Introduction

1.1 General approach

Natural products are compounds produced by living organisms and include both small and macromolecules. All of these compounds are derived, biosynthetically, from precursors called primary metabolites, which include proteins, carbohydrates, and fatty acids.

These metabolites are considered to be the building blocks of life and are necessary for regulating cellular functions.

Some of the small molecules do not have a clearly, defined function.

They are considered secondary metabolites and make up the majority of work on natural products. Secondary metabolites are generally considered to be specific species [1].

One of these natural products are lipids (fixed oils, fats, and waxes) which are esters of long-chain fatty acids and alcohols, or of closely related derivatives.

1.2 lipids

The chief difference between (fixed oils, fats, and waxes) is the type of alcohol, in fixed oils and fats, the alcohol is glycerol, combines with the fatty acids but in waxes, the alcohol has a higher molecular weight, e.g., cetylalcohol [$CH_3(CH_2)_{15}OH$].

Fat and oils are classified according to the presence and number of double bonds in their carbon chain (as shown in fig 1.2) into:

ii) Saturated fatty acids (SFA) contain no double bonds.

ii) Monounsaturated fatty acids (MUFA) contain one, and polyunsaturatedfatty acids (PUFA) contain more than one double bond[1,2].

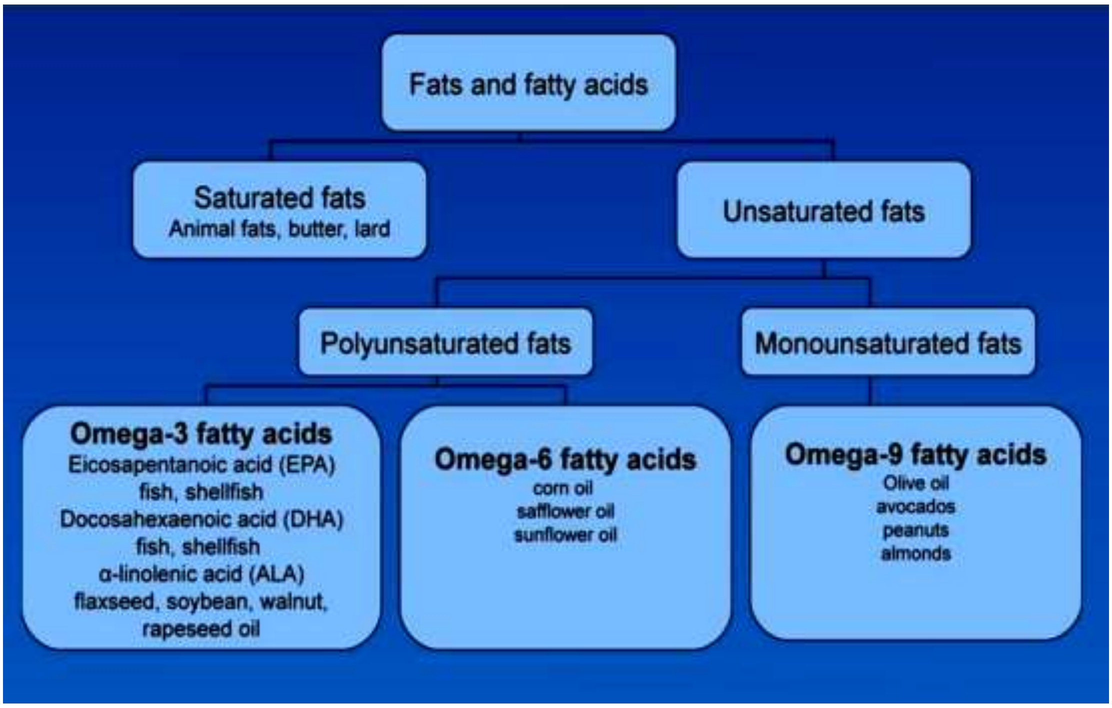


Fig (1.2): Fats and Fatty Acids

PUFA can be further categorized into three main families (fig1.3) according to the position of the first double bond starting from the methyl-end (the opposite side of the glycerol molecule) of the fatty acid chain into:

i) Omega-3 (or n-3) fatty acids which have the first double bond at the third carbon atom and include mainly alpha linolenic acid (ALA) and its derivatives eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

ii) Omega-6 (or n-6) fatty acids have the first double bond at the sixth carbon atom and include mainly linoleic acid (LA) and its derivative arachidonic acid (AA). Omega-9 (or n-9) fatty acids have the first double bond at the ninth carbon atom and include mainly oleic acid.

Fatty acids; on the basis of the ability or not to synthesize from endogenous precursors by animals, and whose deficiency can be reversed by dietary addition, they can be classified as: essential and not essential fatty acid[2].

Plants are capable of synthesizing two kinds of oils: fixed oils and essential oils (also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils). Essential oils (EOs) are consist of complex mixtures of volatile and semi-volatile organic compounds originating from a single botanical source that determines the specific aroma of plants and the flavor and fragrance of the plants, the main differences between fixed oil and essential oil are showed in table 1.1

Table.1.1. Comparing fixed oil with volatile oi

Volatile oil	**Fixed oil**
Also called an essential oil	Also called natural non-volatile oil
Volatile oil can evaporate when placed under room temperature	Fixed oil do not evaporate at room temperature
There is no spot (no permanent stain) left after evaporation	Some type of spot (permanent stain) left after evaporation
They are unable to undergo saponification	Fixed oil can be easily saponified
Mixture of cleoptenes and stearoptenes are termed as volatile oils	Esters of higher fatty acids and glycerin are called as fixed oil
Posses high refractive index	Posses low refractive index
These are optically active	These are optically inactive
Their primary source is leaves, roots, in petals, and bark.	Their major source is seeds of the plant

1.3 Terminology of Fatty acid

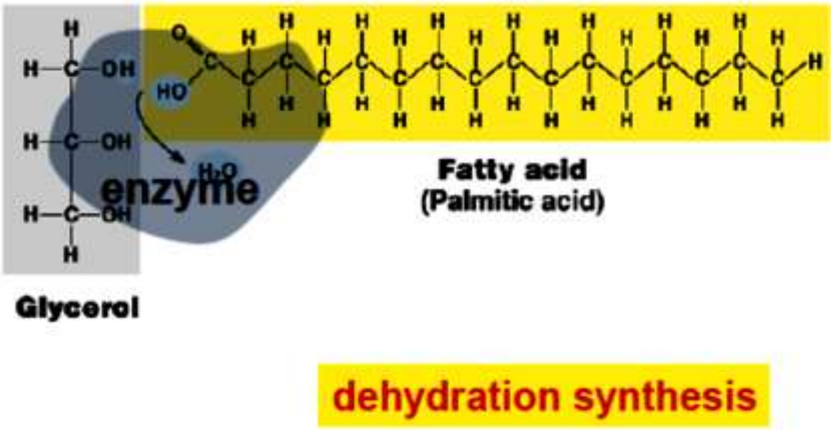
In addition to their formal name, fatty acids are often represented by a shortened numerical name based on the length (number of carbon atoms), the number of double bonds and the omega class to which they belong. Examples of nomenclature are; Linoleic acid (LA), which is also referred to as C18:2 n-6, indicating that it has 18 carbon atoms, 2 double bonds and belongs to the omega-6 fatty acid family. Alpha linolenic acid (ALA), or C18:3 n-3, has 18 carbon atoms, 3 double bonds and belongs to the omega-3 fatty acid family.

