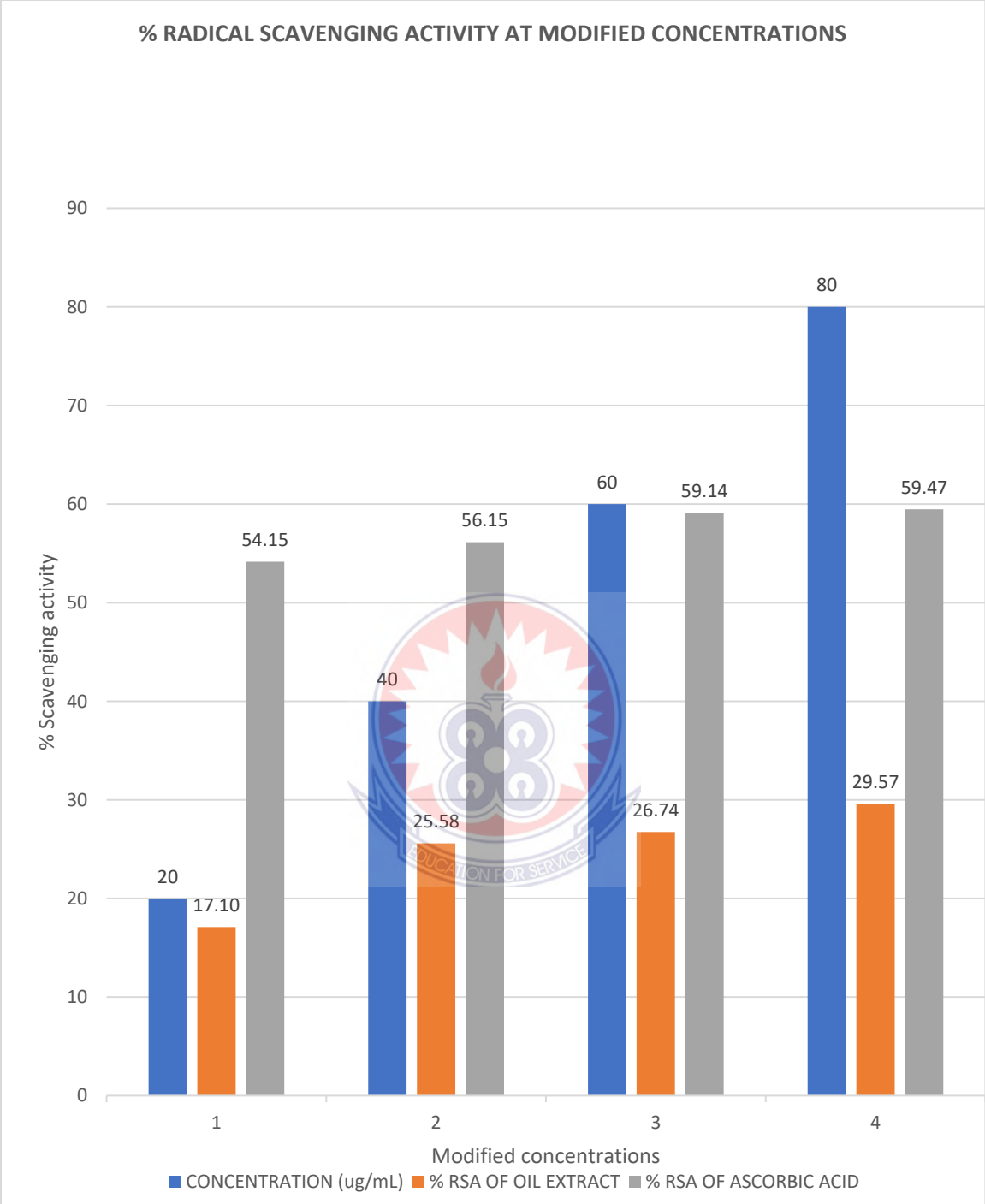

Figure 11: DPPH Radical Scavenging Activity of oil extract and standard ascorbic acid at different concentrations.

$$\%\text{RSA} = \frac{Absorbance\ of\ Control - Absorbance\ of Sample}{Absorbance\ of\ Control} \times 100$$

The **Fig. 11** above shows the comparison of %RSA of both the essential oil extract and the ascorbic acid different concentrations. It could be observed that as the concentration increases, the corresponding %RSA also increases appreciably. This explains the fact that, an enhanced radical scavenging activity of any antioxidant-containing oil or sample, would have its concentration increasing proportionally.

At 95% confidence level, Graphpad analysis of the %RSA of the oil extract and ascorbic acid revealed a p-value of P = 0.003, which is lower than p-value of 0.05. A p-value is a measure of the probability that an observed difference could have occurred between the two means. The lower p-value tells that there is a statistical significance difference between the %RSA of the oil extract and the ascorbic acid. An R^2 = 0.9119 (92%) depicts strong positive correlation between the two means.

