ANTIBACTERIAL ACTIVITY OF *HELIANTHUS ANNUUS* SEEDS EXTRACT ON *SALMONELLA* SPECIES AND *ESCHERICHIA COLI*

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ABSTRACT

Medicinal plants contain active ingredients with therapeutic values. *Helianthus annuus* L (Asteraceae) commonly referred to as "sunflower" has been reportedly used in managing and treating some ailments over the years. This study investigated the *in vitro* and *in vivo* antibacterial effects of the crude extracts of *Helianthus annuus* seeds on *Salmonella spp* and *Eschericha coli.* The phytochemical screening of the methanolic and ethanolic crude extracts revealed the presence of alkaloids, saponins, tannins, flavonoids and phenols. The effect of the crude extracts against the test organisms ranges from 7.33±0.67 mm to 25.67±0.33 mm at concentrations ranging from 60 mg/ml to 480 mg/ml. The minimum inhibitory concentration of the extracts ranges from 0.07±0.03 mg/ml to 2.40±0.06 mg/ml. The minimum bactericidal concentration of the extracts ranges from 0.48±0.01 mg/ml to 2.40±0.12 mg/ml. The acute toxicity study revealed LD_{50}>2000 mg/kg. The sub acute toxicity study showed that there was a significant decrease in Alanine transaminase, Aspartate transaminase and Alanine phosphatase levels in most of the treated groups compared to the control group. Total cholesterol, triglyceride and low density lipoprotein cholesterol were significantly reduced compared to the control while a significant increase in high density lipoprotein was observed in the treatment groups. The serum biochemical protein components were comparable to the control. The result of the haematological study revealed that mean cell volume, haemoglobin and packed cell volume levels were significantly increased in most of the treated groups compared to the control group. There were no features of acute or chronic damage by the extracts on the hepatic and renal tissues. The in vivo antibacterial effect of the extracts revealed decrease in total bacteria load in treated rats. Therefore, *H. annus* seeds extracts possess antibacterial effects against *Salmonella spp and E.coli* and is considered to be relatively safe. Further studies should be carried out to identify the exact active compound or compounds that possess bactericidal activity. There is need to establish the validity and mechanism of action of the hepato-protective effect of *H.annuus* seed extracts inferred in this study.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Medicinal plants contain active ingredients in one or more of its parts with therapeutic value or play the role of precursors for chemo-pharmaceutical synthesis (Bassam, 2012). Over the years, several medicinal plant parts have been utilized in managing and treating certain disease conditions, however only a few have been successfully evaluated and scientifically validated for the claimed effects (Saravanakumar *et al.,* 2010). Among the plants used in traditional medicine is *Helianthus annuus.*

Sunflower (*Helianthus annuus* L) is majorly cultivated in certain areas in the world for its seed, which is an important source of edible oil (Eze *et al.,* 2015). It is a carrier oil and also used in the production of biodiesel and margarine, as it is less expensive compared to olive oil. Sunflowers have the potential to play vital role in phytoremediation as it can be used to extract toxic chemicals like heavy metals, such as lead, arsenic and uranium from soil, as well as engaged in rhizofiltration to neutralize radionuclides and other toxic compounds and materials as well as harmful bacteria from water (Adler, 1996). Extracts from the leave as well as other preparations made from different parts of plant, are used in the treatment of high fevers, as a poultice on sores, swellings, snakebites and spider bites, in the treatment of malaria, lung ailments, diabetes (Saini and Sharma, 2011).

Helianthus annuus seed oil, and herbal tincture have been reported to have anti-inflammatory, antioxidant, antitumor, antipyretic, antihypoglycemic, cathartic, diuretic and antimicrobial activity (Aboki *et al.,* 2012). It has shown antimicrobial properties against diverse microorganism such as *Staphylococcus aureus, Escherichia coli*, *Bacillus subtilis* among others. The effect of polar oil extract from the seeds of sunflower

(*Helianthus annuus*) in Napkin dermatitis and its antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Proteus vulgaris* were studied (Ahmad *et al.,* 2015). Sunflower oil is highly absorbable through the dermal tissues, providing significant nourishment and moisturizing effects. As a result of this, it is therefore, famous as an over-the-counter ingredient as well as ingredients used in the manufacturing of homemade beauty products such as lotions, creams and massage oils. Oil extracted from sunflower plants may also possess a protective barrier property which resists infections in developing infants. Infants administered this oil subcutaneously on a daily basis were 69 % more likely not to develop infections in the hospital (Ahmad *et al.,* 2015).

1.2 Statement of the Research Problem

Antimicrobial resistance (AMR) is a complex and growing international public health problem (WHO, 2015). Antimicrobial resistance is estimated to account for more than 700,000 deaths annually. Antimicrobial resistance is projected to increase to 10 million deaths with estimated cost of about US$100 trillion by the year 2050 (O'Neill, 2016). Antimicrobial resistance affects every country irrespective of income and development (O'Neill, 2016). The rapid spread of multidrug resistant pathogens is of global threat. Drug-resistant pathogens accounts for 214,000 neonatal sepsis leading to deaths annually (Laxminarayan, 2016). Antimicrobial resistance is not restricted to communicable diseases, it also has potential consequences on non-communicable diseases (NCDs). Antimicrobial resistance poses threat to surgical interventions, cancer treatment and organ transplant (Laxminarayan, 2013). About 50 % of pathogens implicated in surgical site infections are resistant to standard prophylactic antibiotics (Teillant *et al.,* 2015). Cancer patients who have undergone chemotherapy also suffer infections caused by pathogens which are resistant to commonly used antibiotics (Teillant *et al.,* 2015).

Treating drug-resistant infections is expensive and takes longer period and has lower success rate than treating drug-susceptible infections. Therefore, AMR negatively impacts on national economic performance (Smith *et al.,* 2015). The challenges of AMR and its magnitude within the African Region including Nigeria have been monitored by surveillance of drug resistance. In the past decades, the widespread of antibiotic used against microbial pathogens of human and animal origin has led to resistance to many antimicrobial agents which constitute a major health threat around the globe (WHO, 2013).

One of the factors contributing to AMR is misuse of antibiotics. However, lack of tests for other infections in patients who test negative for malaria has led to indiscriminate use of antibiotics (Do *et al.,* 2016). According to Leopold *et al.* (2014), there is high level of resistance to commonly used antibiotics in the sub-Saharan African region. For example, 90% of Gram negatives were resistant to chloramphenicol, a commonly used antibiotic while resistance to third-generation cephalosporins (ceftriaxone) was less common (Leopold *et al.,* 2014).

In Nigeria, resistance to Ampicillin increased from 70 to 90 %, Co-trimoxazole from 77 to 85 %, Chloramphenicol from 71 to 77 % and Streptomycin from 71 to 79 % between 1990 and 2000 in finding the root cause of antimicrobial resistance (Ifeanyi, 2012). Methicillin resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Staphylococcus aureus* (VRSA), carbapenem resistant Enterobacteriaceae, Multi drug and extensive-drug resistant *Mycobacterium tuberculosis* (MDR-TB and XDR-TB) have also been reported (Nannini, 2013).

The transfer of genetic elements such as plasmids, transposons, and integrons has contributed to the rapid spread of AMR among bacterial species. According to Harbottle

(2011), AMR genes have been found on mobile elements, leading to multidrug resistance which can be transferred to a susceptible recipient via a single genetic event. The emergence of multidrug-resistant bacteria can only be detected through systematic screening in quality assured microbiology laboratories (Xavier *et al.,* 2010; Liu *et al.,* 2016). The health and economic consequences of AMR has led to collaborative global action to improve access to antimicrobial medicines and to promote availability of new product (Mendelson, 2015). Therefore, the use of herbal traditional medicines has emerged as effective alternatives.

1.3 Justification for the Study

Helianthus annuus has been found to possess broad spectrum of *in vitro* antimicrobial activity against pathogenic microorganisms such as *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Candida albican, Aspergillus fumigatus* and *Rhizopus stolonifera* (Subashini and Rakshitha, 2012; Eze *et al.,* 2015). As evident from the antibacterial study of *H. annuus* seeds by Rubab *et al.* (2016), there are claims that it may contain important chemical substances that confer this plant as medicinal agent possessing antibacterial activity. According to Sharma (2014), *H.annuus* contains various alkaloids, flavonoids, volatile oils and terpenoids which are essential for various antimicrobial activities, antitumor activity and antioxidant activity.

H. annuus seeds are cheap, readily available, accessible and nutritious which possess promising role in variety of infections, inflammations, cancers and cardiac diseases (Ruchika, 2014). Previous studies by Aboki *et al.* (2012) used Soxhlet extraction method and n-hexane (as solvent of extraction) at 60-65˚C, to determine antimicrobial activity of *H. annuus* extracts. In this study, cold maceration method was employed. Several studies

have proved that the methanolic crude extracts of *H. annuus* have antimicrobial activity against wide range of pathogenic microorganisms.

1.4 Aim and Objectives of the Study

The study investigated the antibacterial effect of crude extracts of *Helianthus annuus* seeds on *Salmonella spp* and *Escherichia coli*.

The objectives of the study were to determine the:

i. phytochemical constituents of the crude extracts of *Helianthus annuus* seeds.
ii. *in vitro* antibacterial effect of the crude extracts of *Helianthus annuus* seeds on *Salmonella* spp. and *E.coli*
iii. minimum inhibitory concentration (MIC) and minumum bactericidal concentration (MBC) of the crude extracts of *Helianthus annuus* seeds.
iv. toxicity of the crude extracts of *Helianthus annuus* seeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Medicinal Plant Use

Medicinal plants have undoubtedly been considered by human beings since ancient times. It can be said that before history, the early humans recognized and exploited the plants around them for use as fuel, clothing, shelter and food, they became aware of their properties (Jamshidi-Kia *et al*., 2018). Medicinal plants have been transformed into one of the oldest sciences in countries such as China, Greece, Egypt and India.

The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake (Kelly, 2009). The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: *Rhei rhisoma*, camphor, *Theae folium*, Podophyllum, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra (Wiart, 2006).

The Indian holy books Vedas mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc. The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 prescriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury (Jamshidi-Kia *et al*., 2018).

In Homer's epics The Iliad and The Odysseys, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to.

Some of them were given the names after mythological characters from these epics; for instance, Elecampane (*Inula helenium* L. Asteraceae) was named in honor of Elena, who was the centre of the Trojan War. As regards the plants from the genus Artemisia, which were believed to restore strength and protect health, their name was derived from the Greek word artemis, meaning "healthy." (Toplak, 2005; Jamshidi-Kia *et al*., 2018). Herodotus (500 BC) referred to castor oil plant, Orpheus to the fragrant hellebore and garlic, and Pythagoras to the sea onion (*Scilla maritima*), mustard, and cabbage. The works of Hippocrates (459– 370 BC) contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (*Centaurium umbellatum* Gilib) were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents (Gorunovic and Lukic, 2001; Jamshidi-Kia*et al*., 2018).

There are several other historical reports on the usage of medicinal plants as therapy in managing and treating ailments, not withstanding, today, they are still being used. Medicinal plants have gained international popularity due to its natural source, availability in local communities, affordability and ease of administration. Herbal medicine may be used as alternative treatment in case of adverse effects and drug resistance (Sasidharan *et al.*, 2011; Pandey and Tripathi, 2014; Azwanida, 2015; Ingle *et al.*, 2017).

2.2 Sunflower (*Helianthus annuus* L.) Plant

2.2.1 History of sunflower usage and domestication

Sunflower was domesticated by Native Americans in the Eastern United States in about 3000 BC (Smith, 2006). They used the seeds directly as food and crudely extracted the oil. Native Americans had selected a tall, single-headed variety by the time European

explorers reached North America in the sixteenth century. While sunflower was not the staple that the 'three sisters' (maize, beans, and squash) were, it nonetheless was cultivated by many tribes from eastern North America through the Midwest and as far as northern Mexico. The Native Americans also used sunflower hulls as a source of dye, leaves for herbal medicines, and pollen in religious ceremonies (Seiler *et al*., 2017).

Historical records indicate that the Spanish were the first to introduce sunflower to Europe in the early 1500s (Marek, 2019). Sunflower was initially grown as an ornamental plant. Early English and French explorers also introduced it to their respective countries. From Western Europe, sunflower spread along the trade routes to Egypt, Afghanistan, India, China, and Russia. By the early 1700s, sunflower seeds were eaten as a snack, and in 1716, the first patent for the use of sunflower oil (for industrial purposes) was filed in England. The most significant boost for sunflower as a crop, however, came from the Russian Orthodox Church. Lenten regulations prohibited the consumption of many oily foods, but since sunflower was not specifically listed, the seed and oil became a staple diet item in Russia (Marek, 2019).

Efforts by Russian scientists led to significant crop improvements with oil contents soon exceeding 40 %. While sunflower was grown throughout Western and Eastern Europe, Russia historically was the largest producer with excess of 3 million hectares in the early twentieth century, compared to one-half million hectares for the rest of Europe. Russian immigrants are credited with introducing sunflower to North America. In fact, the open-pollinated variety 'Russian Mammoth' still sold by garden seed firms traces its lineage back to the same-named variety initially introduced in the 1880s. Early cultivation of sunflower in North America was primarily for livestock silage and seed for poultry. By the second half of the twentieth century, improved Russian varieties with oil levels of 45–55 % were available (Putt, 1997). Increased US production of these high-oil

sunflower varieties spurred interest by oil crushers, which led to expanded US production, especially in the Northern Great Plains (North Dakota and Minnesota). The discovery of cytoplasmic male sterility (CMS) by French scientists laid the foundation for the development of sunflower hybrids in the early 1970s. Hybrid sunflower, with higher yields and oil content, more uniformity, and disease resistance, in comparison with open-pollinated varieties, provided the last great impetus in establishing sunflower as a worldwide crop (Fick and Swallers, 1972; Marek, 2019)

2.2.2 Botanical description of the plant

Helianthus annuus L. is a coarse, stout and erect annual plant, up to 1-3 meters high. The roots of the seedling are initially tap rooted, with maturing, it develops into a large fibrous and lateral root. Their stem are 1-6.5 ft. (30-200 cm) tall, hispid, round, branched. The branching are simple to highly branched, each terminating with a composite head (capitulum).

It possesses a large inflorescence (flowering head), and its name is derived from the flower's shape and image, which is often used to depict the sun (Plate I). The flowering head has large, composite heads, solitary at terminal end of peduncle or terminal on a branch, or axillary; composite disk usually 0.8-3.2 in. (2-8 cm) wide or more including rays; peduncles 0.8-8 in. (2-20 cm) long, densely hispid-scabrous. The receptacle are low-convex and chaffy (Khaleghizadeh, 2011).The heads consist of many individual flowers. The ray flowers are sterile with length ranging from 0.6-1.6 inch (1.5-4 cm) long, ligules are yellow in colour. Disc flowers are perfect, corolla lobes are about five in number and are 0.2- 0.3 inch. (5-8 mm) long, tubular, purple-brown to yellow in colour with each floret subtended by a small firm.

Figure 1.0: *Helianthus annuus* L. Flower

Source: (Seiler *et al*., 2017).

Helianthus annuus L lower leaves mostly opposite along stem, upper leaves mostly alternate along stem. Leaf blades narrowly to usually broadly deltoid-ovate, lower ones often cordate, to sub-truncate to broadly cuneate at base, 1.5-8 inch (4-20 cm) long or more, 1.2-6 inch (3-15 cm) wide or more, entire to margins minutely to coarsely serrate, apex acute to abruptly auminate.

Figure 1.1: *Helianthus annuus* L. Leaf

Source: (Seiler *et al*., 2017).

Figure 1.2: Sunflower Field in Bloom

Source: (Seiler *et al*., 2017).

The seeds are in the form of Achenes and are between 0.1-0.3 inches (3-6 mm) long or more, they are narrowly obovate to ovate in shape, more or less four angled and somewhat compressed (Figure 1.3)

Figure 1.3: *Helianthus annuus* L. Seeds

Source: (Seiler *et al*., 2017).

2.2.3 Taxonomy

Table 1: Scientific classification of *Helianthus annuus* L.

Classification	Taxonomy
Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	*Helianthus*
Specie	*Annuus*

Source: (Fabian *et al.,* 2014)

2.2.4 Species of *Helianthus*

There are about 70 species of *Helianthus* which include *H. annuus*L., *H anomalus* Blake, *H. argophyllus* T. & G., *H. bolanderi* A. Gray, *H. debilis* Nutt., *H. deserticola* Heiser, *H. exilis* A. Gray, *H. neglectus* Heiser, *H. niveus* (Benth.) Brandegee, *H. paradoxus* Heiser, *H. petiolaris* Nutt., *H. praecox* Engelm and A. Gray, *H. agrestis* Pollard, *H. arizonensis* R. Jackson, *H. ciliaris* DC., *H. laciniatus* A. Gray, *H. cusickii* A. Gray, *H. gracilentus* A. Gray, *H. pumilus* Nutt., *Atrorubens Corona-solis H. californicus* DC., *H. decapetalus* L., *H. divaricatus* L., *H. eggertii* Small, *H. giganteus* L., *H. grosseserratus* Martens, *H. hirsutus* Raf., *H. maximiliani* Schrader, *H. mollis* Lam., *H. nuttallii* T. & G., *H. resinosus* Small, *H. salicifolius* Dietr., *H. schweinitzii* T. & G., *H. strumosus* L., *H. tuberosus* L., *H. glaucophyllus* Smith, *H. laevigatus* T. & G., *H. microcephalus* T. & G., *H. smithii* Heiser, *H. atrorubens* L., *H. occidentalis* Riddell, *H. pauciflorus* Nutt. (synonym *H. rigidus* Cass.), *H. silphioides* Nutt., *H. angustifolius* L., *H. carnosus* Small, *H. floridanus* A. Gray ex Chapman, *H. heterophyllus* Nutt., *H. longifolius* Pursh, *H. radula*

(Pursh) T. & G. and *H. simulans* E.E. Wats. Howerver, few of these species are of medicinal importance which are *Helianthus annuus L*. and *Helianthus tuberosus* (Al-Snafi, 2018).

2.2.5 Common names of *Helianthus annuus* L.

Arabic: Dawar El Shams, Zahrat El Shams; English: Sunflower; French: Grand soleil, Tournesol; German: Sonnenblume; Hindi: Surajmukhi; Italian: Girasole; Japanese: himawari; Korean: Haebaragi; Portuguese: Girassol; Spanish: Girasol; Swedish: solros (Al-Snafi, 2018).

2.2.6 Biological and chemical composition of *Helianthus annuus* L.

Helianthus annuus seeds are described as achene, a specific type of indehiscent fruit. Individual seeds are approximately 10 to 15 mm long (Muhammad and Muhammad, 2012). The seeds are used as food and poultry feed worldwide. Its oil is used for frying and cooking. The iron-rich sunflower seeds are, by weight, 47 % fat and 24 % protein (Muhammad and Muhammad, 2012). Sunflower seed proteins are characterised by a moderately low level of albumin and high level of globulin proteins. The globulins represent 55 to 60 %, albumins 17-23 %, glutelins 11 to 17 %, Prolamines, 1 to 4 % and the combined non-protein nitrogen and insoluble residue is less than 11 % of the total nitrogen in the meal (Muhammad and Muhammad, 2012).

The phytochemical constituent of methanolic extract of *Helianthus annuus* seeds have been reported to contain carbohydrates, flavanoids, tannins, alkaloids, saponins, phytosterols, steroids and fixed oils (Subashini and Rakshitha, 2012). Allelochemicals in leaves, stems and roots of *Helianthus annuus* were determined using thin layer chromatography for alkaloids and spectrophotometry for phenols and flavonoids. These revealed high content of allelochemicals in leaves compared to roots and stems (Kamal

et al., 2011). The aerial part of *Helianthus annuus* was found to contain an ent-kaurane glycoside named helikauranoside A together with three known ent-kaurane-type diterpenoids: (−)-kaur-16-en-19-oic acid, grandifloric acid, and paniculoside IV (Macias *et al*., 2008)

There are four tocopherol (α, β, γ and δ) isomers present in *Helianthus annuus* seed oil Fiska *et al.,* 2006). Proteins which were light colored were also isolated which contains helianthinin as globulin (Pickardt *et al.,* 2011). A bioflavonoid called Nevadensin, which has significant biological activities including hypotensive, anti-tubercular, antimicrobial, anti-inflammatory, anti-tumour and anti-cancer activities was isolated from *Helianthus annuus* (Pickardt *et al.,* 2011).

2.2.6 Uses of sunflower

2.2.6.1 Uses of sunflower as edible oil

Commercially available sunflower varieties contain from 39 to 49 % oil in the seed. In 1985-86, sunflower seed was the third largest source of vegetable oil worldwide, following soybean and palm (Pilorge, 2020). The growth of sunflower as an oilseed crop has rivaled that of soybean, with both increasing production over 6-fold since the 1930s. Sunflower accounts for about 14 % of the world production of seed oils (6.9 million metric tons in 1985-86) and about 7 % of the oil cake and meal produced from oil seeds. Europe and the USSR produce over 60 % of the world's sunflowers (Dafalla, 2012).

The oil accounts for 80 % of the value of the sunflower crop, as contrasted with soybean which derives most of its value from the meal. Sunflower oil is generally considered a premium oil because of its light color, high level of unsaturated fatty acids and lack of linolenic acid, bland flavor and high smoke points (Hamed *et al*., 2012). The primary fatty acids in the oil are oleic and linoleic (typically 90 % unsaturated fatty acids), with

the remainder consisting of palmitic and stearic saturated fatty acids. The primary use is as a salad and cooking oil or in margarine. In the USA, sunflower oils account for 8 % or less of these markets, but in many sunflower-producing countries, sunflower is the preferred and the most commonly used oil (Gupta, 2002).

High oleic sunflower oil (over 80 % oleic acid) was developed commercially in 1985 and has higher oxidated stability than conventional oil (Purdy, 1985). It has expanded the application of sunflower oils for frying purposes, tends to enhance shelf life of snacks, and could be used as an ingredient of infant formulas requiring stability.

2.2.6.2 Uses of sunflower as meal

Non-dehulled or partly dehulled sunflower meal has been substituted successfully for soybean meal in isonitrogenous (equal protein) diets for ruminant animals, as well as for swine and poultry feeding. Sunflower meal is higher in fiber, has a lower energy value and is lower in lysine but higher in methionine than soybean meal. Protein percentage of sunflower meal ranges from 28 % for non-dehulled seeds to 42 % for completely dehulled seeds. The color of the meal ranges from grey to black, depending upon extraction processes and degree of dehulling (Ogello *et al*., 2017).

2.2.6.3 Industrial applications of sunflower

The price of sunflower oil usually prohibits its widespread use in industry, but there are several applications that have been explored. It has been used in certain paints, varnishes and plastics because of good semi drying properties without color modification associated with oils high in linolenic acid (Gunduz, 2015). In Eastern Europe and the USSR where sunflower oil is plentiful, sunflower oil is used commonly in the manufacture of soaps and detergents. The use of sunflower oil (and other vegetable oils) as a pesticide carrier, and in the production of agrochemicals, surfactants, adhesives,

plastics, fabric softeners, lubricants and coatings has been explored. The utility of these applications is usually contingent upon petrochemical feedstock prices.

Sunflower oil contains 93 % of the energy of US Number 2 diesel fuel (octane rating of 37), and considerable work has been done to explore the potential of sunflower as an alternate fuel source in diesel engines. Blends of sunflower oil and diesel fuel are expected to have greater potential than the burning of pure vegetable oil (Ilkilic, 2008).

2.2.6.4 Uses of sunflower as non-oil seed

The use of sunflower seed for birdfeed or in human diets as a snack has grown consistently over the past 15 years (Putnam *et al*., 1990). Varieties used for non-oil seed purposes are characterized by a larger seed size and require slightly different management practices. During processing, seed is divided into

1) larger seed for in-shell roasting,

2) medium for dehulling, and

3) small for birdseed. However, the standards for different uses vary.

2.2.6.5 Use of sunflower as forage

Sunflower can also be used as a silage crop. It can be used as a double crop after early harvested small grains or vegetables, an emergency crop, or in areas with a season too short to produce mature corn for silage (Putnam *et al*., 1990). Forage yields of sunflower are generally less than corn when a full growing season is available. In one study, sunflower dry matter yields ranged from 2.0 to 3.0 ton/acre compared with 3.1 to 3.8 ton/acre for corn. Moisture content of sunflower at maturity is usually high (80 to 90 %) and would require wilting before ensiling (Heuze *et al.*, 2015).

Nutritional quality of sunflower silage is often higher than corn but lower than alfalfa hay. Crude protein level of sunflower silage is similar to grass hay and higher than corn silage. Generally, crude protein of sunflower decreases and lignin percentage increases after the flowering stage. High plant populations increases fiber and lignin percentage. Seed size does not seem to affect yield or quality (Heuze *et al.,* 2015).

2.2.7 Medicinal uses *Helianthus annuus* (sunflower) plant

Helianthus annuus has been grown widely due to its traditional use as anti-inflammatory, antimalarial, anti-asthmatic, anti-oxidant, anti-tumor and antimicrobial agent (Saini and Sharma, 2011). The Seeds of *Helianthus annuus* is of great medicinal importance. The seeds and flowers of *Helianthus annuus* have been used as excellent source of protein and Vitamins B, D, E and K and also used in the treatment of pulmonary disorders (Saini and Sharma, 2011). Oil extracted from *Helianthus annuus* seeds have been used in treatment of dysentery, dysuria, hemorrhoids, fever, menorrhagia, pleuritis, Inflammation and bronchitis (Sharma *et al.,* 2009).

A decoction made from the leaves of *Helianthus annuus* leaves is used as astringent, diuretic and expectorant (Arshad and Amjad, 2012). The crushed leaves were used as a poultice on sores, swellings, snakebites and spider bites. A decoction of the roots was used as a warm wash on rheumatic aches and pains (Aboki *et al.,* 2012).

The seeds can be made into infusions and used in the treatment of whooping cough (Dwivedi and Sharma, 2014). Seeds are also used as diuretic, expectorant and in treatment of other lung ailments (Kunduraci *et al.,* 2010). The flowers and seeds were used in Venezuela in folk remedies for the treatment of cancer (Dwivedi and Sharma, 2014). The seed oil, and herbal tincture was used as anti-inflammatory, antioxidant,

antitumor, antipyretic, antihypoglycemic, cathartic, diuretic and antimicrobial (Aboki *et al.,* 2012). The following are the specific medicinal properties of *H. annuus*:

2.2.7.1 Antimicrobial activity of *Helianthus annuus*

The antimicrobial activity of methanolic extract of seeds from *Helianthus annuus* was evaluated. The results of antibacterial activity showed high sensitivity to *Salmonella typhi*, moderate sensitivity to *Staphyllococcus aureus* and *Vibrio cholera* and less sensitivity to *Bacillus subtilis* (Subashini *et al.,* 2012). The results of antifungal activity of the extract of *Helianthus annuus* showed high sensitivity to *Rhizopus stolonifer* and *Aspergillus fumigates,* moderate sensitivity to *Candida albicans* and resistant to *Fusarium oxysporum* (Subashini *et al.,* 2012).

