CHAPTER ONE

INTRODUCTION

Peptic ulcer disease (PUD) which includes gastric and duodenal ulcer is the most prevalent gastrointestinal disorder (Valle, 2005). Peptic ulcers are holes or breaks in the protective lining of the duodenum (the upper part of the small intestine) or the stomach (areas that come into contact with stomach acids and enzymes). Gastric ulcer results from persistent erosions and damage of the stomach wall that might become perforated and develop into peritonitis and massive haemorrhage as a result of inhibition in the synthesis of mucus, bicarbonate and prostaglandins (Wallace, 2008). Duodenal ulcers are more common than stomach ulcers. Comparatively rare are esophageal ulcers, which form in the esophagus (or swallowing tube) and are often a result of alcohol abuse (Jiang *et al.*, 2009).

Until the mid-1980s, the conventional knowledge was that ulcers form as a result of stress, a genetic predisposition to excessive stomach acid secretion, and poor lifestyle habits (including overindulging in rich and fatty foods, alcohol, caffeine, and tobacco). It was believed that such influences contribute to a buildup of stomach acids that erode the protective lining of the stomach, duodenum, or esophagus. While excessive stomach acid secretion certainly plays a role in the development of ulcers, a relatively recent theory holds that bacterial infection is the primary cause of peptic ulcers. Indeed, research conducted since the mid-1980s has persuasively demonstrated that the bacterium *Helicobacter pylori* (*H. pylori*) is present in more than 90% of duodenal ulcers and about 80% of stomach ulcers. Other factors also seem to contribute to ulcer formation. Overuse of over-the-counter painkillers (such as aspirin, ibuprofen, and naproxen), heavy alcohol use, and smoking exacerbate and may promote the development of ulcers. Research indicates that heavy smokers are more prone to developing duodenal ulcers than nonsmokers, that people who drink alcohol are more susceptible to esophageal ulcers, and that those who take aspirin

frequently for a long period of time are more likely to develop stomach ulcers than those who don't (Owoyele *et al.*, 2004).

Although ulcers do not always cause symptoms, the most common ulcer symptom is a gnawing or burning pain in the abdomen between the breastbone and the navel. The pain often occurs between meals and in the early hours of the morning. It may last from a few minutes to a few hours. Less common ulcer symptoms include belching, nausea and vomiting, poor appetite, loss of weight, feeling tired and weak. The symptoms of stomach and duodenal ulcers may resemble other digestive conditions or medical problems. People with ulcers may experience serious complications if they do not get treatment. The most common problems include bleeding (as a result of damaged blood vessels), perforation (a hole in the wall of the stomach or duodenum), narrowing and obstruction (swelling which can cause narrow or close intestinal opening) (Fashner and Gitu, 2015).

Africa is endowed with many plants that can be used for medicinal purposes of which they have taken full advantage. In fact, out of the approximated 6400 plant species used in tropical Africa, more than 4000 are used as medicinal plants. Medicinal plants are used in the treatments of many diseases and illnesses, the uses and effects of which are of growing interest to Western societies. Not only are plants used and chosen for their healing abilities, but they also often have symbolic and spiritual significance. For example, leaves, seeds, and twigs that are white, black and red are seen as especially symbolic or magical and possess special properties. Examples of some medicinal plants include Pygeum (*Prunus africana*), *Parquetina nigrescens, Securidaca longepedunculata, Luffa cylindrica* among others. Phytomedicine research is gaining more grounds than ever as majority of people are relying heavily on herbal medicine or plant derived medicines, which occasionally have been shown to be less toxic, relatively safer, easily available and cheaper than the synthetic orthodox medicines (Yakubu *et al.*, 2007). In addition, the medicinal use of plant parts in the management and treatment of diseases has

been an age long practice (Sofowora, 1982). Plants provide a rich source of raw materials for traditional medicine in Africa and other developing parts of the world (Sonibare and Gbile, 2008).

Many medicinal plants are known to exhibit antiulcer activity and are found useful in the treatment of peptic ulcer (Kumar *et al.*, 2011). Some of these medicinal plants have been confirmed scientifically to possess gastro protective and antiulcer property (Farombi and Owoeye, 2011, Kayode *et al.*, 2015). Research into the treatment of ulcer has been intensified and the disease reported to have high recurrence and mortality rates especially in complicated cases. Herbal medicine has also attracted much interest in this area especially herbs from the tropics (Ukwe *et al.*, 2010).

Securidaca longepedunculata, known as violet tree, Rhode's violet, wild vesteria (English), is a shrub or small (2-10 m high) flowering savannah plant. The flowers are sweet scented, bright purple or violet racemes, and the fruit is winged; the plant is widespread throughout tropical Africa. In many parts of Africa, the plant is employed in traditional medicine principally for its psychotropic properties (Winkleman and Dobkin, 1989). Other uses include treatment of various sexually transmitted infections, hernias, coughs, fever, ascariasis, constipation, headaches, rheumatism, stomach ache, malaria, tuberculosis, pain, epilepsy, pneumonia, skin infections, peptic ulcer disease and it is also used as an aphrodisiac for men (Mongalo *et al.*, 2015). Powdered dried root is also used as a pest control agent in stored grain (Belmain *et* al., 2001).

Luffa cylindrica, commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, has been investigated for its anti-inflammatory, anti-fungus, analgesic and sedation, anti-myocardial ischemia, antiulcer, anti-asthma and expectorant effects among others (Partap *et al*., 2012).

1.1 Research justification

The use of medicinal plants and herbal drugs in the management of diseases such as the gastric ulcer disease is rapidly becoming more popular because these herbal drugs and natural remedies are believed to be cheaper and considered to have little or no side effects when compared with their synthetic counterparts. There is also no reported antiulcer activity of the selected plants for this research in literature despite their folkloric use. The traditional claim of the antiulcer property of these plants is not yet validated scientifically as well.

Therefore, this research will:

1. Allow the screening, identification, isolation and structure elucidation of antiulcer compounds from the leaf extract of *Securidaca longepedunculata* and *Luffa cylindrica* thereby adding to the library of new antiulcer agents for the management of peptic ulcer disease.

2. Provide a robust analysis and scientific validation for the folkloric use of the plants in Nigerian ethno medicine.

3. Provide a potentially suitable herbal mixtures or formulations as a natural remedy to peptic ulcer patients especially in the rural areas in Nigeria.

1.2 Hypothesis

The chemical constituents of *Securidaca longepedunculata* and *Luffa cylindrica* are responsible for their antiulcer activity.

1.3 Aim of study

The aim of this research is to validate the antiulcer property, isolate and characterize antiulcer compounds from the leaf extracts of *Securidaca longepedunculata* and *Luffa cylindrica*

1.4 Overall objectives

This study was carried out in order to achieve the following:

To carry out phytochemical screening, *in-vitro* and *in-vivo* antioxidant assays on the plants extracts.

To isolate and characterize the antiulcer constituent of the plants.

To determine the antiulcer property of the isolated compounds from the plant extracts.

Histopathological evaluation of the stomach of all the experimental animals.

1.5 Specific objectives

To screen the selected plants (*Securidaca longepedunculata* and *Luffa cylindrica* for gastroprotective and antiulcer activities and evaluate possible differences in the potency of the antiulcer effects of the different extracts of the plants.

To determine the total protein content and the amount of lipid peroxidation in the stomachs of the experimental rats.

To determine the *in vitro* antioxidant capacity of the leaf extracts of the plants.

To assess the *in vivo* antioxidant property of the leaf extracts of the plants.

To isolate, characterize and identify compounds from the leaf extracts of the plants.

To determine the antiulcer property of the isolated compounds.

Histopathological assessment of the stomach of all the experimental animals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Peptic ulcer

An ulcer is a crater like lesion in a membrane (Helms *et al*., 2006). Peptic ulcer is an open sore in the lining of the upper gastrointestinal tract due to mucosal erosions. Pathophysiology of ulcer is due to an imbalance between the two factors i.e. aggressive (acid, pepsin, *Helicobacter pylori* and Nonsteroidal Anti-inflammatory Drugs, NSAIDs) and local mucosal defensive factors (mucus, bicarbonate, blood flow, prostaglandin, nitric oxide and growth factors (Figure 2.1) (Tulassay and Herszenyi, 2010). An ulcer is a crater like lesion in a membrane (Helms *et al*., 2006). The most common cause of ulcer is *H. pylori*, a bacterium that colonizes the stomach of nearly half the world's population.

2.1.1 Peptic ulcer disease (PUD)

Peptic ulcers are holes or breaks in the protective lining of the duodenum (the upper part of the small intestine) or the stomach (areas that come into contact with stomach acids and enzymes). Abdominal pain in patient with PUD is classically described as gnawing or burning, non-radiating, epigastric pain, which occurs 2-3 hours after meals (when stomach is empty) or at night. The pain is relieved with food or antacids. However, less than 50% of patients with those symptoms are actually found to have peptic ulcer disease. The most discriminating symptom of pain awakening the patient from sleep between 12-3 a.m. affects two-thirds of duodenal ulcer patients and one-third of gastric ulcer patients. However, these same symptoms are also seen in one-third of patients with non-ulcer dyspepsia (Tarnawski *et al.*, 2001). Peptic ulcer occurs mainly due to imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric oxide, peptides, growth factors and injurious factors like acid, pepsin (Hoogerwerf and Pasricha, 2006).

There are two types of peptic ulcer: *gastric ulcer*, which appeared to be due to the damage

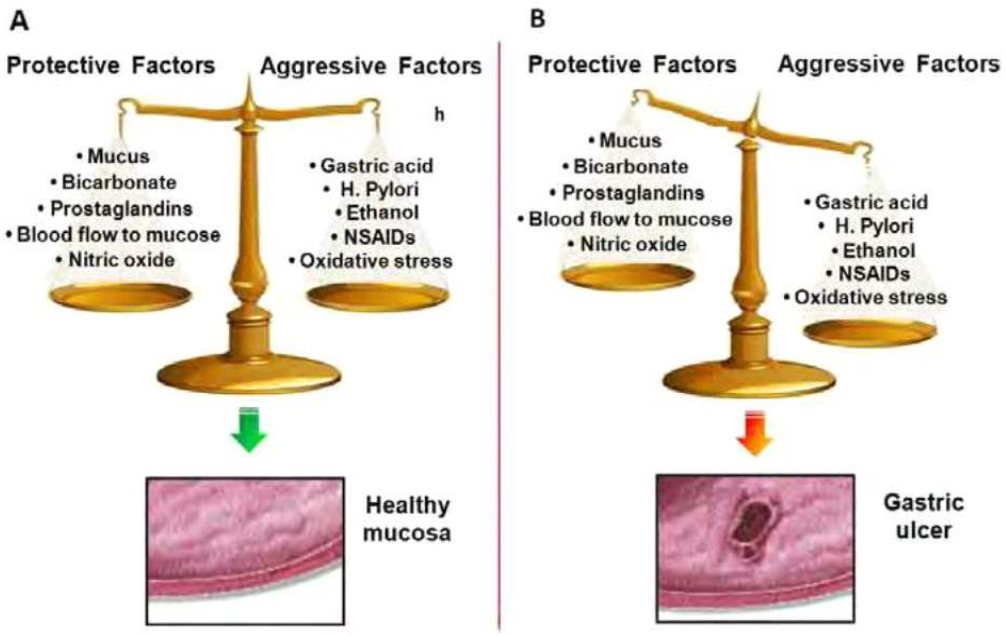


Figure 2.1: Balance between mucosal aggressive and protective factors

(A), imbalance between mucosal aggressive and protective factors (B).