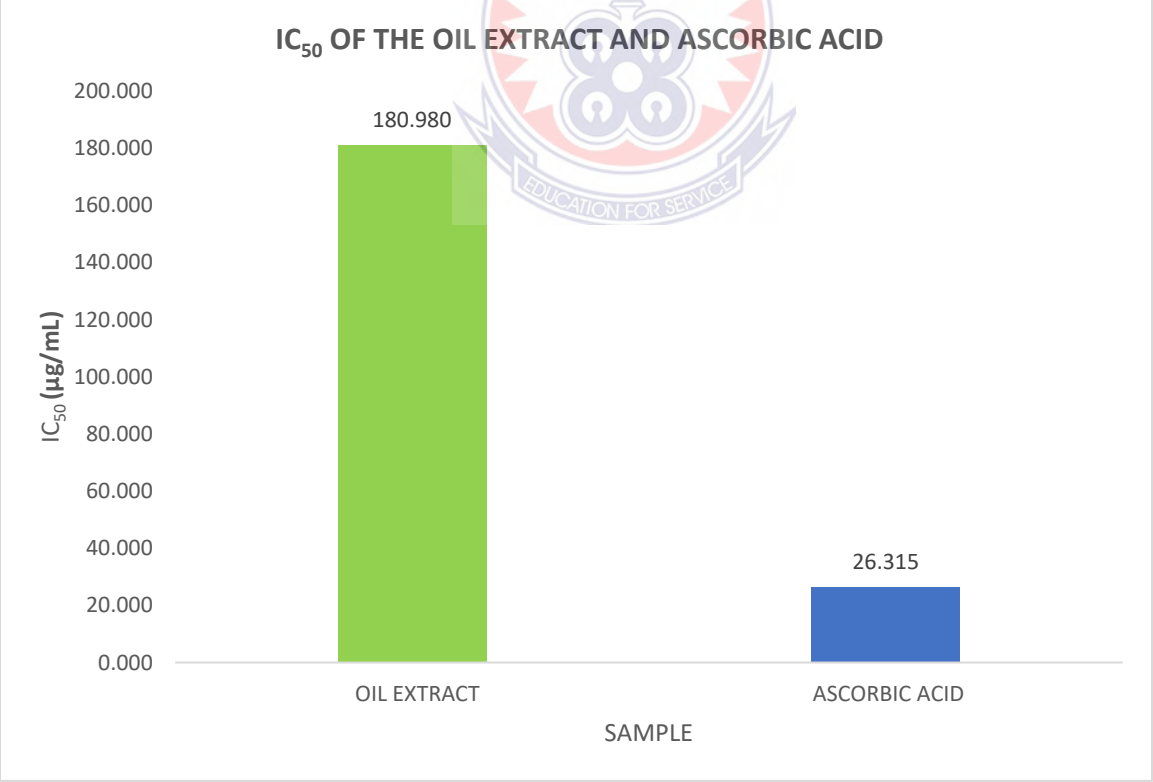

Figure 12: Comparison of the IC_{50} of Lantana camara oil extract and standard ascorbic acid

IC_{50} (Half-maximal Inhibitory Concentration) is the concentration of a substance or drug that can scavenge 50% of DPPH free radical scavenging method (Prior, Wu & Schaich, 2005). The IC_{50} value is inversely proportional to the free radical scavenging activity or antioxidant property of the substance or drug. From **Fig. 12** above**,** the IC_{50} of the *Lantana camara* essential oil extract was estimated to be 180.980 µg/ml, from the linear regression, y = 0.1928x + 15.107 and that of the standard drug ascorbic acid was calculated to be 26.315 µg/ml from, y = 0.09447x + 52.492. This clearly shows that, to inhibit free radical DPPH, higher concentration (180.980 µg/ml) of the essential oil extract is required, whereas one would need a much lower concentration (26.315 µg/ml) of the standard ascorbic acid. **Table 6** compared the IC_{50} values of the oil extract with that of ascorbic acid.

Table 6: Comparison of IC_{50} of the oil extract and ascorbic acid

	OIL EXTRACT (µg/mL)	**ASCORBIC ACID (µg/mL)**
IC_{50}	180.980	26.315

The scavenging activity of free radical in the sample is due to the chemical constituents of the oil with their varied concentration as determined by the GC-MS analysis and some of these compounds known to have antioxidants properties as reported in other research works.

These results of the investigation indicated that oil extracted from *Lantana camara* leaf which are often used in homes as herbal remedies are rich source of appreciable value of polyphenol compounds (Halliwell & Gutteridge, 1990; Valko et al., 2006). They are also one of the most commonly used components of Ayuverdic formulations. The consumption of the oil may have beneficial implications in human health such as treatment and prevention of cancer, cardiovascular diseases and other pathologies by delaying or inhibiting the oxidation of lipids or

other macromolecules and inhibiting the initiation propagation of oxidative chain reactions (Paixao et al., 2007). In addition, the easy availability of the plant oil makes it a promising source of natural antioxidants and other bioactive compounds in foods and pharmaceutical industries.

CHAPTER FIVE

SUMMARY, CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

5.0 Overview

This chapter presents a summary of the findings and conclusion of the study. It also includes contributions of the study, recommendations and finally suggestions for further research.

5.1 Summary of Major Findings

Essential oil was extracted from fresh leaves of *Lantana camara* using hydro-distillation procedure. The essential oil in the distillate was extracted using diethyl ether in separating funnels. The yield of the oil obtained was 12.68g which represented a 2.54% yield. Gas Chromatography -Mass Spectrometry Analysis was carried out on the oil extract using An Agilent Gas Chromatograph 7890A / 5975 C Mass Spectrometer equipped with a Rtx-5mS fused HP-5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25 µm) (J&W, Folsom, CA, USA) and an FID detector were used for the quantitative determination of oil composition. The Total Phenolic Capacity of the essential oil extract was determined by UV-Vis spectrophotometer using the Folin-Ciocalteu's reagent.

The total flavonoid was determined as well as the Total Antioxidant Capacity (TAC) of the essential oil extract of the *Lantana camara* was examined using UV-Vis spectrophotometer according to the phosphomolybdenum assay. Also, the free radical scavenging activity of the essential oil extract was then determined using the DPPH assay. Larvicidal activity was carried out as described by (WHO, 2005) with minor modifications using second instar larvae of the

Culex spp. Same concentrations for the larvicidal activity 1.25mg/ml, 2.5mg/ml, 5.0mg/ml and 10.0mg/ml were prepared and tested on the adult mosquitoes by the feeding assay method.