Antimicrobial activity of oil of *Helianthus annuus* seed was investigated against four bacterial isolates which include *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and one fungal isolate, *Candida albicans (*Aboki *et al.,* 2012). The tested microorganisms were cultured on Nutrient agar (for bacteria at 37 ˚C for 24 h) for the bacteria isolates and on Potatoes Dextrose Agar for the fungal isolates (for fungus at 30 ˚C for 48-72 h). *Bacillus subtillus* had the least mean zone of inhibition of 8 mm, the mean zone of inhibition observed with oil of sunflower seed (at low concentration of 0.2, 0.4, 0.6 mL) of up to 23 mm against C albicans, 25 mm against S aureus, 15 mm with *P. aurenosa* justified that oil of *Helianthus annuus* seed could be used as an antimicrobial agent *(*Aboki *et al.,* 2012).

The antibacterial activity of *Helianthus annuus* leaves was tested on some bacterial pathogens (*Escherichia coli, Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus*. The extracts were prepared using chloroform, N-hexane and methanol. The antibacterial susceptibility testing of the extracts were carried out using the Agar well diffusion

method (Eze *et al.,* 2015). The antibacterial screening showed that the test organisms were all susceptible to the extract. *S. aureus* showed the highest susceptibility to the extracts while *Shigella* spp. showed least susceptibility. The minimum inhibitory concentration of the methanolic fraction of the extract was determined to be 125 μg/ml on all the test organisms which was lower than the MIC of gentamycin (280 μg/ml) (Eze *et al.,* 2015).

The *in vitro* antibacterial activity of the methanol, ethyl acetate and petroleum extracts obtained from the seeds of *H. annuus* was determined against three pathogenic bacteria (*Salmonella typhi*, *Pseudomonas aeruginosa* and *Vibrio cholerea*) (Rubab *et al.,* 2016). The antimicrobial activity was determined by using the standardized disc diffusion method (Zavala *et al.*, 1997; Hossain *et al.*, 2012). The extract concentration able to inhibit bacterial growth was observed through inhibition zone of 8 mm. The methanolic and ethyl acetate extract of *H. annuus* seeds showed high sensitivity to *Salmonella typhi* and moderate sensitivity to *Pseudomonous aeuregenosa*. The petroleum ether extract revealed high sensitivity to *P. aeuregenosa (*Rubab *et al.,* 2016*).*

2.2.7.2 Antiasthmatic effect of *Helianthus annuus*

The effects of *Helianthus annuus* aqueous seed extract was examined on an in vivo anti-asthmatic model of ovalbumin induced mice and their lungs were assessed by hematoxylin and eosin staining. These revealed that the extract has potential in reducing asthma (Heo *et al*., 2008).

2.2.7.3 Antioxidant effect of *Helianthus annuus*

The antioxidant capacity of the striped *Helianthus annuus* seed cotyledon extracts was obtained by extraction with different polarities of solvents by three different in vitro methods which are, ferric reducing/antioxidant power, 2.2-diphenyl-1-picrylhydrazyl radical and oxygen radical absorbance capacity assays. In the three methods, the aqueous extract with a dose 30 μg/ml showed a higher antioxidant capacity value than the ethanolic extract (Giada and Mancini-Filho, 2009). When compared with the synthetic antioxidant butylated hydroxyl toluene, the antioxidant capacity of the aqueous extract varied from 45% to 66% (Giada and Mancini-Filho, 2009). The high antioxidant capacity observed for the aqueous extract of *Helianthus annuus* seed suggests that the intake of this seed may prevent cancer and other oxidative reaction related diseases (Giada and Mancini-Filho, 2009).

The antitumor and antioxidant activity of *Helianthus annuus* was determined. Antioxidant activity results were measured using DPPH and fixed oil of *Helianthus annuus* showed moderate antioxidant activity. Fixed oil of this plant showed no activity when using Iron Chelating Assay (Abushama *et al*., 2014). The antioxidant activity of methanol, chloroform and N-hexane leaves extract was determined using the 2, 2 - diphenyl-1-picrylhydrazyl (DPPH) photometric assay (Eze *et al.,* 2015). The extracts produced a concentration dependent increase in antioxidant activity. The chloroform extract showed greater antioxidant activity while the methanolic extract was least when compared to the ascorbic acid (standard) (Eze *et al.,* 2015).

Based on a study by Rubab *et al.* (2016), the in vitro antioxidant activity of methanolic seed extract of *H. annuus* was determined by DPPH free radical scavenging assay, which demonstrated very significant antioxidant potential compared to standard antioxidant (ascorbic acid). Methanolic extract of *H. annuus* showed 51.57 % DPPH scavenging

activity and the half maximum inhibitory concentration (IC50) was 0.814 whereas 46.66 % DPPH scarvenging activity and half maximum inhibitory concentration (IC50) of 1.0 in case of ascorbic acid (Rubab *et al.,* 2016).

2.3 Research Microorganisms

2.3.1 *Salmonella spp*

Salmonella infection is one of the most common food-borne infections worldwide. *Salmonella* belongs to the family Enterobacteriaceae. It is a Gram-negative, non-spore-forming, rod-shaped and facultative anaerobic bacterium. Some sub species of *Salmonella* ferment lactose. *Salmonella* are hydrogen sulfite producers, they are oxidase-negative and catalase-positive. It hydrolyzes urea, utilizes citrate and decarboxylates lysine as its sole carbon source (Feasey *et al.,* 2012; Andino and Hanning, 2015).

2.3.1.1 Epidemiology of *Salmonella* infection

There is high global burden of morbidity and mortality from human enteric pathogenic bacteria such as *Salmonella* species, despite the presence of antibiotic drugs (Petri *et al.,* 2008; Kirk, *et al.,* 2010; Dixon and Hall, 2015). *Salmonella* infection causes about 2.8 billion cases of diarrhoea annually worldwide. *Salmonella* enterica serovar Typhi (*S. typhi*) which is the causative agent of typhoid fever, is reported to cause 16–33 million infectious cases, with an estimated 500 000 to 600 000 deaths, while nontyphoidal *Salmonella* (NTS) infections account for 90 million cases and 155 000 deaths worldwide annually (Bula-Rodas *et al.,* 2015). The high prevalence of human immunodeficiency virus (HIV) infections in Africa, has led to exercebation of *Salmonella* infections and it has been reported that there are 2000–7500 *Salmonella* infection cases per 100 000 HIV infected adults (Feasey *et al.,* 2012). In Africa, 29.1 % of community- acquired bloodstream infections were attributed to *Salmonella* species (Nam *et al.,* 2015).

2.3.1.2 Sources of infection and mode of transmission

Salmonella are commonly isolated foodborne pathogens, and are predominantly found in include fresh fruits and vegetables (Pui *et al.*, 2011). Farm animals such as swine, poultry and cattle are the prime sources of *Salmonella* infections. The slaughtering process of food animals at abattoirs is also considered one of the important sources of contamination with *Salmonella* (Gillespie *et al.* 2005). The bacterium can be transmitted through faecal–oral routes, where susceptible hosts may acquire *Salmonella* through contaminated foods and water (Ford *et al.*, 2016). The major dissemination is through consumption of uncooked animal food products.

2.3.1.4 Pathogenicity of *Salmonella* infection

When contaminated food or water is ingested, *Salmonella* colonizes the distal ileum and proximal colon (Hocking, 2012; Lonnermark *et al.*, 2015). The infective dose for salmonellosis that is capable of establishing infection in the mucosa of the small intestine ranges from 10^5 to 10^6 cells (Lonnermark *et al.,* 2015). Flagella serve as means of locomotion as well as chemotaxis to target cells, the enterocytes. *Salmonella* cells use type I fimbriae such as long polar fimbriae (Lpf) and thin aggregative fimbriae (Tafi), to adhere to enterocytes. Type IV pili are used by *S. typhi* to attach to host cells (Wagner and Hansel, 2011). Once *Salmonella* has adhered to the host cells on the apical side of M cells or enterocytes, it uses *Salmonella* pathogenicity islands (SPIs) encoded by type III secretion systems (T3SSs) to be phagocytized into the receptive macrophages (Wagner and Hansel, 2011).

Salmonella cells are exocytosed into the interstitial spaces of the lamina propria, where they are randomly picked by macrophages, dendritic cells and polymorphonuclear cells and distributed to the host efferent lymph in the mesenteric lymph nodes and then transported to the spleen and liver through the bloodstream (Velg *et al.*, 2012). The

attachment of *Salmonella* to the receptive epithelial cells and internalization into lamina propria causes inflammatory responses such as, release of pro-inflammatory cytokines which lead to diarrhoea, ulceration and the destruction of the mucosa cells (Velg *et al.*, 2012). Some species of *Salmonella* release enterotoxin and cytotoxin which can cause diarrhoea.

2.3.1.5 Diagnosis of *Salmonella* infection

The widal tube agglutination test has been used for decades. It involves detection of *Salmonella* serovar Typhi and Paratyphi A, with serum that measures agglutinating antibodies to the LPS (O) and flagellar (H) antigens. The efficacy of the Widal test is controversial, due to poor sensitivity and cross-reactivity with other *Salmonella* serovars, resulting in a low predictive value for typhoid fever (Ley *et al.*, 2010; Baker *et al.*, 2010). Non-*Salmonella* infections including malaria and brucellosis were also shown to lead to cross-reactivity in regions where enteric fever is endemic (Baker *et al.*, 2010). Due to low cost and simplicity, Widal test is still commonly used as a diagnostic test in regions that lack advanced laboratory infrastructure (Thriemer *et al.*, 2013). The laboratory diagnosis of typhoid fever is dependent on the detection of bacteria in the blood by PCR or culture. Microbiological culture has excellent specificity with low sensitivity and often requires 24 to 72 h of incubation. Hence, molecular approaches for *Salmonella* identification characterized by high sensitivity and a short time have been developed. PCR-based assays have been clinically validated for diagnosis of gastrointestinal Non Typhoidal *Salmonella* infection (Lin *et al.*, 2011), invasive Non Typhoidal *Salmonella* infection (Tennant *et al.*, 2010) and typhoidal *Salmonella* infection in the blood of patients with enteric fever.

2.3.1.6 Treatment of *Salmonella* infection

Antibiotics are the mainstay in the treatment of infectious diseases and improve health related quality of life, in addition to reducing the mortality associated with bacterial infections. The selectivity of antibiotic drugs against invading bacteria ensures minimal harm to the patients and at the same time ensures maximum eradication of the target bacteria (Nam *et al.,* 2015). Non typhoidal Salmonellosis infections are associated with complications such as meningitis and septicaemia therefore, require treatment with antibiotic drugs, including ciprofloxacin, ceftriaxone and ampicillin (WHO, 2003; Medalla *et al.,* 2012). Infections caused by *S. typhi* and *S. paratyphi* may involve serious complications and require treatment with antibiotics such as cefixime, chloramphenicol, amoxicillin, azithromycin, aztreonam, cefotaxime or ceftriaxone (Kumar and Kumr, 2017).

2.3.1.7 Antimicrobial resistance to *Salmonella*

Antimicrobial drug resistance of non-typhoidal *Salmonella* organisms has been discovered in developed countries as a result of inevitable consequence of the use of antimicrobial drugs in food producing animals. Such drugs may be used either therapeutically or prophylactically, or for growth promotion (feed additives). The first line treatment choices for enteric and non- typhoidal salmonellosis disease were co-trimoxazole, ampicillin or chloramphenicol. However, from the late 1980s, there was an increase in prevalence of resistance to these commonly used antibiotics.

Resistance to commonly used antimicrobials in Typhi and Paratyphi A is a widespread problem in endemic areas and returning travellers (Wain *et al.,* 2015). This led to use of broad spectrum cephalosporins and fluoroquinolones which replaced older agents in the management of *Salmonella* disease (Crump *et al.,* 2011; Msefula *et al.,* 2012)**.** Studies by Wong *et al.* (2014) reported that specific *Salmonella* isolates ST313 were discovered to

be resistant to cephalosporins, tetracyclines, Co-trimoxazole, chloramphenicol and Aminoglycoside such as Streptomicin.

2.3.2 *E. coli* infection

Escherichia coli are a group of gram negative bacteria normally found in the flora of human and animal digestive tracts and as symbionts participating in digestion. *E. coli* are involved in urinary tract infection (UTI), hospital acquired pneumonia (HAP), sepsis, surgical site infection (SSI), gastrointestinal tract infections and hemolytic-uremic syndrome (HUS) (Alkeskas *et al.*, 2015). *Escherichia coli* is the major areobic organism residing in the intestine. It is also found in soil and water as a result of faecal contamination (Tenaillon *et al.*, 2010). Some pathotypes of *E. coli* are known to cause infection of the gastrointestinal system (intestinal pathogenic *E. coli*) while others cause infection outside the gastrointestional system (extraintestinal pathogenic *E. coli*) (Croxen and Finlay, 2010).

2.3.2.1 Epidemiology of *E. coli*

Escherichia coli is the most common cause of urinary tract infection (UTIs) in humans. And is the leading cause of enteric infection and systemic infections (Kim, 2012). The systemic infections include bacteremia, nosocomial pneumonia, cholecystitis, cholangitis, peritonitis, cellulitis, osteomelytis and infectious arthritis. *E. coli* is also the leading cause of neonatal meningitis (Kim, 2012). *E. coli* has been associated with increased morbidity and mortality in recent years (Barton *et al.*, 2011).

Enterotoxigenic *E. coli* (ETEC) is reported to be the most common bacterial enteropathogen isolated in children less than 5 yrs of age in developing countries accounting for about ten thousand deaths each year (Barton *et al.*, 2011). It is also the most common cause of travelers' diarrhea accounting for 10–60 % of infections

depending on the region visited (Barton *et al.,* 2011). Enteroaggregative *E. coli* (EAEC) is the second most common cause of travelers' diarrhea after ETEC. It causes persistent diarrhea in children in developing countries and has been implicated as an important enteric pathogen affecting AIDS patients (Cappello *et al.,* 2011).

2.3.2.2 Sources of infection and mode of transmission

Escherichia coli are the most common member of the Enterobacteriaceae which accounts for 75 - 90 % of Urinary tract infetions in humans (Barton *et al.,* 2011). *E. coli* can be isolated from contaminated food obtained from animal origin like milk (Disassa *et al.,* 2017). Human infection occurs through consumption of contaminated food products such as uncoooked meat or water contaminated with animal or human wastes. It could be through direct person-person spread from poor hygeine (Berger *et al.,* 2010). The potential source of pathogenic *E. coli* is host own intestinal flora, but the infection can also be transmitted through the fecal–oral route or through sexual contact (Terlizzi *et al.*, 2017).

2.3.2.3 Pathogenicity of *E. coli* infection

The factors responsible for the virulence of pathogenic *E.coli* include adhesins, toxins (e.g. alpha-hemolysin, cytotoxic necrotizing factor 1, autotransporter toxins), iron/heme acquisition systems, and iron ion transport. P, S and type 1 fimbriae which are responsible for adhesion to epithelial cells of intestines, kidneys, or lower urinary tract and for stimulating cytokine production by T cells. *E. coli* has the ability to multiply intracelullarly (Baldy-Chudzik *et al.,* 2015). The pathology of *E. coli* (ETEC) occurs through secretion of heat stable toxins STa/STI and STb/STII. STa/STI mimics the intestinal hormone guanylin, binding to and activating intestinal borders guanylate cyclase C receptor, increasing intracellular messenger cyclic GMP. This activates cyclic GMP-dependent protein kinase II leading to phosphorylation of cystic fibrosis

transmembrane regulator and deregulated ion absorption and secretion hence diarrhoea occurs (Baldy-Chudzik *et al.,* 2015).

2.3.2.4 Diagnosis of *E. coli* infections

Escherichia coli grow readily on simple culture media with minimal nutrient of glucose or glycerol. *E. coli* is identified as lactose fermenting gram negative rod. It can grow areobically or anaerobically at 37 ˚C and can be motile or non motile. It is oxidase negative and produces indole. It does not ferment citrate (Croxen and Finlay, 2010). *E. coli* is typed according to their somatic lipopolysaccharide (O), capsular (K) and Flagella (K) antigens. The O:H combination is referred to as serotype. *E. coli* O15; H7 is one of the major serotypes implicated in enterohaemorrhagic *E. coli* infections (Debroy *et al.*, 2011).

2.3.2.5 Treatment of *E. coli* infection

Antimicrobial agents such as β-lactams, fluoroquinolones, aminoglycosides and sulfamethoxazole- trimethoprim are used to treat *E. coli* infections (Pitout, 2012).

2.3.2.6 Antibacterial resistance to *E.coli*

Escherichia coli have become resistant to β-lactam antibiotics due to its outer membrane barrier. The production of β-lactamase is the most important mediator of resistance to broad spectrum of β-lactams (Johnson *et al.,* 2013). β-lactamases constitute different class of enzymes, which are often encoded on plasmids and are most commonly produced by *Enterobacteriaceae.* β-lactamases confer resistance to penicillins and cephalosporins and are the major cause of multidrug resistance in Gram-negative bacteria (Johnson *et al.,* 2013).

The up-regulation of efflux pumps and plasmid mediated resistance mechanisms reduce fluoroquinolone susceptibilities in *E. coli*. Resistance to fluoroquinolones is characterised

by 1-2 point mutation within the quinolone resistant determining regions of genes encoding for DNA gyrase and topoisomerase (Johnson *et al.,* 2013).

2.4 Antibacterial Resistance to infections

Antibiotic drug-resistant bacteria refers to bacteria with the ability to grow or survive in a concentration of antibiotic drug that is normally sufficient to be bactericidal or bacteriostatic (Sabtu *et al.,* 2015). Antibiotic drug resistance may be innate or acquired through exposure of the bacteria to the antibiotic. Conjugation, transduction and transformation are the genetic mechanisms used by bacteria to acquire antibiotic-resistant genes (Sabtu *et al.,* 2015). Resistant bacteria from animals can infect humans by direct contact or through food products of animal origin. There is an increase in multi-resistant strains due to the spread of genes such as plasmids, integrons and transposons which combine with chromosomally encoded resistance genes. Bacteria that have been exposed to low doses of these antibiotics in tissues and products from animals may be less susceptible to drugs therefore, when such bacteria invades the human body through consumption of contaminated foods, they may cause infections that are resistant to many antibiotics (Clauben *et al.,* 2013).

The emergence of major food borne pathogens such as *Salmonella* and *Escherichia coli* have persisted as a major public health concerns and provide evidence of persistence of food borne pathogens despite considerable efforts aimed at prevention and control (Newell *et al.,* 2010). The resistant strains of *Salmonella and E. coli* is mainly promoted by the use of antibiotics in animal feed to promote the growth of food animals, and in veterinary medicine to treat bacterial infections in those animals (Hyeon *et al.,* 2011). The irrational use of antibiotics in food producing animals could result into antibiotic residues in edible tissues and products (Darwish *et al.,* 2013). The rise in antibiotic resistant

pathogens has led to the development of medicinal plants as an alternative method to control pathogenic microorganisms.

2.5 Phytochemical Components

Phytochemicals is obtained (from the Greek word phyto, meaning plant). These are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for man (Hasler and Blumberg, 1999). They also protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack. These phytochemicals contribute to the plant's color, aroma and flavor (Gibson *et al*., 1998). A wide range of dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Phytochemicals are found in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits and seeds (Kochie *et al*., 2010).

Phytochemicals are also known as secondary plant metabolites which possses biological properties such as antioxidant activity and antimicrobial effect. Current researches have shown that many phytochemicals are used to prevent certain diseases in humans (Kochie *et al*., 2010). Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids and chlorophyll's. Secondary constituents are plant substances such as alkaloids, terpenes, flavonoids, plant steroids, saponins, phenolics, and glucosides (Ramawat *et. al.,* 2009). The following are classes of phytochemical compounds.

2.5.1 Classification of phytochemicals

2.5.1.1 Phenolic contents

Phenolic compounds are large and complex group of chemical constituents found in plants (Dai and Mumper, 2010). Phenolics posses several beneficial properties to humans and its antioxidant properties serve as protective role against free radical-mediated disease processes. There are three (3) most important groups of dietary phenolics which are flavonoids, phenolic acids, and polyphenols.

I. Phenolic acids

These are phenolic compounds that contain one carboxylic acid functional group. Naturally occurring phenolic acids contain two diferent carbon frameworks which are the hydroxycinnamic and hydroxybenzoic structures. Hydroxycinnamic acid compounds are produced as simple esters with glucose or hydroxy carboxylic acids. Plant phenolic compounds are different in molecular structure, and are characterized by hydroxylated aromatic rings (Mandal *et al*., 2010).

II. Flavonoids

Flavonoids are polyphenolic compounds that are abundant in nature. About 4,000 flavonoids have been known to occur in nature and are found in vegetables, fruits and beverages like tea, coffee and fruit drinks (Piero *et al*., 2015). Flavoinoids are common in parts of plants normally consumed by humans which include approximately 650 flavones and 1030 flavanols (Piero *et al*., 2015).

Most flavonoids occur naturally in combination with sugar moiety which can be characterized as monoglycosidic or diglycosidic. The glycosidic linkage is usually located at position 3 or 7 and the carbohydrate unit can be L-rhamnose, Dglucose, glucorhamnose, galactose or arabinose (Nyanmai *et al*., 2015).

2.5.1.2 Tannin contents

Tannins are naturally occuring substances that contain diverse oligomers and polymers (Harborne, 1999). They are heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin,), alkaloids, nucleic acids and minerals (Kar, 2007). Based on their structural characteristics, tannins are divided into four (4) major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins (Kar, 2007).

1. Gallotannins are all those tannins in which galloyl units or their meta-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.
2. Ellagitannins are those tannins in which at least two galloyl units are C–C coupled to each other, and do not contain a glycosidically linked catechin unit.
3. Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.
4. Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin.

2.5.1.3 Alkaloid contents

Alkaloids are natural product which contains heterocyclic nitrogen atoms that are basic in character. The name of alkaloids is derived from the word “alkaline” and it is used to describe nitrogen-containing base. The basicity of alkaloids varies depending on the structure of the molecule, presence and location of the functional groups (Sarker & Nahar, 2007). Alkaloids are grouped based on the type of heterocyclic ring system present in the molecule .Alkaloids are named based on the plants they are derived from or from their characteristic physiological activity. Most alkaloids are readily soluble in alcohol, although they are sparingly soluble in water, their salts of are usually soluble

(Firn, 2010). There are various classes of alkaloids according to heterocyclic ring system. They are; pyrrolidine alkaloids, pyridine alkaloids, pyrrolidine-pyridine alkaloids, pyridine-piperidine alkaloids, quinoline alkaloids and isoquinoline alkaloids.

2.5.1.4 Terpenoid contents

Terpenoids are a class of natural products derived from five-carbon isoprene units. Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons. They are found in living things, and therefore considered as the largest group of natural products (Firn, 2010). Terpenoids are used as flavours and fragrances in foods and cosmetics examples menthol and sclareol. Terpenes are present in plants as constituents of essential oils. Their building block is the hydrocarbon isoprene, CH_2=C (CH_3)-CH=CH_2. Terpene hydrocarbons therefore have molecular formula (C_5H_8) n and they are classified according to the number of isoprene units (Martinez *et al*., 2008). They are hemiterpenoids, monoterpenoids: sesquiterpenes, diterpenes, Triterpenes and Tetraterpenoids.

2.5.1.5 Saponin contents

Saponins are a group of secondary metabolites found in plant kingdom. The term saponin is derived from Saponaria vaccaria (Quillaja saponaria), a plant, which is abundant in saponins and was once used as soap (Kar, 2007). Saponins therefore produce foam in water. On hydrolysis, an aglycone is produced, which is called sapogenin. There are two types of sapogenin: steroidal and triterpenoidal. The sugar moiety is attached to C-3 in saponins, because in most sapogenins there is a hydroxylgroup at C-3. There are two major groups of saponins which are; steroid saponins and triterpene saponins. Saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis, they yield aglycones. They are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar organic solvents like benzene and n-hexane (Kar, 2007).

2.5.2 Medicinal properties of phytochemicals

Phenolics

Phenolic compounds have been subjected to Agricultural, biological, chemical and medical studies. Results have shown that phenolic compound posses antioxidant activities which can be used in processed foods as antioxidants. Therefore, the biological activity of these compounds is related to their antioxidant property (Lafay and Gil-Izquierdo, 2008). Phenolics acid have been found to possesses diverse biological activities such as antiulcer, anti- inflammatory, antioxidant (Silva *et al*., 2007), cytotoxic and antitumor, antispasmodic, and antidepressant activities (Ghasemzadeh *et al*., 2010). Flavonoids are group of phenolics with various therapeutic values. Flavonoids have been reported to posses antihyperglycemic effect (Muriithi *et al*., 2015). Flavonoids have been recognised due to their broad biological and pharmacological activities including anti-microbial, cytotoxic, anti-inflammatory and anti-tumor activities. The most prominent property of the flavonoid family is antioxidant property (Shirsat *et. al.*, 2012; Teiten *et. al.,* 2013) which protects humans from oxidative stress damage.

Tannins

In Asian medicine, plants containing tannins are used as astringents, diuretics and in treating diarrhoea, as well as stomach and duodenal tumours (Dolara *et al*., 2005). They are also used as anti- inflammatory, antiseptic and antioxidant (Dolara *et al*., 2005).

Alkaloids

Alkaloids are important in protection and survival of plants. They ensure protection against micro-organisms (such as bacteria and fungi), insects and herbivores (feeding deterrens) and also against other plants by production of allelopathic chemicals (Madziga *et al*., 2010). Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine), antimalarial activity

(quinine), and anticancer actions (vincristine, vinblastine). Some alkaloids are used as CNS stimulant such as caffeine and nicotine, they also posses analgesic activity as seen in morphine (Madziga *et al*., 2010).

Terpenoids

Teroenoids have medicinal properties such as anticarcinogenic (example, taxol), antimalarial (example, artemisinin). Sesquiterpene lactones have been isolated and found to posses antimicrobial (particularly antiprotozoal) and neurotoxic action (Degenhardt, 2003).

Saponins

Saponins are important for therapeutic purposes and have shown hypolipidemic and anticancer activity (Sarker and Nahar, 2007). The two major types of steroidal sapogenin are diosgenin and hecogenin. Steroidal saponins are used in production of sex hormones for clinical use such as progesterone is derived from diosgenin. The most abundant starting material for the synthesis of progesterone is diosgenin isolated from Dioscorea species. Steroidal hormones, such as cortisone and hydrocortisone, can be prepared from the starting material hecogenin, which is isolated from Sisal leaves (Sarker and Nahar, 2007).

2.6 Extraction of Plant Materials

Extraction refers to the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents using standard extraction procedures (Azwanida, 2015). The main purpose of extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). The crude extracts obtained contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids (Sasidharan *et al*., 2011).

Some of these crude extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need to be further processed and incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities (Rungsung *et al*., 2015). Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug. The common extraction methods include;

2.6.1 Types of extraction methods

2.6.1.1 Maceration method

Maceration involved soaking plant materials (coarse or powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation (Bimakr, 2010). This process is intended to soften and break the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or strained by filtration. This method is best suitable for use in case of the thermolabile drugs (Bimakr, 2010).