Fatty acids have a backbone made of carbon atoms. They vary in the number of carbon atoms, and in the number of double bonds between them. For example, butyric acid (C4:0), palmitic acid (C16:0) and arachidic acid (C20:0), contain 4, 16 or 20 carbon atoms in their chain, respectively. Short-chain fatty acids (SCFA) are fatty acids with up to 5 carbon atoms, medium-chain fatty acids (MCFA) have 6 to 12, long-chain fatty acids (LCFA) 13 to 21, and very long chain fatty acids (VLCFA) are fatty acids with more than 22 carbon atoms. The majority of naturally occurring fatty acids, both in the diet and in the body, contain 16-18 carbon atoms.

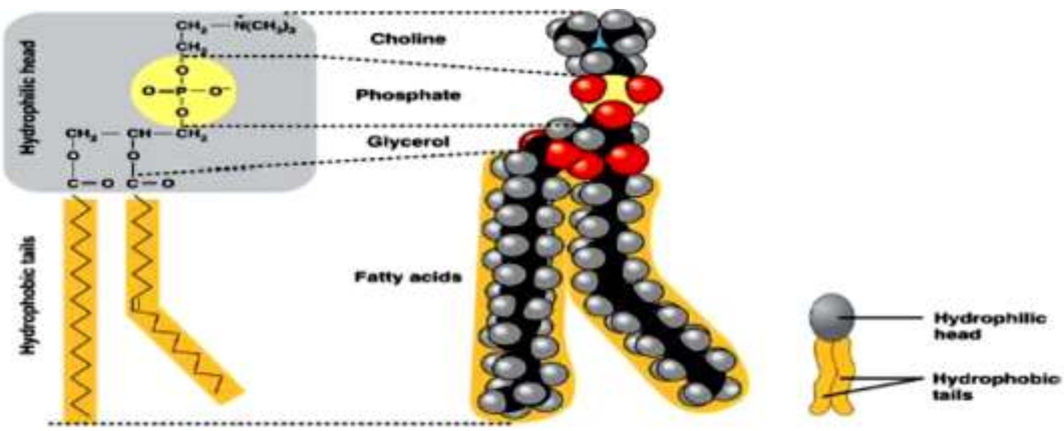
1.4 Structure of fats and oils

Fats and oils are made from two kinds of molecules:

i) glycerol (a type of alcohol with a hydroxyl group on each of its three carbons) and three fatty acids joined by dehydration synthesis.



Since there are three fatty acids attached, these are known as triglycerides, a long hydrocarbon chain is called The "tail" of a fatty acid, and is making it hydrophobic, on other side a carboxyl group is called The "head" of the molecule which is hydrophilic.

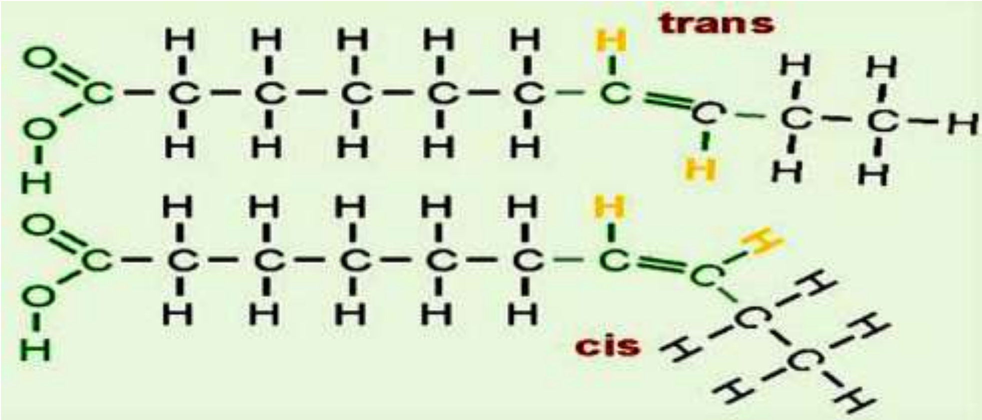


Fats, which are mostly from animal sources, have all single bonds between the carbons in their fatty acid tails. All the fatty acids in these triglycerides contain the maximum possible amount of hydrogens, these would be called saturated fats. The hydrocarbon chains in these fatty acids are, thus, fairly straight and can pack closely together, making these fats solid at room temperature, (solidity of these fats contributes to cardiovascular disease atherosclerosis)[2].

Oils, mostly from plant sources, have some double bonds between some of the carbons in the hydrocarbon tail, causing bends or "kinks" in the shape of the molecules. Because some of the carbons share double bonds, they're not bonded to as many hydrogens as they could if they weren't double bonded to each other. Therefore these oils are called unsaturated fats. Because of the kinks in the hydrocarbon tails, unsaturated fats (or oils) can't pack as closely together, making them liquid at room temperature[2].

(b) Unsaturated fat and fatty acid

In unsaturated fatty acids, there are two ways the pieces of the hydrocarbon tail can be arranged around a C=C double bond (cis and trans). In a cis or trans mode[2].



Naturally-occurring unsaturated vegetable oils have almost all cis bonds, but using oil for frying causes some of the cis bonds to convert to trans bonds, however, if oil is constantly reused, like in fast food French fry machines, more and more of the cis bonds are changed to trans until significant numbers of fatty acids with trans bonds build up. The reason for this concern, is that fatty acids with trans bonds are carcinogenic, or cancer-causing, although most vegetable oils are liquid at ordinary temperatures and most animal fats are solid, there are notable exceptions, such as cocoa butter, which is a solid vegetable oil, and cod liver oil, which is a liquid animal fat[2].

1.5 Sources of fats and oils

Fixed oils and fats (lipids) are present virtually in all the food we eat. It is the only material present in vegetable oils and animal fats, it is an important component of milk and milk–based products such as cream and cheese, eggs and meat, and is even present in leaves and green vegetables. Fixed oil and fat are present in many plants such as castor seed, olive, peanut, soybean, sesame, almond, cotton seed, corn, safflower, cocoa butter, linseed, sunflower, oil palm and shea butter[3].

Dietary fat is transported as free acid to adipose tissue where it is converted to triacylglycerols. Endogenous fat, made mainly in the liver but also in other organs, is exported as very low density lipoprotein (VLDL) into plasma. Cholesterol is carried to peripheral tissue in low density lipoprotein (LDL) and returned to the liver in high density lipoprotein (HDL) which acts as a scavenger for cholesterol[4].

1.6 Role of Fixed Oil and Fats in Health

1.6.1 Source of metabolic energy

Though the presence of fat can contribute substantially to the palatability of the diet, the main nutritional contribution of fixed oil and fat is to supply metabolic energy. The useful energy available to the body (metabolizable energy, ME) depends mainly on the digestibility of the fat and the chain length of the constituent fatty acids. Most common food fats are efficiently digested by most people with insignificant differences in their digestible energies[5].