5.2 Conclusions

Based on the findings of this study, it can be concluded that the leaves of *Lantana camara* contain essential oils with total of 76 compounds identified representing 99.40% of the oil with (E)- Caryophyllene as the predominant compound in the oil extract constituting 20.6%. A comparison of the total phenolic content (TPC) and the total antioxidant capacity (TAC) of the oil extract indicated a slight difference in activity of the TAC than the TPC, meaning there are more phenolic contents present in the oil extract, with a lower antioxidant capacity. It can also be concluded that, the presence of antioxidant properties in the essential oil provides the basis for its (*Lantana camara*) wide use as a medicinal plant with the curative effect against numerous diseases and conditions.

5.3 Recommendations

Research that is time bound is definitely limited to selective explorations. This study was limited by time and therefore recommends that further studies be carried out using varying methods of extraction. The consumption of the oil may have beneficial implications in human health such as treatment and prevention of cancer, cardiovascular diseases and other pathologies by delaying or inhibiting the oxidation of lipids or other macromolecules and inhibiting the initiation propagation of oxidative chain reactions. In addition, the easy availability of the plant oil makes it a promising source of natural antioxidants and other bioactive compounds therefore recommended for the cosmetic and pharmaceutical industries.

5.4 Suggestions for Further Studies

As a result of limited time and financial constrain faced by this study, it is suggested that a comparative study be carried out on the composition and activity of essential oils extracted from leaves of *Lantana camara* using the various extraction methods. Further studies could also be carried out on the other parts of the *Lantana camara* plant. The insecticidal activity of the essential oil extract of *Lantana camara* could be deeply explored and used as a safer and cheap source of insecticide.

REFERENCES

Aamer, R. A., & Emara, H. H. (2016). Effect of Using Cauliflower (Brassica oleracea) to Improve Quality Characteristics of Tuna Fish Burger. *Alexandria Journal of Agricultural Sciences*, *61*(6), 611-626.

Abdollahzadeh, S. H., Mashouf, R. Y., Mortazavi, H., Moghaddam, M. H., Roozbahani, N., & Vahedi, M. (2011). Antibacterial and antifungal activities of Punica granatum peel extracts against oral pathogens. *Journal of Dentistry (Tehran, Iran)*, *8*(1), 1.

Abou-Karam, M., & Shier, W. T. (1990). A simplified plaque reduction assay for antiviral agents from plants. Demonstration of frequent occurrence of antiviral activity in higher plants. *Journal of natural products*, *53*(2), 340-344.

Adorjan, B., & Buchbauer, G. (2010). Biological properties of essential oils: an updated review. *Flavour and Fragrance Journal*, *25*(6), 407-426.

Ahmadifar, E., Falahatkar, B., & Akrami, R. (2011). Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, Oncorhynchus mykiss. *Journal of Applied Ichthyology*, *27*(4), 1057-1060.

Akash, M. S. H., & Rehman, K. (2020). Ultraviolet-visible (UV-VIS) spectroscopy. In *Essentials of Pharmaceutical Analysis* (pp. 29-56). Springer, Singapore.

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi pharmaceutical journal*, *21*(2), 143-152.

Alía, M., Ramos, S., Mateos, R., Bravo, L., & Goya, L. (2005). Response of the antioxidant defense system to tert-butyl hydroperoxide and hydrogen peroxide in a human hepatoma cell line (HepG2). *Journal of biochemical and molecular toxicology*, *19*(2), 119-128.

Ali-Emmanuel, N., Moudachirou, M., Akakpo, J. A., & Quetin-Leclercq, J. (2003). Treatment of bovine dermatophilosis with Senna alata, Lantana camara and Mitracarpus scaber leaf extracts. *Journal of ethnopharmacology*, *86*(2-3), 167-171.

Alok, S., Jain, S. K., Verma, A., Kumar, M., Mahor, A., & Sabharwal, M. (2014). Herbal antioxidant in clinical practice: A review. *Asian Pacific journal of tropical biomedicine*, *4*(1), 78-84.

Amirav, A., Fialkov, A. B., Margolin Eren, K. J., Neumark, B., Elkabets, O., Tsizin, S., ... & Alon, T. (2020). Gas chromatography–mass spectrometry (GC–MS) with cold electron ionization (EI): bridging the gap between GC–MS and LC–MS. *Current Trends in Mass Spectrometry, supplement to LCGC North America*, *18*, 5-15.

André, G., Berentsen, P. B. M., Engel, B., De Koning, C. J. A. M., & Lansink, A. O. (2010). Increasing the revenues from automatic milking by using individual variation in milking characteristics. *Journal of dairy science*, *93*(3), 942-953.

Arumugam, G., Swamy, M. K., & Sinniah, U. R. (2016). Plectranthus amboinicus (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. *Molecules*, *21*(4), 369.

Atarés, L., De Jesús, C., Talens, P., & Chiralt, A. (2010). Characterization of SPI-based edible films incorporated with cinnamon or ginger essential oils. *Journal of food engineering*, *99*(3), 384-391.

Audic, J. L., & Chaufer, B. (2005). Influence of plasticizers and crosslinking on the properties of biodegradable films made from sodium caseinate. *European Polymer Journal*, *41*(8), 1934-1942.