2.6.1.2 Infusion method

This is an extraction process similar to maceration. The drug material is grinded into fine powder, and then placed in a container. The extraction solvent hot or cold is then poured on the drug material, soaked, and kept for a short period of time (Pandey and Tripathi, 2014; Azwanida, 2015; Majekodunmi, 2015; Ingle *et al*., 2017). This method is suitable for extraction of bioactive constituents that are readily soluble. This method is employed for preparation of fresh extract before use. The solvent to sample ratio is usually 4:1 or 16:1 depending on the intended use (Pandey and Tripathi, 2014; Azwanida, 2015; Majekodunmi, 2015; Ingle *et al*., 2017).

2.6.1.3 Digestion method

This extraction method involves the use of moderate heat during extraction. The solvent used for the extraction is poured into a clean container followed by powdered drug material. The mixture is placed over water bath or in an oven at a temperature about 50 ºC (Pandey and Tripathi, 2014; Majekodunmi, 2015; Ingle *et al*., 2017). Heat is required throughout the extraction process to decrease the viscosity of extraction solvent and enhance the removal of secondary metabolites. This method is suitable for plant materials that are readily soluble (Pandey and Tripathi, 2014; Majekodunmi, 2015; Ingle *et al*., 2017).

2.6.1.4 Decoction method

This process involves continuous hot extraction using specified volume of water as a solvent. A dried, grinded, and powdered plant material is placed into a clean container. Water is then poured and stirred. Heat is then applied throughout the process to hasten the extraction (Pandey and Tripathi, 2014; Azwanida, 2015; Majekodunmi, 2015; Ingle *et al*., 2017). The process is lasted for a short duration usually about 15min. The ratio of solvent to crude drug is usually 4:1 or 16:1. It is used for extraction of water soluble and heat stable plant material (Pandey and Tripathi, 2014; Azwanida, 2015; Majekodunmi, 2015; Ingle *et al*., 2017).

2.6.1.5 Percolation method

This involves use of a percolator which is a narrow-cone-shaped glass vessel with opening at both ends. A dried, grinded, and finely powdered plant material is moistened with the solvent for extraction. Solvent is then added, and the mixture is kept for a period of 4h. Subsequently, the content is then transferred into the percolator with the lower end closed and allow to stand for a period of 24 h (Azwanida, 2015; Pandey and Tripathi, 2014; Majekodunmi, 2015). The solvent of extraction is then poured from the top until

the drug material is completely saturated. The lower part of the percolator is then opened, and the liquid allowed to drop slowly. A little quantity of solvent is added continuously, and the extraction takes place by gravitational force, pushing the solvent through the drug material downward (Azwanida, 2015; Pandey and Tripathi, 2014; Majekodunmi, 2015). The addition of solvent is stopped when the volume of solvent added reaches 75 % of the intended quantity of the entire preparations. The extract is separated by filtration followed by decantation. The marc is then expressed and final amount of solvent added to get required volume (Azwanida, 2015; Pandey and Tripathi, 2014; Majekodunmi, 2015).

2.6.1.6 Soxhlet extraction or hot continuous extraction method

This method involves the use of apparatus called Soxhlet extractor made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube, and condenser at the top. A dried, grinded, and finely powdered plant material is placed inside porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed (Doughari, 2012; Hossain *et al*., 2014). The extraction solvent is poured into the bottom flask, followed by the thimble into the extraction chamber. The solvent is then heated from the bottom flask, evaporates, and passes through the condenser where it condenses and flow down to the extraction chamber and extracts the drug. When the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask (Doughari, 2012; Hossain *et al*., 2014). The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind. This method is suitable for plant material which is partially soluble in the chosen solvent and for plant materials with insoluble impurities. However, it is not a suitable method for thermolabile plant materials.

Advantages. Large amount of drug can be extracted with smaller amount of solvent. It is also applicable to plant materials that are heat stable. No filtration is required, and high amount of heat could be applied (Doughari, 2012; Hossain *et al*., 2014). Disadvantage; This method is not suitable for thermolabile materials. It leads to exposure to hazardous liquid organic solvents, with potential toxic emissions during extraction (Doughari, 2012; Hossain *et al*., 2014).

2.6.1.7 Microwave-assisted extraction method

This is an advanced extraction procedure in preparation of medicinal plants. This technique uses mechanism of dipole rotation and ionic transfer by displacement of charged ions present in the solvent and drug material. This method is suitable for extraction of flavonoids. It involves the application of electromagnetic radiation in frequencies between 300 MHz and 300 GHz and wavelength between 1cm and 1 m (Doughari, 2012; Altemimi *et al*., 2017; Ingle *et al* ., 2017). The microwaves applied at frequency of 2450 Hz yield energy between 600 and 700 W. The technique uses microwave radiation to bombard an object, which can absorb electromagnetic energy and convert it into heat. The heat produced facilitates movement of solvent into the drug matrix (Doughari, 2012; Altemimi *et al*., 2017; Ingle *et al.,* 2017). When polar solvent is used, dipole rotation and migration of ions occur, leading to increase solvent penetration and this assist extraction process. However, when nonpolar solvent is used, the microwave radiation released will produce only a little heat hence, this method does not favor use of nonpolar solvents (Doughari, 2012; Altemimi *et al*., 2017; Ingle *et al*., 2017). Advantages; It minimizes solvent and time of extraction as well as increase in the outcome (Doughari, 2012; Altemimi *et al*., 2017; Bhan, 2017; Ingle *et al*., 2017). Disadvantages; This method is suitable only for phenolic compounds and flavonoids.

Compounds such as tannins and anthocyanins may be degraded due to high temperature (Doughari, 2012; Altemimi *et al*., 2017; Ingle *et al*., 2017).

2.6.1.8 Ultrasound-assisted extraction method

This process involves application of sound energy at a very high frequency greater than 20 KHz to disrupt plant cell all and increase the drug surface area for solvent penetration leading to release of secondary metabolites. Plant materials are dried, grinded into fine power, and sieved properly. The prepared sample is then mixed with and appropriate solvent of extraction and packed into the ultrasonic extractor (Pandey and Tripathi, 2014; Azwanida, 2015; Altemimi *et al*., 2017).The high sound energy applied hasten the extraction process by reducing the heat. Advantages; Ultrasound-assisted extraction is applicable to small sample, it reduces the time of extraction and amount of solvent used, and maximizes the yield (Pandey and Tripathi, 2014; Azwanida, 2015; Altemimi *et al*., 2017). Disadvantages. This method is difficult to be reproduced; also, high amount of energy applied may degrade the phytochemical by producing free radical (Pandey and Tripathi, 2014; Azwanida, 2015; Altemimi *et al*., 2017).

2.6.2 Factors to be considered in choosing extraction method

(a) Stability to heat. Heat-stable plant material are extracted using Soxhlet extraction or microwave-assisted extraction, whereas plant materials that are not heat stable are extracted using maceration or percolation (Azwanida, 2015; Majekodunmi, 2015). (b) Nature of solvent. If the solvent of extraction is water, maceration is a suitable method but for volatile solvent percolation and Soxhlet extraction are more appropriate (Azwanida, 2015; Majekodunmi, 2015). (c) Cost of the drug. Cheap drugs are extracted using maceration, whereas costly drugs are preferably extracted using percolation (Azwanida, 2015; Majekodunmi, 2015). (d) Duration of extraction. Maceration is suitable for plant material requiring long exposure to the menstruum, whereas techniques such as

microwave- or ultrasound-assisted extraction are used for a shorter duration (Azwanida, 2015; Majekodunmi, 2015). (e) Final volume required. Large volume products such as tinctures are prepared by maceration, whereas concentrated products are produced by percolation or Soxhlet extraction (Azwanida, 2015; Majekodunmi, 2015). (f) Intended use. Extracts intended for consumption by human are usually prepared by maceration, whereas products intended for experimental testing are prepared using other methods in addition to maceration (Azwanida, 2015; Majekodunmi, 2015)

2.6.3 Solvents used in extraction

The solvent used for the extraction of medicinal plants is known as the menstruum. The choice of solvent depends on factors such as, the type of plant, part of plant to be extracted, nature of the bioactive compounds, and the availability of solvent. Polar solvents such as water, methanol, and ethanol are used in extraction of polar compound, whereas nonpolar solvents such as hexane and dichloromethane are used in extraction of nonpolar compounds (Sasidharan *et al*., 2011; Pandey and Tripathy, 2014; Altemimi *et al*., 2017). During liquid–liquid extraction, the conventional method is to select two miscible solvents such as water dichloromethane, water–ether, and water–hexane. In all the combinations, water is present because of its high polarity and miscibility with organic solvent. The compound to be extracted using liquid–liquid extraction should be soluble in organic solvent but not in water to ease separation (Majekodunmi, 2015). Solvents used in extraction are classified according to their polarity, from n-hexane which is the least polar to water the most polar (Sasidharan *et al*., 2011; Pandey and Tripathy, 2014; Altemimi *et al*., 2017).

Fractionation is a process of separation of plant extracts into various fractions. It further segregates the fractions into portions comprising a number of compounds. The process continues until pure compound is isolated (Doughari, 2012; Banu and Lugas, 2015).

During fractionation, the selected solvent is added according to the order of increasing polarity, starting from n-hexane, the least polar to water with the highest polarity (Pandey and Tripathy, 2014).

2.6.4 Factors to be considered in selecting solvents of extraction

i) Selectivity; the ability of a chosen solvent to extract the active constituent and leave the inert material. (ii) Safety; ideal solvent of extraction should be nontoxic and nonflammable. (iii) Cost; it should be as cheap as possible. (iv) Reactivity; suitable solvent of extraction should not react with the extract. (v) Recovery; the solvent of extraction should be quickly recovered and separated from the extract. (vi) Viscosity; it should be of low viscosity to allow ease of penetration. (vii) Boiling temperature; solvent boiling temperature should be as low as possible to prevent degradation by heat (Sasidharan *et al*., 2017; Pandey and Tripathy, 2014).

2.7 Toxicity Studies

Toxicity studies are carried out for the purpose of evaluation of toxic effects of therapeutic agents or potential toxicants that could pose threat to the lives of humans and animals. The traditional methods of determining toxic effects of chemicals and drugs include acute toxicity study which is carried out to determine the short term toxic effect of a toxicant from (1day to 2 weeks), subacute toxicity study is carried out to know the relative long term effect of a toxicant (4 weeks- 6 months) and Chronic toxicity studies are carried out to know the long term effect of a toxicant (1–1½ years) (Saganuwan, 2012).

2.7.1 Acute Systemic Toxicity

Acute systemic toxicity is used to evaluate the adverse effects that occur following exposure of organisms to a single or multiple doses of a test substance within 24 hours by

a known route (oral, dermal or inhalation) (Saganuwa, 2016). After administration, the test substance is absorbed and distrib¬uted to various parts of the body before it elicits systemic adverse effect. The regulatory body requires the acute toxicity test report for labeling and classification of substances for human use (Peers *et al*., 2012). The LD_{50} test was introduced by Trevan in 1927 and used to estimate the dose of a test substance that produces 50 % death in a given species of animals.

This is the first test conducted for every chemical before further toxicity tests are carried out. It is used to estimate the potential hazards of chemicals on humans. Although its major endpoint is death, non-lethal acute effect may occur as signs of toxicity depending on the chemical being tested (Maheshwari and Shaikh, 2016). Acute toxicity determines adverse effects that might occur due to accidental or deliberate short-term exposure (Clemedson *et al*., 2000).

Therefore, substances with LD50 below 5 mg/ kg are classified to be highly toxic while substances with LD50 above 15,000 mg/kg are termed relatively harmless (Loomis and Hayes, 1996).

a. Lorkes method of acute toxicity

This method was introduced in 1983 and it involves the use of thirteen animals in 2 phases. In the first phase, nine animals are divided into three groups of three animals each and are administered 10, 100 and 1,000 mg/kg body weight of the test substance in order to establish the dose range producing any toxic effect. The number of deaths in each group is recorded after 24 hours. In the second phase, four doses of the test substance are selected based on the result of phase 1 and are administered to four (4) groups of animals (one animal per group). After 24 hours, the number of deaths is recorded and the LD50 is

calculated as the geometric mean of the highest non-lethal dose (a) and the least toxic dose (b). LD50 = √a×b (Lorke, 1983; Enegide *et al*., 2013).

b. Limit Test for acute toxicity

The Limit test is a sequential test that uses a maximum of 5 animals. A test dose of 2000, or exceptionally 5000 mg/kg, may be used. The procedures for testing at 2000 and 5000 mg/kg are slightly different. For limit test at 2000 mg/kg, one animal is dosed at 2000 mg/kg and observed for mortality, if animal dies, the main test is then carried out. If the animal survives, four additional animals will be dosed sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD50 is therefore, greater than 2000 mg/kg if three or more animals survive and the LD50 is less than the test dose (2000 mg/kg) when three or more animals die (OECD, 2001). For limit test at 5000 mg/kg, a dose range of 2000-5000 mg/kg is used exceptionally and only when justified by specific regulatory needs and this should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment. One animal is dosed at 5000 mg/kg, if the animal dies, the main test is conducted to determine the LD_{50}. If the animal survives, two additional animals are dosed. If both animals survive, the LD_{50} is greater than the limit dose and the test is terminated (OECD, 2001).

2.7.2 Subacute toxicity

The subacute toxicity study is conducted for 28 days to examine the toxicity on organs (OECD, 2001). This is carried out in order to choose dosage levels to be used in sub-chronic and chronic toxicity studies. animals are grouped based on experimental design and various concentrations of the test substance is administered. Control groups are included Group 1 could served as control (normal saline) while Groups 2 to 6 can be

administrered 400, 600, 800, 1000 and 1200 mg/kg body weight of the test substance respectively. The animals are then observed every 2 hours for toxic symptoms, signs of poisoning and mortality over a period of 30 days. Animals are then euthanised and organs are assessed (Yeo *et al*., 2012).

2.7.3 Subchronic toxicity Studies

The test is conducted for a period of 90 days (3 months). In this type of study, subchronicity factor gives an indication of the cumulative effects of poisons. It is the ratio of acute LD_{50} to 90 days LD_{50}. A compound though may have low acute toxicity, but has the tendency to accumulate in the body tissues and can cause subacute or chronic toxicity. Such toxicants are termed as cumulative poisons such as lead and fluoride (Saganuwan, 2012). A satellite group may be included in the study protocol, and this group has both a control group and a high dose group (Muralidhara *et al*., 2010). Tissues are collected for gross pathology and histopathology.

2.7.4 Chronic toxicity studies

It is a long term toxicity study that lasts for a long period of time usually 1–2 years. Rodents such as mice and rats are usually used. Chronicity factor gives an indication of the cumulative effects of poisons. It is the ratio of acute LD_{50} to 1–2 years LD_{50}. This type of test can be conducted on drugs developed for terminal diseases such as cancers and AIDs (Saganuwan, 2012). A satellite group may be included in the study protocol. This group has both a control group and a high-dose group. The animals are observed for normal and abnormal body functions and biochemical parameters should be measured. Tissue are collected for gross and histopathology (Jaijoy *et al*., 2010). Carcinogenicity testing is under chronic toxicity testing in which both rodent and non-rodent species of animals are used. The test can be terminated after 1½ years in case of mice and hamsters and after 2 years in case of rats. Haematological analysis is performed in healthy animals

after 1 and 1½ years in mice and rats respectively and the study is terminated. The animals are euthanised for gross pathology and histopathology (Saganuwan, 2012).

CHAPTER THREE

3.0 METHODS

3.1 Collection of plant material

The *Helianthus annuus* seeds were collected from its habitat in Kwara State. It was then transported to Biological Sciences Department, Federal University of Technology Minna for identification and authentication by a Herbarium.

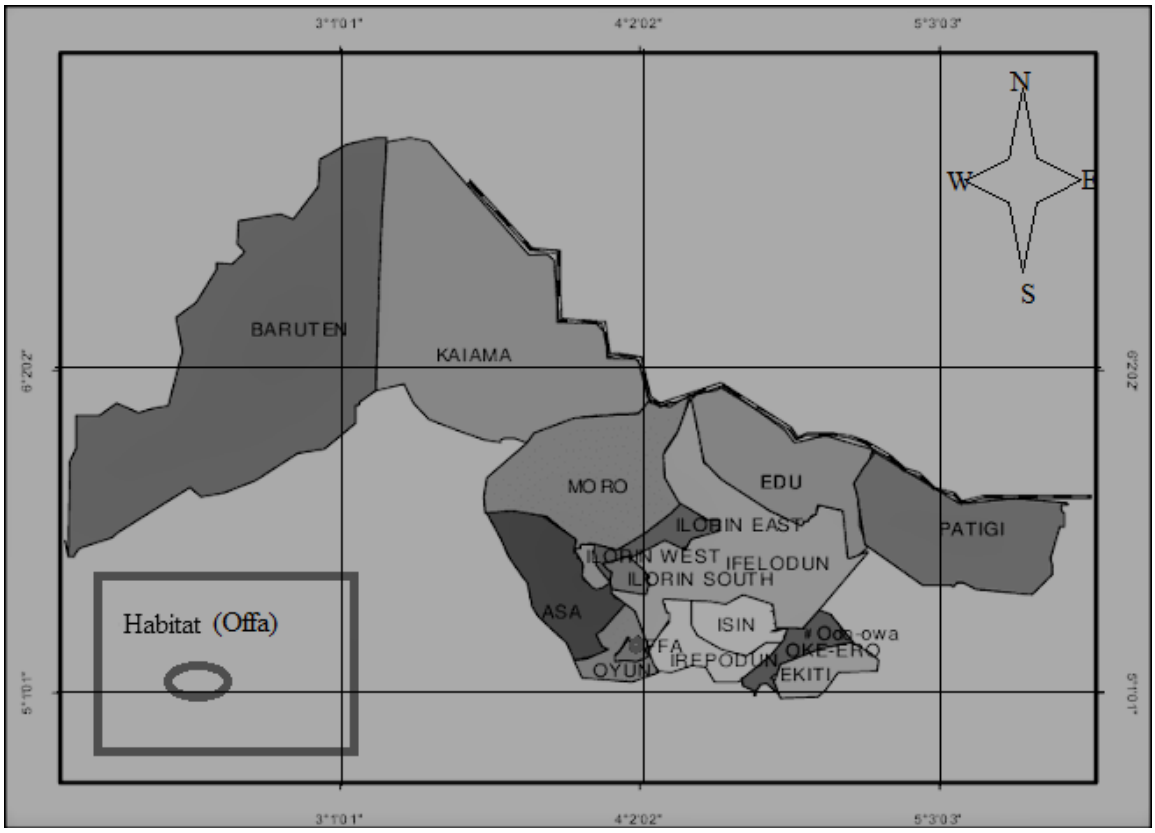

Figure 5: Map of Kwara state showing Offa as the Habitat

Source: (Oye, 2008).

3.2 Experimental animals and management

Male rats with body weight between (108-190 g) were obtained from a commercial breeder in Jos and transported. Animals were housed in the animal house of Federal University of Technology Minna in a temperature-controlled room under 12 hours light and 12 hours dark cycle. Animals had free access to food and water and were acclimatized for a week prior to experiments.

3.3 Sources of Microorganisms

The clinical bacteria isolates used in this study were *Escherichia coli* and *Salmonella spp.* These isolates were obtained from Generel Hospital Minna after acquiring ethical approval from the hospital. Nutrient Broth was used to confirm the organisms by sub-culturing at 37 °C for 18 hours. The organisms were maintained on Nutrient Agar (NA) slant and refrigerated at 4 ˚C (Igbinosa *et al.,* 2009).

3.4 Identification of the Isolates

1. **Gram staining**

A clean glass slide was picked, a drop of normal saline was dropped on the clean glass slide, a loop full of colonies was picked from the nutrients agar culture and was emulsified with the normal saline to form a smear. The smear was dried by using heat fixing method, crystal violet reagent was added to the smear and was allowed for one minute and then, the crystal violet was rinsed with water. Grams iodine solution was added to the smear for a minute and rinsed off with water. Alcohol was added in drops for 25 seconds which serves as decolourizer and was rinsed with water. Safranin solution was added to the smear which serves as counter stain and was allowed for one minute, the safranin solution was rinsed and the smear was allowed to dry (Smith and Hussey, 2016).

2. Microscopic Examination

During the microscopical viewing, the colony that appeareddark purple in colour were considered as Gram positive organism while those that appears pale to dark red in colour were considered as Gram negative organism, also their cellular morphological shape was noted (Smith and Hussey, 2016).

3. Biochemical test

a) Indole test

A loop full of the colonies from the Nutrient Agar plate was picked and inoculated into a pepton water broth. The broth was incubated for five days at 37 ^{0}C. After the incubation period, three drops of kovacs reagent was added into the broth and was shook gently. The presence of the red or pinkish ring top layer indicates the organism to be Indole positive while the absence of it indicates the organism to be indole negative

b) **Methyl Red (MR) test**

A loop full of the colonies from the Nutrient Agar plate was picked and inoculated into the MR-VP broth. The broth was incubated for five days at 37 ^{0}C. After the incubation periods a methyl-red reagent was added to the tube and it was observed for change in colour. When the culture medium turns red after addition of methyl red the organism is MR Positive whilst when the culture medium remains yellow the organism is MR Negative

c) Catalase test

A drop of hydrogen peroxide was dropped in a clean glass slide a colony was picked from Nutrient agar and was emulsify on the slide, It was observed for bubble formation within 5 seconds. Formation of bubbles within five seconds indicates the organism to be catalase positive whilst absent of the bubbles indicate the organism to be catalase negative

d) Oxidase test

A piece of filter paper was placed on a clean a clean petri dish and a 2-3 drops of oxidase reagent (tetramethyl-p- phenylenediamine dihydrochloride). The colonies were picked with a sterile stick and emulsified on the surface of the filter paper. Development of a blue-purple colour within a few seconds ranges from 10-30 seconds indicate the organism to be oxidase positive as whilst absent of such show the organism to be oxidase negative

e) Motility test

Semi agar medium was produced using nutrient broth and agar in a test tube. The organism was stabilized at the center of the media in the test tube using a sterile wire loop. The tube was incubated at 37 °C for 2-7 days within routine observation. Observation of diffused hazy growth that spread throughout the media makes the organism to be motile whist the conferment of growth within the stab line makes the organism to be non-motile.

f) Urease test

Urea agar slant was prepared in a sterile bottle and the test organism was inoculated heavily on the slant surface. The cap of the bottle was left partly loose and the slant was incubated for 5-7 days at 37 °C Development of pink colour within the 7 days incubation period termed the organism to be urease positive whilst absent of pink colour within the incubation period makes the organism to be urease negative.

3.5 Preparation of plant sample

The *Helianthus annuus* seeds were air dried at room temperature for two weeks. The dried seeds were milled to powder and air dried at room temperature for one week.

3.6 Extraction

The method of Shankeshwari *et al.* (2018) was employed to prepare the extracts by cold maceration with slight modification. The powdered seed material 500 g was mixed with 2500 mL in the ratio (1:5 w: v) of 99 % methanol and 99 % ethanol respectively and kept in a conical flask (Hossain *et al.*, 2013). The mixture was stirred thoroughly with a glass rod. The conical flask was kept with intermittent shaking for 72 h. The mixtures were then filtered using Whatman No.1 filter paper. The methanolic and ethanolic filterate were then concentrated by using rotary evaporator and then allowed to dry at room temperature for two weeks. The extracts were then weighed and stored in closed containers at room temperature.

The method of Hossain *et al.* (2014) was employed in fractionation of the methanol crude extract. The methanol crude extract (20 g) was suspended in water (400 mL). Then it was extracted successively with different organic solvents which are hexane and ethyl acetate to obtain hexane, ethyl acetate and residual methanol aqueous fractions respectively. All extracts were filtered separately through Whatman No. 41 filter paper to remove particles. The particle free extracts were evaporated completely at room temperature to obtain dried extracts. The dried extracts were weighed and kept in sterile sample bottles and stored in the refrigerator at 4 °C for further use.

3.7 Phytochemical Screening

Qualitative and quantitative phytochemical tests of each of the seeds extract was carried out to identify the constituents. Standard procedures were followed to detect the presence of Tannins, saponins, alkaloids, phenols and flavonoids (Trease and Evans, 1989; Sofowara, 1993).

3.7.1 Qualitative phytochemical screeningof *H. annuus* extract

i) Saponin

Two milliliters (2mL) of water was added to 0.5 g of the extract. It was shaked and observed for persistence in the foam produced for 10 minutes.

ii) Tannins

Twenty milliliters (20mL) of water was added to the dried powdered sample 0.5 g in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or a blue- black colour indicating the presence of tannins.

iii) Phenols

Ferric Chloride Test: extracts were treated with 3-4 drops of Ferric Chloride solution and observed for the formation of a bluish black colour.

iv) Flavonoid

Extracts were treated with few drops of sodium hydroxide solution and observed for the formation of an intense yellow colour that turned colourless on addition of diluted acid.

v) Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered, the filtrates of the extractwastreated with Mayer's reagent (Potassium Mercuric iodide) and observed for the formation of yellow precipitate.

vi) Diterpenes

Copper acetate test**:** Extracts were dissolved in water and treated with 3-4 drops of Copper acetate solution and observed for the formation of an emerald green colour.

vii) Anthraquinones

One millilitre (1 mL) of the extract was dissolved in 10 mL of benzene in a test tube. The mixture was filtered and to the filtrate, 5 mL of 10 % Ammonia was added, shaked and observed for the appearance of a pink red/violet colour in the ammonia phase.

viii) Cardiac glycosides

Five millilitres (5mL) of each extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, whole in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

3.7.2 Quantitative phytochemical screeningof *H. annuus* extract

i) Saponin determination

The method as described by Harbone (1999) was used. Twenty grams of each plant sample was weighed into a conical flask and 100 cm^3 of 20 % aqueous ethanol were added. The samples were heated over a hot water bath for 4 hour with continuous stirring at 55 ^oC. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40ml over water bath at about 90 ^oC. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer recovered while the ether layer was discarded. The process was repeated. 50 ml of n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated.

ii) Tannin

Tannin was determined using the method outlined by Harbone (1999). Two grams of each plant sample were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 hours. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. Five milliliter of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. Two milliliter of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505 nm.

iii) Alkaloid

Harbone's (1999) method of alkaloid determination was used. Five grams (5 g) of the samples were weighed into 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harbone, 1999).

3.8 Antibacterial assay of seed extracts of *Helianthus annuus*

3.8.1 Reconstitution of extracts

The extracts were reconstituted with DMSO and distilled water to get extract concentrations of 60, 120, 240 and 480 mg/mL. These concentrations were prepared by weighing 0.3, 0.6, 1.2 and 2.4 g of each extract and dissolved in 1 ml of DMSO and 4 mL of distilled water.

3.8.2 Preparation of media

The media was prepared according to the manufacturer's instructions. Twenty-eight grams of Nutrient agar standard measurement was poured into sterile conical flask, 1 Litre of distilled water was added. The prepared media was cork-fitted with cotton wool, foil paper, and masking tape. This was done to avoid removal of the cork when autoclaving. The media was autoclaved for 15 minutes at 121 °C. It was then removed and cooled to 47 °C. The media was mixed properly and poured into sterile petri-dishes close to the flame.

3.8.3 Standardization of inoculum

The test microorganisms (*Escherichia coli* and *Salmonella spp*) three colonies were picked, inoculated into 5 mL of sterile nutrient broth and incubated (18-24 hours) for bacterial growth. Turbidity was adjusted to match 0.5 McFarland's standard (Coyle, 2005).