Source: Tulassay and Herszenyi, 2010

of the lining of the stomach, and *duodenal ulcer*, which was associated with excessive acid secretion by the stomach. Such ulcers do not occur if there was atrophy of the gastric mucosa, when the stomach secretes no acid.

2.1.2 Duodenal ulcer disease

Duodenal ulcer is a deep lesion penetrating through the entire thickness of the gastrointestinal mucosa and muscularis mucosa (Tarnawski *et al*, 2001). It is indisputable that *H. pylori* infection is the most important etiologic factor for gastro duodenal ulcer however neither eicosanoids nor bacterial infection alone or combined could explain the pathogenic effects of the disease due to reoccurrence after cessation of the treatment for each (Khuroo *et al.*, 1989).

Duodenal ulcer is a mucosal erosion of the duodenum, due to multiple causes, including bacteria (Marshall and Warren, 1984), chewing gum, tobacco smoking, not eating properly, spice and chronic stress (Kim *et al.*, 2007).

2.1.3 Gastric ulcer disease

A gastric ulcer, also called stomach ulcer, is a break in the normal gastric mucosa integrity that extends through the muscularis mucosa into the submucosa or deeper (Figure 2.2). Potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products and certain drugs have been implicated in the pathogenesis of gastric ulcer. The

incidence varies with age, gender, geographical location and is associated with severe complications including hemorrhages, perforations, gastrointestinal obstruction, and malignancy. Thus, this clinical condition represents a worldwide health problem because of its high morbidity, mortality and economic loss (Dimaline and Varro, 2007).

Gastric ulcer is a complex pluricausal disease and is known to develop due to imbalance between aggressive and protective factors. Several endogenous and exogenous factors are responsible for gastric ulceration. These include *H. pylori* infection, increased production of gastric acids, pepsin and stomach juices, certain types of medicines, notably the non-steroidal anti-inflammatory drugs (NSAIDs), and even personal factors such as consumption of tobacco, alcohol and caffeine, as well as emotional and physical stresses (Rad *et al.*, 2004).

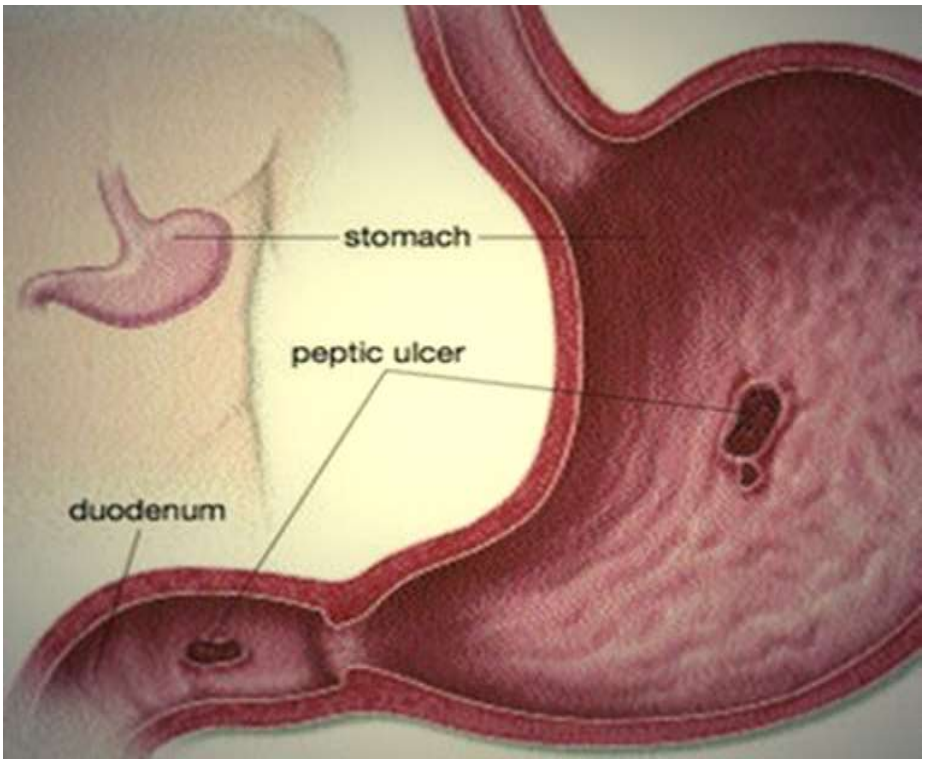


Figure 2.2: Diagram of ulceration in the stomach

Source: (Akhtar *et al*., 1992)

The normal stomach mucosa maintains a balance between protective and aggressive factors. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, use of alcohol and nonsteroidal anti-inflammatory drugs (NSAIDs), as well as infection with microorganisms (*Helicobacter pylori* and others). On the other hand, mucus secretion, bicarbonate production, gastro protective prostaglandin synthesis and normal tissue microcirculation protect against ulcer formation. Although in most cases the etiology of ulcer is unknown yet, it is generally accepted that gastric ulcers are multifactorial and develop when aggressive factors (endogenous, exogenous and/or infectious agents) overcome mucosal defense mechanisms (Tulassay and Herszényi, 2010).

2.2 Development of peptic ulcer

While excessive stomach acid secretion certainly plays a role in the development of ulcers, a relatively recent theory holds that bacterial infection is the primary cause of peptic ulcers. Indeed, research conducted since the mid-1980s has persuasively demonstrated that the bacterium *Helicobacter pylori (H. pylori)* is present in more than 90% of duodenal ulcers and about 80% of stomach ulcers. Other factors also seem to contribute to ulcer formation. Overuse of over the counter painkillers (such as aspirin, ibuprofen, and naproxen), heavy alcohol use, and smoking exacerbate or may promote the development of ulcers. Research indicates that heavy smokers are more prone to developing duodenal ulcers than non-smokers, and people who drink alcohol are more susceptible to esophageal ulcers, and that those who take aspirin frequently for a long period are more likely to develop stomach ulcers than those who do not (Owoyele, 2004).

2.3 Symptoms and signs of peptic ulcer

Moynihan in 1905, an Irish surgeon was the first to relate the clinical symptoms of peptic ulcer disease with the pathological findings (Gibioki, 1987). It has been reported that

small ulcers may not cause any symptoms and large ulcer can cause serious bleeding. The most common symptom is burning pain, especially just below the breastbone. Gastric ulcer pain may be less severe than duodenal ulcer pain and is noticeably higher in abdomen. Eating may increase pain in subjects rather than relieving pain. Other symptoms may include nausea, vomiting and weight loss. Vomiting might be related to partial or complete gastric outlet obstruction. Duodenal ulcer pain may awaken the patients from sleep and also involve burning or gnawing sensation in upper abdomen. Pain in back, lower abdomen or chest area may occasionally arise and occurs when the stomach is empty about two hours after a meal or during the night. Relief frequently occurs after eating. Epigastric tenderness, melena resulting from acute or sub-acute gastrointestinal bleeding and complete gastric outlet obstruction may also occur in ulcer disease (University of Maryland Medical Center, 2008).

2.4 Causes of peptic ulcer

2.4.1 Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are used all around the world. Thus, the drug related problems due to NSAIDS are most common. NSAIDs inhibit the activity of cyclooxygenase (COX), the key enzyme in prostaglandins (PG) production. Various studies indicate that NSAIDs help in the progression of ulceration by overcoming the expression of the enzyme cyclooxygenase (COX) which has been documented to inhibit the conversion of arachidonic acid (AA) to PG's, that impairs the mucosal barrier and results in corrosive action with pepsin thus resulting in the progression of peptic ulcers (Scarpignato and Hunt, 2010). Further, COX-1 inhibition by the NSAID leads to the significant release of the endothelin-1 (ET-1) which is a potent vasoconstriction, which has been shown to induce mucosal injury. NSAIDs by inhibiting the prostaglandins cause the activation of neutrophils and the local release of reactive oxygen species (ROS) and thus initiates the gastric injury (Whittle, 2002). NSAIDs also cause marked reduction in

the mucosal blood flow, mucus- bicarbonate secretions, impaired platelet aggregation, reduced epithelial cell renewal and increased leukocyte adherence that are responsible for pathogenesis of ulceration (Allen *et al.*, 1993).

2.4.2 *Helicobacter pylori*

Helicobacter pylori are the main cause of stomach ulcers. *H. pylori* are gram-negative bacillus, motile, microaerophilic, flagellated and spiral shaped bacteria (Mazumdar *et al.*, 2011). *H. Pylori* cause increased expression of cytokines such as TNF-α in gastritis. IL-1β is also over expressed in the *H. pylori*-induced gastritis (Martin, 1997). *H. pylori*-infected gastric mucosa showed infiltration of polymorphonuclear leukocytes, lymphocytes, monocytes and plasma cells in the lamina propria, and intraepithelial severe neutrophil infiltration (Fan *et al.*, 1996).

2.4.3 Ethanol

The mechanism of ethanol gastric lesions is varied, including the depletion of gastric mucus content, damaged mucosal blood flow and mucosal cell injury. It has been documented that ethanol causes severe damage to the gastrointestinal mucosa, starts with microvascular injury results in increase vascular permeability, edema formation and epithelial lifting. Szabo et al., suggested that after intragastric administration of ethanol a rapid and time dependent release of endothelin-1 into the systemic circulation preceded the development of the hemorrhagic mucosal erosion by vasoconstriction (Szabo *et al.*, 2010). Ethanol also initiates apoptosis, which lead to cell death (Vidya *et al.*, 2001).

2.4.4 Nitric oxide (NO)

Nitric oxide has been involved in gastric ulceration and shown to diminish neutrophil adhesion, raise gastric blood flow and mucus secretion (Li and Wallace, 2000) and promotion of angiogenesis (Takeuchi *et al.*, 1995). Nitric oxide inhibits gastric secretion by inhibition of histamine release from enterochromaffin-like cells (Kato *et al.*, 1998).

Nitric Oxide has been studied to play an important role in GI mucosal defense and the pathogenesis of mucosal injury (Wallace and Miller, 2000).

2.4.5 Tumor necrosis factor (TNF-α)

Tumor necrosis factor (TNF-α) also plays a vital role in ulcer progression. TNF-α is capable of inhibiting gastric secretion and parietal cell apoptosis induction by a nuclear factor- κB (NF- kb) (Boraschi *et al*, 1998). Synthetic anti-ulcer drugs like H_2 blocker, Proton pump inhibitor (PPI) and cytoprotectants are available in the market for the management of ulcers, but they possess major adverse drug reaction (ADR) that restrict their use, herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects (Arul *et al.*, 2005).

2.5 Role of reactive oxygen species (ROS) and free radicals

Oxidative stress has evolved as one of the major pathogenic factors that directly impaired cells functions, promotes cellular organelles damage in the cells, including particularly, mitochondria, lysosomes, and nucleus. Since, reactive oxygen species (ROS) could directly disrupt the mitochondrial membrane that subsequently lead to the release of cytochrome C; the later becomes a part of apoptosome complex or in an additive way leads to membrane rupture of the lysosomes which leads to the release of cathepsins, the later activate caspase and apoptosis cascade, and finally leads to cell death via apoptosis. On the contrary, the cells protect themselves against the destructive effects of ROS through scavenging them by enzymes defense system, or by the antioxidant activities of the dietary compounds (Gislason, 1995). Several studies concluded that polyunsaturated fatty acids are the most vulnerable to free radical attacks and the initial products of lipid peroxidation are conjugated diene hydroperoxides. Although, ROS production is difficult to measure in biological tissues, there are various indirect manifestations of oxidative stress, including lipid peroxidation, DNA oxidation, protein oxidation, and a shift in the redox states of thiol or disulfide redox couples. The lipid peroxidation and appearance of

lipid free radicals and malondialdehyde (MDA) in the blood and gastric juice could result from ROS-initiated chain reactions or initiated by indirect mechanisms that suppress the antioxidant capacity in both blood and gastric wall to scavenge ROS. The fundamental primary product is lipid hydroperoxides, which are capable of initiating lipid peroxidation chain reaction and decompose giving rise to secondary oxidation products including: aldehydes, hydrocarbons, acids, ketones and higher polymers. Among these, is MDA that is mutagenic and carcinogenic, and its reaction with thiobarbituric acid is a marker for lipid peroxidation (Dotan *et al*, 2004). The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells may contribute to gastric mucosal damage. Therefore, by scavenging free radicals, the reactive oxygen metabolites might be useful by protecting the gastric mucosa from oxidative damage or by accelerating healing of gastric ulcers (Lutnicki *et al.*, 2001).