1.6.2 In growth and development

Some lipids that are needed for tissue growth and development can be synthesized by the cells themselves. However, the fatty acids linoleic (18:2n–6) and α–linolenic (18:3n–3) cannot be made by animal cells[5] and these ‘essential fatty acids’ must be supplied by the diet [6]. Once incorporated into cells they may be further elongated and desaturated to longer chain polyunsaturated products. From conception, the cells of the growing foetus need to incorporate fixed fat and oil into their rapidly proliferating membranes. The foetus is totally dependent on the placental transfer of substrates from the mother’s circulation, which is then elaborated into lipids[5].

1.6.3 Provision of essential fatty acids (EFA)

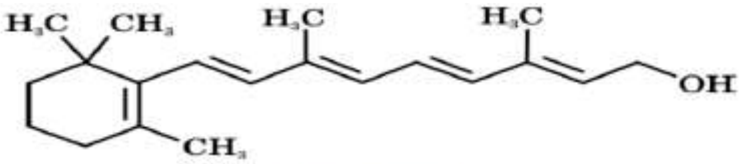
Biochemically, EFA deficiency is characterized by changes in the fatty acid composition of many tissues, especially their biological membranes, whose function is impaired and, in the mitochondria, the efficiency of oxidative phosphorylation is much reduced . Each family of unsaturated fatty acids (n–3, n–6, n–9) is biochemically distinct and its members cannot be interconverted in animal tissues. The ‘parent’ or ‘precursor’ fatty acid of the n–9 family, oleic acid, can originate from the diet or from biosynthesis in the body, whereas linoleic and α–linolenic acids, the precursors of the n–6 and n–3 families, respectively, must be obtained from the diet [7]. They are thus essential nutrients.

1.6.4 Supply of Fat–Soluble Vitamins

The fat–soluble vitamins, found in mainly fatty foods, are classified as vitamins A, D, E and K. In contrast to EFA, which are required in gram quantities, fat–soluble vitamins are required in microgram quantities per day[5]. Although some fat is necessary in the diet to improve the absorption and utilization of fat–soluble vitamins, there is little evidence that, within the normal range of fat intakes, the amount of dietary fat significantly affects the utilization of fat–soluble vitamins. However, when fat absorption is impaired, insufficient fat–soluble vitamins may be absorbed, leading to a deficiency state. This could occur when the secretion of bile salts is restricted (as in biliary obstruction), when sections of the gut have been removed or damaged by surgery or in diseases, such as tropical sprue and cystic fibrosis, all of which are associated with poor intestinal absorption [6-8]. When people have access to varied diets, there is less likelihood of dietary deficiencies of fat soluble vitamins. However, some fat–soluble vitamins are present in a limited number of foods; inevitably, when food supplies are limited by economic conditions, deficiency diseases can then become a significant problem.

1.6.4.1 Vitamin A

Vitamin A (retinol) is found only in animal fats. Plant materials, such as dark green leaves and vegetable oils, like palm oil, contain a precursor, â–carotene (pro vitamin A), which can serve as a source of retinol in the body [9]. After absorption, or conversion from carotene, retinol is esterified mainly with palmitic acid (irrespective of the composition of the dietary esters) in the enterocytes in a reaction catalyzed by one of two microsomal enzymes [10].



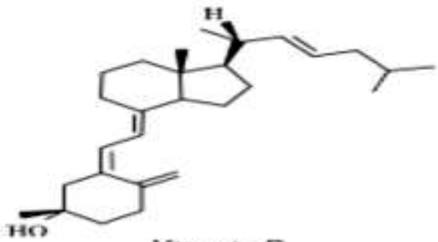
Vitamin A

Transport in the blood requires hydrolysis of the ester followed by binding of retinol to a specific transport protein, retinol binding protein. Retinol is delivered to target tissues (*e.g.* visual cells) by interaction of the retinol binding protein to cell–surface receptors. Vitamin A, with its analogues and metabolites, has several distinct functions: in the visual process, in cellular differentiation, embryogenesis and in the immune response.

1.6.4.2 Vitamin D

Vitamin D is the generic name for two sterols with the property of preventing the disease rickets. Ergocalciferol (vitamin D2) is formed by irradiation of the plant sterol, ergosterol is the main dietary source of vitamin D. Cholecalciferol (vitamin D3) is produced in the skin by ultraviolet irradiation of 7–dehydrocholesterol present in the skin surface lipids. It is the main source of the vitamin for most human beings [11]. Dietary sources are fish liver oils (*e.g.* cod), eggs, liver and some fat spreads. The parent forms of vitamin D are biologically inactive. The active metabolites are formed in the liver and kidneys[11] .

After absorption from the diet or formation in the skin, vitamin D is carried in the circulation bound to a specific transport protein of the α–globulin class.

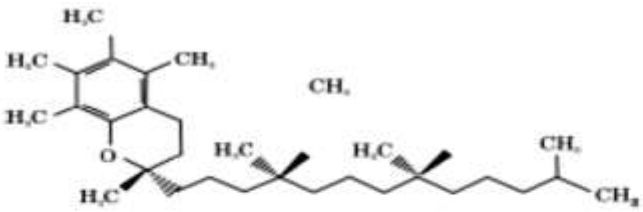


Vitamin D

1.6.4.3 Vitamin E

Vitamin E activity is possessed by eight tocopherols and tocotrienols.

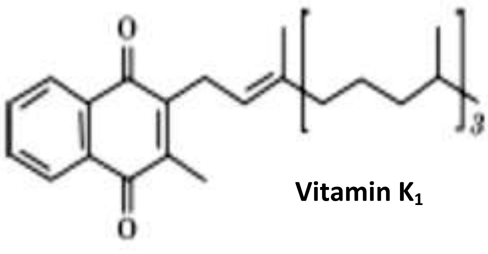
α –Tocopherol is the most potent of these, the other compounds having between 10% and 50% of its activity. α–Tocopherol is also the most abundant form of vitamin E in animal tissues, representing 90% of the mixture. The form used in commercial preparations is synthetic racemic α–tocopherol , often in the acetylated form as a protection from oxidation [12]. In 1922, the Americans, Evans and Bishop, discovered that vitamin E prevented sterility in rats reared on fat–deficient diets fortified with vitamins A and D . Because vitamin E is so widespread in foods and like all other fat–soluble vitamins is stored in the body, deficiency states are rarely if ever seen, possible exceptions Vitamin E (α-tocophenol) being in premature infants with low fat stores and in people with severe malabsorption [13]. The richest sources are vegetable oils, cereal products and eggs. Vitamin E is carried in the blood by the plasma lipoproteins.



Vitamin E (α-tocophenol)

1.6.4.4 Vitamin K

Vitamin K is the generic name given to a group of compounds, having in common a naphthoquinone ring system (menadione) with different side chains. Plants synthesize phylloquinone (vitamin K1) with a phytyl side chain identical to that in chlorophyll. Bacteria synthesize menaquinones (vitamin K2) the side chains of which comprise 4–13 isoprenyl units. Major dietary sources of vitamin K1 are fresh green leafy vegetables, green beans and some seed oils. Cereals are poor sources and, of animal foods, only beef liver is a significant source. Vitamin K2 is found in fermented foods, such as cheeses and yoghurt and in ruminant liver. Bacteria in the human gut synthesize menaquinones, but there is uncertainty about their bioavailability and hence their significance as a source of vitamin K [14].