Aziz, Z. A., Ahmad, A., Setapar, S. H. M., Karakucuk, A., Azim, M. M., Lokhat, D., ... & Ashraf, G. M. (2018). Essential oils: extraction techniques, pharmaceutical and therapeutic potential-a review. *Current drug metabolism*, *19*(13), 1100-1110.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of food engineering*, *117*(4), 426-436.

Baaloudj, O., Assadi, I., Nasrallah, N., El Jery, A., Khezami, L., & Assadi, A. A. (2021). Simultaneous removal of antibiotics and inactivation of antibiotic-resistant bacteria by photocatalysis: A review. *Journal of Water Process Engineering*, *42*, 102089.

Barreto, D. V., Barreto, F. C., Liabeuf, S., Temmar, M., Lemke, H. D., Tribouilloy, C., ... & European Uremic Toxin Work Group (EUTox. (2010). Plasma interleukin-6 is independently associated with mortality in both hemodialysis and pre-dialysis patients with chronic kidney disease. *Kidney international*, *77*(6), 550-556.

Barreto, F. S., Sousa, E. O., Rodrigues, F. F. G., Costa, J. G. M., & Campos, A. R. (2010). Antibacterial activity of Lantana camara linn lantana montevidensis brig extracts from cariri-ceara, Brazil. *Journal of young pharmacists*, *2*(1), 42-44.

Barreto, I. (2010). Dynamic capabilities: A review of past research and an agenda for the future. *Journal of management*, *36*(1), 256-280.

Barroso, F. G., de Haro, C., Sánchez-Muros, M. J., Venegas, E., Martínez-Sánchez, A., & Pérez-Bañón, C. (2014). The potential of various insect species for use as food for fish. *Aquaculture*, *422*, 193-201.

Baser, K. H. C., & Buchbauer, G. (2009). *Handbook of essential oils: science, technology, and applications*. CRC press.

Basile, A., Jiménez-Carmona, M. M., & Clifford, A. A. (1998). Extraction of rosemary by superheated water. *Journal of Agricultural and Food Chemistry*, *46*(12), 5205-5209.

Basu, K. (2006). Gender and say: A model of household behaviour with endogenously determined balance of power. *The Economic Journal*, *116*(511), 558-580.

Begum, S., Wahab, A., Siddiqui, B. S., & Qamar, F. (2000). Nematicidal constituents of the aerial parts of Lantana camara. *Journal of Natural Products*, *63*(6), 765-767.

Belmain, S. R., Neal, G. E., Ray, D. E., & Golob, P. (2001). Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food and chemical toxicology*, *39*(3), 287-291.

Bevilacqua, G., Czakon, M., van Hameren, A., Papadopoulos, C. G., & Worek, M. (2011). Complete off-shell effects in top quark pair hadroproduction with leptonic decay at next-to-leading order. *Journal of High Energy Physics*, *2011*(2), 1-30.

Bidel, S., Hu, G., & Tuomilehto, J. (2008). Coffee consumption and type 2 diabetes—An extensive review. *Central European Journal of Medicine*, *3*(1), 9-19.

Boehm, K., Büssing, A., & Ostermann, T. (2012). Aromatherapy as an adjuvant treatment in cancer care–a descriptive systematic review. *African Journal of Traditional, Complementary and Alternative Medicines*, *9*(4), 503-518.

Bonita, J. S., Mandarano, M., Shuta, D., & Vinson, J. (2007). Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies. *Pharmacological research*, *55*(3), 187-198.

Bounatirou, S., Smiti, S., Miguel, M. G., Faleiro, L., Rejeb, M. N., Neffati, M., ... & Pedro, L. G. (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et Link. *Food chemistry*, *105*(1), 146-155.

Brenes, A., & Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. *Animal feed science and technology*, *158*(1-2), 1-14.

Buchbauer, G. (2010). Biological activities of essential oils. *Handbook of Essential Oils: Science, Technology, and Applications; Baser, KHC, Buchbauer, G., Eds*, 235-280.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*, *94*(3), 223-253.

Chamorro, E. R., Zambón, S. N., Morales, W. G., Sequeira, A. F., & Velasco, G. A. (2012). Study of the chemical composition of essential oils by gas chromatography. *Gas chromatography in plant science, wine technology, toxicology and some specific applications*, *1*, 307-324.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-based complementary and alternative medicine*, *2014*.

Cherubini, E., & Conti, F. (2001). Generating diversity at GAB Aergic synapses. *Trends in neurosciences*, *24*(3), 155-162.

Chrissie, W. (1996). The Encyclopedia of Aromatherapy. *Vermont: Healing Arts Press*, 16-21.

Cieślik, E., Gręda, A., & Adamus, W. (2006). Contents of polyphenols in fruit and vegetables. *Food chemistry*, *94*(1), 135-142.

Dandlen, S. A., Lima, A. S., Mendes, M. D., Miguel, M. G., Faleiro, M. L., Sousa, M. J., ... & Figueiredo, A. C. (2010). Antioxidant activity of six Portuguese thyme species essential oils. *Flavour and fragrance Journal*, *25*(3), 150-155.

David, A. V. A., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy reviews*, *10*(20), 84.

Dawidowicz, A. L., Rado, E., Wianowska, D., Mardarowicz, M., & Gawdzik, J. (2008). Application of PLE for the determination of essential oil components from Thymus vulgaris L. *Talanta*, *76*(4), 878-884.

De Castro, M. L., Jiménez-Carmona, M. M., & Fernandez-Perez, V. (1999). Towards more rational techniques for the isolation of valuable essential oils from plants. *TrAC Trends in Analytical Chemistry*, *18*(11), 708-716.