3.8.4 Preparation of disc

The paper disc diffusion method described by Doughari *et al.* (2007); Adebayo and Adegoke (2008) was employed.Whatsmann filter papers (6 mm) in diameter were cut into round pieces with the aid of a puncher to obtain disks. Fifty (50) pieces of the disks were transferred into labelled sterile bottles and sterilized in hot air oven at 45 ˚C for three (3) days. Five (5 mL) of each of the prepared extracts was transferred into the corresponding labelled bottles containing sterile disks. The disks were dried in hot air oven at 45 ˚C for 1-2 hrs. The control disk was prepared by dissolving 500 mg of ciprofloxcin in 5 mL of distilled water and transfering into a sterile bottle containing sterile disk. The disk was dried at 45 ˚C for 1-2 hrs.

3.9 *In vitro* evaluation of antibacterial activity

The crude extracts of *Helianthus annuus* seeds (methanol and ethanol) as well as fractions of the crude extracts were evaluated in vitro for antibacterial activity against *Escherichia coli* and *Salmonella spp.* Muella Hinton Agar (38 g) was added to 1 Litre of distilled water in a conical flask and stirred. It was then autoclaved at 121 ˚C and cooled to 50 ˚C. A portion of the medium (20 mL) was poured into sterile Petri dishes and allowed to solidify (Daniyan *et al.*, 2011).

A loopful colony of each test organism was sub-cultured on Nutrient broth and incubated at 37 °C for 24 hours. This broth culture was spread on the entire plate with the aid of a swab stick to ensure uniform growth. The disk impregnated with various concentration of the extracts were placed on the surface of the medium at four points equidistant from one another. The control disk was also placed on the surface of the medium in a different plate. The plates were allowed to stand for 2 hrs and then incubated for 24 hours at 37 °C. Zones of inhibition were observed and measured. Antimicrobial assays were carried out in triplicate and the mean diameter was calculated.

3.9.1 Minimum inhibitory concentration (MIC) of extracts

The Minimum inhibitory concentration (MIC) of extracts against the test organisms were determined using the broth dilution method as described by Daniyan *et al.* (2011). Using test tubes, 8 mL of the nutrient broth was dispensed into various labelled test tubes and autoclaved for 15 minutes. Using two (2 mL) of the initial concentration of the extracts, broth dilution was carried out in 8 folds and a control test tube containing 2 mL of the extract and 8 mL of broth was set aside. The bacteria suspension of the test organisms diluted at 10^6 Cfu/ml was inoculated into each tube .The test tubes were sealed with foil paper, cotton wool, and masking tape and then incubated at 37 ℃ for 18-24 hours. The

least concentration of the extract that showed less turbidity was taken as the minimum inhibitory concentration (Abalaka *et al.*, 2011).

3.9.2 Minimum bactericidal concentration (MBC) of the extracts

The 18-24 hrs broth culture in the test tubes used for determination of minimum inhibitory concentration were then streaked on nutrient agar plate and incubated for 24 hrs. The lowest concentration of the extract indicating a bactericidal effect after incubation was regarded as the Minimum Bactericidal Concentration (MBC) (Aboada *et al.*, 2006).

3.10 Toxicity studies

3.10.1 Acute toxicity (Limit test)

The acute oral toxicity of the plant extracts was determined, using the method described by Organization of Economic and Cooperative Development OECD (2000). The limit test was used at 2000 mg/kg. Oral route of administration was used. Animals were fasted overnight for about 16 hrs prior to dosing. A group of 5 rats per extract received oral dose of 2000 mg/kg of the extract. The treated rats were observed within 24 hrs for mortality and general behaviour. The number of dead animals was recorded and the lethal dose (LD50) was calculated using the formula below:

$LD_{50} \sqrt{(D_0 \times D_{100})}$

Where D_0 = Dosage of 0% mortality, D_{100} = Dosage of 100% mortality

The animals were observed closely at 0 hr, 4 h, 24 h and 14 days for any delayed toxic reaction such as tremors, convulsion and irritability. The number of death was recorded.

3.10.2 Subacute toxicity studies

The subacute toxicity studies was carried out according to Chung-Tack *et al.* (2015) with slight modifications. In the subacute toxicity studies, the extracts were administered once a day for twenty eight (28) days without fasting. The rats were weighed on weekly basis and dosing was based on recently measured body weight. The rats were grouped into four groups, each group containing four rats. Group one (1) rats were administered distilled water which served as the control group. Group two (2) to four (4) were administered different concentrations of the methanol crude extract at 10 mg/kg, 300 mg/kg and 600 mg/kg respectively. This process was repeated for the grouping of the other three extracts (methanol-nhexane fraction, ethanolcrude and ethanol hexane fraction). All the animals were observed once daily for clinical signs and twice daily for mortality and morbidity throughout the study. On the twenty eight day, the rats were euthanised and blood samples were taken for haematological and biochemical analysis (Peter and Kwiterovich, 2004). The rats were dissected and organs (liver and kidney) were taken for histopathological examinations (Ateeq, 2015).

3.10.3 Biochemical analysis

3.10.3.1 Determination of total cholesterol

Total cholesterol level was estimated by cholesterol oxidase peroxidase methodology using a commercial kit (AGAPE, Switzerland) with the help of UV-visible spectrophotometer at wavelength of 505 nm *in vitro* quantitative determination of cholesterol in serum or plasma. The reagent is prepared by dissolving contents of reagent 2 (R2) with the amount of reagent 1 (R1) indicated on the vial label,linear up to 500 mg/dl, extended stability reconstituted reagent stable up to 90 days when stored at 2-8 ^{O}C (Peter and Kwiterovich, 2004).

Clinical Significance

It is the main lipid found in the blood, bile and brain tissues. It is also one of the most important steroids of the body and is a precursor of many steroid hormones. Two thirds of cholesterol present in the blood is esterified. The liver metabolizes the cholesterol and it is transported in the blood stream by lipoproteins. Increased levels are found in hypercholesterolemia, hyperlipidemia, hypothyroidism, uncontrolled diabetes nephritic syndrome and cirrhosis. Decreased levels are found in malabsorption, malnutrition, hyperthyroidism, anaemia and liver diseases (Peter and Kwiterovich, 2004).

Principle

Enzymatic determination of total cholesterol is based on the following reactions.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol esterase}} \text{4-cholesten-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Red quinine} + 4H_2O$$

Laboratory Procedure

1. To each tubes Add 1000 µl of Working Reagent to Blank, Standard and Sample (test) tubes

2. Add 10 µl of Standard reagent to the standard test tube

3. Add 10 µl of sample (test) to the sample test tube

4. Mix, and incubate for 5 min. at 37 °C. Measure the Absorbance of sample and standard against reagent blank (Peter and Kwiterovich, 2004).

Calculation

Total Cholesterol conc. (mg/dl)

$$= \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 200$$

Normal range: 150-220 mg/dl (Peter and Kwiterovich, 2004).

3.10.3.2 Determination of serum triglyceride (TG)

Serum triglyceride level was estimated by GPO-TOPS (glycerol-3-phosphate oxidase) methodology using a commercial kit (AGAPE, Switzerland) with the help of UV-visible spectrophotometer at wavelength of 546 nm *in vitro* quantitative determination of triglyceride in serum or plasma. The reagent is ready to use, linear up to 1000 mg/dL, extended stability up to the expiry date stated on the label, when stored at 2-8 °C (Peter and Kwiterovich, 2004).

Clinical Significance

Triglycerides are simple lipids, formed in the liver by glycerol and fatty acids. They are transported by VLDL, LDL and constitute about 95 % of fat, stored as source of energy in the tissue and plasma. Increased levels are found in hyperlipidemia, diabetes, nephritic syndrome and hypothyroidism. Increased levels are risk factor for arteriosclerotic coronary disease, peripheral vascular disease, acute pancreatitis and hyperlipoproteinaemia. Decreased levels are found in malnutrition and hyperthyroidism (Peter and Kwiterovich, 2004).

Principle

Enzymatic determination of triglyceride is based on the following reactions.

$$TGL + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{glycerol + fatty acids}$$

$$\text{glycerol + ATP} \xrightarrow{\text{Glycerol kinase Mg2+}} \text{glycerol-3-phosphate + ADP}$$

glycerol-3-phosphate oxidase

Glycerol-3-Phosphate + O_2----------> Dihydroacetonephosphate + $2H_2O_2$

peroxidase

$2H_2O_2$ + 4-Aminoantipyrine + TOPS----------> violet coloured complex

Laboratory Procedure

1. To each tubes Add 1000 µl of Working Reagent to Blank, Standard and Sample (test) tubes

2. Add 10 µl of Standard reagent to the standard test tube

3. Add 10 µl of sample (test) to the sample test tube

4. Mix and incubate for 5 min. at 37 °C. Measure the Absorbance of sample and standard against reagent blank.

Calculation

Triglyceride conc. (mg/dl)

$$\text{Triglyceride conc. (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 200$$

Normal range: 60-165 mg/dl (Peter and Kwiterovich, 2004).

3.10.3.3 Determination of serum Low Density Lipoprotein (LDL-C)

Clinical Significance

Blood total cholesterol levels have long been known to be related to coronary heart disease (CHD). In recent years, in addition to total cholesterol, low density lipoprotein cholesterol (LDL-C) has become an important tool used to assess an individual risk of developing CHD since a strong negative relationship between LDL-C concentration and the incidence of CHD was reported. LDL cholesterol acts as a key factor in the pathogenesis of atherosclerosis and coronary artery disease (Peter and Kwiterovich, 2004).

Principle

The LDL-C Direct is a homogenous assay for directly measuring LDL-C levels in serum or plasma without the need for any off-line pretreatment or centrifugation. In the first reaction, non LDL unesterified is consumed by peroxidase in the presence of 4-AAP (aminoantipyrin) to yield a colorless product. The second reagent consists of a detergent capable of solubilizing LDL specifically. Cholesterol esterase and chromogenic coupler react with this solubilize LDL-C to develop color. The intensity of color formed is directly proportional to the concentration of LDL-C (Peter and Kwiterovich, 2004).

Laboratory Procedure

Differential measurement

1. To each tubes Add 270 µl of Reagent, R1 to Blank, Calibrator and Sample (test) tubes

2. Add 3 µl of Calibrator reagent to the Calibrator test tube

3. Add 3 µl of sample (test) to the sample test tube

4. Mix and incubate for 5 min at 37 °C. measure the absorbance (OD1) at 546V nm/660 nm.

5. To each tubes Add 90 µl of Reagent, R2 to Blank, Calibrator and Sample (test) tubes

6. Mix and incubate for 5 min at 37 °C. measure the absorbance (OD2) at 546 nm/660 nm.

Calculation

$$\text{LDL-C conc. (mg/dl)} = \frac{(OD2 - OD1)\ \text{sample}}{(OD2 - OD1)\ \text{calibrator}} \times \text{Calibrator conc}$$

Laboratory Procedure

1. To each tubes Add 450 µl of Reagent, R1 to Blank, Calibrator and Sample (test) tubes

2. Add 5µl of Calibrator reagent to the Calibrator test tube

3. Add 5 µl of sample (test) to the sample test tube

4. Mix and incubate for 5 min at 37 °C

5. To each tubes Add 150 µl of Reagent, R2 to Blank, Calibrator and Sample (test) tubes

6. Mix and incubate for 5 min at 37 °C. measure the absorbance of calibrator and sample against reagent blank at 546 nm/660 nm (Peter and Kwiterovich, 2004).

Calculation

$$\text{LDL-C conc.} = \frac{\text{Absorbance of sample}}{\text{Absorbance of calibrator}} \times \text{Calibrator conc}$$

3.10.3.4 Determination of Total protein

Total protein level was estimated by direct Biuret methodology using a commercial kit (AGAPE, Switzerland) with the help of UV-visible spectrophotometer at wavelength of 546 nm *in vitro* quantitative determination of Total Protein in serum or plasma. Reagent is ready to use, linear up to 15g/dl, extended stability reagent stable up to expiry date when stored at room temperature and standard at 2-8 °C (Peter and Kwiterovich, 2004).

Clinical Significance

Proteins form the major portion of dissolved substances in the plasma. They form the basic structural components of the body. They constitute the enzymes present in our body and also act as secondary source of energy. The other functions include distribution of water, buffering, transport of various components, defense and coagulation of blood in our body. Increased levels are found in dehydration and myeloma. Decreased levels are

found in the liver disorders, nephritic syndrome, malnutrition and protein due to haemorrhage (Peter and Kwiterovich, 2004).

Principle

Colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional o the protein concentration (Peter and Kwiterovich, 2004).

Laboratory Procedure

1. To each tubes Add 1000 µl of Reagent to Blank, Standard and Sample (test) tubes

2. Add 20 µl of Standard reagent to the standard test tube

3. Add 20 µl of sample (test) to the sample test tube

4. Mix, and incubate for 10 min. at 37 °C. Measure the Absorbance of sample and standard against reagent blank (Peter and Kwiterovich, 2004).

Calculation:

$$\text{Total protein conc. (g/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 6$$

Normal range: 6.2-8.0 mg/dl (Peter and Kwiterovich, 2004).

3.10.3.5 Determination of Serum Bilirubin Concentration

Biosystems diagnostic kit (Barcelona Spain) was used in the determination of total bilirubin. The working reagent for total bilirubin determination was prepared by transfering the content of reagent BT vial into a reagent AT bottle followed by proper mixing.One hundred (100 µl) of distil water, serum sample and standard were pipetted

into the respective test tubes of blank, sample blank/sample and standard. One mL (1 mL) of reagent AT was pipetted into sample bank test tube followed by addition of 1ml of working reagent into reagent blank, sample and standard test tubes. All the mixtures were allowed to stand for 2 mins at room temperature after thorough mixing before the absorbance (A) of the sample blank and the sample were taken at 540 nm against the distilled water and reagent blank respectvely. The bilirubin concentration was calculated as shown below:

$$\text{Bilirubin Conc. (mg/dl)} = \frac{A_{\text{Sample}} - A_{\text{Sample Blank}}}{A_{\text{Standard}}} \times \text{Standard conc.}$$

The Bilirubin in the serum sample reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560 nm in the aqueous solution. The intensity of the colour produced is directly proportional to the amount of direct bilirubin concentration present in the sample. The total bilirubin value represents the sum of the bilirubin glucuronide (direct) and the unconjugated (indirect) bilirubin. The colour produced measured at 560 nm is proportional to the amount of the total bilirubin concentration present in the sample (Peter and Kwiterovich, 2004).

3.10.3.6 Estimation of Urea Concentration

Urea concentration was determined by using a randox commercial kit. The reagent R1 was prepared by addition of the content of R1a into R1b bottle followed by gently mixing while the content of R2 and R3 were mixed with 660 ml and 750 ml of distilled water respectvelly. Ten microlitres (10 µl) of each sample, distil water and standard was added to the sample, blank and standard tubes followed by the addition of 100 µl of R1 each of the test tubes. The mixtures were properly mixed and incubated at 37 °C for 10 mins after which 2.5 ml of both reagent R2 and R3 was added.The resultant mixtures were

incubated at 37 °C for 15 mins. The absorbance of the samples (A_{Sample}) and standard ($A_{Standard}$) against the blank and the concentration of urea was calculated as shown below:

$$\text{Urea concentration (mg/dl)} = \frac{A_{sample}}{A_{Standard}} \times \text{Standard Conc.}$$

The principle involves the hydrolysis of urea in the serum into ammonia in the presence of urease enzyme. The ammonia was then measured photometrically at 546 nm by Berthelots reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenol (blue compound)}$$

3.11 Preparation of Mcfarland standard and Standardization of innoculum

Macfarland standard is a chemical solution of barium chloride and sulfuric acid. The chemical reaction between two chemicals produce a fine precipitate of barium sulfate. The turbidity of McFarland standard is visually comparable to a bacteria suspension. McFarland turbidity standard (0.5 MFU) was prepared by mixing 0.05 ml of 1 % $Bacl_2$ and 9.95 mls of 1 % H_2SO_4 to obtain solution of 0.5 optical density. 0.5 McFarland turbidity standard provides an optical density comparable to the density of bacterial suspension with a 1.5×10^8colony forming unit (CFU/mL). Bacteria Suspension was prepared by picking colonies of 18-24 hrs culture of test organisms and transferred into sterile nutrient broth and incubated for 2-3hrs. The turbidity was adjusted to match McFarland standard which contains 1.5×10^8 CFU/ml.

3.12. *In vivo* antibacterial activity of extracts

Determination of the antibacterial activity was done as reported by Hosseinzadeh *et al*. (2007) with little modifications. The rats were grouped into eight groups of eight (8) rats each. In each group, four rats were infected orally with 1.0 mL (10^6 CFU/mL) of

overnight broth culture of *E. coli* and the other four with 1.0 mL (10^6 Cfu/mL) of overnight broth culture of *Salmonella spp* and then kept in separate cages. After Seven days, blood samples were taken from the animal's tail and cultured on nutrient broth. The overnight blood cultures were inoculated on specific medium for *E. coli* (Eosin methylene blue agar) and *Salmonella spp* (*Salmonella Shigella* agar) respectively. Growth was observed for both *Salmonella spp* and *E. coli* and colonies were counted. Twenty-four hours (24 hrs) later, each group received different treatments. Groups were designed as follows:

Table 3.1: Experimental Design

Group	Administration
Group 1 (Negative control)	(a) received 2ml of distilled water and *Salmonella spp* (b) received 2ml of distilled water and *Escherichia coli*
Group 2 (Positive Control)	(a) received 20mg/kg of ciprofloxacin and *Salmonella spp* (b) received 20mg/kg of ciprofloxacin and *Escherichia coli*
Group 3 Methanol crude extract	(a) received 100mg/kg of methanol crude extract and *Salmonella Spp* (b) received 100mg/kg of methanol crude extract and *Escherichia coli*
Group 4 Methanol crude extract	(a) received 200mg/kg of methanol crude extract and *Salmonella Spp* (b) received 200mg/kg of methanol crude extract and *Escherichia coli*
Group 5 Methanol crude extract	(a) received 400 mg/kg of methanol crude extract and *Salmonella Spp* (b)received 400 mg/kg of methanol crude extract and *Escherichia coli*
Group 6 Methanol nhexane fraction	(a) received 100mg/kg of nhexane fraction of methanol and *Salmonella Spp* (b) received 100mg/kg of nhexane fraction of methanol and *Escherichia coli*
Group 7 Methanol nhexane fraction	(a) received 200mg/kg of nhexane fraction of methanol and *Salmonella Spp* (b) received 200mg/kg of nhexane fraction of methanol and *Escherichia coli*
Group 8 Methanol nhexane fraction	(a) received 400mg/kg of nhexane fraction of methanol and *Salmonella Spp* (b) received 400 mg/kg of nhexane fraction of methanol and *Escherichia coli*

Treatment duration was for fourteen (14) days. Animals were allowed access to food and water throughout the treatment period. On the 15th day, blood samples were collected from each group of animals to assess the bacteria load by culturing on specific media.

3.13 Data Analysis

The data collected were analysed using Statistical Package for the Social Sciences (SPSS) version 20. The results were evaluated using Analysis of Variance (ANOVA) and were presented as the mean value ± SEM (standard error of mean). Differences among

the means for the groups were assessed using the Duncan's Multiple Range Test to determine which mean values were significantly different at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

Table 4.1: Qualitative Phytochemical Components of *Helianthus annuus* Seed Extracts

	Methanol extracts of *H. annuus*				Ethanol extracts of *H. annuus*			
Components	MCR	MHXN	META	MAQS	ECR	EHXN	EETA	EAQS
Alkaloids	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+
Steroids	+	+	+	-	+	+	+	-
Tannins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Terpennoids	+	+	+	-	+	+	+	-
Anthraquinones	-	-	-	-	-	-	-	-
Cardiac glycoside	-	-	-	-	-	-	-	-

Note: -absent, + present;

KEY: MCR= methanol crude extract, **MHXN**= nhexane fraction of methanol, **META**= ethylacetate fraction of methanol, **MAQS**= aqueous fraction of methanol, **ECR**= ethanol crude, **EHXN**= n-hexane fraction of ethanol, **EETA**=ethylacetate fraction of ethanol, **EAQS**= aqueous fraction of ethanol

4.1.1 Phytochemical Contents of *Heliantus annuus* seeds extract

The results in Table 4.1 show the qualitative phytochemical constituents of *Helianthus annuus* seed. The results showed that alkaloids, saponins, tannins, flavonoids and phenols were present in all of portions extracted with methanolic and ethanolic extracts while anthraquinones and cardiac glycoside were absent. Steroids and terpennoids were present in all, except in aqueous methanolic (MAQS) and ethanolic (EAQS) fractions.

Table 4.2: Quantitative Phytochemical (mg/100 g) Components of *Helianthus annuus* Seed

Extract	Phenols	Flavonoids	Tannins	Saponins	Alkaloids
MCR	310.58±0.02^{d}	23.08±0.03^{g}	21.92±0.00^{e}	252.14±0.28^{d}	34.26±0.29^{f}
MAQS	440.16±0.03^{g}	16.63±0.02^{d}	6.94±0.01^{ab}	270.58±0.02^{f}	31.89±0.21^{e}
META	490.61±0.02^{h}	13.85±0.05^{c}	7.07±0.00^{b}	190.77±0.07^{c}	22.61±0.53^{b}
MHXN	263.22±0.37^{c}	12.02±0.06^{b}	23.12±0.01^{f}	386.18±0.31^{h}	29.01±0.21^{d}
ECR	312.37±0.33^{e}	20.24±0.06^{f}	23.17±0.05^{f}	255.91±0.15^{e}	35.92±0.42^{g}
EAQS	200.36±0.87^{a}	20.66±0.39^{f}	9.01±0.51^{c}	157.92±0.57^{a}	38.91±0.53^{h}
EETA	323.41±1.34^{f}	18.49±0.39^{e}	6.29±0.36^{a}	181.03±0.53^{b}	27.22±0.65^{c}
EHXN	214.24±0.17^{b}	9.39±0.28^{a}	19.94±0.11^{d}	330.48±1.19^{g}	17.82±0.54^{a}

Values are presented as Mean ± Standard Error of Mean of triplicate determinations. Values in a column with different superscripts are significantly different at $p< 0.05$. While **MCR**= methanol crude extract, **MHXN**= nhexane fraction of methanol, **META**= ethylacetate fraction of methanol, **MAQS**= aqueous fraction of methanol, **ECR**= ethanol crude, **EHXN**= nhexane fraction of ethanol, **EETA**=ethylacetate fraction of ethanol, **EAQS**= aqueous fraction of ethanol.

The results in Table 4.2 show the quantitative phytochemical components analysis of *Helianthus annuus*seeds. There was significant difference ($p<0.05$) among all the constituents (phenols, flavonoids, tannins, saponins and alkaloids) extracted using the various solvents. Phenols was significantly ($p < 0.05$) higher in the portion extracted with aqueous ethylacetate (META) and aqueous methanolic (MAQS) fractions while it was significantly lower in aqueous ethanolic (EAQS) and n-hexane (EHXN) fractions. Flavonoids was quantitatively and significantly ($p < 0.05$) highest in crude methanolic (MCR) extracts followed by aqueous (EAQS) and crude (ECR) ethanol. It was lowest in n-hexane (EHXN) fraction of the ethanol. Tannins was significantly ($p < 0.05$) higher in crude (ECR) and n-hexane methanol (MHXN) fractions and ethyacetate fraction of ethanol (EETA). Saponins was significantly ($p<0.05$) highest in n-hexane fraction of methanol (MHXN) followed by n-hexane fraction of ethanol (EHXN) while it was lowest in aqueous fraction of ethanol (EAQS). Alkaloids was highest in aqueous fraction of ethanol (EAQS) and lowest in n-hexane fraction of ethanol (EHXN).

Table 4.3: Cultural growth characteristics on selective media, microscopic morphological characteristics and Gram staining Reaction

Organism	Cultural Growth characteristics	Morphological characteristics	Gram staining
Salmonella spp	Colourless colonies with black center on SSA	Rod shaped	Negative
Escherichia coli	Metallic green sheen colonies on EMB	Rod shaped	Negative

Key: SSA=Salmonella Shigellaagar, **EMB=**Eosine Methylene blue agar

4.1.2 Cultural, morphological and growth characteristics of organisms

Table 4.3 shows the cultural growth characteristics, morphological characteristics and Gram stain reaction of the test organisms. *Salmonella spp* forms colourness colonies with black centre on *Salmonella Shigella* agar, its rod shaped and Gram negative while *Escherichia coli* forms metallic green sheen colonies on Eosine Methylene blue agar, its rod shaped and gram negative on staining

Table 4.4: Biochemical characteristics of the test organisms

Organisms	Indole	Methyl-red	Catalase	Motility	Oxidase	Urease
Salmonella spp	-	+	-	+	-	-
Escherichia coli	+	+	-	+	-	-

Key; + present, -ve absent

4.1.3 Biochemical characteristics of the test Organisms

Table 4.4 shows the biochemical characteristics of the test organisms. *Salmonella spp* and *Escherichia coli* tested positive to indole, methyl red, and catalase. They were both motile while they tested negative to oxidase and urease.

Table 4.5: Zones of inhibition (mm) of *Helianthus annuus* Seed Extracts against *Salmonella spp.*

Extract	Zone of inhibition (mm)			
	60 mg/mL	120 mg/mL	240 mg/mL	480 mg/mL
MCR	18.50±0.50^{b}	20.33±0.33^{b}	23.33±0.88^{a}	25.67±0.33^{a}
MAQS	N.I	N.I	N.I	N.I
META	N.I	N.I	N.I	N.I
MHXN	17.33±0.67^{b}	19.33±0.67^{b}	21.33±0.67^{a}	23.33±1.67^{a}
ECR	12.67±0.67^{a}	17.33±1.45^{ab}	20.33±1.20^{a}	22.00±1.53^{a}
EAQS	N.I	N.I	N.I	N.I
EETA	N.I	N.I	N.I	N.I
EHXN	12.33±0.33^{a}	14.33±1.45^{a}	19.00±0.58^{a}	21.67±0.33^{a}
Positive control (Ciprofloxacin) at 100 mg/mL	32.67±2.91^{c}	32.67±2.91^{c}	32.67±2.91^{b}	32.67±2.91^{b}

Values are presented as Mean ± Standard Error of Mean of triplicate determinations. Values in a column with different superscripts are significantly different at $p< 0.05$. While **MCR**= methanol crude extract, **MHXN**= nhexane fraction of methanol, **META**= ethylacetate fraction of methanol, **MAQS**= aqueous fraction of methanol, **ECR**= ethanol crude, **EHXN**= nhexane fraction of ethanol, **EETA**=ethylacetate fraction of ethanol, **EAQS**= aqueous fraction of ethanol. **N.I = No Inhibition.**

4.1.4 *In vitro* Antibacterial study

The results in Table 4.5 show the zone of inhibition of different solvents extraction of *Helianthus annuus* seed against *Salmonella spp.* The aqueous fraction of methanol (MAQS), ethyacetate fraction of ethanol (META), aqueous fraction of ethanol (EAQS) and ethylacetate fraction of ethanol (EETA) showed no zone of inhibition. Crude

methanolic extract (MCR), n-hexane fraction of methanol (MHXN), crude ethanol extract (ECR) n-hexane fraction of ethanol (EHXN) and Ciprofloxacin which serves as positive control showed zone of inhibition against *Salmonella spp*.