Vitamin K_1

Free phylloquinone is absorbed from the small intestine with about 80% efficiency, but only about a tenth of the vitamin K1 present in dark green leafy vegetables is absorbed. Vitamin K is carried in chylomicrons and delivered mainly to the liver, in chylomicron remnants [15]. About two–thirds of the vitamin K intake is soon excreted from the body (into the faeces via the bile) and as there is no evidence of an enterohepatic circulation, it is presumed that a constant intake is essential.

1.6.5 Improvement in food palatability

Fats contribute to palatability mainly in two ways. Firstly by responses to their texture in the mouth (sometimes called mouth feel) and secondly by olfactory responses, namely taste in the mouth and aroma or odour in the nose; together, these are called flavour [16]. Fat is, on the one hand, a source of taste and aroma compounds and, on the other, a medium that regulates the distribution of these compounds between water, fat and vapour phases, which influence their perception by the sense organs. Some flavour compounds result from the decomposition of lipids by lipolysis, oxidation and microbial or thermal degradation. These processes may produce free fatty acids, aldehydes, ketones, lactones and other volatile compounds. PUFA play a dominant role because of their susceptibility to oxidation [17].

1.6.6 Role in immune function

The immune system has evolved to protect the body from constant attack by infectious organisms and other nonself-molecules that could have detrimental effects on the host. It involves complex interactions between different types of cells, which produce substances that are toxic to invading pathogens[18]. Lymphocytes are key players in the immune system. B–lymphocytes produce antibodies against specific antigens located on the surface of invading organisms (humeral immunity).

T–lymphocytes do not produce antibodies. They recognize peptide antigens attached to major histocompatibility complex proteins on the surfaces of so–called antigen– presenting cells. In response to antigenic stimulation, they secrete cytokines (*e.g.* interleukins, interferon, transforming growth factor, etc.), whose function is to promote the proliferation and differentiation of T– lymphocytes as well as other cells of the immune system [19]**.** These include B–lymphocytes, monocytes, neutrophils, natural killer cells and macrophages. Whereas life would hardly be possible without this well–integrated system, failure adequately to regulate these processes can lead to damage to the body's own tissues as seen in severe inflammation and in autoimmune diseases[19].

1.7 Role of fixed oil and fats in diseases

Fat is an essential part of the diet and is linked to good health as well as to disease. It is important to optimize the quality and the quantity of fat consumed in relation to other aspects of lifestyle.

1.7.1 Obesity

A growing number of persons are either overweight or obese, probably as a consequence of imbalance over many years between increased caloric intake and decreased energy requirement resulting from more sedentary and less active lifestyles. In tackling this problem, attention is focused on fixed fat and oil because it is the most energy–dense of our nutrients[20]**.** The problem of obesity is partly genetic (40–70%) and partly environmental (food intake and physical inactivity) [7]. In 1991, deaths associated with obesity in the United States (300 000) were second only to deaths associated with smoking (400000) and it is likely that in the intervening years these numbers have become closer. Obesity is a potent risk factor for type–2 diabetes, hypertension and dyslipidemia [21]. In Europe, obesity Figures are also on the increase and it is reported that 20 per cent of men and 25 per cent of women in the UK are obese, that these levels have tripled in the last twenty years and that 9000 deaths a year are associated with this condition at a cost of £2.5 billion. Obesity is a major public health problem throughout Europe, especially among women in Southern and Eastern European countries. These countries are also among the highest for cardiovascular disease (CVD)[22]. In Europe, the treatment of obesity–related diseases accounts for eight per cent of all medical costs.

1.7.2 Coronary Heart Disease

Cardiovascular diseases (CVD) is a broad term embracing diseases of the blood vessels of the heart, brain (cerebrovascular disease, stroke) and the limbs (peripheral vascular disease). CVD is usually a culmination of atherosclerosis (accumulation of material–plaque–in the walls of arteries of cells comprising connective tissue, lipids, calcium and debris resulting from cellular breakdown) and thrombosis [21]. Coronary heart disease (CHD) is a major cause of death in the developed world with a peak age of death of 70–74 for men and 75–79 for women. There are three stages in the development of CHD namely; injury of coronary arteries, fibrous plaque formation and thrombosis leading to heart attack or stroke [6]. The following have been recognised as risk factors; high blood pressure, high levels of plasma LDL (low density lipoprotein) cholesterol, low levels of plasma HDL (high density lipoprotein) cholesterol, high levels of plasma fibrinogen and low levels of plasma antioxidants [23]. The lipid hypothesis in respect of CHD is concerned with the relationship of blood cholesterol and saturated fatty acids (SFA) with CHD mortality. Diets with a high content of fat/SFA/cholesterol lead to high concentrations of total cholesterol in the blood and especially of LDL–cholesterol which results in a high morbidity and mortality from CHD[8]. However, reducing the amount of fat/SAF/cholesterol in the diet reduces the concentration of cholesterol in the blood and especially in the LDL. This results in a lower risk of CHD and eventually a fall in morbidity and mortality [24].

1.7.3 Diabetes

Diabetes mellitus is a chronic disease in which the metabolism of sugars (and of fats and proteins) is disturbed by lack of or by decreased activity of the hormone insulin, produced by the endocrine part of the pancreas.

Its main characteristic is an increase in the level of blood sugar provoking acute symptoms such as thirst, frequent voiding and weight loss [21]. The incidence of this disease is increasing all over the world and it is predicted that it will affect 210 million people by 2010. Diabetes is an independent risk factor for CVD. It is suggested that individuals with normal body weight and normal lipid levels should limit fat intake to less than 30 per cent total energy with saturated fatty acids restricted to 10 per cent, polyunsaturated acids to less than 10 per cent, and monounsaturated acids at 10–15 per cent. Those with elevated LDL levels should reduce saturated acids to seven per cent and cholesterol intake to less than 200 mg/day. It is known that the various desaturases involved in the conversion of C18 polyunsaturated fatty acids to the important acids of longer chain length are decreased in diabetic patients. As a consequence, the phospholipids in tissue lipids contain more saturated and monounsaturated acids and less polyunsaturated fatty acids (PUFA)[22]. This, in turn, affects membrane fluidity and eicosanoid production.

1.7.4 Inflammatory diseases

Inflammation is characterised by swelling, redness, pain and heat in localised areas of the body. These symptoms result from a series of interactions between cells of the target tissue, cells of the immune system, their products such as eicosanoids, cytokines, immune globulins and blood components[20]. The eicosanoids are all regulators of inflammation affecting vascular permeability, vasodilation and vasoconstriction, platelet aggregation and deaggregation, further eicosanoid synthesis and serve as chemotactic agents [25]. Dietary lipids (fixed oils and fats) modulate inflammation and influence the course of diseases such as arthritis, psoriasis, asthma, and inflammatory bowel disease mainly through changing the production of eicosanoids particularly by macrophages and neutrophils. These effects are the consequence of changing n–6/n–3 ratios. Inflammation is a component of a range of acute and chronic human diseases characterised by the production of inflammatory cytokines, amino–acid–derived eicosanoids, inflammatory mediators such as platelet activating factor, and adhesion molecules [26]. Polyunsaturated fatty acids of the n–3 series act directly by inhibiting amino acid metabolism and indirectly by altering the expression of inflammatory genes [27]. They are considered to be of therapeutic value for a variety of acute and chronic inflammatory conditions.