Devaraj, S., Xu, D. Y., & Jialal, I. (2003). C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells: implications for the metabolic syndrome and atherothrombosis. *Circulation*, *107*(3), 398-404.

Díaz, J., Bernal, A., Pomar, F., & Merino, F. (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (Capsicum annuum L.) seedlings in response to copper stress and its relation to lignification. *Plant Science*, *161*(1), 179-188.

Doustkhah, E., Hassandoost, R., Khataee, A., Luque, R., & Assadi, M. H. N. (2021). Hard-templated metal–organic frameworks for advanced applications. *Chemical Society Reviews*, *50*(5), 2927-2953.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews*.

Dua, H. S., Joseph, A., Shanmuganathan, V. A., & Jones, R. E. (2003). Stem cell differentiation and the effects of deficiency. *Eye*, *17*(8), 877-885.

Edris, A. E. (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(4), 308-323.

El Gharras, H. (2009). Polyphenols: food sources, properties and applications–a review. *International journal of food science & technology*, *44*(12), 2512-2518.

Evans, D. S. (2009). The online advertising industry: Economics, evolution, and privacy. *Journal of economic perspectives*, *23*(3), 37-60.

Fabra, M. J., Talens, P., & Chiralt, A. (2008). Tensile properties and water vapor permeability of sodium caseinate films containing oleic acid–beeswax mixtures. *Journal of Food Engineering*, *85*(3), 393-400.

Faridi, Z., Liberti, L., Shuval, K., Northrup, V., Ali, A., & Katz, D. L. (2008). Evaluating the impact of mobile telephone technology on type 2 diabetic patients' self-management: the NICHE pilot study. *Journal of evaluation in clinical practice*, *14*(3), 465-469.

Fernandes, E., dos Santos, C., & Milidiú, R. L. (2012, July). Latent structure perceptron with feature induction for unrestricted coreference resolution. In *Joint Conference on EMNLP and CoNLL-Shared Task* (pp. 41-48).

Field, M. (2003). Intestinal ion transport and the pathophysiology of diarrhea. *The Journal of clinical investigation*, *111*(7), 931-943.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, *80*(13), 1925-1941.

Geethalakshmi, R., Sakravarthi, C., Kritika, T., Arul Kirubakaran, M., & Sarada, D. V. L. (2013). Evaluation of antioxidant and wound healing potentials of Sphaeranthus amaranthoides Burm. f. *BioMed research international*, *2013*.

George, D. R., Smith, T. J., Shiel, R. S., Sparagano, O. A. E., & Guy, J. H. (2009). Mode of action and variability in efficacy of plant essential oils showing toxicity against the poultry red mite, Dermanyssus gallinae. *Veterinary parasitology*, *161*(3-4), 276-282.

Goñi, P., López, P., Sánchez, C., Gómez-Lus, R., Becerril, R., & Nerín, C. (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food chemistry*, *116*(4), 982-989.

Guardiola-Albert, C., Díez-Herrero, A., Amerigo Cuervo-Arango, M., Bodoque, J. M., García, J. A., Naranjo-Fernández, N., & Aroca-Jiménez, E. (2020). Analysing flash flood risk perception through a geostatistical approach in the village of Navaluenga, Central Spain. *Journal of Flood Risk Management*, *13*(1), e12590.

Gülçin, I. (2008). Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent. *Journal of enzyme inhibition and medicinal chemistry*, *23*(6), 871-876.

Hagstrum, D. W., & Subramanyam, B. (2016). Stored-product insect resource. AACC International. *Inc., St. Paul, Minnesota, USA*, 509.

Hanif, M. A., Nisar, S., Khan, G. S., Mushtaq, Z., & Zubair, M. (2019). Essential oils. In *Essential oil research* (pp. 3-17). Springer, Cham.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of nutritional biochemistry*, *13*(10), 572-584.

Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., ... & von Wright, A. (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. *Journal of agricultural and food chemistry*, *46*(9), 3590-3595.

Hemalatha, K. S., Rukmani, K., Suriyamurthy, N., & Nagabhushana, B. M. (2014). Synthesis, characterization and optical properties of hybrid PVA–ZnO nanocomposite: a composition dependent study. *Materials Research Bulletin*, *51*, 438-446.

Herbert, J. M., Maffrand, J. P., Taoubi, K., Augereau, J. M., Fouraste, I., & Gleye, J. (1991). Verbascoside isolated from Lantana camara, an inhibitor of protein kinase C. *Journal of Natural Products*, *54*(6), 1595-1600.

Hsin, J. P., & Manley, J. L. (2012). The RNA polymerase II CTD coordinates transcription and RNA processing. *Genes & development*, *26*(19), 2119-2137.

Huang, K. Y., Lee, D., Nakigudde, J., Cheng, S., Gouley, K. K., Mann, D., ... & Mendelsohn, A. (2019). Use of technology to promote child behavioral health in the context of pediatric care: a scoping review and applications to low-and middle-income countries. *Frontiers in psychiatry*, *10*, 806.

Hurrell, R. F. (2003). Influence of vegetable protein sources on trace element and mineral bioavailability. *The Journal of nutrition*, *133*(9), 2973S-2977S.

Hussain, Z., Gimenez, F., Yi, D., & Rubin, D. (2017). Differential data augmentation techniques for medical imaging classification tasks. In *AMIA annual symposium proceedings* (Vol. 2017, p. 979). American Medical Informatics Association.

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in microbiology*, *3*, 12.

Jo, M. H., Grose, J. E., Baheti, K., Deshmukh, M. M., Sokol, J. J., Rumberger, E. M., ... & Ralph, D. C. (2006). Signatures of molecular magnetism in single-molecule transport spectroscopy. *Nano Letters*, *6*(9), 2014-2020.