2.6 Role of antioxidant enzymes

Antioxidants acts as radical scavengers, which inhibits lipid peroxidation and other free radical-mediated processes, and therefore they protect the human body from several diseases attributed to the reactions of radicals. Numerous substances have been suggested to act as antioxidants. Various phenolic antioxidants such as flavonoids, tannins, coumarins, xanthenes and, more recently, procyanidins have been shown to scavenge free radicals in a dose-dependent manner and therefore are viewed as promising therapeutic drugs for free radical pathologies (Czinner *et al.*, 2001). Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called free radicals such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite, which results in oxidative stress leading to cellular damage (Mattson and Cheng, 2006). Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Uddin *et al.*, 2008; Jayasri *et al.*, 2009). Antioxidants exert

their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a fairly 'stable radical'. The free radicals are metastable chemical species, which tend to trap electrons from the molecules in the immediate surroundings. These radicals if not scavenged effectively in time, they may damage crucial biomolecules like lipids, proteins including those present in all membranes, mitochondria and, the DNA resulting in abnormalities leading to disease conditions (Uddin *et al*, 2008). Thus, free radicals are involved in a number of diseases including: tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. Parkinsonism, Alzheimer's diseases), AIDS and even early senescence (Uddin *et al.*, 2008, Kayode *et al*., 2009, Kayode *et al*., 2015). The human body produces insufficient amount of antioxidants, which are essential for preventing oxidative stress. The body's own natural antioxidant defenses such as glutathione or catalases (Sen, 1995) can remove free radicals generated in the body. Therefore, this deficiency had to be compensated by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, β-carotene and natural products in plants (Rice- Evans *et al.*, 1997).

Plants contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity (Cai and Sun, 2003). Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics, which possess the ability to scavenge the free radicals in human body. Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reduction in the occurrence of many diseases (Anderson and Teuber, 2001). Many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to protective effects *in vivo* (Rice-Evans, 1997; Jayasri *et al*., 2009). The enzymatic and non-enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbic acid (vitamin C), α -tocopherol (vitamin E), glutathione (GSH), β-carotene, and vitamin A, which can be evaluated using easy photometric assays (Stahl *et al*., 1998). There is a balance between both the activities and the intracellular levels of these antioxidants that are essential for the survival of

organisms and their health (Aleryani *et al*., 1998).

2.6.1 Antioxidant enzymes

2.6.1.1 Superoxide dismutase

Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPX reactions (Teixeira *et al.*, 1998).

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2$$

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) (Sun *et al.*, 1995). SOD destroys $O_2^{\bullet -}$ by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates (Meier *et al.*, 1998). All types of SOD bind single charged anions such as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe-, Mn^{-} or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibited by N_3^-, CN^- (Leone *et al.*, 1998), and by F^- (Vance and Miller, 1998).

2.6.1.2 Catalase

Catalase is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa (Aebi, 1980). CAT reacts very efficiently with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity:

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

$$ROOH + AH_2 \rightarrow H_2O + ROH + A$$

In animals, hydrogen peroxide is detoxified by CAT and by GPX. Catalase protects cells

from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Survival of rats exposed to 100% oxygen was increased when liposomes containing SOD and CAT were injected intravenously before and during the exposure (Turrens *et al*., 1984). The increased sensitivity of transfected CAT-enriched cells to some drugs and oxidants is attributed to the property of CAT in cells to prevent the drug-induced consumption of O_2 either for destroying H_2O_2 to oxygen or for direct interaction with the drug (Speranza *et al.*, 1993). There are five GPX isoenzymes found in mammals. Although their expression is ubiquitous, the levels of each isoform vary depending on the tissue type. Cytosolic and mitochondrial glutathione peroxidase (cGPX or GPX1) reduces fatty acid hydroperoxides and H_2O_2 at the expense of glutathione (Imai *et al.*, 1998).

2.6.2 *In vitro* antioxidants

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions or from exogenous factors (Cerutti, 1991). *In vivo*, some of these ROS play a positive role such as energy production, phagocytosis, regulation of cell growth and intercellular signaling or synthesis of biologically important compounds (Halliwell, 1997). However, ROS may also be very damaging; they can attack lipids in cell membranes and also attack DNA, inducing oxidations that cause membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity and also cause DNA mutation leading to cancer (Tyagi *et al*., 2010). A potent scavenger of these species may serve as a possible preventive intervention for free radical-mediated diseases (Ames *et al*., 1995). Recent studies showed that a number of plant products including polyphenolic substances (e.g. flavonoids and tannins) and various plant or herb extracts exert antioxidant actions (Tyagi *et al*., 2010). In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule (Rachh *et al*., 2009). It has been suggested that fruits, vegetables,

natural plants contain a large variety of substance called phytochemicals, which are present in plants and are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of peroxidant metals, quenchers of singlet oxygen and so on (Ebadi, 2002). The antioxidants can interfere with the oxidation process by reacting with free radicals (Gupta *et al*., 2004). Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity (Kumaran and Karunakaran, 2007). Antioxidants principles from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance (Tyagi *et al*., 2010).

Flavonoids are ubiquitous in the higher plants and play an ecological as well as physiological role. The isoflavones are responsible for the chemical signaling involved in leguminous root node formation (Reynertson *et al*., 2005). Natural antioxidants from fruits and vegetables provide a measure of protection that slows the process of oxidative damage, and are implicated as the protective constituents of these foods (Hollman and Katan 1999). Research in natural antioxidants is becoming increasingly important both in understanding the beneficial aspects of plant foods and in improving the quality of fatty foods. Antioxidants are routinely used by the industry to prevent the oxidation of food in storage and inhibit rancidity. The well-known vitamin antioxidants in food include ascorbic acid, b-carotene and a-tocopherol. Many clinical and epidemiological studies have sought to demonstrate the efficacy of these vitamins in preventing a wide variety of diseases (Reynertson *et al*., 2005). However, some of these studies failed to show significant antioxidative protection *in vitro* (Scheen 2000), which suggests that vitamins obtained via whole food or by a balanced diet may be more effective than supplements, possibly through synergistic interactions with other compounds. Fruits and vegetables are high in flavonoid content; flavonoids impart color and taste to flowers and fruits, and it is estimated that humans consume between a few hundred milligrams and one gram of flavonoids every day (Hollman and Katan 1999). Flavonoids appear in blood plasma at

pharmacologically active levels after eating flavonoid-rich foods but do not accumulate in the body (Hollman and Katan 1999). Consuming flavonoids regularly increases longevity by reducing inflammation and contributing to the amelioration of atherosclerosis from coronary heart disease (CHD) (Frankel *et al*. 1993a). The range of flavonoid biological activity is large; in addition to scavenging free radicals and ROS, flavonoid actions include anti-inflammatory, antiallergenic, antiviral, antibacterial, antifungal, antitumor, and antihemorrhagic (Reynertson *et al*., 2005). Flavonoids also inhibit a number of enzymes, including aldose reductase, a-glucosidase, xanthine oxidase, monooxygenase, lipoxygenase and cyclooxygenase (Yoshikawa *et al*., 1998). Plant polyphenols interact with LDL, enriching and protecting it from oxidation when entering the bloodstream. The so-called "French Paradox" refers to the fact that despite the high fat content of the French diet, there is a lower incidence of CHD in France than in countries where fat intake is similar. This has been attributed to the high polyphenolic content of red wine and other fruits and vegetables (Frankel *et al*., 1993a).

Some flavonoids act as antifeedants to herbivorous pests. The isoflavones are responsible for the chemical signaling involved in leguminous root node formation (Harborne and Baxter 1999). Studies suggest that the antioxidant potential of phenolics is mainly due to their ability to act as reducing agents (Vinson et al. 1999b). It is well established that the efficacy of flavonoids as antioxidants stems from the number and position of the hydroxyl substitutions on the basic structure; an increase in number of hydroxyl groups is directly correlated with increasing activity, and the 3', 4'-dihydroxy substitution is significant (Rice-Evans *et a*l,. 1997; Reynertson *et al*., 2005).

2.7 Role of digestive enzymes and hormones

Digestive enzymes such as histamine mediate a wide range of cellular responses, including allergic and inflammatory reactions, gastric acid secretion and possibly neurotransmission in parts of the brain. Additionally, it is secreted by mast cells as a result

of allergic reactions or trauma. Pharmacologically, histamine produces vasodilation and an increase in permeability of blood vessel walls that may contribute to gastric hemorrhage (Hung and Wang, 2004). In experimental animal, increased mucosal histamine has been reported to elicit gastric secretion and mucosal lesion (Andersson *et al*., 1990). Since, histamine may cause increase in gastric mucosal permeability to electrolytes and renders the stomach more susceptible to acid-induced damage (Gislason *et al*., 1995). The role of histamine in the secretion of acid from acid-producing parietal cells is widely reported (Tairov *et al*., 1984). Where, histamine activates histamine-2 receptors on the acid-producing parietal cells to stimulate acid production, the over production of acid inhibits through low antral pH gastrin release from G-cells, thus preventing the stimulatory effect of gastrin on enterochromaffin-like (ECL) cells and further histamine release (El-Omar *et al*., 1997). This inhibitory control is mediated via the release of somatostatin from D-cells situated in close proximity to the G-cells (Zhao, 2003). Gastrin and somatostatin are gastrointestinal hormones closely related to the function of the gastrointestinal system. In rats with ulcer, the gastrin level in the plasma, gastric juice and the antral mucosa tissues increase, while the somatostatin level declines (Sun *et al*., 2002, Hiruma-Lima *et al*., 2006). The gastrin secreted by antral G cells is the principal stimulant of the gastric acid secretion. Duodenal ulcer patients were shown to have a larger capacity to secrete acid under maximal stimulation by gastrin (McColl *et al*., 2000).

Somatostatin, a regulatory peptide, acts on multiple targets throughout the body, including the gastrointestinal tract. This peptide has been reported to exert potent inhibitory effects on gastric acid, pepsin and gastrin secretion (Sun *et al*., 2002). Several studies have shown a possible influence of a local decrease in gastric somatostatin in the physiologic characteristics of peptic ulcer disease. For the digestive system, as for virtually all other organs, somatostatin action has been generally characterized as inhibitory. For example, somatostatin strongly inhibits secretion of a number of hormones such as gastrin, motilin, cholecystokinin, insulin, glucagon, and reduces gastric acid and secretion (Hiruma-Lima *et al*., 2005).