Among the four extracts that have inhibitory activities, methanol crude extract (MCR) and n-hexane fraction of methanol (MHXN) have significantly ($p < 0.05$) higher inhibitory activities compared to crude ethanol extract (ECR) and n-hexane fraction of ethanol (EHXN) at 60 mg/mL. Their inhibitory activities were however, lower compared to ciprofloxacin (100 mg/mL) used as standards or positive control. Similar result was obtained at 120 mg/mL, but at dose 240 mg/mL and 480 mg/mL there was no significant ($p<0.05$) difference among the four extracts. However, there was significant difference ($p<0.05$) between the inhibitory activities of those four extracts and the ciprofloxacin (100 mg/mL) used as standard or positive control. The inhibitory activities at the difference concentration increases with increase in concentration for all the extracts.

Table 4.6: Zones of Inhibition (mm) of *Helianthus annuus* Seed Extracts against *Escherichia coli*

Extract	Zone of inhibition (mm)			
	60 mg/mL	120 mg/mL	240 mg/mL	480 mg/mL
MCR	8.50±0.50[a]	10.00±0.00[a]	10.67±0.67[a]	11.67±0.67[a]
MAQS	N.I	N.I	N.I	N.I
META	N.I	N.I	N.I	N.I
MHXN	7.33±0.67[a]	11.00±0.58[a]	15.33±0.33[b]	17.00±0.58[b]
ECR	N.I	N.I	N.I	N.I
EAQS	N.I	N.I	N.I	N.I
EETA	N.I	N.I	N.I	N.I
EHXN	N.I	N.I	N.I	N.I
Positive control (Ciprofloxacin) at 100 mg/mL	11.67±0.88[b]	11.67±0.88[a]	11.67±0.88[a]	11.67±0.88[a]

Values are presented as Mean ± Standard Error of Mean of triplicate determinations. Values in a column with different superscripts are significantly different at $p < 0.05$.**KEY:MCR**= methanol crude extract, **MHXN**= nhexane fraction of methanol, **META**= ethylacetate fraction of methanol, **MAQS**= aqueous fraction of methanol, **ECR**= ethanol crude, **EHXN**= nhexane fraction of ethanol, **EETA**=ethylacetate fraction of ethanol, **EAQS**= aqueous fraction of ethanol, **N.I = No Inhibition**

The results in Table 4.6 showed the zone of inhibition of *Helianthus annuus* seed of the various extracts against *Escherichia coli.* The aqueous fraction of methanol (MAQS), ethyacetate fraction of ethanol (META), aqueous fraction of ethanol (EAQS), crude ethanol extract (ECR), n-hexane fraction of ethanol (EHXN) and ethylacetate fraction of ethanol (EETA) showed no zone of inhibition. Methanolic Crude extract (MCR), n-hexane fraction of methanol (MHXN) and ciprofloxacin which serves as positive control showed inhibitory activities against *Escherichia coli.*

There was no significant ($p<0.05$) difference between methanol crude extract (MCR) and n-hexane fraction of methanol (MHXN) at 60 mg/mL and 120 mg/mL, but the difference was significant ($p<0.05$) at 240 mg/mL and 480 mg/mL. Ciprofloxacin (100 mg/mL) was significantly ($p<0.05$) higher at 60 mg/mL compared to methanol crude extract (MCR) and n-hexane fraction of methanol (MHXN), but there was no significant ($p<0.05$) difference between ciprofloxacin control, methanol crude extract (MCR) and n-hexane fraction of methanol (MHXN) at 120 mg/mL. Extracts from n-hexane fraction of methanol (MHXN) had significantly ($p<0.05$) higher zone of inhibition at 240 mg/mL and 480 mg/mL when compared to MCR and the ciprofloxacin (standard or control) while there was no significantly difference ($p<0.05$) between the control (ciprofloxacin) and methanol crude extract (MCR) at those concentrations. Zone of inhibition also increased with increase in concentrations across the two extracts of *Helianthus annuus* seed that shows inhibitory activities.

Table 4.7: MIC (mg/ml) of the *Helianthus annuus* Seed Extracts against *Salmonella spp* and *E. coli*

Extracts	*Salmonella*	*E. coli*
Methanol Crude (**MCR)**	0.46 ± 0.01^{b}	0.19 ± 0.01^{b}
Methanol n-hexane (**MHXN**)	0.48 ± 0.02^{b}	0.96 ± 0.01^{c}
Ethanol Crude (**ECR**)	0.07 ± 0.03^{a}	_
Ethanol n-Hexane (**EHXN)**	2.40 ± 0.06^{c}	_
Ciprofloxacin 20 (Standard)	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}

Values are presented as Mean ± Standard Error of Mean of three replicates. Values in a column with different superscripts are significantly different at $P < 0.05$**. KEY:MCR**= methanol crude extract, **MHXN**= nhexane fraction of methanol, **ECR**= ethanol crude, **EHXN**= nhexane fraction of ethanol

The results in Table 4.7 showed comparison of the minimum inhibitory concentration (MIC) of *Helianthus annuus* seed extracts on both *Salmonella spp* and *Escherichia coli.*

The minimum inhibitory concentration (MIC) of methanol crude, methanol n-hexane and ethanol crude and ethanol n-hexane extracts against *Salmonella spp.*, were significantly ($p<0.05$) higher than the standard drug except that of ethanol crude extract (0.07±0.03 mg/ml) which was comparable to the standard drug (0.02±0.00 mg/ml). The n-hexane fraction of ethanol (EHXN) had significantly ($p<0.05$) higher MIC value (2.40±0.06 mg/ml) against *Salmonella spp.* than the other extracts. The MIC of the extracts against *E. coli* showed significantly higher values than that of the standard drug.

Table 4.8: MBC (mg/ml) of the *Helianthus annuus* Seed Extracts against *Salmonella spp* and *E. coli*

Extract	*Salmonella*	*E. coli*
Methanol Crude (**MCR)**	2.40 ± 0.12^{d}	0.96 ± 0.02^{b}
Methanol n-hexane (**MHXN**)	2.40 ± 0.17^{d}	4.80 ± 0.12^{c}
Ethanol Crude (**ECR**)	0.48 ± 0.01^{b}	_
Ethanol n-Hexane (**EHXN)**	1.20 ± 0.12^{c}	_
Ciproloxaxin 20	0.18 ± 0.02^{a}	0.25 ± 0.05^{a}

Values are presented as Mean ± Standard Error of Mean of three replicates. Values in a column with different superscripts are significantly different at $P < 0.05$**KEY:MCR**= methanol crude extract, **MHXN**= nhexane fraction of methanol, **ECR**= ethanol crude, **EHXN**= nhexane fraction of ethanol

Table 4.8 Presents the minimum bactericidal concentration (MBC) of *Helianthus annuus* seed extracts on *Salmonella spp* and *Escherichia coli.* The MBC values of the extracts against *Salmonella spp* range from 0.18 mg/ml for the standard drug to 2.40±0.12 mg/ml for MCR and MHXN. The MBC of ethanol hexane (EHXN) was lower than that of MCR and MHXN while that of ethanol crude (ECR) was lower than EHXN but statistically different ($p<0.05$) from the standard (Ciprofloxacin). Similarly, none of the extracts had MBC on *E. coli*comparable ($p<0.05$) to the standard drug, however, Methanol Crude (MCR) and ethanol crude (ECR) had MBC significantly ($p<0.05$) lower

than was observed for nhexane fraction of ethanol (EHXN) (4.80±0.12 mg/ml) and nhexane fraction of methanol (MHXN) (4.80±0.12 mg/ml).

Table 4.9: Safe dose Determination (LD_{50}) of *Helianthus annuus* Seed Extracts

Extracts	Number of rats	Concentration of extracts (mg/kgbw)	Number of deaths
MCR	5	2000	0/5
MHXN	5	2000	0/5
ECR	5	2000	0/5
EHXN	5	2000	0/5

KEY: MCR= methanol crude extract, **MHXN**= nhexane fraction of methanol, **ECR**= ethanol crude, **EHXN**= nhexane fraction of ethanol

4.1.5 Acute Toxicity Studies

The results in Table 4.9 show the LD_{50} also referred to as safe dose of *Helianthus annuus* seed extracts from crude methanol, ethanol as well as n-hexane fraction of the methanol and ethanol. There was no mortality at 2000 mg/kgbody weight concentration of the extracts for all of the four extracts.

Table 4.10: Subacute Toxicity studies of *Helianthus annuus* Seeds Extracts

Groups	Extracts	Concentrations (mg/kg bw)	Number of deaths
1	Distilled water	10	1/4
2	Methanol crude	10	0/4
		300	1/4
		600`	0/4
3	Ethanol crude	10	0/4
		300	0/4
		600	0/4
4	Methanol	10	0/4
	hexane	300	0/4
		600	0/4
5	Ethanol hexane	10	1/4
		300	2/4
		600	0/4

4.1.6 Subacute Toxicity Studies

The results in Table 4.10 show the sub-acute toxicity studies of *Helianthus annuus* seeds extracts the test organism. At 10 mg/kg bw, one out of four (1/4) mortality was recorded for distilled water and ethanol hexane administered groups. There was no mortality at the same concentration for crude methanol, crude ethanol and n-hexane methanol administered groups. At 300 mg/kgbw, there was one out of four (1/4) mortality for crude methanol, two out of four (2/4) for hexane ethanol while there was no mortality for crude ethanol and methanol hexane. At 600 mg/kgbw, there was no recorded mortality for any of the extracts.

Table 4.11: *In vivo* Antibacterial Activityof *Helianthus annuus* Seeds Extracts against *Salmonella spp* and *Escherichia coli*

Groups	Subgroups	Treatment	No of deaths
1	1a	Distilled water + *Salmonella spp*	0/4
	1b	Distilled water +*Escherichia coli*	1/4
2	2a	Ciprofloxacin 20 mg/kg+ *Salmonella spp*	1/4
	2b	Ciprofloxacin + *Escherichia coli*	2/4
3	3a	Methanol crude 100 mg/kg + *Salmonella spp*	0/4
	3b	Methanol crude 100 mg/kg + *Escherichia coli*	0/4
4	4a	Methanol crude 200 mg/kg + *Salmonella spp*	0/4
	4b	Methanol crude 200 mg/kg + *Escherichia coli*	1/4
5	5a	Methanol crude 400 mg/kg + *Salmonella spp*	1/4
	5b	Methanol crude 400 mg/kg + *Escherichia coli*	0/4
6	6a	Methanol hexane 100 mg/kg + *Salmonella spp*	0/4
	6b	Methanol hexane 100 mg/kg + *Escherichia coli*	0/4
7	7a	Methanol hexane 200 mg/kg + *Salmonella spp*	0/4
	7b	Methanol hexane 200 mg/kg + *Escherichia coli*	0/4
8	8a	Methanol hexane 400 mg/kg + *Salmonella spp*	1/4
	8b	Methanol hexane 400 mg/kg + *Escherichia coli*	0/4

The results in Table 4.11 show the *in vivo* antibacterial activityof *Helianthus annuus* seeds extracts against four (4) test organism. As distilled water gave no mortality for *Salmonella spp* but gave one mortality *E. coli*. At 20 mg/kg Ciprofloxacin gave one mortality for *Salmonella spp* and two for *E. coli* while there was no mortality for crude methanol for both organisms. At 100 mg/kg crude methanol gave no mortality for either

of the organisms, but at 200 mg/kg there was one mortality for *E. coli.* The 400 mg/kg crude methanol gave one mortality for *Salmonella spp* but there was no mortality for *E. coli* at the same concentration. The 100 mg/kg and 200 mg/kg methanol-hexane gave no mortality for the two organisms. However, at 400 mg/kg there was one (1) mortality for *Salmonella spp* while there was no mortality for *E. coli.*

Table 4.12: Serum Enzymes Biochemical Parameters of Rats treated with *H. annuus* Seed Extracts for 28 Days as compared to the Control Group

Dose (mg/kgbw)	Biochemical parameters				
	TP(g/L)	**ALB(g/L)**	**ALT(IU/L)**	**AST(IU/L)**	**ALP(IU/L)**
MC10	26.15 ± 1.39^{b}	15.45 ± 1.80^{bc}	35.44 ± 2.48^{bcd}	16.60 ± 1.18^{c}	120.29 ± 2.05^{ab}
MH10	30.27 ± 1.38^{c}	19.04 ± 1.46^{c}	31.94 ± 1.51^{b}	21.48 ± 1.14^{de}	129.29 ± 2.10^{bc}
EC10	21.01 ± 1.51^{a}	11.09 ± 0.76^{a}	36.05 ± 2.02^{bcd}	23.01 ± 0.97^{de}	128.32 ± 4.43^{abc}
EH10	23.04 ± 0.88^{ab}	15.43 ± 1.41^{bc}	39.54 ± 1.10^{cde}	23.53 ± 2.14^{de}	160.07 ± 3.33^{g}
MC300	22.43 ± 1.03^{ab}	16.10 ± 0.84^{bc}	35.62 ± 1.13^{bcd}	11.36 ± 0.68^{a}	134.85 ± 1.75^{cd}
MH300	32.98 ± 1.56^{cd}	21.07 ± 1.46^{de}	30.84 ± 2.08^{ab}	19.18 ± 0.72^{cd}	151.76 ± 1.47^{fg}
EC300	22.42 ± 1.03^{ab}	15.95 ± 0.80^{bc}	41.06 ± 1.65^{d}	24.73 ± 1.64^{e}	120.42 ± 3.76^{ab}
EH300	31.66 ± 1.15^{c}	20.49 ± 1.19^{de}	34.95 ± 1.19^{bcd}	21.14 ± 1.14^{de}	138.23 ± 3.29^{cd}
MC600	23.17 ± 1.45^{ab}	15.95 ± 2.09^{bc}	25.64 ± 1.89^{a}	11.88 ± 1.18^{ab}	135.27 ± 2.52^{cd}
MH600	37.53 ± 0.46^{e}	23.37 ± 0.70^{e}	32.90 ± 1.60^{bc}	15.74 ± 1.21^{bc}	118.87 ± 2.45^{a}
EC600	25.13 ± 0.98^{b}	16.03 ± 1.44^{bc}	37.28 ± 1.70^{bcde}	23.91 ± 0.85^{e}	118.37 ± 3.32^{a}
EH600	35.62 ± 1.12^{de}	22.05 ± 0.92^{de}	33.17 ± 3.43^{bc}	16.30 ± 2.22^{c}	140.37 ± 3.20^{de}
NC	22.38 ± 1.31^{ab}	12.58 ± 0.72^{ab}	42.99 ± 2.77^{e}	21.15 ± 1.30^{de}	148.60 ± 4.79^{ef}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p< 0.05$. While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, EC= Ethanol crude extract, EH= n-Hexane fraction of ethanol crude extract, NC= normal control. TP=Total protein, ALB= Albumin, ALT=Alanine transaminase, AST=Aspartate transaminase and ALP=Alanine phosphatase

The results in Table 4.12 show the biochemical parameters of the rats treated with the extract of *Helianthus annuus* seed. The biochemical parameters showed significant difference among the extracts from the difference solvents. Crude methanol extracts showed decrease in the total protein with increase in concentration and was significantly ($p < 0.05$) higher at 10 mg/kg body weight. At 10, 300 and 600 mg/kg body weight, total protein concentration was significant ($p<0.05$) higher n-hexane fraction of the crude methanol extracts. The total protein also increased with increase in concentration for the same n-hexane fraction of the crude methanol extracts and the fraction performs better than the negative control. Similar result was obtained for crude ethanol which showed significantly ($p<0.05$) higher total protein concentration at 600 mg/kg body weight. The n-hexane fraction of the crude ethanol extract showed the same trend. When all the extracts were compared, the highest total protein concentration were obtained at 600 mg/kg body weight in n-hexane fraction of methanol crude extract (MHXN) and n-Hexane fraction of ethanol crude extract (EHXN) while the least were obtained at 10 mg/kg body weight crude ethanol extract, negative control, 300 mg/kg body weight crude ethanol extract and 300 mg/kg body weight crude methanol extract.

Albumin concentration at 10 mg/kg body weight was significantly ($p<0.05$) highest in the n-hexane fraction of methanol crude extract (MHXN) compared to other fractions at the same concentration. The albumin concentration increases with increase in concentration for the same fraction of the n-hexane fraction of crude methanol extract. The least albumin concentration was obtained in crude ethanol extract, but the albumin increased with increase in concentration. The alanine transferase (ALT) activity was significantly ($p<0.05$) higher for the n-Hexane fraction of crude ethanol extract at 10 mg/kg body weight but significantly ($p<0.05$) least in n-Hexane fraction of crude methanol extract. At 300 mg/kg body weight, alanine transferase (ALT) activity was significantly ($p<0.05$)

higher for crude ethanol extract and significantly lowest ($p < 0.05$) in crude methanol extract. At 600 mg/kg body weight, alanine transferase (ALT) activity was significantly ($p < 0.05$) higher for crude ethanol extract (ECR) and significantly ($p < 0.05$) lowest in n-hexane fraction of crude methanol extract (MHXN). The overall comparison showed that negative control had the highest alanine transferase activity which was significantly ($p < 0.05$) higher.

Aspartate transaminase activity decreased with increase in concentration while alanine phosphatase increased with increase in concentration. Although there were significant ($p<0.05$) differences among the treated groups and the control in some cases, they were still within the normal range.

Table 4.13: Serum Lipid Biochemical Parameters of Rats treated with *H. annuus* Seeds Extracts for 28 Days as compared to the Control Group

Dose (mg/kgbw)	Biochemical parameters (mmol/L)			
	TC	**TRIG**	**HDL-C**	**LDL-C**
MC10	142.99 ± 2.25^{c}	131.90 ± 1.16^{ab}	100.23 ± 1.22^{c}	109.58 ± 2.46^{cde}
MH10	146.33 ± 3.24^{cd}	141.58 ± 2.45^{cd}	119.42 ± 2.59^{de}	104.10 ± 2.62^{bcd}
EC10	154.53 ± 1.39^{e}	126.16 ± 1.29^{a}	82.31 ± 3.48^{a}	109.23 ± 3.76^{cde}
EH10	164.56 ± 2.51^{f}	131.84 ± 3.15^{ab}	111.36 ± 4.28^{d}	113.43 ± 3.53^{e}
MC300	146.47 ± 1.94^{cd}	143.71 ± 2.46^{de}	115.93 ± 2.68^{de}	111.97 ± 2.72^{de}
MH300	127.85 ± 1.69^{b}	144.55 ± 2.19^{def}	141.02 ± 2.77^{fg}	82.54 ± 1.66^{a}
EC300	144.57 ± 3.67^{cd}	126.75 ± 3.57^{a}	88.89 ± 0.97^{ab}	101.72 ± 1.77^{bc}
EH300	151.58 ± 1.93^{de}	135.83 ± 3.17^{bc}	124.67 ± 2.50^{e}	95.99 ± 2.10^{b}
MC600	131.91 ± 1.74^{b}	151.72 ± 1.06^{f}	138.53 ± 2.54^{f}	103.69 ± 2.86^{bcd}
MH600	116.57 ± 3.78^{a}	149.00 ± 1.80^{ef}	147.25 ± 3.86^{g}	77.30 ± 3.10^{a}
EC600	144.68 ± 2.63^{cd}	150.04 ± 1.84^{ef}	93.44 ± 2.34^{bc}	98.50 ± 1.81^{b}
EH600	147.61 ± 0.99^{cde}	151.29 ± 1.16^{f}	122.96 ± 3.24^{e}	84.20 ± 2.10^{a}
NC	174.83 ± 2.58^{g}	146.65 ± 2.15^{def}	95.44 ± 1.61^{bc}	114.21 ± 2.24^{e}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p< 0.05$. While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, EC= Ethanol crude extract, EH= n-Hexane fraction of ethanol crude extract, NC= normal control. TC=Total cholesterol, TRIG=Triglycerides, HDL-C= high density lipoprotein cholesterol and LDL-C=Low density lipoprotein cholesterol

The results in Table 4.13 shows the lipid component of the Biochemical Parameters of rats treated with *H. annuus* seeds extracts for 28 days compared to the control groups. The total cholesterol ranges from 116.57 mg/g found in the group administered 600 mg/kg body weight of n-hexane fraction of crude methanol extract to 174.83 mg/g found in the group administered the control. Total cholesterol was significantly ($p<0.05$) higher in the negative control group as compared to the treated groups. Among the treated groups, the group treated with 10 mg/kg body weight had the highest cholesterol level while the group treated with 600 mg/kg body weight of n-hexane fraction of crude methanol extract had significantly ($p<0.05$) lower value of total cholesterol (116.57±3.78 mg/g).

Triglyceride ranges from 126.16 mg/g found in 10 mg/kg body weight crude ethanol to 151.72 mg/g found in the group administered 600 mg/kg body weight. The triglyceride components in the groups administered 600 mg/kg body weight of crude methanol, crude ethanol, n-hexane fraction of crude methanol and n-Hexane fraction of crude ethanol extracts were significantly higher ($p<0.05$) than the control group while the control group was significantly ($p<0.05$) higher than the other groups treated with extracts concentrations lower than 300 mg/kg body weight.

The high-density lipoproteins cholesterol (HDL-C) ranged from 82.31 mg/g found in the group of rat administered 10 mg/kg body weight to 147.25 mg/g obtained for the group administered 600 mg/kg body weight of n-hexane fraction of crude methanol extract. The 300 and 600 mg/kg body weight groups of n-Hexane fraction of crude ethanol extract of *H. annuus* seeds were significantly higher ($p<0.05$) compared to the control group. The groups treated with 10 mg/kg body weight crude ethanol and 300 mg/kg body weight crude ethanol had significantly lower ($p<0.05$) HDL-cholesterol level compared to the control group.

The results of the low-density lipoprotein cholesterol (LDL-C) showed the LDL-cholesterol ranges from 82.54 mg/g for group administered 300 mg/kg body weight n-hexane fraction of crude methanol extract to 114.21 mg/g for group administered the negative control. The normal control group had the highest value which was significantly ($p<0.05$) higher compared to all the other group except the group administered 10 mg/kg body weight n-Hexane fraction of crude ethanol extract.

Table 4.14: Protein Components of the Serum Biochemical Parameters of Rats treated with *H. annuus* Seeds Extracts for 28 Days as compared to the Control Group

Dose (mg/kg bw)	Biochemical parameters (mg/dL)				
	Creatinine	**Total bilirubin**	**Indirect bilirubin**	**Uric acid**	**Urea**
MC10	7.74 ± 0.68^{bc}	0.83 ± 0.04^{ab}	0.33 ± 0.04^{a}	7.74 ± 0.68^{abc}	72.43 ± 2.60^{a}
MH10	8.89 ± 0.71^{bcd}	1.04 ± 0.05^{cd}	0.69 ± 0.05^{de}	11.37 ± 1.30^{efg}	72.85 ± 2.01^{a}
EC10	12.17 ± 1.38^{de}	0.77 ± 0.07^{a}	0.51 ± 0.10^{bc}	8.74 ± 0.63^{abcde}	75.31 ± 2.08^{a}
EH10	14.00 ± 1.35^{e}	1.31 ± 0.04^{e}	0.90 ± 0.02^{f}	12.20 ± 0.95^{fg}	77.00 ± 1.26^{a}
MC300	6.33 ± 0.38^{ab}	1.12 ± 0.08^{d}	0.56 ± 0.02^{bcd}	8.42 ± 0.34^{abcd}	69.83 ± 2.35^{a}
MH300	11.11 ± 0.71^{cde}	1.00 ± 0.05^{bcd}	0.63 ± 0.05^{cd}	9.63 ± 0.41^{cdef}	74.18 ± 2.73^{a}
EC300	10.96 ± 0.97^{cde}	0.86 ± 0.07^{abc}	0.55 ± 0.07^{bcd}	7.31 ± 0.97^{abc}	71.47 ± 2.11^{a}
EH300	21.08 ± 1.54^{g}	0.99 ± 0.08^{bcd}	0.50 ± 0.02^{bc}	9.07 ± 0.47^{bcde}	77.50 ± 1.95^{a}
MC600	4.30 ± 0.47^{a}	1.45 ± 0.06^{e}	0.49 ± 0.01^{bc}	6.36 ± 0.44^{ab}	73.64 ± 1.90^{a}
MH600	10.63 ± 0.98^{cde}	1.08 ± 0.06^{d}	0.78 ± 0.02^{ef}	12.49 ± 1.65^{fg}	78.03 ± 3.41^{a}
EC600	17.11 ± 0.83^{f}	0.74 ± 0.03^{a}	0.50 ± 0.02^{bc}	5.86 ± 0.54^{a}	73.46 ± 3.72^{a}
EH600	13.03 ± 1.49^{e}	0.87 ± 0.03^{abc}	0.61 ± 0.01^{bcd}	10.98 ± 1.07^{defg}	71.81 ± 2.21^{a}
NC	17.79 ± 1.40^{f}	0.86 ± 0.07^{abc}	0.48 ± 0.02^{b}	12.69 ± 1.13^{g}	76.60 ± 1.76^{a}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p< 0.05$. While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, EC= Ethanol crude extract, EH= n-Hexane fraction of ethanol crude extract, NC= normal control

The results in Table 4.14 show protein components of the serum biochemical parameters of rats treated with *H. annuus* seeds extracts for 28 days as compared to the control group. The creatinine component ranges from 4.30 mg/g to 21.08 mg/g among the rat groups treated with the various extract of *H. annuus* seed. The rat group administered 300 mg/kg body weight n-hexane fraction of crude ethanol had significantly ($p<0.05$) higher serum creatinine level compared to the control group. However, the creatinine level in the control group was significantly ($p<0.05$) higher than the rest of treated groups except the group administered 600 mg/kg body weight of crude ethanol which had the same level of significant ($p< 0.05$) with the control group.

Total bilirubin ranges from 0.77 mg/g in the group administered 10 mg/kg body weight crude ethanol to 1.45 mg/g for the group administered 600 mg/kg body weight crude methanol. There was significant ($p<0.05$) difference among the various groups with some groups sharing the same level of significant ($p< 0.05$). The group administered 10 mg/kg body weight n-hexane fraction of the crude methanol, 10 mg/kg body weight n-hexane fraction of crude ethanol, 300 mg/kg body weight of crude methanol, 300 mg/kg body weight of n-hexane fraction of crude methanol, 600 mg/kg body weight of crude methanol and the group administered 600 mg/kg body weight had significantly ($p<0.05$) higher over the control. The control group and the group treated with 300 mg/kg weight crude ethanol and 600 mg/kg body weight of n-hexane fraction of crude ethanol had the same level of total bilirubin. The remaining groups had significantly ($p< 0.05$) lower total bilirubin when compared to control groups.

The indirect bilirubin concentration ranges from 0.33 mg/L in group administered 10 mg/kg body weight crude methanol to 0.90 mg/L in group administered n-hexane fraction of the crude ethanol extract. The indirect bilirubin level was significantly ($p<0.05$) higher in all the other groups except the group administered 10 mg/kg body weight crude

methanol extract. Uric acid concentration ranges from 5.86 mg/L in the group administered 600 mg/kg body weight crude ethanol to 12.69 mg/L in the control groups. There was significant difference ($p< 0.05$) among the various groups, and the control group had significantly ($p<0.05$) higher uric acid concentration compared the rest of the group. Urea ranges from 69.83 mg/L in the group administered 300 mg/kg body weight of crude methanol to 78.03 mg/L in the group administered 600 mg/kg body weight of n-hexane fraction of crude ethanol extract. There was no significant ($p< 0.05$) difference in the urea concentration for all the groups including the control group.