Juven, B. J., Kanner, J., Schved, F., & Weisslowicz, H. (1994). Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *Journal of applied bacteriology*, *76*(6), 626-631.

Kaliora, A. C., Dedoussis, G. V. Z., & Schmidt, H. (2006). Dietary antioxidants in preventing atherogenesis. *Atherosclerosis*, *187*(1), 1-17.

Karadag, A., Ozcelik, B., & Saner, S. (2009). Review of methods to determine antioxidant capacities. *Food analytical methods*, *2*(1), 41-60.

Kasali, A. A., Ekundayo, O., Paul, C., Koenig, W. A., Eshilokun, A. O., & Yadua, P. (2004). Essential oil of Lantana camara L. var. aculeata from Nigeria. *Journal of essential oil Research*, *16*(6), 582-584.

Katz, T. M., Miller, J. H., & Hebert, A. A. (2008). Insect repellents: historical perspectives and new developments. *Journal of the American Academy of Dermatology*, *58*(5), 865-871.

Khan, I. U., Sajid, S., Javed, A., Sajid, S., & Shah, S. U. (2017). Comparative diagnosis of typhoid fever by polymerase chain reaction and widal test in Southern Districts (Bannu, Lakki Marwat and DI Khan) of Khyber Pakhtunkhwa, Pakistan. *Acta Sci. Malaysia*, *1*(2), 12-15.

Kirkman, H. N., & Gaetani, G. F. (2007). Mammalian catalase: a venerable enzyme with new mysteries. *Trends in biochemical sciences*, *32*(1), 44-50.

Kohen, R., & Nyska, A. (2002). Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic pathology*, *30*(6), 620-650.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *Journal of clinical pathology*, *54*(5), 356-361.

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., & Yildirim, A. (2005). Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera essential oils. *Journal of agricultural and food chemistry*, *53*(24), 9452-9458.

Koroch, A. R., Rodolfo Juliani, H., & Zygadlo, J. A. (2007). Bioactivity of essential oils and their components. *Flavours and fragrances*, 87-115.

Kumadoh, D., Archer, M. A., Yeboah, G. N., Kyene, M. O., Boakye-Yiadom, M., Adi-Dako, O., ... & Mintah, S. O. (2021). A review on anti-peptic ulcer activities of medicinal plants used in the formulation of Enterica, Dyspepsia and NPK 500 capsules. *Heliyon*, *7*(12), e08465.

Kumar, K., Dasgupta, C. N., & Das, D. (2014). Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. *Bioresource technology*, *167*, 358-366.

Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology*, *91*(3), 453-462.

Lang, G., & Buchbauer, G. (2012). A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review. *Flavour and Fragrance Journal*, *27*(1), 13-39.

Li, F. S., & Weng, J. K. (2017). Demystifying traditional herbal medicine with modern approach. *Nature plants*, *3*(8), 1-7.

Limberger, R. P., Apel, M. A., Sobral, M., Moreno, P. R., Henriques, A. T., & Menut, C. (2001). Chemical composition of essential oils from some Campomanesia species (Myrtaceae). *Journal of Essential Oil Research*, *13*(2), 113-115.

Lingamaneni, K., Rao, A. L., & Mishra, U. S. (2011). Antibacterial and analgesic activity of leaves of Lantana camara. *International Journal of Phytomedicine*, *3*(3), 381.

Lopez-Reyes, J. G., Spadaro, D., Prelle, A., Garibaldi, A., & Gullino, M. L. (2013). Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits in vivo. *Journal of Food Protection*, *76*(4), 631-639.

Lu, J., & Holmgren, A. (2014). The thioredoxin antioxidant system. *Free Radical Biology and Medicine*, *66*, 75-87.

Mahdi-Pour, B., Jothy, S. L., Latha, L. Y., Chen, Y., & Sasidharan, S. (2012). Antioxidant activity of methanol extracts of different parts of Lantana camara. *Asian Pacific journal of tropical biomedicine*, *2*(12), 960-965.

Mansoori, A., Singh, N., Dubey, S. K., Thakur, T. K., Alkan, N., Das, S. N., & Kumar, A. (2020). Phytochemical characterization and assessment of crude extracts from Lantana camara L. for antioxidant and antimicrobial activity. *Frontiers in Agronomy*, *2*, 582268.

Marcus, M., Yasamy, M. T., van Ommeren, M. V., Chisholm, D., & Saxena, S. (2012). Depression: *A global public health concern*.

Mawal, S., & Patil, S. (2019). Assessment of allelopathic potential of Lantana species on some selected agricultural crops. *Assessment*, *4*(1).

Mayee, R., Rawat, S., Thosar, A., Atre, K., & Mane, P. (2011). Development and Validation of HPLC Method for Determination of Diclofenac Sodium by Tape Stripping Method. *Asian Journal of Pharmaceutical & Biological Research (AJPBR)*, *1*(3).

Mekem Sonwa, M. (2000). *Isolation and structure elucidation of essential oil constituents: comparative study of the oils of Cyperus alopecuroides, Cyperus papyrus, and Cyperus rotundus* (Doctoral dissertation, Staats-und Universitätsbibliothek Hamburg Carl von Ossietzky).

Meyer-Warnod, B. (1984). Natural essential oils: extraction processes and application to some major oils. *Perfumer & flavorist*, *9*(2), 93-104.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish journal of environmental studies*, *15*(4).

Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological reviews*, *52*(4), 673-751.

Mishra, K., Boynton, L., & Mishra, A. (2014). Driving employee engagement: The expanded role of internal communications. *International Journal of Business Communication*, *51*(2), 183-202.