2.8 Role of prostaglandins and other lipid mediators of inflammation

The major prostaglandins (PGs) produced by the human and rodent gastric mucosa are PGE2 and PGI2, with lesser amounts of PGF2α and PGD2 also being detectable. Thromboxane has also been detected in the gastric mucosa, but much of what can be measured is actually from platelets within the gastric microcirculation (Calam, 1997). The prostaglandin receptors that mediate many of the effects of prostanoids on mucosal defense have been characterized using pharmacological probes and knock-out mice. Several plants containing high amounts of lipids have been shown to possess anti-ulcer activity in several experimental ulcer models. Among these, the saponins, isolated from the rhizome of *Panax japonicus* and the fruit of *Kochia scoparia* (which contain approximately 20% of saponins) have been demonstrated to possess protective properties against ethanol-induced and indomethacin-induced gastric damages. Their protective activities were not due to inhibition of gastric acid secretion but probably due to activation of mucous membrane protective factors. Aescin, a mixture of saponins from the seeds of *Aesculus hippocastanum* (horse chestnut) has been shown to possess antiulcer activity in CRS and PL ulcer models, which is in part, due to inhibition of gastric acid and pepsinogen secretion. There is an increased change of the mucosal blood flow around the ulcer in experimental animals (Skarstein, 1979). (Skarstein (1979) also suggested that there is an increase in the concentration of prostaglandins in ulcerative regions, when compared to other parts of the gastric mucosa, since prostaglandin causes vasodilation. The larger blood supply seems to reflect the active re-epithelization, which requires an abundant supply of glucose and oxygen (Hiruma-Lima *et al*., 2006).

2.9 Management strategies and antiulcer drugs

Management of peptic ulcer disease continues to evolve because of the emergence of various novel therapeutic agents, advancements in several operative techniques and pharmacological oriented strategies. With the development of various therapies, as well as recognition and understanding of *H. pylori* infection along with mechanism, the management of ulcer has been largely successful. Several drugs are extensively used for

acidity reduction in peptic ulcer, gastro esophageal reflux disease and in many forms of gastritis as well as treating of *H. pylori* infection. Various therapeutic agents utilized in management of ulcer and in the regimens for treating *H. pylori* infection includes H2 blockers, proton pump inhibitors, antacids, prostaglandins etc.

2.9.1 H2 receptor antagonists

Until the mid-1970s, there was no effective medical treatment for duodenal ulcer. The outlook changed in November 1976, with the emergence of cimetidine, the first histamine H2 receptor antagonist, which dramatically transformed management resulting in swift symptom relief, ulcer healing and marked reduction in relapse. Various other H2 antagonists, which are available, include cimetidine, ranitidine, famotidine and roxatidine amongst others; others are also marketed in several countries. These drugs are competitive inhibitors of histamine at the H2 receptor, thus suppressing gastrin stimulated acid secretion and proportionately reducing gastric juice volume. Histamine-mediated pepsin secretion is also decreased. Long-term continuous maintenance treatment with H2 receptor antagonists for five or more years effectively prevents ulcer recurrence in the majority of patients and significantly reduces the risk of ulcer complications. In addition, maintenance treatment has proved to be safe and is well tolerated by patients.

2.9.2 Proton pump inhibitors

The first proton pump inhibitor (PPI) on the US market, omeprazole, appeared in 1988. This approval paved the way for the sequential introduction of other PPI congeners such as pantoprazole, lansoprazole rabeprazole, esomeprazole magnesium, the S-isomer of omeprazole etc. These are most potent suppressors of gastric acid secretion and inhibit gastric H+K+-ATPase enzyme (proton pump). In typical doses, these drugs diminish the daily production of acid (basal and stimulated) by 80 to 95%. PPI are prodrugs that require activation in an acid environment. After absorption into the systemic circulation, the prodrug diffuses into parietal cells of the stomach and accumulates in the acidic secretory canaliculi. The activated form then binds covalently with sulfhydryl groups of cysteine's in the H+ K+-ATPase, irreversibly inactivating the pump molecule.

Pantoprazole was first approved for use in the treatment of gastritis and duodenal ulcer in Germany in early 1994. A single intravenous bolus of 80 mg of pantoprazole inhibits acid production by 80 to 90% within an hour, and this inhibition persists for up to 21 hours, permitting once-daily dosing to achieve the desired degree of hypochlorhydria. The FDA-approved dose of intravenous pantoprazole for gastro esophageal reflux disease is 40 mg daily for up to 10 days.

2.9.3 Antacids

Antacids neutralize gastric acid and reduce pepsin activity. These agents relieve symptoms, promote ulcer healing, and decrease recurrence. They are relatively inexpensive. The optimal antacid regimen for ulcer healing generally includes 10 to 30 ml of liquid or 2 to 4 tablets 1 h and 3 h after each meal and at bedtime. The total daily dosage of antacids should provide 200 - 400 mEq neutralizing capacity. However, antacids have been superseded by acid suppressive therapy in the treatment of peptic ulcer and are used only for short-term symptomatic relief.

2.9.4 Anticholinergics

Although anticholinergic medications inhibit basal and meal-stimulated gastric acid secretion, they do so at a substantially lower rate than do other antisecretory agents. In addition, significant adverse effects of nonselective anticholinergic agents limit their use in ulcer disease.

Misoprostol, sucralfate, carbenoxolone and colloidal bismuth: Naturally, occurring prostaglandins have been shown to heal peptic ulcer in almost non-antisecretory doses. However, these compounds are rapidly metabolized when given orally, and they cause abdominal cramps, diarrhea, and uterine contractions. Therefore, in order to develop therapeutically effective prostaglandins, chemical and structural modifications are required. Significant number of prostaglandins analogues has been synthesized in search for a longer duration of action, increased potency, and greater pharmacological specificity. Prostaglandins especially misoprostol which is a synthetic prostaglandin E1 analogue protects against peptic ulcers by reducing gastric acid secretion, increasing bicarbonate

production, and boost production of gastric mucus, a natural defense against peptic ulcers. Sucralfate is a sucrose-aluminum complex that dissociates in the stomach, rapidly reacting with hydrochloric acid to form a thick, pasty substance that adheres to the gastric mucosa, especially to ulcer. By binding to the ulcer, sucralfate protects the ulcer from damaging effects of acid, pepsin and promoting healing. Carbenoxolone is a derivative of glycyrrhizic acid, a constituent of liquorice. It exerts minimum inhibitory effect on gastric acid secretion. It inhibits pepsin activity, stimulates mucus secretion and reduces gastric epithelial cell loss. Tripotassium dicitrate bismuthate is colloidal bismuth. At low pH, it binds to ulcerated mucosa and forms a protective layer lasting for about 6 h. It should therefore, be given before meals and at bedtime (Dotan, 2004).

2.9.5 Side effects of some antiulcer drugs

Antiulcer drugs such as pepcis, zantac, tagamet and others have been known to posses one side effect or the other (Dotan, 2004).

Below is a list of some of the antiulcer drugs and their side effects:

Axid (nitzatidine) - Diarrhea, headache, nausea and vomiting, sore throat.

Carafate (sucralfate) - Constipation, insomnia, hives, upset stomach, vomiting.

Cytotec (misoprostol) - Cramps, diarrhea, nausea, gas, headache, menstrual disorders (including heavy bleeding and severe cramping).

Pepcid (famotidine)-Constipation or diarrhea, dizziness, fatigue, fever.

Prilosec (omeprazole)-Nausea and vomiting, headache, diarrhea, abdominal pain.

Tagamet (cimetidine)-Headache, breast development in men, depression and disorientation.

Zantac- (ranitidine hydrochloride) Headache.

2.10 Role of natural products/medicinal plants

Around the world, many medicinal plants are used to treat peptic ulcer in folk medicine by different tribes and culture. Some of these medicinal plants are collected from the wild or in gardens close to their houses. The plant or parts of plant used vary from people to people. Some of these plants used for the management of peptic ulcer in folklore include *Ficus tinctoria* G.Forst. ssp. *F.gibbosa* (Blume.) Moraceae (Raghunathan, 2017), *Adansonia digitate, Balsamodendron mukul, Berberis aristata* (Vimala and Shoba, 2014), *Artocarpus heterophyllus* (Lam) (Lawal *et al*., 2010), *Glycyrrhiza glabra* L. (Alzweiri *et al*., 2011), *Litsea cubeba* (Lour.) Pers. (Adhikari *et a*l., 2019) amongst others.

Medicinal plants have been a valuable source of therapeutic agents to treat various disorders including gastric/peptic ulcers. Recent progress in the understanding of the pathogenesis of gastric ulcer has led to the development of newer and better drug therapy (Pakodi, 2000).

2.10.1 Medicinal plants with antiulcer properties

Mentha piperita L.*:* - Peppermint, scientifically known as *Mentha piperita* consists of the fresh plant of the *Mentha piperita,* belonging to family Labiatae. Evaluated aqueous extract of leaves at the dose of 250 mg/kg b.w and 500 mg/kg b.w. separately was effective against ethanol, pylorus ligation and by necrotizing agent induced ulcer in rats (Al-Mofleh *et al*., 2006).

Azadirachta indica A. Juss*:* - Neem, scientifically known as *Azadirachta indica*. Neem consists of all aerial parts of plant known as *Azadirachta indica*, belonging to family Meliaceae. Evaluated aqueous extract of Neem bark at the dose of 30 mg/kg b.w. 60mg/kg b.w. separately was effective against ulcer in rats (Bandyopadhyay *et al*., 2004).

Curcuma longa L.*:* - Indian saffron and Turmeric, scientifically known as *Curcuma longa* consists of dried as well as fresh rhizomes of plants known as *Curcuma longa*, belonging to the family Zingiberaceae. Evaluated ethanolic

extract of Tumeric dried rhizomes at the dose of 125 mg/kg b.w, 250 mg/kg b.w., 500 mg/kg b.w. separately was effective against ethanol, and indomethacin induced gastric ulcer in rats (Rafatullah *et al*., 1990).

Ocimum sanctum L*.:* - Tulsi, scientifically known as *Ocimum sanctum* consist of dried and fresh leaves of *Ocimum sanctum*, belonging to family Labiatae. Evaluated aqueous extract of Tulsi leaves at the dose of 100 mg/kg b.w. and 200 mg/kg b.w. separately was effective against ethanol-induced gastric ulcer in rats (Ghangale *et al*., 2009).

Zingiber officianale Roscoe*:* - Ginger, scientifically known as *Zingiber officinale* consists of rhizomes of Zingiber officinale, belonging to family Zingiberaceae. Evaluated ethanolic extract of ginger rhizomes at the dose of 100 mg/kg b.w 200 mg/kg b.w, and 400 mg/kg b.w separately was effective against indomethacin-induced ulcer in rats (Anosike *et al*., 2009).

Andrographis paniculata Wall*:* - Kirayat, scientifically known as *kalmegh* consists of dried leaves and tender shoots of the plant known as *Andrographis paniculata* belonging to family Acanthaceae. Evaluated ethanolic extract of kalmegh dried whole aerial part at the dose of 200 mg/kg b.w. was effective against cysteamine induced duodenal ulcer in rats (Saranya and Geetha, 2011).

Terminalia chebula Retz.*:* - Myrobalans, scientifically known as *Terminalia chebula.* Myrobalans are the dried fruits of *Terminalia chebula* belonging to family combretaceae. Evaluated Methanolic extract of Myrobalan dried fruits at the dose of 250mg/kg b.w. and 500mg/kg b.w. separately was effective against ethanol and pylorus ligation induced ulcer in rats (Raju *et al*., 2009).

Momordica charantia L.: - Karela, scientifically known as *Momordica charantia* consists of fresh green fruits. It belongs to the family Cucurbitaceae. Evaluated alcoholic and aqueous extract of karela fruits at the dose of 200 mg/kg b.w. and 400 mg/kg b.w. separately was effective against aspirin, stress and pylorus ligation induced ulcer model in rats (Venkatrao *et al*., 2011)

Glycyrrhiza glabra L.*:*- Liquorice, scientifically known as *Glycyrrhiza glabra*

consists of the dried unpeeled roots and stolons belongs to the family Leguminosae. Evaluated methanolic extract of Liquorice roots at the dose of 200 mg/kg b.w, and 800 mg/kg b.w. separately was effective against ethanol-induced ulcer in rats (Mostafa *et al*., 2004).