1.7.5 Cancer

Cancer represents the uncontrolled proliferation of cells. It arises from mutations in genes that normally control cell division or promote programmed cell death (apoptosis). The development of cancer involves a number of stages. Initiation is the initial process of DNA mutation; promotion is a stage at which a potentially cancerous cell, through accumulation of further mutations, becomes a fully cancerous cell (BNF, 1992). Then there is a stage of progression, or development by cell division, following which the cancerous cells may spread and invade other tissues (metastasis). Fixed oil and fats are involved in cancer in many ways. Dietary lipids, including micronutrients such as fat–soluble antioxidants, may play a role in predisposing to, or protecting from cancer. The development of cancer involves cellular changes that include alterations to lipid components of the cell (and this may be relevant to the design of new treatments). Finally, there may be a role for certain dietary lipids in the control or treatment of certain cancers or aspects of cancer [27]. The evidence is strong for a link between adiposity and breast cancer and for endometrial cancer, both in post–menopausal women [25]. The most consistent theory implicates the production of oestrogens by the excess adipose tissue. Adipose tissue is an important site for the conversion of androgens to oestrogens, oestradiol and oestrone. The development of some forms of breast and endometrial cancer is stimulated by oestrogens, and hence the association with dietary fat or obesity. The expression of variant forms of glycolipids on cell surfaces may offer a means of directing specific drugs to kill the tumour cells. In addition, several potential new treatments based on phospholipid analogues have been developed and in some cases tested in clinical trials [28]. These compounds are intended to incorporate into target membranes, either the cell membrane or intracellular membranes such as the endoplasmic reticulum. Thus, their mode of action differs from conventional anti–cancer drugs that target DNA replication [18] . Several types of compounds have been tested, although most are analogues of phosphatidylcholine or of lysophosphatidylcholine. Fixed oil and fats are believed to act in diverse ways, all of which could be seen as targeting cells with a high rate of cell division. They may act by disrupting signal transduction and signalling pathways, *e.g.* inhibition of the phosphoinositide–specific phospholipase C with suppression of the diacylglycerol–protein kinase C pathway, or inhibition of phosphocholine cytidylyltransferase leading to inhibition of phosphatidylcholine biosynthesis de novo [29]. These compounds have shown great promise in cellular systems, but their clinical application has been limited by problems of toxicity to the intestinal tract (whose cells are also characterized by a high rate of division) [30].

1.8 Toxic Fixed Oils and Fats

Though fixed oil and fats serve as one of the main components of human diets, however some of them portend danger to man as a result of their toxicity. Toxic fixed oil and fats include ones containing cyclopropenes, long–chain monoenes, trans–unsaturated fatty acids and lipid peroxides.

1.8.1 Cyclopropenes

Of the fatty acids, those containing a cyclopropene ring have been considered toxic as a result of their ability to inhibit the Ä9–desaturase. One result of this is to alter membrane permeability as seen in 'pink–white disease'. If cyclopropene fatty acids are present in the diet of laying hens, the permeability of the membrane surrounding the yolk is increased, allowing release of pigments into the yolk [14]. Cottonseed oil is the only important oil in the human diet that contains cyclopropene fatty acids. However, their concentration in the natural oil is low (0.6–1.2%) and is reduced still further to harmless levels (0.1–0.5%) by processing. There has been no evidence that consumption of cottonseed oil in manufactured products has had any adverse nutritional effects [31].

1.8.2 *Long–chain monoenes*

The long–chain monoenoic acid, erucic acid is present in high concentration (up to 45% of total fatty acids) in the seed oil of older varieties of rape (*Brassica napus*) [31]**.** After about a week feeding of rats on 5% rapeseed oil containing diet, their hearts contained three to four times as much lipid as normal hearts and although, with continued feeding, the size of the fat deposits (referred to as a lipidosis) decreased, other pathological changes were noticeable. These included the formation of fibrous tissue in the heart muscle. The biochemistry of the heart muscle was also affected [13]. Mitochondrial oxidation of substrates, such as glutamate, was reduced and the rate of ATP biosynthesis was impaired. The degradation of triacylglycerols that contain erucic acid is somewhat slower than with fatty acids of normal chain length and this may have contributed to the accumulation of lipid deposits [11].

1.8.3 Trans–unsaturated fatty acids

Some epidemiological studies have shown an apparent statistically significant correlation between intakes of certain types of trans–unsaturated fatty acids and increased risk of coronary heart disease. Critical evaluation of all such studies lead to the conclusion that an association between trans–unsaturated fatty acid consumption and coronary heart disease has not been demonstrated; nor is there any reliable evidence linking trans–unsaturated fatty acid intake with cancer or other chronic diseases. A theoretical basis for some of the claimed effects of trans–unsaturated fatty acids on heart disease has been discussed in terms of their influence on plasma lipoproteins and on components of the blood–clotting system [28]**.** While there is no conclusive evidence for the latter, there is an effect of trans–unsaturated fatty acids in raising LDL–cholesterol and decreasing HDL–cholesterol concentrations in the blood. High concentrations of trans–unsaturated fatty acids may adversely affect the metabolism of the parent essential fatty acids, linoleic and α– linolenic acids to their long–chain metabolites by inhibiting desaturase activity or competing with normal substrates for desaturases. In this way, they may raise the dietary requirement for essential fatty acids. This kind of 'nutritional imbalance' must be distinguished, however, from direct and specific toxic effects [27].

1.8.4 Lipid peroxides

Lipid peroxides may be formed from poly–unsaturated fatty acids when they are in contact with oxygen. When lipid hydroperoxides are ingested, they are rapidly metabolized in the mucosal cells of the small intestine to various oxyacids that are then rapidly oxidized to carbon dioxide [5]. High concentrations of lipid peroxides in the gut may damage the mucosa and potentiate the growth of tumours. However, there is scanty evidence for the absorption of unchanged hydroperoxides nor for their incorporation into tissue lipids. Hydroperoxyalkenals, lower molecular weight breakdown products of lipid hydroperoxides, are absorbed and may be toxic. Rats given diets enriched in these compounds manifested increased liver weight, increased concentrations of malondialdehyde, peroxides and other carbonyl compounds and decreased concentrations of α–tocopherol and linoleic acid in tissues [6].

1.9 Methods of extracting essential oils

The extraction of essential oils from plant material can be achieved by various methods, of which hydro-distillation, steam and steam/water distillation are the most common method of extraction, other methods include solvent extraction, aqueous infusion, cold or hot pressing, effleurage, supercritical fluid extraction and phytonic process[21-25]. The chemical composition of the oil, both quantitative and qualitative, differs according to the extraction technique. For example, hydro-distillation and steam-distillation methods yield oils rich in terpene hydrocarbons. In contrast, the super-critical extracted oils contained a higher percentage of oxygenated compounds [32].

Some of the extraction methods are given below:

1.9.1 Maceration

In this process, the whole or coarsely powdered plant material is placed in a stopper container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marceration (the damp solid material) is pressed, and the combined liquids are clarified by filtration [33].