Table 4.15: Serum Biochemical Mineral Components of Rats treated with *H. annuus* Seeds Extracts for 28 Days as compared to the Control Group

Dose (mg/kg bw)	Biochemical parameters (mEq/L)			
	Sodium	**Potassium**	**Chloride**	**Bicarbonate**
MC10	172.00±3.97^{de}	8.00±0.07^{a}	88.23±2.66^{abc}	36.89±1.34^{ab}
MH10	163.77±2.05^{bc}	10.99±0.96^{bc}	92.04±2.77^{bcd}	35.13±1.73^{ab}
EC10	179.01±2.72^{ef}	11.45±1.15^{c}	93.83±2.14^{cde}	41.63±2.43^{bc}
EH10	174.86±2.54^{de}	12.09±1.01^{cd}	88.45±1.09^{abc}	49.46±1.55^{e}
MC300	181.98±2.18^{f}	12.55±1.16^{cd}	80.43±2.25^{a}	40.36±1.83^{abc}
MH300	157.70±1.70^{b}	13.12±0.87^{cd}	105.00±4.30^{f}	33.44±2.24^{a}
EC300	178.98±0.96^{ef}	11.47±0.78^{c}	86.66±1.99^{abc}	35.17±2.21^{ab}
EH300	157.51±1.64^{b}	13.22±0.64^{cd}	82.96±2.55^{ab}	42.05±2.03^{bcd}
MC600	158.65±2.19^{b}	14.74±0.63^{d}	85.79±2.63^{abc}	44.15±1.89^{cde}
MH600	146.99±2.17^{a}	10.63±0.98^{abc}	90.03±2.48^{bc}	36.27±1.64^{ab}
EC600	168.12±3.34^{cd}	10.92±1.00^{bc}	101.94±2.15^{ef}	38.84±2.73^{abc}
EH600	162.92±2.64^{bc}	14.58±1.02^{d}	99.57±3.56^{def}	48.77±2.94^{de}
NC	178.33±2.55^{ef}	8.49±0.62^{ab}	90.39±4.72^{bc}	51.02±3.26^{e}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p< 0.05$. While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, EC= Ethanol crude extract, EH= n-Hexane fraction of ethanol crude extract, NC= normal control

The results in Table 4.15 show the serum mineral component of the biochemical parameters of rats treated with *H. annuus* seed extracts. The sodium level in the serum ranges from 146.99 mg/L in the group administered 600 mg/kg body weight of n-hexane fraction of crude methanol to 181.98 mg/L in group administered 300 mg/kg methanol crude (MCR). The group administered 300 mg/kg body weight crude methanol had significantly ($p<0.05$) higher sodium concentration compared to the control group. The control group, the group administered 300 mg/kg body weight of crude ethanol and 10 mg/kg body weight shared the same significant ($p< 0.05$) level and were significantly higher over the other group except the group administered 300 mg/kg body weight of crude methanol. Potassium concentration in the rats administered the various extract of *H. annuus* seed ranges from 8.00 to 14.74 mg/L. The potassium concentration was significantly lower in the group administered 10 mg/kg body weight of crude methanol compared to the rest of the groups. In the same manner, the potassium concentration was significantly lower in the control group when compared to the rest of the group other than the group administered 10 mg/kg body weight which was significantly lower.

The chlorine concentration ranges from 80.43 mg/L to 105.00 mg/L. The control group and the group administered 600 mg/kg body weight of n-hexane fraction of the crude methanol shared the same level of significant. They were significantly higher compared to the group administered 10 mg/kg body weight crude methanol, 300 mg/kg body weight of crude methanol and the group of rats administered 300 mg/kg body weight of n-hexane fraction of the crude ethanol. However, they were significantly lower when compared to the rest of the groups. The bicarbonate was lower among the treated groups compared to the control group. It ranges from 33.44 mg/L to 51.02 mg/L found in the control group. The bicarbonate concentration was significantly higher in the control over all the other groups except the group administered 10 mg/kg body weight of n-hexane fraction of

crude ethanol which share the same significant level as the control group.

Table 4.16: Haematological values of Rats treated with *Helianthus annuus* Seed Extracts for 28 Days compared to the Control Group

	Haematological parameters			
Dosage (mg/kg bw)	**Hb(g/dL)**	**PCV(%)**	**MCV(fL)**	**MCH(pg)**
MC10	16.40±1.31^{ab}	40.00±1.15^{ab}	47.00±2.31^{bc}	20.50±1.44^{abcd}
MC300	20.11±1.92^{abcd}	41.50±3.75^{ab}	47.50±1.44^{bcd}	17.50±0.87^{a}
MC600	22.40±1.30^{cd}	34.50±2.60^{a}	56.50±1.44^{f}	20.50±2.02^{abcd}
MH10	23.47±1.24^{d}	40.00±0.58^{ab}	48.50±2.02^{cd}	22.50±1.44^{abcde}
MH300	20.37±0.84^{abcd}	39.50±2.02^{ab}	54.50±2.02^{ef}	25.00±1.15^{cde}
MH600	21.10±1.34^{bcd}	45.50±2.02^{bc}	53.00±1.73^{def}	19.00±1.15^{ab}
EC10	22.11±1.93^{cd}	39.50±1.44^{ab}	48.50±2.02^{cd}	19.50±2.02^{abc}
EC300	24.59±1.56^{d}	36.88±1.09^{a}	46.00±1.91^{bc}	26.25±1.11^{ef}
EC600	22.53±1.14^{cd}	41.00±2.31^{ab}	50.50±2.02^{cde}	22.50±2.60^{abcde}
EH10	21.53±1.91^{cd}	40.50±3.75^{ab}	42.50±2.02^{ab}	19.00±2.31^{ab}
EH300	18.32±1.20^{abc}	51.50±4.50^{c}	37.75±1.75^{a}	27.00±1.00^{e}
EH600	23.50±1.25^{d}	40.00±1.73^{ab}	39.50±0.87^{a}	21.50±2.02^{abcde}
NC	15.95±0.97^{a}	37.50±1.44^{a}	37.50±0.87^{a}	23.50±2.02^{bcde}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$. Key: MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, EC= Ethanol crude extract, EH= n-Hexane fraction of ethanol crude extract, NC= normal control. Hb=haemoglobin, PCV=Packed cell volume, MCV=Mean cell volume and Mean cell Haemoglobin

The results in Table 4.16 show the hematological parameters of the rats treated with *Helianthus annuus* seed extracts. The hemoglobin value was significantly ($p<0.05$) lower in the control group compared to the treatment groups. The same trend was observed in the value of the packed cell volume (PCV). The control group had significantly ($p<0.05$) lower packed cell volume (PCV), except that the group shared the same significant ($p< 0.05$) level with the rat group administered 600 mg/kg body weight of crude methanol and 300 mg/kg body weight of crude ethanol.

The MCV values ranges from 37.50 mg/L to 56.50 mg/L. The least value of MCV was obtained for the control group which significantly ($p<0.05$) differs from the treatment groups except for the groups treated with 300 and 600 mg/kg body weight of n-Hexane fraction of crude ethanol extract. The control group shared the same level of significance ($p< 0.05$) with the 300 and 600 mg/kg body weight of n-Hexane fraction of crude ethanol extract and they were significantly ($p< 0.05$) lower than the rest of the groups. MCH values ranges from 17.50mg/L in the group administered 300 mg/kg body weight of crude methanol to 27.00 mg/L in the group treated with 300 mg/kg body weight of n-Hexane fraction of crude ethanol extract. The MCH of the control group was significantly ($p<0.05$) higher in some cases and significantly ($p<0.05$) lower in others while sharing the same level of significant ($p< 0.05$) with the other treatment groups.

Table 4.16: Haematological values of Rats treated with *Helianthus annuus* Seed Extracts for 28 Days compared to the Control Group

	Haematological parameters			
Dosage (mg/kg bw)	**MCHC(g/dL)**	**RBC(10^{12}/L)**	**PLC(10^{6}/L)**	**TWBC(10^{12}/L)**
MC10	42.00±3.46^{b}	7.70±0.69^{abc}	149.00±3.46^{ab}	7.15±0.43^{bc}
MC300	38.50±3.75^{ab}	9.95±0.26^{def}	148.50±0.87^{ab}	9.40±0.52^{de}
MC600	41.50±2.60^{b}	8.70±0.52^{bcde}	153.00±2.31^{b}	10.95±0.78^{ef}
MH10	42.00±1.15^{b}	7.00±0.81^{ab}	153.00±1.73^{b}	7.25±0.95^{bcd}
MH300	43.50±3.18^{b}	8.50±0.35^{abcd}	154.50±2.02^{b}	7.30±0.81^{bcd}
MH600	40.50±2.60^{b}	7.75±0.61^{abc}	148.50±6.06^{ab}	11.55±0.49^{f}
EC10	39.50±1.44^{ab}	6.60±0.58^{a}	142.00±1.73^{a}	7.90±0.17^{bcd}
EC300	42.38±2.13^{b}	8.11±0.64^{abcd}	147.13±3.56^{ab}	6.06±0.83^{ab}
EC600	32.00±1.73^{a}	10.85±0.95^{f}	155.50±2.02^{b}	4.80±0.17^{a}
EH10	36.50±1.44^{ab}	8.30±0.52^{abcd}	149.00±2.31^{ab}	8.55±0.43^{cd}
EH300	37.50±3.50^{ab}	8.08±0.58^{abcd}	147.50±2.50^{ab}	6.93±0.53^{bc}
EH600	40.50±1.44^{b}	10.55±0.49^{ef}	150.50±3.75^{ab}	7.05±0.95^{bc}
NC	42.50±1.44^{b}	9.55±0.55^{cdef}	146.00±1.73^{ab}	9.45±0.61^{de}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$. Key: MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, EC= Ethanol crude extract, EH= n-Hexane fraction of ethanol crude extract, NC= normal control. MCHC=Mean cell haemoglobin concentration, RBC=Red blood cells, PLC=Platelet count and TWBC= Total white blood cells

The results in Table 4.16 show the hematological parameters of the rats treated with *Helianthus annuus* seed extracts. MCHC values ranged from 32.00 % in the group treated with 600 mg/kg body weight of crude ethanol to 43.50 % in the group treated with 300 mg/kg body weight of n-hexane fraction of crude methanol. The MCHC of the control group was not significantly ($p<0.05$) different from most of the treated groups except in the groups treated with 300mg/kg body weight of crude methanol, 300 and 600 mg/kg body weight of crude ethanol as well as 10 and 300 mg/kg body weight of n-Hexane fraction of crude ethanol extract where it was significantly ($p<0.05$) higher.

The red blood cell (RBC) of the rats ranges from 6.60 in the group treated with 10 mg/kg body weight of crude ethanol extracts to 10.85 in the group treated with 600 mg/kg body weight of crude ethanol extract. The RBC value of the control group was significantly ($p<0.05$) higher than most of the treatment groups except in the groups treated with 300 mg/kg body weight of crude ethanol extract. It however shared the same level of significance ($p< 0.05$) with the group treated with 600 mg/kg body weight of n-Hexane fraction of crude ethanol extract.

The platelet count (PLC) value ranges from 142.00 mg/L in group treated with 10 mg/kg body weight of crude ethanol to 155.50 mg/L in the group treated with 600 mg/kg body weight of crude ethanol. Only the platelet of the group treated with 10 mg/kg body weight of crude ethanol was significantly ($p<0.05$) lower than the control group. The remaining groups either shared the same significant level ($p<0.05$) with or had significantly ($p<0.05$) higher PLC than the control group. The groups with platelet values significantly ($p<0.05$) higher than the control includes the groups treated 600 mg/kg body weight of crude methanol, 10 and 300 mg/kg body weight of n-hexane fraction of crude ethanol extracts and 600 mg/kg body weight of crude ethanol extract.

The total white blood cells (TWBC) ranges from 4.80 mg/L in the group treated with 600 mg/kg body weight of crude ethanol to 11.55 mg/kg body weight of n-hexane fraction of crude ethanol. The TWBC was significantly ($p<0.05$) higher in the group treated with the control compared to most of the other groups treated with the various extract except in the group administered 300 and 600 mg/kg body weight of crude methanol and 600 mg/kg body weight of n-hexane fraction of the crude ethanol. While the group treated with 300 mg/kg body weight of crude methanol shared the same level of significance ($p< 0.05$) with the control group, the 600 mg/kg body weight of crude methanol and 600 mg/kg body weight of n-hexane fraction of the crude ethanol were significantly ($p<0.05$) higher than the control groups

Table 4.17: Relative Body Weight of Rats infected with *Escherichia coli* and treated with *H. annuus* Seed Extracts

	Body weight (g)		
Dose (mg/kg bw)	***E. coli***		
	WEEK-0	WEEK-1	WEEK-2
MC100	115.75±21.10[a]	120.40±8.75[ab]	140.16±8.76[a]
MC200	106.23±27.27[a]	114.02±13.31[a]	129.24±10.07[a]
MC400	120.21±26.71[a]	123.94±20.17[ab]	147.53±17.61[a]
MH100	116.01±30.09[a]	115.32±20.71[a]	131.10±17.30[a]
MH200	100.08±14.29[a]	114.25±9.84[a]	129.94±8.92[a]
MH400	104.01±17.81[a]	112.72±17.00[a]	130.58±16.84[a]
Cipro 20	106.23±23.37[a]	129.37±12.35[ab]	145.09±14.88[a]
Negative	164.74±32.72[a]	177.82±36.64[b]	174.62±39.91[a]

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$. While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, negative = distilled water.

The results in Table 4.17 show relative body weight of rats infected with *Escherichia coli* and treated with *H. annuus* seeds extracts. There was no significant difference ($p< 0.05$) in the rat treated with the various extracts, the negative and the positive control groups at the start of the administration at week 0 with the group infected with *E. coli* but there was significant ($p<0.05$) difference at week 1. The relative body weight of the group administered with the negative control was significantly higher ($p<0.05$) over the other groups. The relative body weight of the group administered 20 mg/kg body weight ciprofloxacin positive control and the groups administered 100 and 400 mg/kg body weight shows no significant difference ($p< 0.05$), but were significantly difference ($p<0.05$) from the other groups and the negative control. At week 2, the significant

($p<0.05$) difference observed in the relative body weight in week 1 was no longer visible for rats infected with *E. coli*.

Table 4.18: Relative Body Weight of Rats infected with *Salmonella spp* and treated with *H. annuus* Seed Extracts

	Body weight (g)		
Dose (mg/kg bw)	***Salmonella sp.***		
	WEEK-0	WEEK-1	WEEK-2
MC100	116.70 ± 21.58^{ab}	138.78 ± 17.95^{ab}	155.70 ± 15.16^{b}
MC200	115.97 ± 2.42^{ab}	138.60 ± 3.80^{ab}	151.76 ± 2.41^{b}
MC400	90.07 ± 12.82^{ab}	109.57 ± 14.79^{ab}	129.11 ± 11.64^{ab}
MH100	132.27 ± 15.74^{ab}	140.69 ± 15.69^{ab}	144.83 ± 17.11^{b}
MH200	158.78 ± 30.05^{b}	172.20 ± 27.53^{b}	163.39 ± 22.23^{b}
MH400	160.82 ± 23.74^{b}	164.98 ± 18.41^{b}	157.35 ± 12.86^{b}
Cipro 20	82.69 ± 13.29^{a}	91.02 ± 10.45^{a}	116.53 ± 7.66^{a}
Negative	145.63 ± 25.34^{b}	143.93 ± 26.61^{b}	152.61 ± 19.95^{b}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p<0.05$. While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, negative = distilled water.

The results in Table 4.18 show relative body weight of rats infected with *Salmonella spp* and treated with *H. annuus* seeds extracts There were significant ($p<0.05$) difference in the relative body weight of rats infected with *Salmonella spp* at week 0 and 1 but the significant difference ($p<0.05$) also was no longer visible at week 2. The relative body weight of the group administered 200 mg/kg body weight and 400 mg/kg body weight of n-hexane fraction of crude methanol extracts were significantly ($p<0.05$) higher over the

rest of the groups including the control groups at week 0 for the groups infected with *Salmonella spp.* Similar results were replicated at week 2 as earlier observed.

Table 4.19: Total Bacteria Load (*Salmonella spp* and *Escherichia coli*) in Rats at 7 days before treatment with *H. annuus* Seed Extracts

Colony Forming Units ($\times 10^6$CFU/mL)	
E.coli	*Salmonella sp.*
12.00 ± 1.73^{a}	16.00 ± 2.89^{a}
12.00 ± 0.58^{a}	18.50 ± 0.87^{a}
17.00 ± 1.15^{b}	17.00 ± 3.46^{a}
13.00 ± 1.73^{ab}	17.00 ± 2.31^{a}
13.50 ± 0.87^{ab}	13.50 ± 0.87^{a}
9.50 ± 0.29^{a}	14.00 ± 1.73^{a}
13.00 ± 2.31^{ab}	14.00 ± 2.31^{a}
13.50 ± 0.29^{ab}	15.00 ± 1.15^{a}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates.Values with different superscripts in a column are significantly different at $p<0.05$.MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, negative = distilled water.

The results in Table 4.19 show the colony forming unit of *E. coli* and *Salmonella spp* before treatment with extracts from *Helianthus annuus* seed using different solvents, ciprofloxacin and the negative control. The colony forming units ranges from 9.50 ± 0.29^{a} to 17.00 ± 1.15^{b} ($\times 10^6$CFU/mL) for groups infected with *E. coli*. The group to be treated with Methanol crude had significantly ($p<0.05$) higher colony forming unit compared to other groups. While other groups share the same level of significance. There was no significant difference in the colony forming units of *Salmonella* as all the groups share equal level of significance ($p<0.05$).

Table 4.20: Total Bacteria Load (*Salmonella spp* and *Escherichia coli*) in Rats after treatment with *H. annuus* Seed Extracts

Dose (mg/kg bw)	Colony Forming Unit ($\times 10^6$CFU/mL)	
	E. coli	*Salmonella sp.*
Negative	13.50 ± 2.02^{d}	10.50 ± 2.02^{c}
Ciprofloxacin	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MC100	4.00 ± 0.58^{c}	2.00 ± 0.58^{b}
MH100	1.50 ± 0.29^{b}	0.00 ± 0.00^{a}
MC200	1.00 ± 0.58^{b}	0.00 ± 0.00^{a}
MH200	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MC400	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
MH400	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p< 0.05$.While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, negative = distilled water.

The results in Table 4.20 show the colony forming unit of *E. coli* and *Salmonella spp* treated with extracts from *Helianthus annuus* seed using different solvents, ciprofloxacin and the negative control. A dose dependent effect of the plant extract against both bacteria was observed. The total bacteria load decreased with increasing plant extract concentration. The negative control had significantly ($p<0.05$) higher colony forming unit for both *E. coli* and *Salmonella spp* compared to the other extracts. The crude methanol extract had four (4) units for *E coli* and two (2) for *Salmonella spp* while n-hexane fraction of crude methanol extract at 100 mg/kg body weight and crude methanol at 200 mg/kg body weight concentration had 1.5 ± 0.29 and 1 ± 0.58($\times 10^6$CFU/mL) respectively. The other extracts at different concentration had no colony forming unit (Significantly lower than the negative control but comparable to the standard drug group).

Table 4.21: Relative Body Weight of Rats treated with *H. annuus* Seed Extracts

Dose (mg/kgbw)	Body weight (g)				
	Week-0	**Week-1**	**Week-2**	**Week-3**	**Week-4**
MC10	88.56±17.71[a]	95.19±18.26[a]	104.43±18.37[a]	109.96±17.19[a]	122.00±14.28[a]
MH10	92.79±7.73[a]	102.27±7.02[a]	110.66±6.81[a]	118.76±6.33[a]	127.63±5.34[ab]
EC10	89.49±10.93[a]	100.80±11.45[a]	111.95±12.53[a]	122.81±12.63[a]	134.41±13.23[ab]
EH10	104.65±5.81[a]	112.02±4.51[a]	124.25±4.52[a]	131.96±3.80[a]	141.08±4.06[ab]
MC300	98.73±4.13[a]	105.56±5.31[a]	116.12±6.03[a]	125.29±7.31[a]	134.19±7.25[ab]
MH300	91.66±7.09[a]	101.79±6.55[a]	111.79±6.03[a]	121.99±5.36[a]	128.15±4.55[ab]
EC300	102.38±16.46[a]	115.46±17.46[a]	126.52±19.90[a]	140.77±19.36[a]	154.09±18.39[b]
EH300	104.11±9.35[a]	113.38±9.12[a]	124.53±8.59[a]	135.39±8.57[a]	145.81±8.32[ab]
MC600	87.96±10.93[a]	97.68±10.91[a]	106.73±9.89[a]	117.40±9.99[a]	128.66±10.03[ab]
MH600	80.50±5.75[a]	88.84±6.62[a]	100.45±5.68[a]	109.59±4.83[a]	119.36±4.19[a]
EC600	98.05±8.17[a]	106.15±7.98[a]	116.32±5.93[a]	123.44±5.86[a]	131.91±5.90[ab]
EH600	89.80±6.15[a]	98.95±5.48[a]	111.14±5.05[a]	120.18±4.12[a]	128.67±3.90[ab]
CONTROL	94.26±5.66[a]	103.24±3.74[a]	112.83±3.00[a]	120.83±2.76[a]	129.49±1.91[ab]

Values are presented as Mean ± Standard Error of Mean (SEM) of three replicates. Values with different superscripts in a column are significantly different at $p < 0.05$. While MC= Methanol crude extract, MH= n-hexane fraction of methanol crude extract, EC= Ethanol crude extract, EH= n-Hexane fraction of ethanol crude extract

The results in Table 4.21 show the relative body weight of rats treated with the various extract of *H. annuus* seeds compared to control group. Although the groups administered 10 mg/kg body weight n-Hexane fraction of crude ethanol and 300 mg/kg body weight n-Hexane fraction of crude ethanol extracts had higher relative body weight at the start of the administration compared to the control and the other groups, there was no significant difference ($p< 0.05$) in the relative body weight of the rats at week 0 when the treatment started. The trend continued up to the third week. However, there was significant difference ($p<0.05$) among the various groups treated with difference extract of *H. annuus* seeds at week four (week 4) which marked the end of the treatment. The relative body weight of the group treated with crude ethanol was significantly ($p<0.05$) higher over all the other extracts including the control group. The control shared the same properties with most of the extract except the 300 mg/kg body weight crude ethanol, 10 mg/kg body weight crude methanol and 600 mg/kg body weight n-hexane fraction of crude methanol extract. The relative body weight of the group administered 10 mg/kg body weight crude methanol and 600 mg/kg body weight n-hexane fraction of crude methanol extracts were significantly ($p<0.05$) lower than the control group.

4.1.7 Histopathology Results

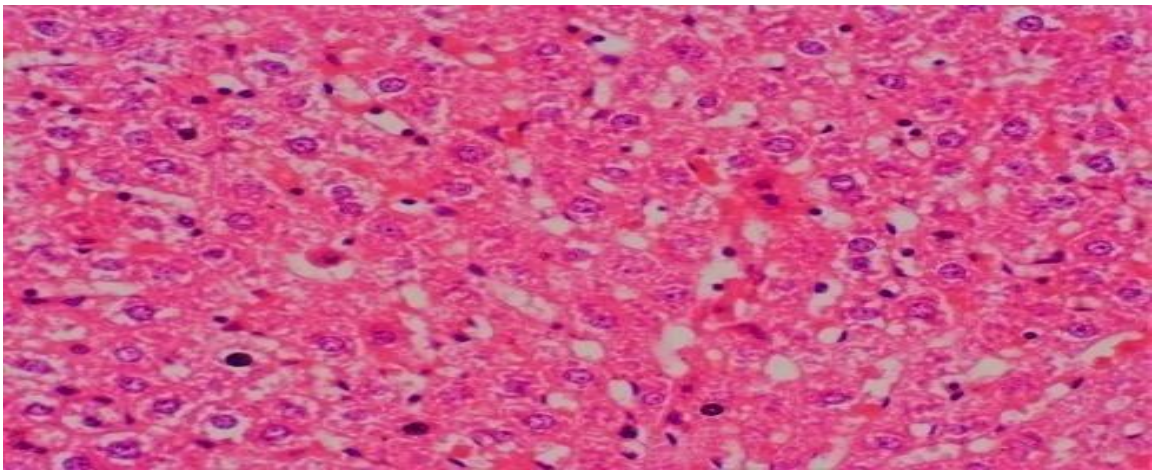

Plate I: Histopathology of the Liver of Rat Treated with Negative Control (NC-L)

The result in Plate I shows the histopathology of the liver of rat treated with the negative control. The section shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes. There are foci showing lymphocytic infiltration of the portal tracts and the few are seen in the sinusoids. There are no features of acute or chronic toxicity or damage.

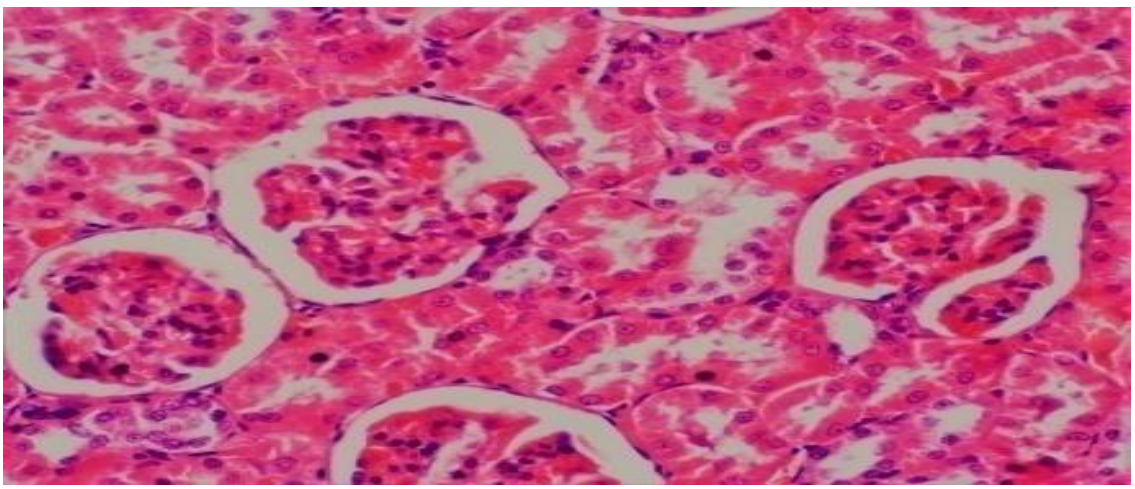

Plate II: Histopathology of the Kidney of Rat Treated with Negative Control (NC-K)

The result in Plate II shows the histopathology of the kidney of rat treated with Negative control. The section shows renal tissue with preserved architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no features of acute or chronic toxicity or damage.