Emblica officinalis Geart..:- (Amla), scientifically known as *Emblica officinalis* consists of the dried, as well as fresh fruits of the plant (*Phyllantus emblica* Linn). It belongs to the family Euphorbiaceae. Evaluated methanolic extract of amla fruit at the dose of 250mg/kg b.w. and 500mg/kg b.w. separately was effective against indomethacin and pylorus ligation induced ulcer in rats (Al-rehaily *et al*., 2002)

Withania somnifera L. :- Ashwagandha, scientifically known as *Withania somnifera* consists of dried roots and stem bases It belongs to the family Solanaceae. Evaluated methanolic extract of ashwagandha root at the dose of 100 mg/kg b.w. was effective against stress and pylorus ligation induced ulcer in rats (Bhatnagar *et al*., 2005).

Parquetina nigrescens : Kayode *et al*, observed that pretreatment with *P. nigrescens* was found to exact a significant gastro protective and antiulcer effect partly by protecting against the ethanol-induced ulcerogenic effects in experimental rats and probably through the induction of antioxidant enzymes in experimental animals (Kayode *et al*., 2009).

Securidaca longepedunculata Fres. and *Luffa cylindrica* (L.) M.Roem: *Securidaca longepedunculata* (SL) and *Luffa cylindrica* (LC) are medicinal plants commonly used in combination with other recipes in the treatment of many ailments in traditional medicine among the Yoruba ethnic group in Nigeria. The antiulcer effects of the leaf extracts (methanol, ethyl acetate and hexane extracts) of SL and LC in rats has been examined. The antiulcer effects of the extracts were assessed using ethanol-induced gastric ulcer model of ulceration in rats. The vehicle, test drug or plant extracts were administered 50 min prior to ulcerogenic procedure. Ulceration was induced in animals with 1 mL absolute ethanol one hour before being sacrificed. Ulcer index, protein content and lipid peroxidation activity of the gastric tissues were

determined. The standard antiulcer drug, Cimetidine (50 mg/kg b.w), used as negative control, has percentage protection of 59.4% and the methanol (150 and 300 mg/kg b.w), ethyl acetate (150 and 300 mg/kg b.w) and hexane (150 and 300 mg/kg b.w) extracts of SL showed (68.8% and 78.1%), (93.8% and 87.5%) and (71.9% and 75.0%), protection, respectively. Similarly, the 150 mg/kg b. w ethyl acetate extract of LC gave 78.1% inhibition while the 300 mg/kg ethyl acetate extract revealed 87.5% protection. Further, investigation showed that the extracts increased the protein content in the gastric tissues, which suggests an ability to stimulate tissue regeneration. Lipid peroxidation levels were significantly ($p<0.05$) lowered in the pretreated groups. Taken together, the study demonstrated that the leaf extracts of SL and LC are possible potent gastro protective and antiulcer agents against ethanol-induced gastric ulcer thus providing evidences that may justify their ethno medicinal uses asantiulcer agents (Kayode *et al*., 2015)

2.10.2 Phytochemicals or secondary metabolites with antiulcer effects

Examples of compounds with antiulcer effects have been isolated from medicinal plants (Table 2.1) while the mechanism of action of some secondary metabolites from medicinal plants with antiulcer properties have also been established and reported (Table 2.2) (Oyagi, 2010).

Table 2.1: Antiulcer constituents isolated from medicinal plants

Plant Name	Family	Compounds	Reference
Aralia elata (Miq.) Seem	Araliaceae	Araloside	Lee *et al*., 2005
Tithonia diversifolia (Hemsl.) A.Gray	Asteraceae	Tagitinin C	Sanchez-Mendoza *et al*., 2011

Jasminum Grandiflorum L	Oleaceae	Methyl jasmonate	Umamaheswari et al., 2007
Phyllanthus niruri L	Euphorbiaceae	4-methoxy-securinine	Okoli *et al*., 2009
Qualea grandiflora Aubl.	Vochysiaceae	Sitosterol	Hiruma-Lima *et al*., 2006
Tectona grandis L.f.	Verbenaceae	Verbascoside (phenylethanoid)	Singh *et al*., 2010
Cucumis sativus L.	(Cucurbitaceae)	9-beta-methyl-19-norlanosta-5-ene (cucurbitane glycoside)	Dhiman *et al.,* 2000
Sargassum Micracanthum (Kützing) Endlicher	Sargassaceae	Plastoquinones	Mori *et al*., 2006

Table 2.2: Some secondary metabolites with antiulcer effects

Chemical Class	**Compound**	**Model**	**Activity**	**Mechanism of Action**
Caroteneoids/ Polyisoprenoids	teprenone	rats/aspirin + HCl, CRS, acetic acid, C-48/80	prophylactic + healing	preservation of mucus, antioxidative
Sesquiterpenoids	plaunotol	rats/C-48/80, indomethacin	Prophylactic	mucosal PG enhancement, antioxidative

Diterpenoids	*trans*-DHC	mouse, rats/ stress, ethanol + HCl	Prophylactic	--
Triterpenoids	glycyrrhizic acid	rats/ indomethacin	Healing	PG synthesis, mucus promotion, cell proliferation
Phenols	eugenol	rats/EtOH	Prophylactic	--
Quinones	Garcinol	rat / indomethacin, stress	Prophylactic	Antioxidative

2.11 *In vivo* models in gastro protection studies

Peptic ulcers can be induced by physiological, pharmacological or surgical manipulation in several animal systems. However, rodents are the most commonly used as *in vivo* experimental models. The principles of those that are most frequently used by researchers investigating the gastro protective effects of plants or herbal remedies, along with their underlying mechanisms of action, are described below.

2.11.1 Absolute ethanol-induced gastric lesions

Gastric mucosal injury may occur when noxious substances such as gastric acid and HCl secretion into the gastric lumen (DeFoneska and Kaunitz, 2010) impair defense mechanisms. Administration of absolute ethanol by gavage has long been used as a reproducible method to induce gastric injury in experimental animals (Kayode et al., 2009; Kayode et al., 2015; Thabrew and Arawwawala, 2016). Ethanol can promote the development of gastric lesions by exposing the mucosa to the hydrolytic and proteolytic actions of hydrochloric acid and pepsin (Glavin and Szabo, 1992). Moreover, ethanol can stimulate gastric acid secretion, resulting in microvascular injuries that facilitate vascular permeability, through reflex release of gastrin and histamine from sensitive nerve terminals present in the gastric mucosa. It is known that intra-gastric administration of ethanol results in gastric mucosal injury characterized by disturbances in microcirculation, mast cell secretary products, inhibition of prostaglandin synthesis, reduction in mucus production and reactive species (Samonina et al., 2004). Ethanol is also known to increase cellular oxidative stress (Repetto and Llesuy, 2002) and produce alterations in gastric cell calcium levels (Wong et al., 1991) that may lead to the pathogenesis of gastric mucosal injury.

The variety of damaging effects mediated by ethanol has been exploited in developing the ethanol induced gastric ulcer model for testing the gastro protective potential of various plants/ natural compounds. However, because this model is independent of gastric acid secretion, it is not suitable to evaluate protection against ulceration dependent on acid secretion. Because ethanol can directly enhance the levels of free radicals that can mediate alterations in cell structure and function or contribute to other mechanisms that

support oxidative damage (Samonina et al., 2004) and can also mediate direct toxic effects on the gastric mucosa resulting in reduced secretion of bicarbonates and gastric mucous production, it is more appropriate to use this model for evaluating the gastro protective potential of test materials that have cytoprotective and/or antioxidant activities . To induce ulcers with ethanol, rats that have been fasted for 24-36 h are pretreated with vehicle, extracts, or reference drug orally. After 1h, ulcers are induced by administration of absolute ethanol orally and kept for another hour after which rats are sacrificed, stomachs excised and severity of ulceration measured (Kayode et al., 2015; Thabrew and Arawwawala, 2016).

2.11.2 HCl-ethanol induced gastric lesions

This model may be considered to be an advanced model of the absolute ethanol induced ulcer model discussed before. Instead of ethanol only, a mixture of HCl and ethanol are used to induce ulceration. Gastric lesions induced by HCl/ethanol are due to direct necrotizing action on the gastric mucosa. Combination of ethanol with HCl is considered to accelerate the progress of ulcerogenesis and enhance gastric injury (Oates and Hakkinen, 1988). In this model, 18-24 h fasted mice are pretreated with vehicle, extracts, or reference drug orally. After 1 h, gastric lesions are induced by administrating the ethanol/HCl mixture. The animals are euthanized after 30 min or 1 h, stomachs excised and severity of ulceration measured (Thabrew and Arawwawala, 2016).

2.11.3 Water-immersion stress or cold-resistant stress induced gastric ulcers

Gastric ulcers induced by water –immersion stress or cold resistant stress in rats or mice are known to resemble human peptic ulcers, both grossly and histopathologically (Konturek *et al*., 2003). In water-immersion stress model, animals are fasted for a period of 24-36 h prior to the experiment and treated with vehicle or test drug or reference drug.

After 30 min animals are placed individually in each compartment of a stress cage, immersed vertically up to the xyphoid level in a water bath, and kept for 7 h to induce stress ulcer. After 7 h animals are sacrificed and ulceration quantified. In cold resistant stress model animals are fasted for a period of 18 h prior to the experiment treated with vehicle or test drug or reference drug. After 1 h, rats are individually restrained in plastic cages in a refrigerator for 2-4 h and sacrificed. Finally, stomachs are taken out and severity of ulceration measured (Thabrew and Arawwawala, 2016).

2.11.4 Pylorus-ligated-induced peptic ulcer

The ligation of the pylorus end of the stomach causes accumulation of gastric acid in the stomach, which in turn produces ulcers. Ulcers result from a breakdown of the gastric mucosal barrier resulting from auto digestion of the gastric mucosa. This model is useful for evaluating the (a) cytoprotective effects of drugs that increase secretion of mucus and (b) anti-secretory drugs that reduce secretion of gastric aggressive factors such as acid and pepsin. Pylorus ligation is performed according to the method described by Shay *et al*. (1945) with slight modifications. In brief, animals fasted for 48 h, are pretreated with vehicle, extracts, or reference drug orally, and after 1 h pyloric end of the stomach is ligated under ether anesthesia. Stomach is then placed carefully in the abdomen and the wound sutured by interrupted sutures. Animals are sacrificed after 4 h and their stomachs are removed and longitudinally excised along the greater curvature. The inner surface is then examined for ulcerative lesions. The gastric volume and acidity are then assessed according to method described by Shay *et al*. (1945).

2.11.5 Non-steroidal anti-inflammatory drugs (NSAID's) induced gastric ulcers

Gastric ulcers are known to be induced by excessive use of many Non-Steroidal Anti-Inflammatory Drugs (NSAID's) such as indomethacin, aspirin and ibuprofen (Rainsford, 1987). This property has been exploited for the development of NSAID's induced gatric ulcer models in rats. This is one of the most commonly used models for investigating

antiulcer properties of test agents. NSAID's induce gastric ulcers by inhibiting prostaglandin synthesis via the cyclooxygenase pathway (Rainsford, 1987; Wallace, 2000). In the stomach, prostaglandins protect against mucosal injury by stimulating bicarbonate and mucus secretion, maintaining mucosal blood flow and regulating mucosal cell turnover and repair (Lamarque, 2004). NSAID's can also induce mucosal damage by enhancement of reactive oxygen free radical production and neutrophil infiltration (Lamarque, 2004). Furthermore, NSAID's (especially those of acidic nature) can exert direct cytotoxic effects on epithelial cells and disrupt surface active phospholipids on the mucosal surface, independent of effects on synthesis of prostaglandins, thus making the mucosa more susceptible to damage by luminal acid (Wallace, 2008). This model is therefore useful for evaluating the ability of antisecretory and cytoprotective agents to protect against gastric ulcer development. To evaluate gastro protective potential of test agents, rats fasted for 24-36 h are orally administered the selected NSAID dissolved in an appropriate vehicle (e.g. water, 1% carboxymethyl cellulose) and after 1 h treated with the different doses of the test agent. After 4 h post treatments with the test agent, rats are sacrificed, stomachs excised and severity of ulceration measured (Thabrew and Arawwawala, 2016).