1.9.2 Cold Pressing

Cold pressing is used to extract the essential oils from citrus rinds such as orange, lemon, grapefruit and bergamot. This method involves the pressing of the rind at about 120 degrees F to extract the oil. . The result is a watery mixture of essential oil and liquid. It is important to note that oils extracted using this method have a relatively short shelf life, so make or purchase only what you will be using within the next six months[34].

1.9.3 Solvent extraction

A hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solution is filtered and concentrated by distillation, a substance containing (resinoid), or a combination of wax and essential oil (known as concrete) remains. From the concentrate, pure alcohol (or any other suitable solvent) is used to extract the oil. When the solvent evaporates, the oil is left behind. This is not considered the best method for extraction as the solvents can leave a small amount of residue behind which could cause allergies and effect the immune system[34].

1.9.4 Enfleurage

Enfleurage is an intensive and traditional way of extracting oil from flowers. The process involves layering fat over the flower petals. After the fat has absorbed the essential oils, alcohol is used to separate and extract the oils from the fat. The alcohol is then evaporated and the essential oil collected[33].

1.9.5 Hydrodistillation

Some process becomes obsolete to carry out extraction process like Hydro Distillation which often used in primitive countries. The risk is that the still can run dry, or be overheated, burning the aromatics and resulting in an Essential Oil with a burnt smell. Hydro distillation seems to work best for powders (i.e., spice powders, ground wood, etc.) and very tough materials like roots, wood, or nuts[34].

1.9.6 CO_2 and Supercritical CO_2 extraction

The most modern technologies, carbon dioxide and supercritical carbon dioxide extraction involve the use of carbon dioxide as the 'solvent' which carries the essential oil away from the raw plant material. The lower pressure CO_2 extraction involves chilling carbon dioxide to between 35 and 55 degrees F, and pumping it through the plant material at about 1000 psi. The carbon dioxide in this condition is condensed to be a liquid. Supercritical CO_2 extraction (SCO_2) involves carbon dioxide heated to 87 degrees F and pumped through the plant material at around 8,000 psi – under these conditions; the carbon dioxide is likened to a 'dense fog' or vapor. With release of the pressure in either process, the carbon dioxide escapes in its gaseous form, leaving the essential oil behind. The usual method of extraction is through steam distillation. After extraction, the properties of a good quality essential oil should be as close as possible to the "essence" of the original plant. The key to a 'good' essential oil is through low pressure and low temperature processing. High temperatures, rapid processing and the use of solvents alter the molecular structure and will destroy the therapeutic value and alter the fragrance [34].

1.9.7 Turbo distillation extraction

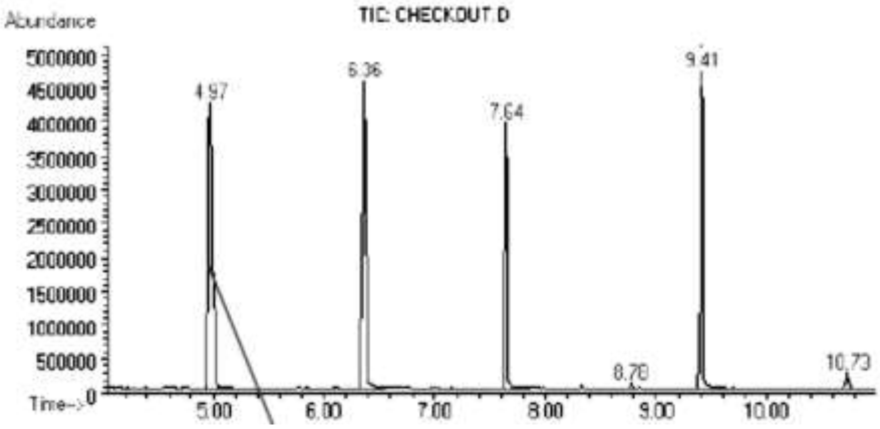
Turbo distillation is suitable for hard-to-extract or coarse plant material, such as bark, roots, and seeds. In this process, the plants soak in water and steam is circulated through this plant and water mixture. Throughout the entire process, the same water is continually recycled through the plant material. This method allows faster extraction of essential oils from hard-to-extract plant materials[33].

1.9.8 Steam Distillation

Most commonly, the essence is extracted from the plant using a technique called distillation. One type of distillation places the plants or flowers on a screen. Steam is passed through the area and becomes "charged" with the essence. The steam then passes through an area where it cools and condenses. This mixture of water and essential oil is separated and bottled. Since plants contain such a small amount of this precious oil, several hundred pounds may need to produce a single ounce[34].

1.10 Gas chromatography mass spectrometry (GC/MS)

GC/MS is a technique combining the separation power of GC and the identifying power of MS. First the GC separates mixes of chemical into individual components then the MS fragments the chemicals into unique patterns or spectra. As the individual compounds elute from the GC column, they enter the electron ionization (mass spec) detector. There, they are bombarded with a stream of electrons causing them to break apart into fragments. These fragments can be large or small pieces of the original molecules. The computer records a graph for each scan. The x-axis represents the M/Z ratios. The y-axis represents the signal intensity (abundance) for each of the fragments detected during the scan. This graph is referred to as a mass spectrum (MS).



Fig(1.5):Chromatogram generated by a GC

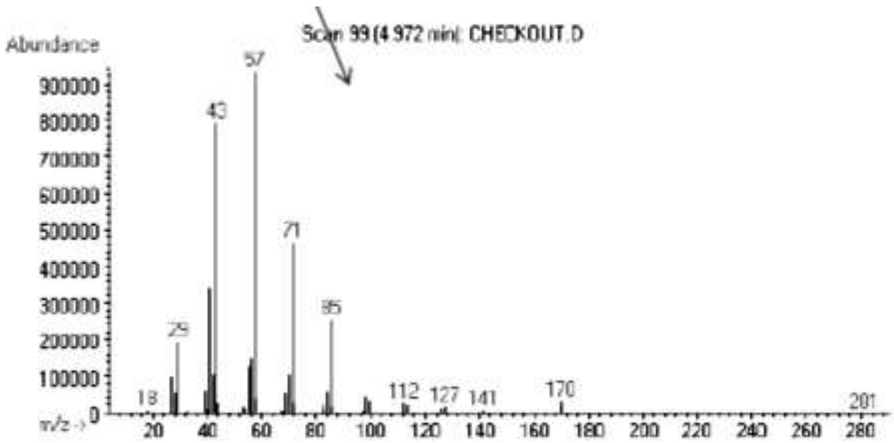


Fig (1.6): Mass – spectrum[35]

1.10.1 Uses of GC/MA

- Identification and quantitation of volatile and semi volatile organic compounds in complex mixtures.

- Determination of molecular weights and (sometimes) elemental compositions of unknown organic compounds in complex mixtures.

- Structural determination of unknown organic compounds in complex mixtures both by matching their spectra with reference spectra and by a priori spectral interpretation.

In addition to sample preparation time, the instrumental analysis time usually is fixed by the durationof the gas chromatographic run, typically between 20 and 100 min[35].

For the above variety uses , GC/MS can be used in many fields[36-43].