Mohamed, T. O. I. (2019). *Determination of Correlation between Platelet Parameters and Complications of Type2 Diabetes Mellitus in Sudanese Patients in Khartoum State* (Doctoral dissertation, Sudan University of Science & Technology).

Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of agricultural and food chemistry*, *57*(5), 1655-1666.

Morton, W. M., Ayscough, K. R., & McLaughlin, P. J. (2000). Latrunculin alters the actin-monomer subunit interface to prevent polymerization. *Nature cell biology*, *2*(6), 376-378.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of pharmaceutical and biomedical analysis*, *41*(5), 1523-1542.

Naeem, A., Abbas, T., Ali, T. M., & Hasnain, A. (2018). Essential oils: Brief background and uses. *Ann. Short Rep*, *1*(6).

Okur, M. E., Ayla, Ş., Çiçek Polat, D., Günal, M. Y., Yoltaş, A., & Biçeroğlu, Ö. (2018). Novel insight into wound healing properties of methanol extract of Capparis ovata Desf. var. palaestina Zohary fruits. *Journal of Pharmacy and Pharmacology*, *70*(10), 1401-1413.

Olmedo, R. H., Nepote, V., & Grosso, N. R. (2013). Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils. *LWT-Food Science and Technology*, *53*(2), 409-417.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of agricultural and food chemistry*, *50*(11), 3122-3128.

Oussalah, M., Caillet, S., Saucier, L., & Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157: H7, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes. *Food control*, *18*(5), 414-420.

Owolabi, O. J., Omogbai, E. K., & Obasuyi, O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. *African Journal of Biotechnology*, *6*(14).

Paixao, N., Perestrelo, R., Marques, J. C., & Câmara, J. S. (2007). Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. *Food Chemistry*, *105*(1), 204-214.

Panteleon, V., Kostakis, I. K., Marakos, P., Pouli, N., & Andreadou, I. (2008). Synthesis and free radical scavenging activity of some new spiropyranocoumarins. *Bioorganic & medicinal chemistry letters*, *18*(21), 5781-5784.

Patel, M. R., Mahaffey, K. W., Garg, J., Pan, G., Singer, D. E., Hacke, W., ... & Rocket AF Steering Committee. (2011). Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *New England Journal of Medicine*, *365*(10), 883-891.

Perumalla, A. V. S., & Hettiarachchy, N. S. (2011). Green tea and grape seed extracts—Potential applications in food safety and quality. *Food Research International*, *44*(4), 827-839.

Polidori, M. C. (2003). Antioxidant micronutrients in the prevention of age-related diseases. *Journal of Postgraduate Medicine*, *49*(3), 229.

Poulose, S. M., Miller, M. G., & Shukitt-Hale, B. (2014). Role of walnuts in maintaining brain health with age. *The Journal of nutrition*, *144*(4), 561S-566S.

Pour, B. M., & Sasidharan, S. (2011). In vivo toxicity study of Lantana camara. *Asian Pacific Journal of Tropical Biomedicine*, *1*(3), 230-232.

Pour, B. M., Latha, L. Y., & Sasidharan, S. (2011). Cytotoxicity and oral acute toxicity studies of Lantana camara leaf extract. *Molecules*, *16*(5), 3663-3674.

Prakash, A., & Rao, J. (2018). *Botanical pesticides in agriculture*. CRC press.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*, *53*(10), 4290-4302.

Purkayastha, M. D., Kalita, D., Mahnot, N. K., Mahanta, C. L., Mandal, M., & Chaudhuri, M. K. (2012). Effect of l-ascorbic acid addition on the quality attributes of micro-filtered

coconut water stored at 4 C. *Innovative Food Science & Emerging Technologies*, *16*, 69-79.

Queen, J. P., Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge university press.

Ratnam, D. V., Ankola, D. D., Bhardwaj, V., Sahana, D. K., & Kumar, M. R. (2006). Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *Journal of controlled release*, *113*(3), 189-207.

Rigotti, G., Marchi, A., Galie, M., Baroni, G., Benati, D., Krampera, M., ... & Sbarbati, A. (2007). Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plastic and reconstructive surgery*, *119*(5), 1409-1422.

Roberts, T. V., Lawless, M., Bali, S. J., Hodge, C., & Sutton, G. (2013). Surgical outcomes and safety of femtosecond laser cataract surgery: a prospective study of 1500 consecutive cases. *Ophthalmology*, *120*(2), 227-233.

Rojas, M. C., & Brewer, M. S. (2008). Effect of natural antioxidants on oxidative stability of frozen, vacuum-packaged beef and pork. *Journal of Food quality*, *31*(2), 173-188.

Roudier, F., Ahmed, I., Bérard, C., Sarazin, A., Mary-Huard, T., Cortijo, S., ... & Colot, V. (2011). Integrative epigenomic mapping defines four main chromatin states in Arabidopsis. *The EMBO journal*, *30*(10), 1928-1938.

Sadaka, F., Nguimjeu, C., Brachais, C. H., Vroman, I., Tighzert, L., & Couvercelle, J. P. (2014). WITHDRAWN: Review on antimicrobial packaging containing essential oils and their active biomolecules.

Sako, A., Semdé, S., & Wenmenga, U. (2018). Geochemical evaluation of soil, surface water and groundwater around the Tongon gold mining area, northern Côte d'Ivoire, West Africa. *Journal of African Earth Sciences*, *145*, 297-316.

Sarma, R., & Baruah, J. B. (2010). Variation in coordination modes of aromatic N-oxides in lanthanum (III) coordination polymers. *Journal of Coordination Chemistry*, *63*(3), 457-463.