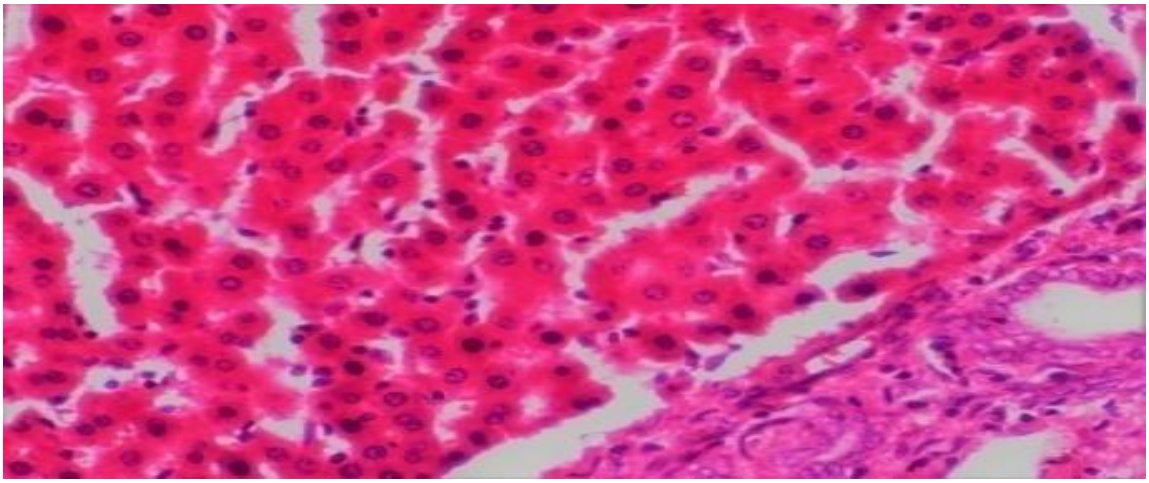

Plate III: Histopathology of Liver of Rat Treated with n-hexane Fraction of Crude Methanol Extractat 10 mg/kg (MH10 L)

The result in Plate III shows the histopathology of the liver of rat treated with n-hexane fraction of crude methanol extract at 10 mg/kg (MH10L). The section shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes. There are foci showing lymphocytic infiltration of the portal tracts and the few are seen in the sinusoids. There are no features of acute or chronic damage.

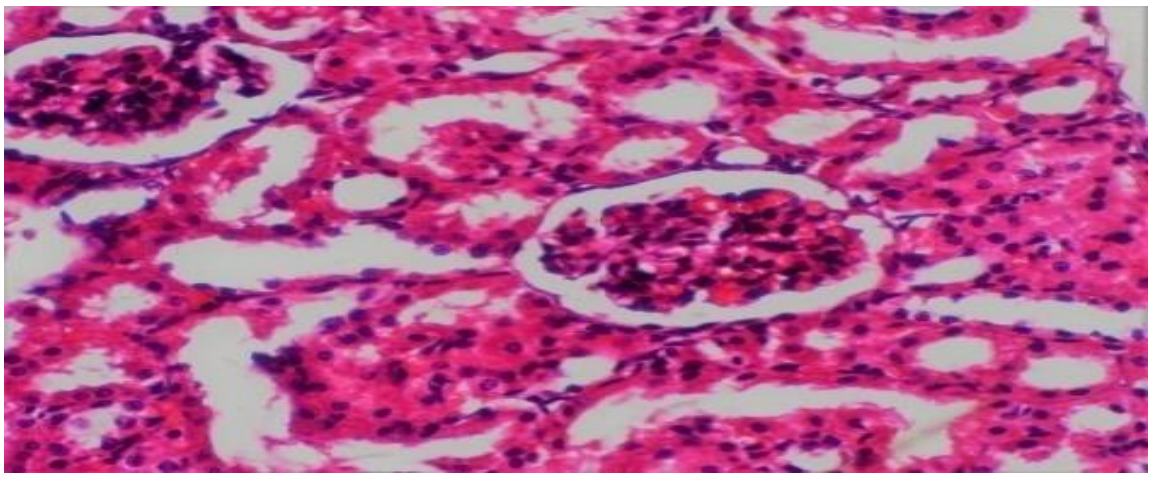

Plate IV: Histopathology of Kidney of Rat Treated with n-hexane Fraction of Crude Methanol Extract at 10 mg/kg (MH10 K)

The result in Plate IV shows the histopathology of the kidney of rat treated with n-hexane fraction of crude methanol extract at 10 mg/kg (MH10 K). The section shows renal tissue with preserved architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no features of acute or chronic damage.

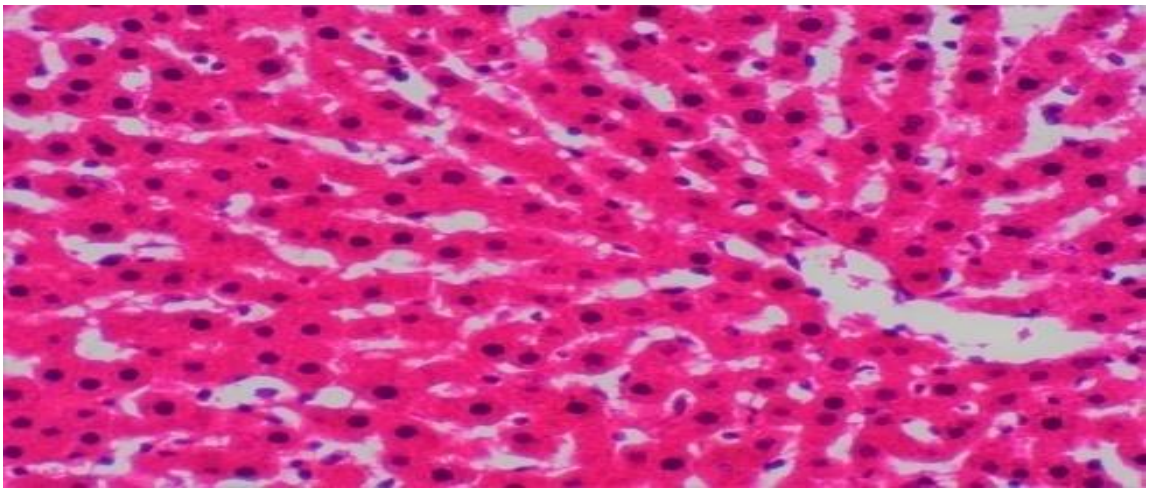

Plate V: Histopathology of the Liver of Rat Treated with n-hexane Fraction of Crude Methanol Extract at 300 mg/kg (MH300K).

The result in Plate V shows the histopathology of the liver of rat treated with n-hexane fraction of crude methanol extractat 300 mg/kg (MH300 L). The section shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes. There are foci showing lymphocytic infiltration of the portal tracts and the few are seen in the sinusoids. There are no features of acute or chronic damage.

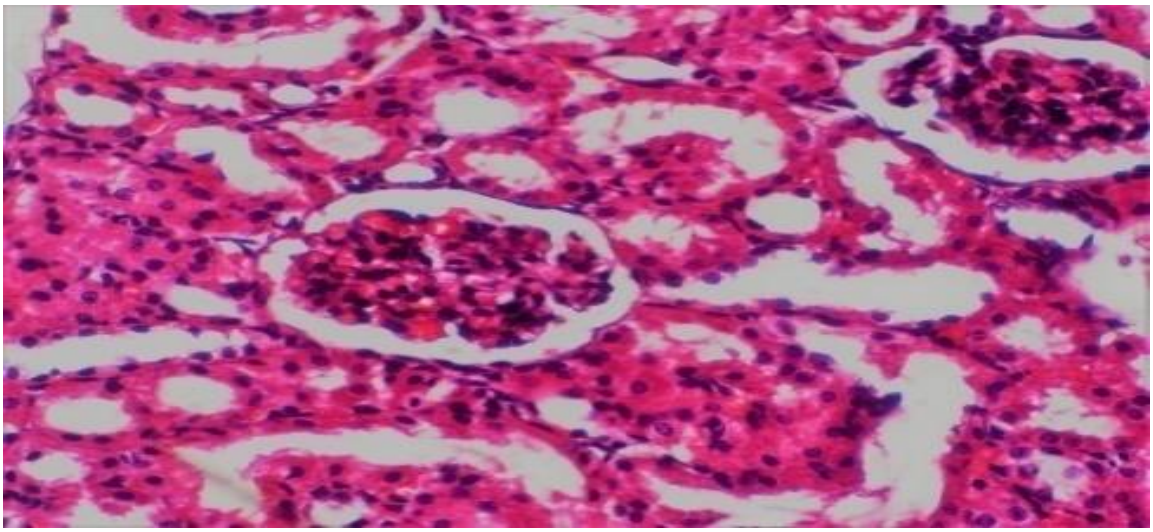

Plate VI: Histopathology of the Kidney of Rat Treated with n-hexane Fraction of Crude Methanol Extractat 300 mg/kg (MH300 K)

The result in Plate VI shows the histopathology of the kidney of rat treated with n-hexane fraction of crude methanol extractat 300 mg/kg (MH300 K). The section shows

renal tissue with preserved architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no features of acute or chronic damage.

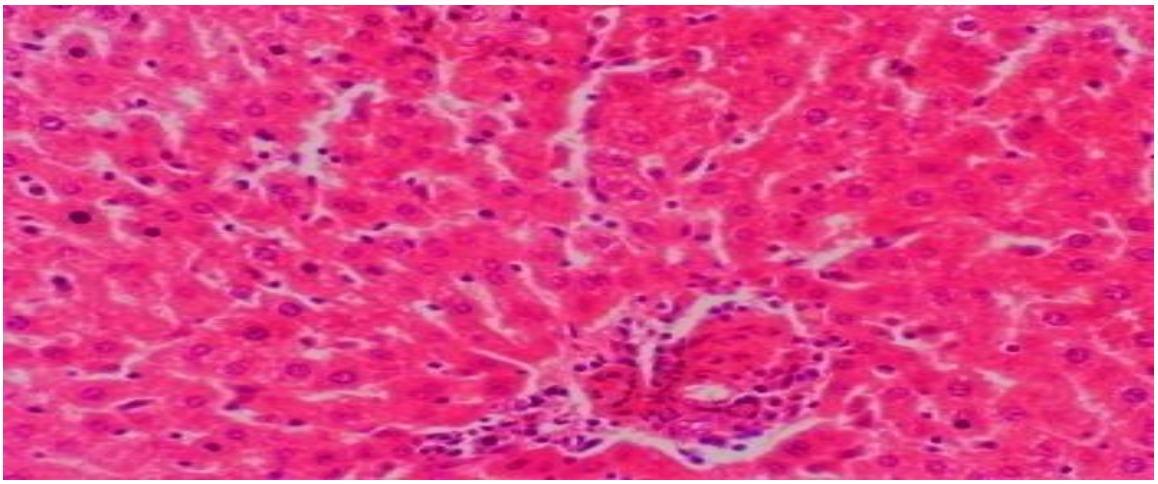

Plate VII: Histopathology of the Liver of Rat Treated with n-hexane Fraction of Crude Methanol Extract at 600 mg/kg (MH600L)

The result in Plate VII shows the liver histopathology of rat treated with n-hexane fraction of crude methanol extract at 600 mg/kg (MH600 L). The section shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes. There are foci showing lymphocytic infiltration of the portal tracts and the few are seen in the sinusoids. There are no features of acute or chronic damage.

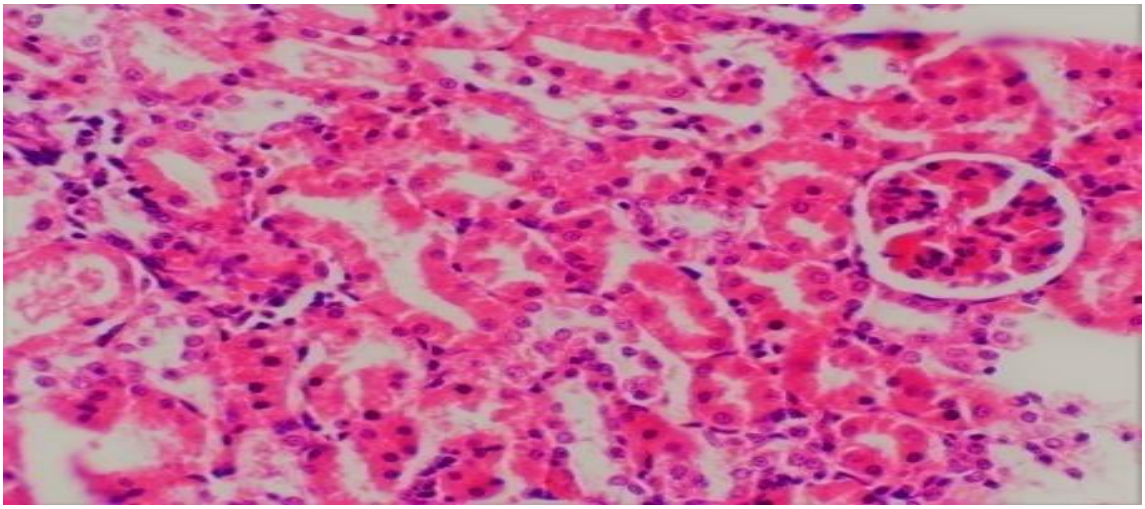

Plate VIII: Histopathology of the Kidney of Rat Treated with n-hexane Fraction of Crude Methanol Extract at 600 mg/kg (MH600K)

The result in Plate VIII shows the histopathology of the kidney of rat treated with n-hexane fraction of crude methanol extractat 600 mg/kg (MH600 K). The section shows

renal tissue with preserved architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no features of acute or chronic damage.

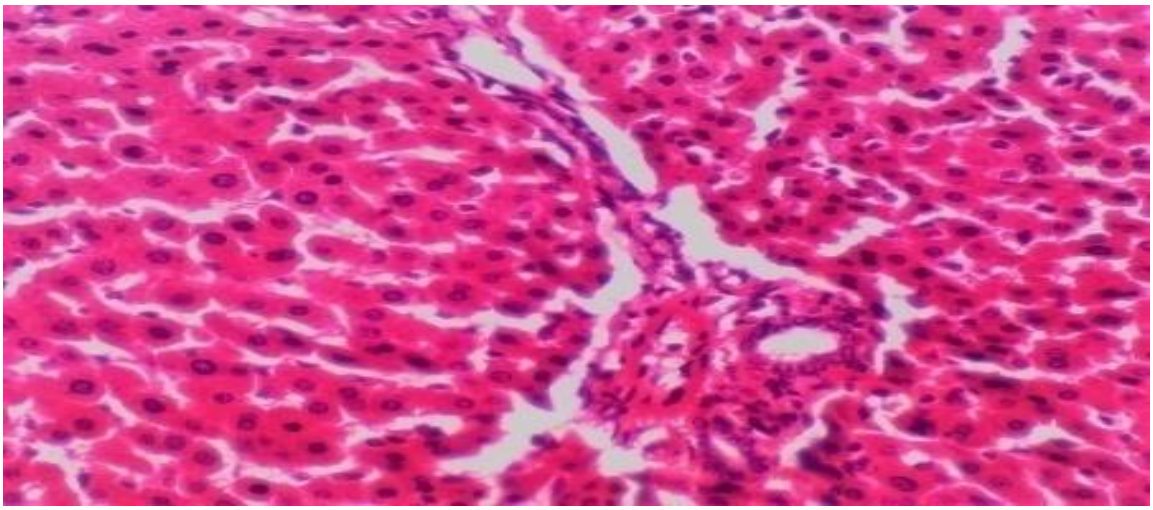

Plate IX: Histopathology of the Liver of Rat Treated with Crude Methanol Extractat 10 mg/kg (MC10L)

The result in Plate XIII shows the histopathology of the liver of rat treated with crude methanol at 10 mg/kg (MC10 L). The section shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes. There are foci showing lymphocytic infiltration of the portal tracts and the few are seen in the sinusoids. There are no features of acute or chronic damage.

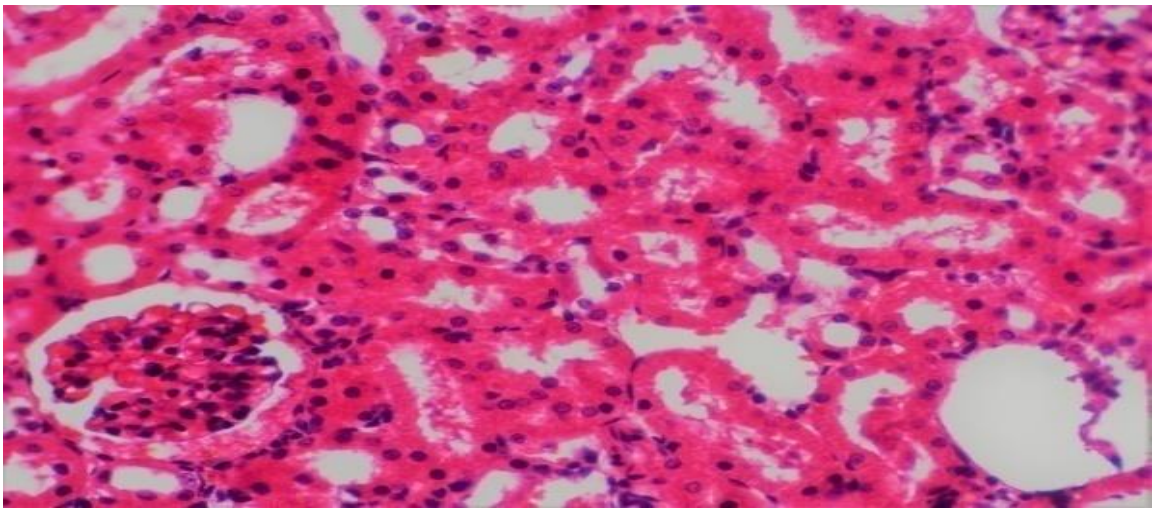

Plate X: Histopathology of the Kidney of Rat Treated with Crude Methanol Extractat 10 mg/kg (MH10K)

The result in Plate X shows the histopathology of the kidney of rat treated with crude methanol at 10 mg/kg (MC10 K). The section shows renal tissue with preserved

architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no features of acute or chronic damage.

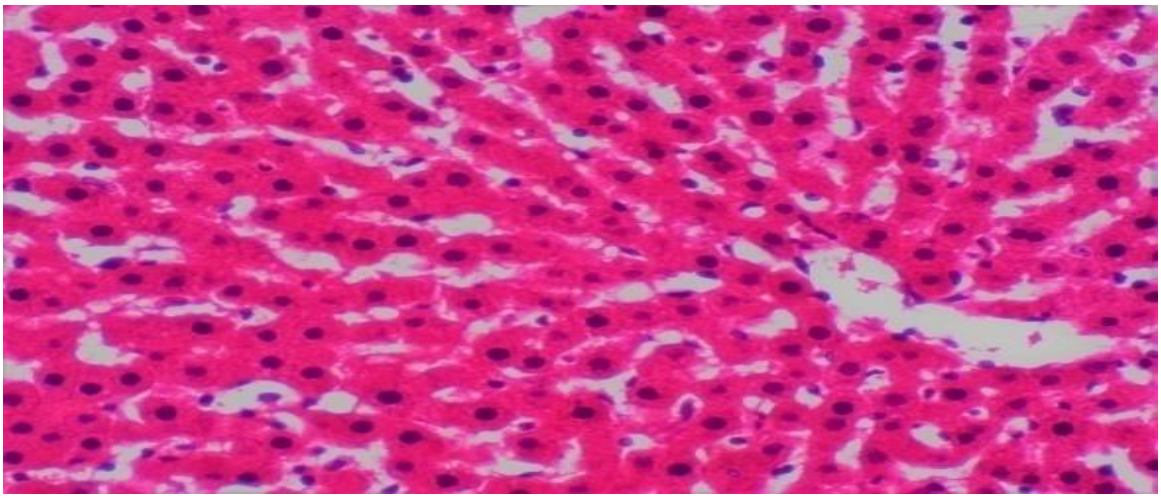

Plate XI: Histopathology of the Liver of Rat Treated with Crude Methanol Extract at 300 mg/kg (MH300L)

The result in Plate XI shows the histopathology of the liver of rat treated with crude methanol at 300 mg/kg (MC300 L). The section shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes. There are foci showing lymphocytic infiltration of the portal tracts and the few are seen in the sinusoids. There are no features of acute or chronic damage.

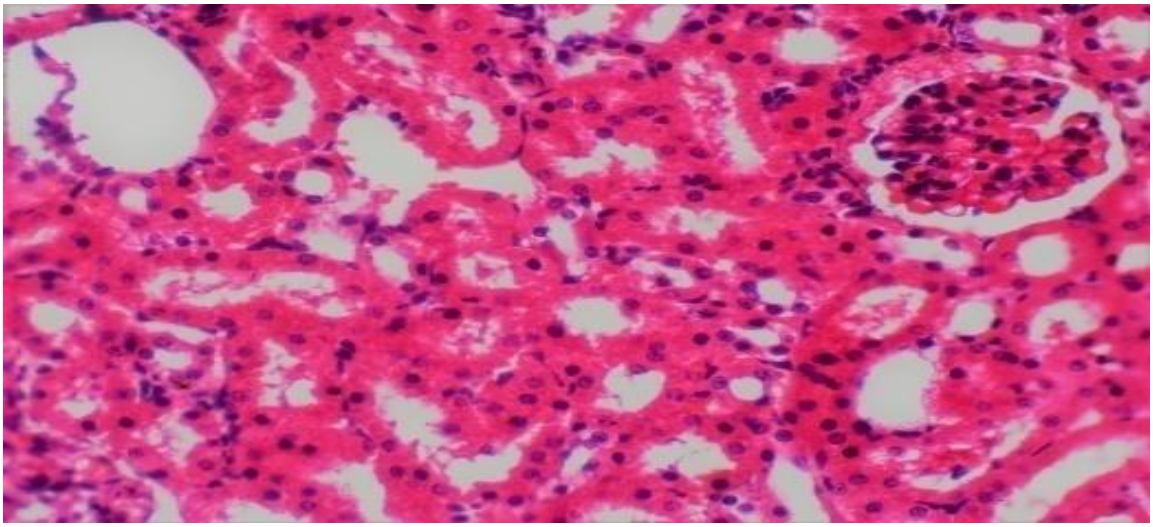

Plate XII: Histopathology of the Kidney of Rat Treated with Crude Methanol Extract at 300 mg/kg (MH300K)

The result in Plate XII shows the histopathology of the kidney of rat treated with crude methanol at 300 mg/kg (MC300 K). The section shows renal tissue with preserved

architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no features of acute or chronic damage.

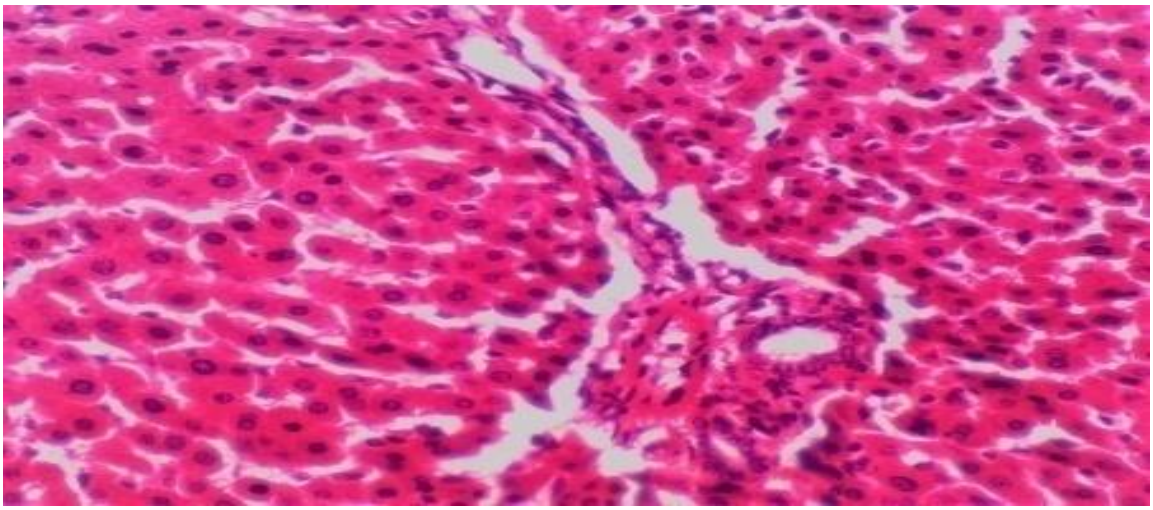

Plate XIII: Histopathology of the Liver of Rat Treated with Crude Methanol Extractat 600 mg/kg (MH600L)

The result in Plate XIII shows the histopathology of the Liver of rat treated with crude methanol at 600 mg/kg (MC600 L). The section shows hepatic tissue with fairly preserved architecture and composed of centrilobular distribution of hepatocyte with cytoplasmic clearing and eosinophilic granular structures in the cytoplasm. The sinusoids appear dilated in areas.

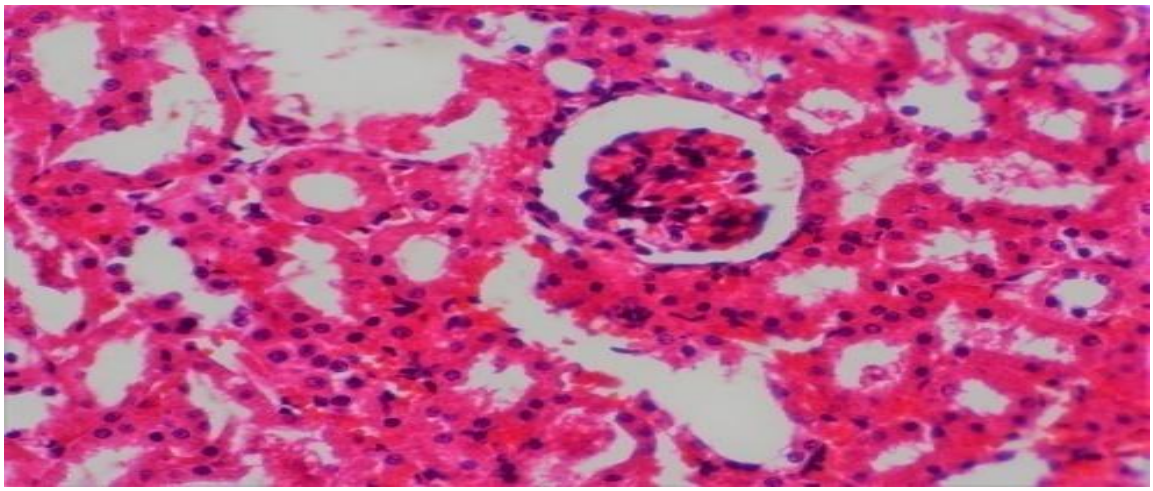

Plate XIV: Histopathology of the Kidney of Rat Treated with Crude Methanol Extractat 600 mg/kg (MH600K)

The result in Plate XVIII shows the histopathology of the Kidney of rat treated with crude methanol at 600 mg/kg (MC600 K). The section shows renal tissue with preserved

architecture composed of normal glomeruli, tubules and unremarkable interstitium. There are no features of acute or chronic damage.