2.11.6 Histamine-induced gastric ulcers

Histamine is known to be one of the important factors mediating formation of gastric ulcers, and this is the basis of the histamine induced gastric ulcer model (Adinortey *et al*., 2013). Histamine released from mast cells binds with receptors present on the surface of parietal cells and causes activation of adenylate cyclase, which converts ATP into c-AMP. This conversion is responsible for enhanced secretion of HCl from parietal cells (Sander *et al*., 2006). Histamine also has vasodilating ability and reduces mucus production. These pharmacological effects of histamine have been exploited in producing the histamine-induced ulcer model. This model is useful for evaluating the antisecretory effects of drugs and other agents that function as H2-receptor antagonists. To induce ulcers with histamine, animals fasted for 18- 24 h are subcutaneously administered

histamine phosphate. The animals are sacrificed after 2 h or 4 h following histamine administration and the stomachs dissected out after pylorus and cardiac ligation (Shan *et al*., 2006). The gastric content is collected into a centrifuge tube for determination of pH and total acidity. This model has been used to evaluate gastro protection by fixed oil (3 mL/kg) (Singh and Majumdar, 1999) and cold ethanolic leaf extract of Ocimum sanctum Linn. (50, 100 mg/kg) (Dharmani *et al*., 2004). Gastric ulceration was induced by intraperitoneal administration of histamine acid phosphate using male and female guinea pigs (300-350 g). Omeprazole (10 mg/kg p.o) was used as reference drug (Dharmani *et al*., 2004).

2.11.7 Serotonin-induced gastric ulcers

Serotonin is a vasoconstrictor and ulceration induced by serotonin is believed to arise from a disturbance of gastric mucosal microcirculation (Singh and Majumdar, 1999). In this model, rats fasted for 24 h are pretreated with vehicle, extracts, or reference drug. After 30 min serotonin, creatinine sulphate (20-50 mg/kg) is administered subcutaneously and animals sacrificed. Their stomachs longitudinally excised after 6-18 h along the greater curvature. The inner surface is then examined for ulcerative lesions.

2.11.8 Acetic acid-induced gastric ulcers

Takagi *et al*. (1969) developed a model for inducing chronic gastric ulcers in rats by sub-mucosal injection of acetic acid. These ulcers resemble chronic ulcers in humans both grossly and histologically. To overcome certain problems, the original method has undergone several modifications and the one that is currently most favoured is that developed by Okabe and Pfeiffer (1972). This method involves application of acetic acid solution intraluminally. The acetic acid induced ulcer model is most suitable for evaluating the effects of various test agents on the healing process of chronic peptic ulcers and also for screening their antisecretory and cytoprotective effects.

Diethyldithiocarbamate induced gastric ulcers: The diethyldithiocarbamate model is useful to assess whether antioxidant activity is a mechanism by which a test agent mediates gastro protection (Oka *et al*., 1990) and to evaluate the cytoprotective potential of test agents. Antral lesions are induced by diethyldithiocarbamate through the mobilization of superoxide and hydroxyl radicals (Salim, 1991). In this model, animals are fasted for 24 h and water withheld for 2 h before commencement of experiment. Acute glandular lesions are then induced by subcutaneous injection of 1 mL of diethyldithiocarbamate in saline followed by oral dose of 1 mL of 0.1N HCl (Thabrew and Arawwawala, 2016).

2.12 *Securidaca longepedunculata*

The genus *Securidaca* comprises about 80 species, characterized by papillionaceous purplish flowers and mostly scandent shrubs and lianas, which produce compounds known as securixanthones with antimicrobial and antioxidant properties (Da Costa *et al*., 2013, Mongalo *et al*., 2015). Although protected under provincial and national legislation, *S. longepedunculata* stem bark and roots are still found amongst the most traded medicinal plants in Africa (Tabuti *et al*., 2012). The species is threatened by various anthropogenic and environmental conditions including seasonal fires, droughts, and debarking (Oni *et al*., 2014). According to Baloyi *et al*. (2012), seeds of *S. longepedunculata* grow better at a soil depth of 4 cm between 20 and 30ºC. The seedling survival rate declines with increasing concentrations of gibberellic acid in a growing medium with a compost manure (Mongalo *et al*., 2015).

2.12.1 Taxonomy of *Securidaca longepedunculata* Fres.

Kingdom: Plantae
Division: Phyta
Class: Angiosperms

Order: Fabales

Family: Polygalaceae

Genus: Securidaca

Specie: *Securidaca longepedunculata*.

2.13 Botanical description and distribution

Securidaca longepedunculata Fresen, (synonyms *Securidaca longepedunculata* var. *longepedunculata* or *Elsota longepedunculata*, family Polygalaceae) (Figure 2.3) is a small tree up to 6 meters high with a pale grey, smooth bark and oblong, more or less hairless alternate leaves that are variable in size and shape and crowded towards the stem tips (Van Wyk *et al*., 2009). Clustered flowers are small, pink to lilac or purple in colour, sweet scented and are produced in early summer. Fruits are a round nut, heavily veined, occasionally smooth, oblong, purplish green when young and possess a membranous wing of about 4 cm long (Coates-Palgrave, 2005). The species is mostly distributed in various tropical African countries, including Angola, Benin, Botswana, Burundi, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Sudan, Tanzania, Uganda, Zambia, Zimbabwe, Mozambique, as well as in the North West and Limpopo Provinces of South Africa (Mongalo *et al*., 2015).

Figure 2.3: *Securidaca longepedunculata* collected at Adesiyan Village, Ido Local Government Area (LGA), Oyo State (Date of collection: January, 2014).

2.14 Local names

In South African languages, the plant species is called violet tree, fiber tree, or Rhodesian violet (English), In other African countries, various names in different cultural and ethnic groups have been used Amharic (esamanahi); Arabic (saggat,alali); Lozi (mwinda); Lunda (mutata); Nyanja (mwinda,mpuluka); Bemba (mupapi); Luganda (lilo); Hausa (uwar magunguna,sanya); Mandinka (yodo,juto,jodo); Shona (mufufu); Swahili (muteya, mzigi, Chipvufana, mufufu, munyapunyapu, munyazvirombo, mutangeni, umfufu); Tigrigna (shotora); Tongan (njefu,bwazi,mufufuma); Wolof (fouf); Yoruba (ipeta). (Orwa *et al*., 2009, Mongalo *et al*., 2015).

2.15 Ethnomedicinal uses

In many parts of Africa, the plant (*Securidaca longepedunculata*) is employed in traditional medicine principally for its psychotropic properties (Winkleman and Dobkin, 1989). Other uses include treatment of various sexually transmitted infections, hernias, coughs, fever, ascariasis, constipation, headaches, rheumatism, stomach ache, malaria, tuberculosis, pain, epilepsy, pneumonia, skin infections, peptic ulcer disease and it is also used as an aphrodisiac for men (Kayode *et al.*, 2015, Mongalo *et al.*, 2015). Powdered dried root is also used as a pest control agent in stored grain (Belmain *et* al., 2001). Due to the presence of saponins, bark root bark and crushed seeds gives a soapy solution in water and are used as soap for washing or bleaching clothes (Orwa *et al*., 2009). Fresh

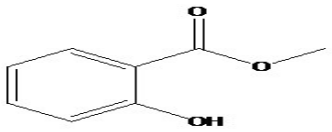
leaves of *Securidaca longepedunculata* are made into paste with little or no water along with the bark of *Gardenia erubescens* and applied externally twice a day for sixty-three days to treat skin cancer (Mustapha, 2013a; Mongalo *et al*., 2015). Moreover, fresh leaves of *Securidaca longepedunculata* are made into paste with little or no water along with leaves of *Jussiaea suffruticosa* and shea butter and the resulting mixture is applied externally, twice a day to treat a variety of skin infections. Dry leaves of *Securidaca longepedunculata* are also ground into powder and put into the fire and the resulting smoke is inhaled to treat headaches while the boiled leaves are taken orally for contraceptive purposes (Mustapha, 2013b). The leaves of *Securidaca longepedunculata* are either chewed fresh or both orally and nasally administered to treat epilepsy, headaches, stomach ache, infertility, snakebite, toothache and to expel the placenta (Augustino *et al*., 2011; ; Mongalo *et al*., 2015).

2.16 Phytochemistry

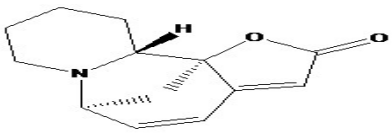
The volatile oil of the roots of *Securidaca longepedunculata* contains large amounts of methyl salicylate (Van Wyk *et al*, 2005). The report agrees with those of Jayasakara *et al*. (2002) and Lognay *et al*. (2000), which revealed that the major component (over 90%) of the volatile material from the root bark is methyl-2-hydroxybenzoate (methyl salicylate) **(1)**. Furthermore, securinine **(2)**, presenegenin, 2-hydroxybenzoate esters such as methyl 2-hydroxy-6 methoxybenzoate and its benzyl analogue were also reported. In general, most classes of compounds have been isolated from the roots, using variety of solvents. This may well explain the ethnomedicnal uses and hence the biological activity studied on the plant species.

The aqueous root of *Securidaca longepedunculata* and ethanol extracts yielded alkaloids, cardiac glycosides, flavonoids, saponins, tannins, volatile oils, terpenoids and some steroids (Gbadamosi, 2012) while chloroform and ethanol extracts indicated flavonoids, saponins, coumarins, tannins and alkaloids (Adebayo and Osman, 2012). The ethyl acetate fraction of the root of *Securidaca longepedunculata* contained compounds such as

1,5-dihydroxy 3,4,6,7,8-pentamethoxyxanthone, 1,7-dihydroxyxanthone, 5-O-prenyl-1-hydroxy 2,3,6,7,8-pentamethoxyxathanthone, 2-hydroxy-1, 7-dimethoxyxanthone, β Sitosterol **(3)**, 1,7-dihyroxy-4-methoxyxanthone, quercetin-3-O-β-galacto-pyranoside and 3-hydroxy-6-methoxysalicylic acid (Meli *et al*., 2007). The compounds 1,3,6,8 tetrahydroxy-2,5-dimethoxyxanthone and 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone were also isolated from the acetone extract of the fresh root bark (Meyer *et al*., 2008). Moreover, the hexane extract of the root indicated the presence of dihydroxy-2, 3, 6, 7, 8-pentamethoxyxanthone, 2-hydroxy-l, 7 dimethoxyxanthone and 1,6-dihydroxy-xanthone (Lannang *et al*., 2006). The water and aqueous methanol extracts from the root yielded a variety of compounds in varying amounts, including gallic acid, chlorogenic acid, caffeic acid, epicatechic acid, rutin, p-coumaric acid, cinnamic acid, apigenin, quercetin glucosyl and quercetin dihydrate (Muanda *et al*., 2010). Four highly oxygenated xanthones, muchimangins A-D, with a diphenylmethyl substituent have also been isolated from the root as minor constituents (Dibwe *et*