1.11 Antibacterial

Accurate determination of bacterial susceptibility to antibiotics is essential to the successful management of bacterial infections and to the comparative analysis of antimicrobial agents. This can be done by a number of techniques, which include the disc diffusion method, the broth dilution assay and the E tests. The effectiveness of antibiotics can be assessed by their ability to suppress bacterial growth, described by the MIC, or by their ability to kill bacteria, characterized by the minimal lethal concentration (MLC). MIC is usually derived by means of tests in solid media, whereas both MIC and MLC can be determined in broth dilution assays. A number of reports have been dedicated to comparing the effectiveness of these methods[44].

The agar diffusion techniqu is commonly used for determination of MIC in solid media. It involves the application of antibiotic solutions of different concentrations to cups, wells or paper discs, placed on the surface of or punched into agar plates seeded with the test bacterial strain. Antibiotic diffusion from these sources into the agarose medium leads to inhibition of bacterial growth in the vicinity of the source and to the formation of clear ’zones’ without bacterial lawn. The diameter of these zones increases with antibiotic concentration. The value of MIC is determined as the zero intercept of a linear regression of the squared size of these inhibition zones, x, plotted against the natural logarithm of the antibiotic concentration, c:

$$\ln(\mathrm{MIC}) = \ln(c) - \frac{x^2}{4Dt} \qquad (1)$$

Here, D is the diffusion coefficient, presumed to be independent of concentration, and t the time of antibiotic diffusion.

This analysis is based on a solution of a differential equation describing free diffusion in one dimension[45]:

$$D\frac{\partial^2 c(x,t)}{\partial x^2} - \frac{\partial c(x,t)}{\partial t} = 0 \qquad (2)$$

where c(x,t) describes the dependence of antibiotic concentration on distance from the source and on time. This approach has been applied successfully to studies of two-dimensional diffusion of dyes and antibiotics, most notably penicillins[5].

1.12 The Target Plants

1.12.1 *Hibiscus asper* Hook.

The genus *Hibiscus* Linnaeus belongs to the family Malvaceae. It comprises about 250 species distributed in tropical and subtropical areas[46]. H. asper was first described in 1849 from the specimen collected in Sierra Leone[47]. It is widely distributed throughout tropical Africa, grows in fallow fields, grassland and edges of gallery forest. It is a perennial herb whose stems are with five prickles, simple or stellate hairs up to 2 m tall. Its leaves are alternate, and stipules up to 6 mm long.

- Plant taxonomy:

Kingdom: Plantae

Order: Malvales

Family: Malvaceae

Subfamily: Malviodeae

Tribe: Hibisceae

Genus : Hibiscus

Species: H. Asper

In Nigeria, the calyx and leaf of *H. asper* are commonly used in soups as vegetables in the middle belt and north eastern regions while it is largely used in the tropical regions of Africa as potent sedative, restorative tonic, anti-inflammatory, anti-depressive, anti-anaemic drug as well as in the management of jaundice [48] . It was reported that the leaf is highly recommended by traditional practitioners for the treatment of abscesses, urethritis, joint pain, male infertility and skin infections in western region of Cameroon[49]. The plant is also used in veterinary medicine in the management of cutaneous infections of the domestic animals and as an anti-parasitic drug. Although the plant is used as food and herbs by indigenous people but there is scarcity of scientific information on its nutritional and chemical components. Many studies were carried out to described and identified the characterization of component of different parts of H. asper, one of these studies was designed to screen the leaf and calyx of *H. asper* for their mineral, proximate and phytochemical components as well as antioxidant activity with a view to providing more scientific information on its nutritional and therapeutic potentials[50].

1.12.2 *Merremia (Ipomea) Dissecta*:

The genus *Merremia* is one of the largest genera of *Convolvulaceae* and consists of around 650 species of climbers and consists of ca 650 species of climbers and shrubs. The plant is Perennial climbers, with herbaceous stems, sparsely hirsute to glabrous with long yellow hairs. Leaves petiolate; petiole up to 7-10 cm long, with sparsely long yellow hairs. Leaf-blades palmately 5-lobed, with the two basal lobes forked unequally, 8 cm long, 9-10.5 cm wide[51].

The genus *Ipomoea* L. is distributed in different regions of the world **[53]**. Over half of these are distributed in tropical America, and others in the Pacific basin and in Africa, Asia and Australia.

- Scientific classification

Kingdom : Planate

Order : Solanales

Family : Convolvulaceae

Genus : Merremia

Species: Dissecta

Synonym(S): Convolvulus dissectus, ipomea dissecta, ipomea sinauta, operculina dissecta

A teaspoon of infusion of leaves in one cup of water is taken as sedative and in urinary tract infections. Application of leaf juice externally is an effective remedy for herpes. Leaf juice is also applied on scabies and skin diseases. Boiled tubers are eaten whereas fresh are poisonous[53].

Cold infusion is anatural remedy for giddiness and is given as a treatment for chest complaints in children[54]. It has been reported that infusion crushed leaves is used for chest problems, and inflammation and work as emollients and sedatives at the same time[55]. Hot infusion is used to relieve urinary infections. The plant has been employed to treat snakebite and intoxication in Africa[56]. It is also used to treat sprains[57]. A decoction of the plant is considered as an effective external remedy for scabies and itches[58]. One of studies related this plant revealed the presence of alkaloids, glycosides, tannins, saponnins, phytosterols which are important in medicines[59,60].

1.12.3 *Cucumis prophetarum L.*

Cucumis prophetarum is a tropical speciesof plants of the family Cucurbitaceae . It is called "Cucumber of the Prophet" [(145)] in Arabic , Shari-al-deep, Haiduq and Musht-al-zahb. The fruit of this cucumber is yellow and green when it's not ripe . The skin that protects a gelatinous pulp containing the seeds, is strewn with thorns evoking those of the horse chestnut bug . It measures 10 to 25 cm .

Cucumis prophetarum spreads from Africa and the Arabian Peninsula to India, wildly distributed in north Hijaz, Najd, northern and eastern regions of Saudi Arabia[62] .

- Plant taxonomy

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Violales

Family: Cucurbitaceae

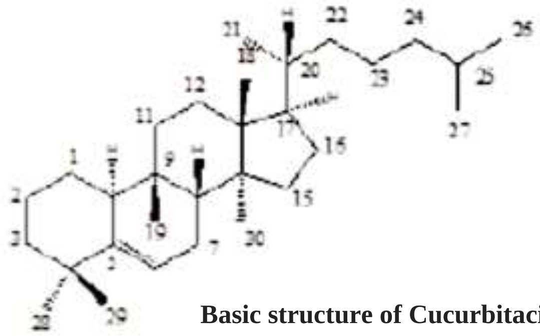
Genus: cucumis

Species: prophetarum.

the oil extracted from the seed of cucumis prophetarum is used locally in Sudan for tawing animals skin and for treating some skin diseases , generally in Ethiopia; cucumis prophetarum is used in traditional medicine for the treatment of rabies and while giving birth to a child, it helps to remove the placenta quickly1. The genus Cucumis prophetarum L also used in traditional medicine in Middle East, and North Africa as abortifacient, and antihepatotoxic in the treatment of liver diseases [63]. Moreover the secondary metabolites such as class of Cucurbitacins isolated from the genus exhibits anti-inflammatory, analgesic, antimicrobial, anti-helminthic hepato-protective, cardiovascular, and anti-diabetic effects, antioxidant activity and anticancer effects[61]. The ethanol extract of aerial parts produced contraction of isolated rabbit duodenum, which was antagonized by atropine exhibiting a muscarine type cholinergic activity. It also produced a significant fall in blood pressure rabbit. The plant exhibited mollucicidal activity against Biomphalaria alexandrina snails in bait formulations[64]. The chloroform extract showed neuromuscular blocking activity on muscles. The extract also exhibited antimicrobial activity against Bacillus subtilis in MIC of 2 mg/ml [63].