4.2 Discussion

Helianthus annuus seed had been previously reported to contain a good number of phytochemicals. Subashini and Rakshitha (2012) reported that methanolic extract of *Helianthus annuus* seeds contain carbohydrates, flavanoids, saponins, phytosterols, tannins, alkaloids and steroids. The qualitative results in Table 4.1 confirmed some of the claims made by Subashini and Rakshitha (2012). The *Helianthus annuus* seed extracts contain alkaloids, saponins, steroids, tannins as well as others like flavonoids, phenols and terpennoids were present. Although, *Helianthus annuus* seed had been reported in some to contain anthraquinone and cardiac glycosides, they were absent from the finding of this study which is in line with the finding of Verma *et al*. (2017) where these two components were also absent. This variation may be attributed on the type of extracting solvent employed, the method of extraction engaged or the plant part used.

The significantly high phenol yield obtained from the quantitative phytochemical analysis of *Helianthus annuus* seed extracted with ethylacetate (META) and aqueous methanolic (MAQS) fractions showed that ethylacetate and aqueous methanol were better solvent to extract the phenol component of the seed. Crude methanol, crude ethanol and aqueous fraction of ethanol were better in the extraction of flavonoids and tannins because they gave significantly higher yield. The n-hexane fraction of methanol and n-hexane fraction of ethanol were better solvent for the extraction of *Helianthus annuus* seed as they yield significantly higher quantitatively. Crude, ethylacetate and aqueous fraction of methanol were better solvent for the extraction of alkaloids. The biological activities that plant extracts elicit are basically a function of the array of phytochemicals

which act individually or synergistically to show a broad spectrum of pharmacological activities.

The inhibitory activities of *Helianthus annuus* seed extracted from different solvents against *Salmonella spp* revealed that the aqueous fraction of methanol (MAQS), ethyacetate fraction of ethanol (META), aqueous fraction of ethanol (EAQS) and ethylacetate fraction of ethanol (EETA) had no zone of inhibition. This means that the above extracts are not effective against *Salmonella spp*. However, crude methanolic extract (MCR), n-hexane fraction of methanol (MHXN), crude ethanol extract (ECR) n-hexane fraction of ethanol (EHXN) and ciprofloxacin had inhibitory activity against *Salmonella spp.*

Among the four extracts that have inhibitory activities, methanol crude extract (MCR) and n-hexane fraction of methanol (MHXN) have significantly higher inhibitory activities compared to crude ethanol extract (ECR) and n-hexane fraction of ethanol (EHXN). Their inhibitory activities were however, lower compared to ciprofloxacin used as standards or positive control. The larger zone of inhibition around an antibiotic-containing disk, according to Kirby-Bauer Disk Susceptibility (2021) indicates that the bacteria or the test organisms are more sensitive to the antibiotic in the disk. This means the ciprofloxacin control was more effective against *Salmonella spp* compared to the extracts. Inhibitory activities increased with increase in concentration for all the extracts. This means increasing concentration of the various extracts of *Helianthus annuus* seed will increase effect against *Salmonella spp.*

The aqueous fraction of methanol (MAQS), ethylacetate fraction of ethanol (META), aqueous fraction of ethanol (EAQS), crude ethanol extract (ECR), n-hexane fraction of ethanol (EHXN) and ethylacetate fraction of ethanol (EETA) had no zone of inhibition

against *Escherichia coli.* This means these solvent extraction of the *Helianthus annuus* seed were not potent against *Escherichia coli.* Crude methanolic extract (MCR), n-hexane fraction of methanol (MHXN) and ciprofloxacin which serves as positive control had inhibitory activities against *Escherichia coli.* This means extracting *Helianthus annuus* seed with crude methanol and n-hexane fraction of methanol will be effective against *Escherichia coli.*

Ciprofloxacin was more effective against *Escherichia coli* compared to methanol crude extract (MCR) and n-hexane fraction of methanol (MHXN) at 60 mg/ml because it had larger value of inhibitory zone (Kirby-Bauer Disk Susceptibility Test, 2021). At 120 mg/ml, MCR and MHXN were at the same level of significance with the control.The inhibitory zones for MHXN at 240 mg/ml and 480mg/ml was significantly higher than the control. The zone of inhibition also increased with increase in concentrations of *Helianthus annuus* seed extract which means *Helianthus annuus* seed extracts will be more potent against *Escherichia coli* at higher concentrations.

The minimum inhibitory concentration (MIC) value (which is the least concentration that have inhibitory effects) observed in methanol crude extract against *E. coli* and ethanol crude extract against *Salmonella spp* which was comparable to the standard drug (Ciprofloxacin) corresponds to that observed in the study of Subashini and Rakshitha, (2012) who evaluated the antioxidant as well as antimicrobial activity of methanolic extract of seeds from *Helianthus annuus* L. The authors reported that the seed extract showed high sensitivity to *Salmonella typhi.* Similarly, report from the study of Liu *et al*. (2016) showed that sunflower essential oil (SEO) had a higher antibacterial effect against P. aeruginosa, *E. coli* and *S. aureus* than monomer mixtures and each monomers. The work of Eze *et al*. (2015) on in vitro antibacterial, antioxidant characteristics of *Helianthus annuus* leave extract on some infectious causative bacteria revealed an MIC

that ranged from 125-500 ug/ml *against E. coli, Staphylococcus aureus, Salmonella enterica* and *Shigella* spp.

The significant lower minimum bactericidal concentration (which represents the least concentration that can kill a test organism) value of ethanol crude extract against *Salmonella spp.* and methanol crude extract against *E. coli* were more significant compared to other extracts. In consonance to the present study, Adetunji *et al*. (2010), reported similar MBC of ethanol extract against *Staphylococcus aureus, Aspergilus niger* and *Candida albicans*. Also in agreement with the present study, Aboki *et al*. (2012) who evaluated the physicochemical and anti-microbial properties of sunflower (*Helianthus annuus* L.) seed oil revealed a low MBC value (that is, high bactericidal effect) of the extract against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Candida albicans*.

The inhibitory and bactericidal effect of *H. annuus* extracts as observed in the present study may be due to the presence of various phytoconstituent (active ingredients) available in the plant sample which act against the test bacteria. It has been suggested that some of the chemical constituents of plants extracts (terpenoid, alkaloid and phenolic compounds) interact with some enzymes as well as proteins present on the cell membrane of the microbial cell. This then triggers disruption and disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for aminoacids biosynthesis (Burt, 2004; Gill and Holley, 2006). Some other researchers have attributed the inhibitory effect of these plant extracts to the hydrophobicity characters of these plants extracts which enable them to react with protein of microbial cell membrane and mitochondria distrupting their structures and changing their permeability (Tiwari *et al*., 2013). Tannins and flavonoids have also been reported to possess as well as induce antibacterial activity against microbes as a result of their ability to inactivate microbial

adhesions, enzymes, cell envelope transport proteins (Cowan, 1999; Ashraf *et al*., 2018). Several alkaloids have been reported to interfere with DNA, telomeres, telomerase, topoisomerase, the cytoskeleton or protein biosynthesis as a result inducing apoptosis (Wink, 2015; Eid *et al*., 2012; El-Readi *et al*., 2013; Noureini and Wink, 2014; Wink and Schimmer, 2018).

It was observed that ethanol extract of *Helianthus annuus* seed showed higher inhibitory and bactericidal effects on *Salmonella spp* while methanol crude extracts showed higher inhibitory and cidal effect on *E. coli* than other extracts. This may be attributed to the differences in nature as well as concentration of the active ingredients present in them (Evans, 2009: Eze *et al*., 2015). Also, the method of extraction, part of the plant material being extracted as well as the type of extracting solvent may play vital roles in the varying degrees of antimicrobial activities elicited by the extracts.

The acute toxicity studies revealed the extracts of *Helianthus annuus* seed were non-toxic to the test organisms (rats) as most of the extracts did not record any mortality. Hence both the aqueous and organic extract are in category 5 of Global Harmonization System (>2000-5000 mg/kg b.w.t) (Onzago *et al*., 2014). This is in agreement with the study of Onoja *et al*. (2018) who reported an LD50 of hydromethanol extract of *Helianthus annuus* to be greater than 2000 mg/kg bw. Extracts of *H. annuus* have been reported to be used as ingredient in snack bar, bread roll, chocolates, edible oil, creams among others (Adeleke and Babalola, 2020). However, only mild reactions were recorded among few individuals that consumed products containing *Helianthus annuus* (cometic ingredient review, 2015). Sunflower seed/oil have been reported to have loads of health benefits which are attributed to its nutritional composition which include high concentration of monounsaturated and polyunsaturated fats, proteins, tocopherols, phytosterols, copper, zinc, folate, iron and vitamin B possessing antimicrobial, antidiabetic, anti-inflammatory,

antihypertensive, and antioxidants (Nandha *et al*., 2014; Adeleke and Babalola, 2020). The toxicity of certain plant materials or extracts may be due to the presence of an active constituents lethal to animals or substances which interact with others in the system to elicit harmful effects.

The sub-acute toxicity test which lasted for twenty eight days revealed 50 % mortality for group administered 300 mg/kg body weight of n-hexane fraction of the crude ethanol. There was also one-out-four mortality record for 300 mg/kg body weight of crude methanol. Although, the acute toxicity test showed that the plant extracts were safe, a large possibility exists that the presence of the solvent used for extraction might have elicited complications that resulted to death over the period of administration. The death observed in the group (negative control) administered 10 mg/kg body weight of distilled water may be termed as a chance event. Generally, It is noteworthy, that the non-toxicity and antibacterial potential of this plant extract may be taken advantage of by incorporating them into food, skin care, oral health products.

The in vivo antibacterial activity of *Helianthus annuus* seed extracts against *Salmonella spp* and *Escherichia coli* is shown in the total bacterial load present in the treated groups. The significant similarity in bacterial load between the extracts of *H. annuus* (400 mg/kg of methanol crude extract and n-hexane methanol fraction) and the standard drug (Ciprofloxacin) as well as the significant lower bacterial load shows that the extracts have high antibacterial features and may be used as potential treatment against *E. coli* and *Salmonella* infections.The zero bacterial load at higher concentrations indicates that the plant extract has bactericidal effect as the standard drug.

The standard drug (Ciprofloxacin) is a flouroquinolone which has been evaluated to be particularly effective against gram-negative bacteria at lower concentrations than the

other agents with similar antibacterial spectra (Masadeh *et al*., 2015). It is used in the treatment of several infections such as cystitis, urinary tract infections, sinustitis and certain bacterial infections among others (Castro *et al*., 2013). All the extracts of *H. annuus* in the present study contained alkaloid which has quinolone. The major mechanism of action of quinolones is inhibition of nucleic acid synthesis. Here, DNA gyrase which is responsible for supercoiling and uncoiling of DNA strands, involved in repair, replication as well as transcription is inhibited (Wink, 2015). It has been reported that quinolones induce changes in the permeability of membranes and hence disrupt the cell integrity, allows the efflux of the constituents of cytoplasm and the death of cells (Nam *et al*., 2015).

The histopathology results revealed that there was neither liver nor kidney damage to the various groups administered different concentration of various extracts. This was indicated by the fact that both the structure of the liver and kidney as well as their minor organelles were well preserved. This agreed with the earlier result that *Helianthus annuus* seed was non-toxic even at doses as high as 2000 mg/kg body weight.

The lipid component of the biochemical parameters revealed that some of the extracts had significantly lower total cholesterol, triglycerides and low-density lipoproteins cholesterol (LDL-C). Hyperlipidemia is one among the major factors that predisposes subjects to cardiovascular diseases (Piche *et al*., 2018). High levels of LDL-C and total cholesterol have tendency to promote atherosclerosis, hence, any intervention that lower the concentrations may be a potential route to prevent or manage cardiovascular related diseases. Evidence from the present study suggests *Helianthus annuus* seed extracts can lower total cholesterol, triglycerides and low-density lipoprotein cholesterol slightly. The presence of certain active compounds (like phenols) in the extracts of *H. annuus* may backup the evidence of *H. annuus* having hypolipidemic effects. Polyphenols have been

reported to possess cholesterol lowering effects. They bind to cholesterol and bile acids form complexes as such enhancing their evacuation through feaces. This in turn reduce the formation of micelle while lipid uptake from intestine to the blood is reduced (Sommella *et al*., 2019). Flavonoids have been reported to have many health benefits as an antioxidant (Guo *et al*., 2017), and can serve as inhibitor for low-density lipoprotein (LDL) oxidation, and as a scavenger for DPPH radical activity (Lee *et al*., 2005; Grassi *et al.,* 2010).

Gou *et al*. (2017) reported that *Helianthus annuus* seed extracts have hepatoprotective and lowers the risk of diabetes, arteriosclerosis and hypertension. Among the four lipid components analysed, only high-density lipoprotein cholesterol (HDL-C) was significantly higher in the test group as compared to the control group. Increase in the high-density lipoprotein had been linked to prevention of cardiovascular diseases according to the report of Connelly *et al*. (2016).

The serum protein component of the biochemical parameters was all within the acceptable level even though there was significant difference among the various extracts at different concentrations. This implied that *Helianthus annuus* seed extracts had no overall negative impact on the treated experimental rats. Alagawany *et al*. (2015) stated that *Helianthus annuus* seed extracts had no effect on protein biochemical parameters. The richness of sunflower in sulfur-rich proteins are ideal for different human metabiological needs such as insulin production, muscular and skeletal cell development, and antioxidants (Gou *et al*., 2017).

There was no marked significant difference in mineral constituents of the biochemical parameters as the varied within the normal range. This supported the earlier finding that *Helianthus annuus* seed extracts are rich in nutrients. Gou *et al*. (2017) reported that

Helianthus annuus seed extracts help to maintain serum mineral level thereby helping to maintained health and boost the immune system (Nandha, 2014). This benefit may be linked to the presence of phytochemical like flavonoids. Flavonoids are essential metabolites found in the sunflower family. Non-nutrient phytochemicals with antioxidant potential in diet are dominated by flavonoid and isoflavone consumption. Arai *et al*. (2000), demonstrated that consumption of high flavonoids and isoflavones lowers the incidence of coronary heart disease as HDL-C carries cholesterol away from the blood back to the liver. Isoflavone is a phytoestrogen that has been linked to a variety of health benefits, including antioxidation (Gou *et al*., 2017).

The significantly higher total proteins (EHXN and MHXN treated groups) and the albumin observed in the test groups showed that there was no cells destruction and muscle wasting. The activities of the liver enzymes were within normal range showing there was no oxidative stress or liver damage. This may be due to rich antioxidants in *Helianthus annuus* seeds. Antioxidants have long been known to protect cells from oxidative damage and reduce the risk of chronic diseases (Gou *et al*., 2017). Certain enzymes (catalase, glutathione dehydrogenase amongst others), peptides (reduced glutathione), carotenoids, and phenolic compounds are all examples of natural antioxidants of tocopherols, flavonoids and phenolic acids found in *Helianthus annuus* seed. Natural antioxidants and antiglycatives are more efficient in treating and preventing diabetes (Xi *et al*., 2008) by preventing the formation of reactive oxygen species (ROS), which trigger a variety of biochemical pathways linked to diabetic complications.

The significant change in weight with time observed in all the treatment groups for both *Salmonella spp* and *E. coli* infected rats as well as in comparison with the control groups agrees with the report of Onoja *et al*. (2015). Contrary to the result obtained in this work, Leverrier *et al*. (2019) reported that different concentrations of *H. annuus* extracts

induced decrease in weight of the subjects. They found that, it decreased fat and brown adipose tissue, in high-fat-diet-fed rats by activation of liver Adenosine monophosphate-activated protein kinase (AMPK) pathway in a dose-dependent manner. However, there was no significant difference in the weight of treated rats in this study. Although, the presence of certain phytochemicals have been reported to induce weight gain. Terpenes (Diterpene), a phytochemical present in *H. annuus* can act directly on adenylate cyclase (Tucci, 2010). Adenylate cyclase is an enzyme that activates cyclic adenosine monophosphate (cAMP). In turn cAMP promotes lipolysis which increases the body's basal metabolic rate, and increases utilisation of body fat (Litosch *et al*., 1982; Tucci, 2010).

The levels of enzymes (AST, ALT and ALP) in serum are markers used to ascertain function state as well as injury in some organs. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum (Adeyemi *et al*., 2015), hence an ectoenzyme of the plasma membrane. This enzyme is often employed in assessing the integrity of the plasma membrane, as its presence in either the tissue and/or serum would indicate probable damage to the plasma membrane of the cell. Whenever an increase in ALP level ensue, it may indicate possible damage of the membrane, this is being that ALP is a membrane bound enzyme (Kumar and Gill, 2018). High levels of serum ALP activity is usually noticed in liver damage, cancer and heart infections.

The transaminase (AST and ALT) enzyme are markers for heart and liver integrity as well as functions (Adeniyi *et al*., 2010). These enzymes are majorly released from hepatocytes that have been injured or damaged (Biegus *et al*., 2016). Elevation of these enzymes in the serum have been reported to indicate cellular damage, tissue necrosis, as well as a calculated risk for cardiovascular diseases, with higher risk of cardiovascular disease and elevated myocardial infarction being attributed to elevation of ALT and AST

respectively. Tissue injury and damage are majorly as a result of inflammation caused by certain reactions, interactions between the tissue in question and other substances.

The significant reduction in concentration of AST, ALT and ALP among rats administered extracts of *H. annuus* compared to the negative control, indicates that the plant extracts have anti-hepatotoxic and tissue healing properties. This may be due to the activities of certain active components present in the plant extract. Certain phenolic compounds have been described to possess anti-inflammatory potential. They inhibit NF-κB activation and reduce pathological inflammatory conditions. For example, resveratrol (Kowluru and Kanwar, 2007) and kaempferol (Luo *et al*., 2015) have been reported to inhibits the activation of NF-κB by downregulating IKK phosphorylation, resulting in the suppression of inflammatory cytokines, such as TNF-α, IL-12, and IL-6.

The importance of hematological parameters as indices of physiological as well as pathological status for animals and humans can in no way be played down. Hence, it is employed as an investigative and diagnostic tool for disease. The significant increase observed in some haematological (Hb and MCV) indices of some of the treatment groups compared to the normal control group indicates is not in agreement with the report of Onoja *et al*. (2018) who investigated on the ameliorative effects of *Helianthus annuus* against nephrotoxic, cardiac and haematological disorders in albino rats. The authors reported that there was no significant difference in haemoglobin (Hb) concentration and MCV between the control and treatment groups. The significant increase in PCV level in groups administered 600 mg/kg bw of n-hexane fraction of methanol crude extract and n-hexane fraction of ethanol crude extract compared to other treatment and control groups suggests that at a high concentration, the plant may be used to ameliorate anaemia (anti-anaemic). This feature may be attributed to its enhancement of erythropoietin synthesis by the kidney. Onoja *et al*. (2018) reported that *H. annuus* may possess kidney damage

reversal potential. In agreement to the present study, Adesina *et al*. (2017) reported non-significant difference in MCH and MCHC values of different groups fed different percentages of *H. annuus* inclusion into diet. Similarly, the work of Onoja *et al*. (2015) on the ameliorative effects of *H. annuus* on some diseased state in rats agree with the findings in this study as the haematological parameters (MCH and MCHC) were comparable to the control groups.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the findings of this study, it can be concluded that *Helianthus annuus* seed extracts contained alkaloids, saponins, steroids, tannins, flavonoids, phenols and terpennoids while anthraquinone and cardiac glycosides were absent.

The crude methanolic extract (MCR), n-hexane fraction of methanol (MHXN), crude ethanol extract (ECR) n-hexane fraction of ethanol (EHXN) had inhibitory activity (zone of inhibition) against *Salmonella spp* while crude methanolic extract (MCR), n-hexane fraction of methanol (MHXN) had inhibitory activities (zone of inhibition) against *Escherichia coli.*

The inhibitory activity in each case was lower compared to that of ciprofloxacin used as control. Also, the extract of *Helianthus annuus* seed have bactericidal effect against *Salmonella spp* and *Escherichia coli.* Ethanol crude extract of *H. annuus* had the lowest MIC and MBC (that is, most effective against the bacteria) which was closest to the standard drug.

The *in vivo* antibacterial study which was depicted in total bacteria load in infected rats showed that there was no bacteria load (CFU) in rats infected *Salmonella spp* treated with 100, 200 and 400 mg/kg bw which were comparable to the standard drug (ciprofloxacin) group. Meanwhile, only 100 mg/kg bw of Methanol crude extract group had (2) colonies of *Salmonella spp*. The total bacteria load decreased with increasing plant extract concentration in rats infected with *E.coli* and treated with methanol crude and methanol hexane fraction. There were no colonies found at higer concentrations, the

extracts of *H. annuus* may therefore be presumed to have more antibacterial potential against *Salmonella spp* than *Escherichia coli* in vivo.

Most biochemical parameters were within normal range even among the group of rats. Most of the plant extracts significantly reduced total cholesterol, LDL-C, triglycerides, ALT, AST, ALP but increased HDL-C. This may therefore presents the extracts of *H. annuus* to possess anti lipidemic as well as hepato protective properties. As a result, the extracts of *H. annuus* may be useful in cardiac related diseases. The usage of this plant extract will be without fear because it is safe, as the LD_{50} was recorded to be greater than 2000 mg/kg bodyweight. Moreso, it was observed that the plant extract may possess growth/weight inducing properties as there was progressive weight gain among all the treatment groups through time. The haematological study revealed that MPV, Hb and PCV Levels were increased in most *H. annuus* extracts treated groups. No damage was observed to either the kidney or liver.

5.2 Recommendations

This study investigated the *in vivo* antibacterial effect of different extracts of *Helianthus annuus* seed and found inhibitory as well as bactericidal effect against *Salmonella spp* and *Escherichia coli* in some of the fractions. Hence, the following recommendations can be made based on observations from the study:

i. Fractions of *Helianthus annuus* seed extracts that showed bactericidal activity should be further isolated and characterised to identify the exact active compound or compounds.
ii. Fractions of the *Helianthus annuus* seed extract that recorded mortality for either *Salmonella spp* or *Escherichia coli* should be systematically studied to establish whether those mortality or mortalities were chance event or actual potency of those fractions against the test organisms.

iii. The consumption of *Helianthus annuus* seeds is therefore encouraged due to the antibacterial activity inferred in this study.

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APPENDIX A (MATERIALS)

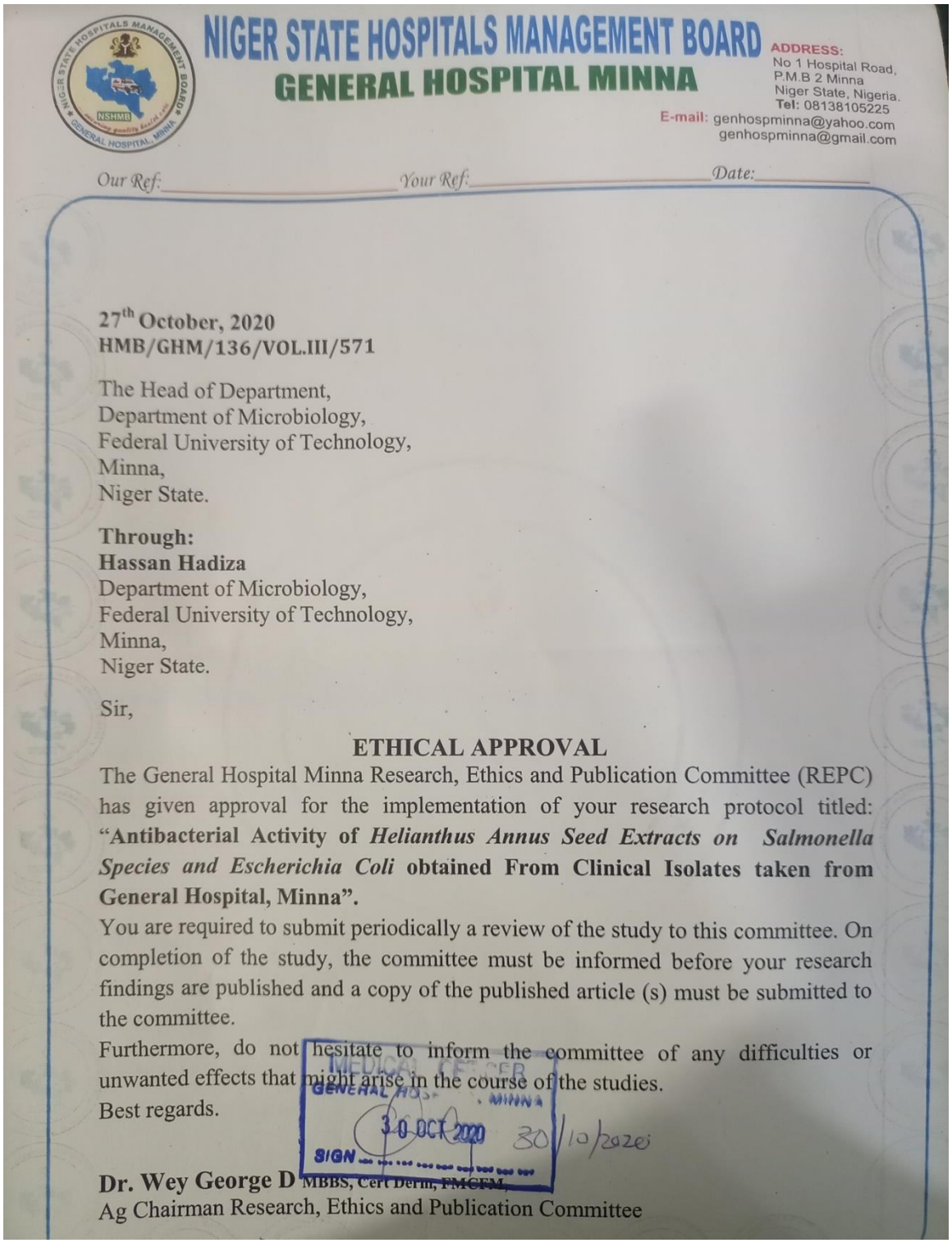
1. Apparatus

Petri dishes, syringes, gloves, beakers, and conical flasks, test tubes, cotton wool, glass rod, masking tape, permanent marker, whatsmann filter paper and foil paper.

2. Chemicals and Reagents

The methanol, ethanol, ethylacetate and hexane used were of analytical grade and were products of BDH Chemicals Ltd, Poole, England. Dimethylsulfoxide a product of Guangdong Guanghua Science Technology Co. Ltd, China. Crystal violet, iodine, alcohol, safranin and methyl-red reagent.

APPENDIX B (ETHICAL APPROVAL)



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Our Ref: _____ Your Ref: _____ Date: _____

27th October, 2020
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The Head of Department,
Department of Microbiology,
Federal University of Technology,
Minna,
Niger State.

Through:
Hassan Hadiza
Department of Microbiology,
Federal University of Technology,
Minna,
Niger State.

Sir,

ETHICAL APPROVAL

The General Hospital Minna Research, Ethics and Publication Committee (REPC) has given approval for the implementation of your research protocol titled: "**Antibacterial Activity of *Helianthus Annus Seed Extracts on Salmonella Species and Escherichia Coli* obtained From Clinical Isolates taken from General Hospital, Minna".**

You are required to submit periodically a review of the study to this committee. On completion of the study, the committee must be informed before your research findings are published and a copy of the published article (s) must be submitted to the committee.

Furthermore, do not hesitate to inform the committee of any difficulties or unwanted effects that might arise in the course of the studies.

Best regards.

MEDICAL RECORD
GENERAL HOSP. MINNA
30 OCT 2020
SIGN

30/10/2020

Dr. Wey George D MBBS, Cert Derm, FMCFM,
Ag Chairman Research, Ethics and Publication Committee