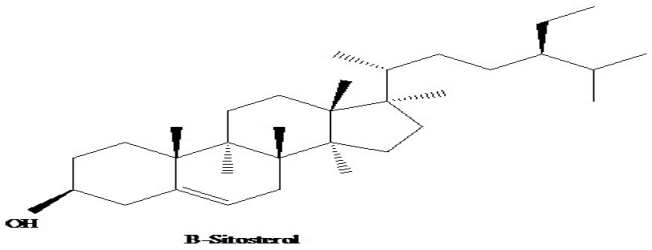
Methyl salicylate



Securinine

Methyl salicylate Securinine

(1) **(2)**



β-Sitosterol

(3)

al., 2012). The dichloromethane extract of the root bark yielded 4-methoxy-benzo[1,3] dioxol-5-yl-phenyl methanone and three other known compounds namely 1,7-dihydroxy-4-methoxyxanthone, benzyl-2 hydroxy-6-methoxybenzoate and methyl-2-hydroxy-6-

methoxybenzoate (Joseph *et al*., 2006). The chloroform extract of the root of *Securidaca longepedunculata* contained compounds such as 2 methoxy-3,4-methylenedioxybenzophenone, benzyl 2-hydroxy-6- methoxybenzoate, 6-hydroxy-2-methoxy benzoic acid, 1,6,8-trihydroxy-2,3,4,5-tetramethoxyxanthone,1,6-dihydroxy-2,3,4,5,8-pentamethoxyxanthone,8-hydroxy-1,4,5,6tetramethoxy-2,3

methylenedioxyxanthone,4,6,8-trihydroxy,1,2,3, tetramethoxyxanthone, 4,8 dihydroxy-1,2,3,5,6-pentamethoxyxanthone, benzyl 3hydroxy-2-methoxybenzoate and some other xanthones (Dibwe *et al*., 2013). Triterpene saponins such as 3-O-β-D-glucopyranosyl presenegenin 28-O-β-D apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-Lrhamnopyranosyl-(1→2)-{4-O-[(E)-3,4,5-trimethoxycinnamoyl]}-β-D-fucopyrano ester and three other related esters have been isolated from the 70% aqueous methanol root extract (Mitaine-Offer *et al*., 2010). Besides sinapoic acid, caffeic acid, 4,5-dicaffeoyl-D-quinic acid, 3,4,5-tricaffeoyl-D quinic acid and a considerable number of monosaccharide and polysaccharide conjugates, the methanol extract of the stem bark revealed the two bitter principles β-D-(3,4-disinapoyl)(fructofuranosyl-α-D-(6-sinapoyl)glucopyranoside and β-D-(3 sinapoyl) (fructofuranosyl-α-D-(6-sinapoyl)glucopyranoside (De Tommasi *et al*., 1993). Although many compounds have been isolated from the root and root bark, there is a need to further explore the phytochemistry of the stem bark and leaves of the species.

2.17 Pharmacological actions of *Securidaca longepedunculata*

2.17.1 Antioxidant activity

A 70% methanol extract of the leaf of *Securidaca longepedunculata* exhibited an IC_{50} of 79.35 µg/ml against 2,2 diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical (Karou *et al*., 2012), while the essential oil of the root bark exhibited an IC_{50} of 500 mg/L (Alitonou *et al*., 2012). The aqueous methanol extract (50%) of the root bark exhibited an IC_{50} of 1.351 and 9.48 µg/ml against ABTS and DPPH respectively (Muanda *et al*., 2010). Although the extraction methods of the leaf and root bark were slightly different, these results may well suggest that the root bark extract quenches DPPH much better than the leaf extract. A variety of compounds belonging to a variety of classes reported in the phytochemistry section of this paper may play a role in the antioxidant properties of the species. The antioxidant activity may be of greater important in preventing oxidative stress which may be involved in many fatal infections and diseases (Mongalo *et al*.,

2015).

2.17.2 Anti-inflammatory properties

The 50% aqueous methanol extract of *Securidaca longepedunculata* showed good anti-inflammatory activity in a dose dependent manner by exhibiting reduction of NO production in macrophages stimulated with LPS/IFN-gamma yielding 51.3% inhibition at a concentration of 150 µl (Muanda *et al*., 2010). The methanol extracts, petroleum ether and methanol fractions obtained from solvent extraction of the root bark were also investigated for anti-inflammatory properties using topical edema of the mouse ear model (Okoli *et al*., 2005). The petroleum ether fraction, methanol fraction and methanol extract revealed 65.63, 53.13 and 40.63% inhibition respectively. The extracts of these species exhibited good anti-inflammatory activity in different models. Interestingly, the water extracts, namely decoctions and infusions, are commonly applied in African indigenous medicine for treating various infections (Mongalo *et al*., 2015).

2.18 *Luffa cylindrica*

Luffa cylindrica (Linn) M.Roem (Synonym: *L.aegyptica* ex Hook) family: Cucurbitaceae. Common Names: sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd. *Luffa cylindrica* (Figure 2.4) is a large climbing vine, with a thin but very tough light green, succulent stem, attaining a length of 10-30 feet. Luffa is a subtropical plant, which requires warm summer temperatures and a long frost-free growing season when grown in temperate regions. The leaves are alternate, palmately lobed, of a light green color, and almost destitute of taste. The flowers are monoecious, petals five, united below into a bell shaped corolla; anthers cohering in a mass; ovary two celled, style slender, stigmas three, both male and female flowers are on the same plant and are pollinated by bees. The fruit is elliptical ovate, fleshy and dehiscent with a green epidermis, longitudinally marked with black ridges varying from 10-15 in a number; under each of

these

Figure 2.4*:* Leaves of *Luffa cylindrica* collected at Igboloye, Ota, Ogun State (Date of collection: January, 2014).

ridges is found a tough woody fiber. It is generally 2-3 inches in diameter and 15-18

inches in length. The exterior is green, sometimes molted and smooth. The genus *Luffa* comprises five species of tropical vines, four native to the Old World and one, *L. operculata*, to the New World. Two species, *L. aegyptiaca* and *L. acutangula*, include domesticated plants that are now widespread in the tropics. Interspecific hybrids are sterile or nearly so. Intraspecific hybrids within *L. acutangula* and *L. aegyptiaca* are fertile, but the hybrid within *L. operculata is sterile*. Phenetic and cladistic analyses indicate that the species are well differentiated with *L. echinata* the most distinct. The fruit of these species is cultivated and eaten as a vegetable. The fruit must be harvested at a young stage of development to be edible. The vegetable is popular in China and Vietnam (Oboh and Aluyor, 2009, Sangh *et al*., 2012).

2.18.1 Taxonomy

Luffa cylindrica (L.) Roem.
Kingdom: Plantae
Division: Mangoliophyta
Class: Mangoliosida
Order: Cucurbitales
Family: Cucurbitaceae
Genus: *Luffa*
Specie: *Cylindrica*
(Kirtikar and Basu, 1973)

2.18.2 Vernacular names

Hindi: *Ghiatarui,* Yoruba: *Kankan ayaba*

2.19 Geographical source

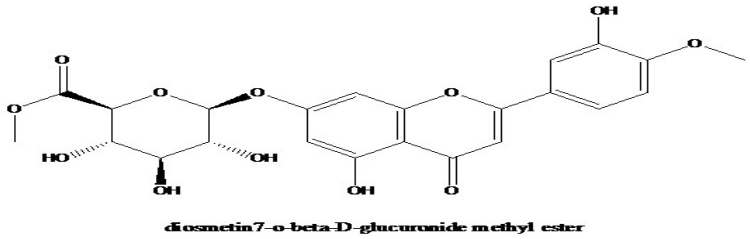
Luffa cylindrica is a sub-tropical plant, which requires warm summer temperatures and long frost-free growing season when grown in temperate regions. It is an annual climbing plant, which produces fruit containing fibrous vascular system. It is a summer season vegetable. It is difficult to assign with accuracy the indigenous areas of Luffa species.

They have a long history of cultivation in the tropical countries of Asia and Africa. Indo-Burma is reported to be the center of diversity for sponge gourd. The main commercial production countries are China, Korea, India, Japan and Central America (Oboh and Aluyor, 2009).

2.20 Phytochemistry

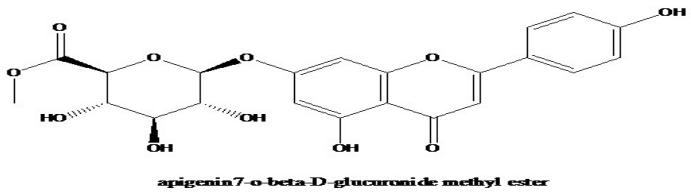
The fruit of *Luffa cylindrica* contains triterpenoid saponins: lucyosides A, B, C, D, E, F, G, H, I, J, K, L, M, ginsenosides Re, Rg1, etc. The leaf contains triterpenoid saponins: lucyin A, lucyosides G, N, O, P, Q, R, 21β- hydroxyoleanoic acid, 3-O-β-Dglucopyranosyl- maslinic acid (Liang, *et al*., 1993), ginsenosides Re, Rg1(Liang, *et al*., 1993), flavonoids:apigenin (Khan *et al*.,1992), etc. The seed contains polypeptides: luffins P1, S (Li *et al*., 2003), luffacylin (Parkash *et al*., 2002) among others. Du Q *et al*., 2006 has carried out Hydrophilic antioxidant constituents in the fruits of the vegetable *Luffa cylindrica* (L.) which were separated by an antioxidant-guided assay, which was evaluated by radical scavenging effect on the DPPH radicals and concluded that the consumption of sponge gourds can supply some antioxidant constituents to human body.
Anamika Khajuria and co-workers (2007) isolated two triterpenoids sapogenins 1 and 2 from the ethanolic extract of the defatted powdered seed of *L. cylindrica*. Yoshikawa *et al.* (1991) isolated two new fibrinolytic saponins, Lucyoside N and P from the seeds of *Luffa cylindrica* Roem. On the basis of chemical and spectral evidence, Lucyoside N was characterized as 3-O-beta-D-galactopyranosyl-(1----2)-beta-Dglucuronopyranosyl- 28O-beta-D-xylopyranosyl-(1----4)-[beta-D-glucopyranosyl-(1----3)]-alpha-L-rhamnopyranosyl-(1----2)alpha- arabinopyranosyl quillaic acid. Lucyoside P was characterized as a gypsogenin glycoside with the same sugar moiety as Lucyoside. Mohammad Hussain and others (2009) isolated 3-hydroxy-1-methylene-2, 3, 4, 4 tetrahydroxynapthalene-2-carbaldehyde (1), 22, 23-dihydroxy spinasterol (2) from petroleum ether extract of the fruits of *Luffa cylindrica*. The structures of the isolated compounds were elucidated by extensive spectroscopic studies including IR and high field NMR analyses. Petroleum ether extract (i.e. crude extract) of the fruits of *Luffa cylindrica* exhibited mild to moderate antimicrobial activity (Hossain *et al*., 2010). Other

compound previously isolated from *Luffa cylindrica* include diosmetin 7-o-beta D-glucuronide methyl ester **(4)**, apigenin 7-o-beta D-glucuronide methyl ester **(5)**, and luteolin 7-o-beta D-glucuronide methyl ester **(6)** (Parkash *et al*., 2002).