Sánchez-García, S., Heredia-Ponce, E., Juárez-Cedillo, T., Gallegos-Carrillo, K., Espinel-Bermúdez, C., De La Fuente-Hernández, J., & García-Peña, C. (2010). Psychometric properties of the General Oral Health Assessment Index (GOHAI) and dental status of an elderly Mexican population. *Journal of public health dentistry*, *70*(4), 300-307.

Saxena, A., Tripathi, R. M., Zafar, F., & Singh, P. (2012). Green synthesis of silver nanoparticles using aqueous solution of Ficus benghalensis leaf extract and characterization of their antibacterial activity. *Materials letters*, *67*(1), 91-94.

Saxena, M., Saxena, J., & Khare, S. (2012). A brief review on: Therapeutical values of Lantana camara plant. *International Journal of Pharmacy & Life Sciences*, *3*(3).

Schmaal, L., Hibar, D. P., Sämann, P. G., Hall, G. B., Baune, B. T., Jahanshad, N., ... & Veltman, D. J. (2017). Cortical abnormalities in adults and adolescents with major

depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group. *Molecular psychiatry*, *22*(6), 900-909.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods*, *18*, 820-897.

Sharma, P. P., Gupta, S. C., & Foster, G. R. (1995). Raindrop-induced soil detachment and sediment transport from interrill areas. *Soil Science Society of America Journal*, *59*(3), 727-734.

Singab, A. N., Youssef, F. S., & Ashour, M. L. (2014). Medicinal plants with potential antidiabetic activity and their assessment. *Med Aromat Plants*, *3*(151), 2167-0412.

Silvestri, V., Barrowdale, D., Mulligan, A. M., Neuhausen, S. L., Fox, S., Karlan, B. Y., ... & Ottini, L. (2016). Male breast cancer in BRCA1 and BRCA2 mutation carriers: pathology data from the Consortium of Investigators of Modifiers of BRCA1/2. *Breast Cancer Research*, *18*(1), 1-13.

Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M., & Knez, Ž. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food chemistry*, *89*(2), 191-198.

Sonibare, O. O., & Effiong, I. (2008). Antibacterial activity and cytotoxicity of essential oil of Lantana camara L. leaves from Nigeria. *African Journal of Biotechnology*, *7*(15).

Sousa, E. O., Miranda, C. M., Nobre, C. B., Boligon, A. A., Athayde, M. L., & Costa, J. G. (2015). Phytochemical analysis and antioxidant activities of Lantana camara and Lantana montevidensis extracts. *Industrial Crops and Products*, *70*, 7-15.

Stevanovic, A., Stevanovic, J., Zhang, K., & Batterman, S. (2009). Optimizing traffic control to reduce fuel consumption and vehicular emissions: Integrated approach with VISSIM, CMEM, and VISGAOST. *Transportation Research Record*, *2128*(1), 105-113.

Stocker, R., & Keaney Jr, J. F. (2004). Role of oxidative modifications in atherosclerosis. *Physiological reviews*, *84*(4), 1381-1478.

Swamy, M. K., & Sinniah, U. R. (2016). Patchouli (Pogostemon cablin Benth.): botany, agrotechnology and biotechnological aspects. *Industrial Crops and products*, *87*, 161-176.

Tsedaley, B. (2015). Integrated management of potato tuber moth (Phthorimaea operculella)(Zeller) in field and storage. *Journal of Biology, Agriculture and Healthcare*, *5*(3), 134-144.

Turek, C., & Stintzing, F. C. (2013). Stability of essential oils: a review. *Comprehensive reviews in food science and food safety*, *12*(1), 40-53.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology*, *39*(1), 44-84.

Valko, M., Rhodes, C. J. B., Moncol, J., Izakovic, M. M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*, *160*(1), 1-40.

Wachira, J. K., Larson, M. K., & Harris, W. S. (2014). n-3 Fatty acids affect haemostasis but do not increase the risk of bleeding: clinical observations and mechanistic insights. *British Journal of Nutrition*, *111*(9), 1652-1662.

Wolf, K., Mazo, I., Leung, H., Engelke, K., Von Andrian, U. H., Deryugina, E. I., ... & Friedl, P. (2003). Compensation mechanism in tumor cell migration: mesenchymal–amoeboid transition after blocking of pericellular proteolysis. *The Journal of cell biology*, *160*(2), 267-277.

World Health Organization. (2018). Global report on insecticide resistance in malaria vectors: 2010–2016.

Zadak, Z., Hyspler, R., Ticha, A., Hronek, M., Fikrova, P., Rathouska, J., ... & Stetina, R. (2009). Antioxidants and vitamins in clinical conditions. *Physiol Res*, *58*(1), S13-S17.

Zénabou, S., Jean, K., Gilles, F., Cheikna, Z., Marius, S. K., & Alfred, T. S. (2018). Chemical composition, antioxidant and antimicrobial activities of Lantana camara Linn leaves essential oil from Burkina Faso. *GSC Biological and Pharmaceutical Sciences*, *5*(3).

Zhu, Y., Chen, G., Lv, F., Wang, X., Ji, X., Xu, Y., ... & Gao, G. (2011). Zinc-finger antiviral protein inhibits HIV-1 infection by selectively targeting multiply spliced viral mRNAs for degradation. *Proceedings of the National Academy of Sciences*, *108*(38), 15834-15839.

Zulueta, A., Esteve, M. J., Frasquet, I., & Frígola, A. (2007). Vitamin C, vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain. *Food Chemistry*, *103*(4), 1365-1374.