The previous phytochemical reports have shown the presence of cucurbitacin A and B, sterol from aerial parts, and saponins/ triterpenes from the fruits [(65-67)].

Other related study revealed that cucurbitacins were isolated as constituents of Cucumis prophetarum L.: such as Cucurbitacin B, isocucurbitacin B, dihydrocucurbitacin B, cucurbitacin E, isocucurbitacin E, dihydrocucurbitacin E, isocucurbitacin D, dihydroisocucurbitacin D, cucurbitacin I, dihydrocucurbitacin I, cucurbitacin Ql, and dihydrocucurbitacin Ql, Cucurbitacin P, Cucurbitacin O [68].



Basic structure of Cucurbitacins

1.12.4 Citrullus lanatus sub sp. Lanatus

There is a long-standing confusion and debate about the taxonomic names of Citrullus species, subspecies and varieties as the main characteristics of each of these subdivisions were poorly documented[69].

The genus Citrullus Schrad. ex Eckl et Zeyh. Includes seven species: C. lanatus (Thunb) Matsum. Et Nakai , found in tropical and subtropical climates worldwide; C. amarus Schrad. also known as C. lanatus var. caffrorum (Alef) Fosb, it is the preserving melon grown in Southern Africa and called tsamma melon ; C. mucosospermus Fursa, it also-called egusi melon, C. colocynthis (L.) Schrad. Which is a perennial species growing in sandy areas throughout northern Africa and adjacent Asia; C. ecirrhosus Cogn., another perennial wild species C. rehmii De Winter, an annual wild species and C. naudinianus (Sond.) Hook.f., from the Namib-Kalahari region[70-73].

Most of the leaf traits were highly variable among group of accessions indicating that various types of leaves are observed in Citrullus spp.

Citrullus lanatus a fruit crop, is herbaceous creeping plant belong to the family cucurbitaceae. It is mainly propagated by seeds and thrives best in warm areas. It is a tropical plant and requires a lot of sunshine and high temperature of over 25°C for optimum growth. plant thrives best in a drained fertile soil of fairly acidic nature. It can be grown along the coastal areas of Ghana, the forest zone and especially along river beds in the Northern Savannah areas [74].

- Botanical taxonomy

Kingdom: Plantae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: Citrullus

Species: C. lanatus

Sub sp : lanatus (wild)

Botanical Name: Citrullus lanatus

Herbal products are globally important because of their low side effect, affordability, accessibility. C. lanatus is a famous fruit in indigenous system of folk medicine and also known to contain bioactive compounds such as triterpenes, sterols, vitamin, cucurbitacin and mineral. Fruit is used in cooling, strengthening, aphrodisiac and blood purifier. Citrullus lanatus seeds are highly nutritious; they contain good amount of protein, fat, vitamins and minerals the seeds are used to prepare snacks, mix into flour and used for sauces. The seeds are used to treatment of urinary tract infection, bed wetting, dropsy and renal stones[75].

One of related studies was done by Gupta Alka, Singh Anamika and Prasad Ranu[75], and showed that citrullus lanatus seeds are a good source of many nutrients like vitamin, minerals which are essential for our body weight and is also a good source of water which can leave cooling effect in our body. Watermelon seeds have been used for its pharmacological activities like antibacterial, antifungal, antimicrobial, antiulcer and anti-inflammatory values since centuries. These watermelon seeds can provide medicinal, economic and health benefit if they consumed freshly or utilized in food products. But there is limited literature on the nutritional and antioxidant properties of watermelon seeds and there is wide scope of investigation.

Table 1.7. proximate contents of Citrullus lanatus [76]

S. No	Parameters	Values
1	Moisture	6.4%
2	Fat	47.1%
3	Protein	68.4%
4	Fiber	1.2%
5	Ash	2.6%
6	Carbohydrates	25.1%

Table 1.8. Phytoconstituents of Citrullus lanatus [77-86]

Part	Constituents
seeds	Lycopene, beta carotene, xanthophylls, phenolic, vitamin C proteins, flavonoids, riboflavin, polyphenolic compounds, terpenes; steroids, cellulose; carbohydrates, amino acids, minerals including: Na, Ca, and Mg
Seed oil	Polyunsaturated fatty acids, higher fatty acids, saturated fatty oil acids

1.12.5 Cleome gynandra

Cleome genus includes 601 plant species from the family Cleomaceae. Of more than 600 plants, 206 (34.3%) plants are having accepted species names. It known as African cabbage , spider wisp, cat's whiske. It is an annual wildflower native to Africa but has become widespread in many tropical and sub-tropical parts of the world. It is an erect, branching plant generally between 25 cm and 60 cm tall. Its sparse leaves are each made up of 3–5 oval-shaped leaflets. The flowers are white, sometimes changing to rose pink as they age. The seed is a brown 1.5 mm diameter sphere. The leaves and flowers are both edible. The leaves have a strong bitter, sometimes peppery flavor similar to mustard greens. Typically, the leaves and shoots are eaten boiled or in stews, so they are form an important part of diets in Southern Africa[87].

C. gynandra is typically well-known herb in southern Africa. The species is also native to the following regions/countries[88].

- Northern Africa: Egypt and Mauritania
- Western Africa: Cameroon, Ghana, Guinea, Côte d'Ivoire, Mali, Niger, Nigeria, and Sierra Leone
- Central Africa: Angola, Burundi, and Zaire Eastern
- African Islands: Madagascar, Mauritius, Reunion, and Seychelles
- Middle East: Oman and North Yemen
- Asia: Afghanistan Afghanistan, Borneo, India, Java, Malaysia, Moluccas, Philippines, Sri Lanka, Sulawesi, and Thailand
- Australasia: Fiji.

- Taxonomic position of *Cleome gynandra L*.

Kingdom: Plantae

Division: Angiosperms

Class: Dicotyledones

Order: Capparidales (Capparales)

Family: Cleomaceae

Genus: *Cleome*

Species: *Gynandra*.

Cleome gynandra Linn. is a well-known medicinal plant with traditional and pharmacological importance. A good number of secondary plant metabolites have also been isolated from different parts of *C. gynandra*. It has been reported that the plant possesses a huge range pharmacological applications, such as anti-inflammatory, free radical scavenging, anticancerous, immunomodulator, and antidiabetic properties. The published literature also showed an enormous amount of phytochemicals endorsement[89].

Aim of work

This work was aimed to:

- Extract fixed oil from some Sudanese medicinal plants.

- Conduct a GC/MS analysis.

- To evaluate antimicrobial potential of the extracted oils.