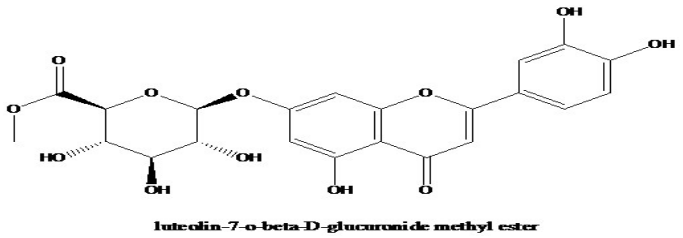
Diosmetin 7-o-beta-D-glucuronide methyl ester

(4)



Apigenin 7-o-beta-D-glucuronide methyl ester

(5)



Luteolin 7-o-beta-D-glucuronide methyl ester

(6)

2.21 Biological and medicinal uses of *Luffa cylindrica*

Earlier studies have shown that *Luffa cylindrica* possesses heptatoprotective, anesthetic activity, anti-inflammatory, anthelmintic, antimicrobial, and anticancer and enzyme inhibitory effects.

The plant is used as a bitter tonic, emetic, diuretic and purgative and it is useful in the treatment of asthma, skin diseases and splenic enlargement. It is used internally for rheumatism, backache, internal hemorrhage, chest pains as well as hemorrhoids. Young fruit can be eaten raw like cucumber or cooked like squash, while the young leaves; shoots, flower buds, as well as the flowers can be eaten after being lightly steamed (Sangh *et al*., 2012). The seeds can be roasted as a snack, or pressed to produce oil. Externally, it is used for shingles and boils. The dried fruit fibers are used as abrasive sponges in skin care, to remove dead skin. The fruits are anthelmintic, carminative, laxative, depurative, emollient, expectorant, tonic and galactagogue and are useful in fever, syphilis, tumours, bronchitis, splenopathy and leprosy. The vine is most commonly grown for the fibrous interior of the fruits. Kernel of seed is expectorant, demulcent and used in dysentery. Seed oil is used in leprosy and skin diseases. Fruit is intensely bitter

and fibrous. It has purgative property and is used for dropsy, nephritis, chronic bronchitis and lung complaints. It is also applied to the body in putrid fevers and jaundice (Muthumani *et al*., 2010; Sangh *et al*., 2012)

2.22 Pharmacological actions of *Luffa cylindrica*

Studies on the pharmacological actions of *Luffa cylindrica* revealed that the plant has anti-inflammatory, antifugal, antibacterial and brochodilatory activities, anti-myocardial ischemia, anti-hypertriglyceride, anti-allergy, analgesic, sedative, immunostimulatory and expectorant effects (Parkash *et al*., 2002; Muthumani *et al*., 2010, Sangh *et al*., 2012). Muthumani *et al* has carried out phytochemical screening and Anti-inflammatory, Bronchodilator and Antimicrobial activities of the seeds of *Luffa cylindrica* and concluded all the extracts revealed the presence of sugar, protein, alkaloids, flavonoids, sterols and glycosides as major constituents (Muthumani *et al* 2010). The extract of *Luffa cylindrica* showed very high degree of antifungal activity (Sangh *et al*., 2012).

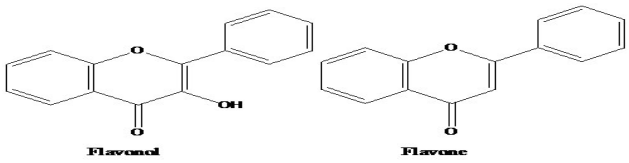
2.23 Secondary metabolites

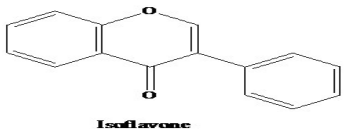
Plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites: *primary metabolites* involved directly in growth and metabolism (carbohydrates, proteins and lipids) and *secondary metabolites* which are considered as end products of primary metabolism but not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins, terpenes, flavonoids, saponnins, tannins, and anthraquinones etc). By definition, secondary metabolites are not essential for the growth, development and survival of a plant, but rather are required for the interaction of plants with their environment (Kutchan and Dixon, 2005). Secondary metabolites are also of interest because of their use as dyes, fibres, glues, oils, waxes, flavouring agents, drugs and perfumes, and they are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides (Dewick, 2002). Based on their

biosynthetic origins, plant secondary metabolites can be divided into three major groups: (i) flavonoids and allied phenolic and polyphenolic compounds, (ii) terpenoids and (iii) nitrogen-containing alkaloids and sulphur-containing compounds (Crozier *et al.,* 2006).

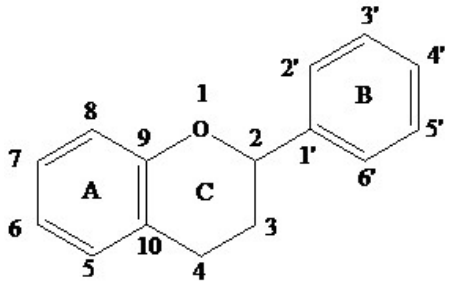
2.23.1 Flavonoids

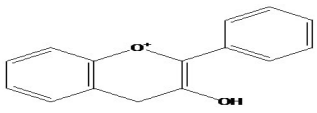
Flavonoids are polyphenolic compounds comprising fifteen carbons, with two aromatic rings connected by a three-carbon bridge (Figure 2.5). They are the most numerous of the phenolics and are found throughout the plant kingdom (Harborne, 1993). They are present in high concentrations in the epidermis of leaves and the skin of fruits and have important and varied roles as secondary metabolites. In plants, flavonoids are involved in such diverse processes as UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance (Pierpoint, 2000). The main subclasses of flavonoids are the flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins. Other flavonoid groups, which quantitatively are in comparison minor components of the diet, are dihydroflavonols, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones and aurones. The basic flavonoid skeleton can have numerous substituents. Hydroxyl groups are usually present at the 4_, 5 and 7 positions. Sugars are very common with the majority of flavonoids existing naturally as glycosides. Whereas both sugars and hydroxyl

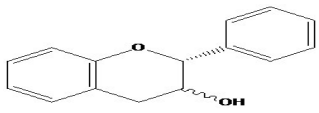


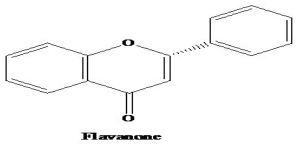


Flavonol Flavone Isoflavone









Anthocyanidin Flavan-3-ol Flavanone

Figure 2.5: Generic structures of the major flavonoids obtained from plants

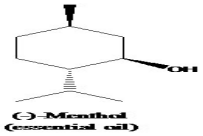
groups increase the water solubility of flavonoids, other substituents, such as methyl groups and isopentyl units, make flavonoids lipophilic.

Stanislavov and Nikolova reported that oral administration of L-arginine in combination with pycnogenol causes a significant improvement in sexual function in men with erectile dysfunction without any side effects (Stanislavov and Nikolova, 2003). Pycnogenol is a mixture of flavonoid compounds extracted from pine tree bark and is available as a dietary supplement (Huynth and Teel, 1999).

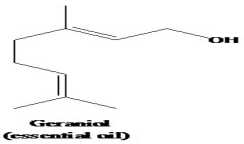
2.23.2 Terpenes

The terpenes, or isoprenoids, are one of the most diverse classes of metabolites. Although the final chemical structures of the terpenes are as diverse as their functions, all terpenes are derived from a sequential assembly of molecular building blocks, each of which consists of a branched chain of five carbon atoms. Some of the major families of terpenes are –(-) menthol (essential oil) **(7)**, geraniol (essential oil) **(8)**, (+)-o-pinene (from pine resin) **(9),** artemisinin (anti-malarial) **(10)**, cholesterol **(11)**, gibberellin A_1 **(12)**, β-sitosterol **(13)** and phytol (side chain of chlorophyll) **(14)** (Dewick, 2001). Classically it was thought that the terpenes were assembled from isoprene, hence their alternative name of isoprenoids. It is now known that the actual five-carbon building blocks in vivo are the interconvertible isomers isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These two building blocks are condensed together in a sequential fashion by the action of enzymes called prenyltransferases. The products include geranyl, farnesyl and geranylgeranyl pyrophosphates, squalene and phytoene, which are the direct precursors of the major families of terpenes (Figure 2.12.2). Subsequent modifications to the carbon backbone (typically by enzyme-catalysed cyclization, oxidation and skeletal rearrangement steps) give rise to the multitude of isoprenoid structures. There are over 30, 000 terpenes (Buckingham, 2004), mainly of

plant origin, encompassing flavours and fragrances, antibiotics, plant and animal hormones, membrane lipids, insect attractants and antifeedants, and mediators of the essential electron-transport processes which are the energy-generating stages of respiration and photosynthesis (Buckingham, 2004)



(-)-Methol
(essential oil)



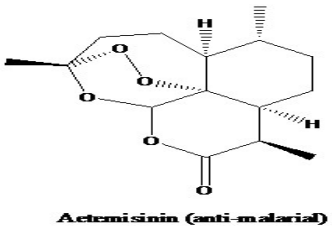
Geraniol
(essential oil)

(+)-o-pinene
(from pine resin)

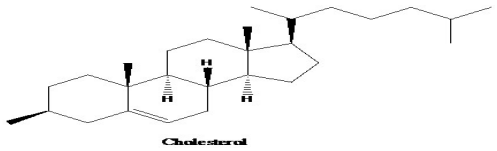
(-)-Methol (essential oil) **(7)**

Geraniol (essential oil) **(8)**

(+)-o-pinene (from pine resin) **(9)**

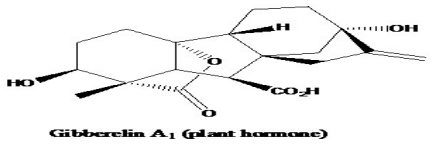


Artemisinin (anti-malarial)

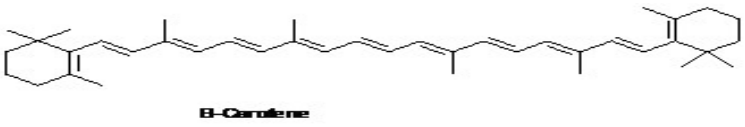


Artemisinin (anti-malarial) Cholesterol

(10) **(11)**



Gibberelin A_1 (plant hormone)



β - Carotene

(13)

Phytol
(side chain of chlorophyll)

Phytol
(side chain chlorophyll)
(14)

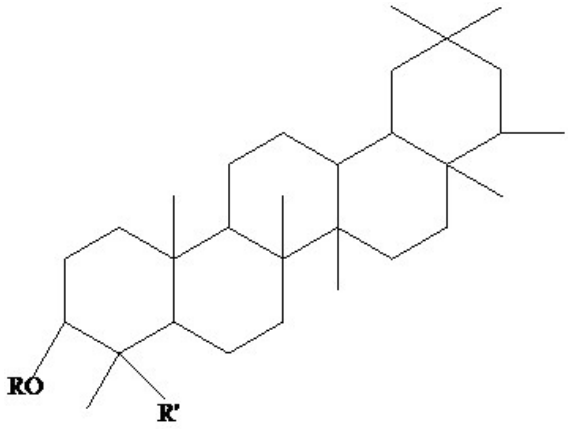
2.23.3 Alkaloids

Alkaloids are natural nitrogen-containing secondary metabolites mostly derived from amino acids and found in about 20% of flowering plants. They are not limited to plants but also occur in marine organisms, insects, microorganisms and some animals. Alkaloids

are significant for the protection and survival of plants because they ensure their survival against microorganisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrents) (Wink, 2003). Due to their potent biological activity, many of the approximately 12 000 known alkaloids have been exploited as pharmaceuticals, stimulants, narcotics and poisons (Wink, 1998). Plant-derived alkaloids currently in clinical use include the analgesics morphine and codeine, the anti-neoplastic agent vinblastine, the gout suppressant colchicine, the muscle relaxants (+)-tubocurarine and papaverine, the anti-arrhythmic ajmaline, and the sedative scopolamine. Other well-known alkaloids of plant origin include caffeine, nicotine, and cocaine, and the synthetic *O*-diacetylated morphine derivative heroin (Wink, 1998).

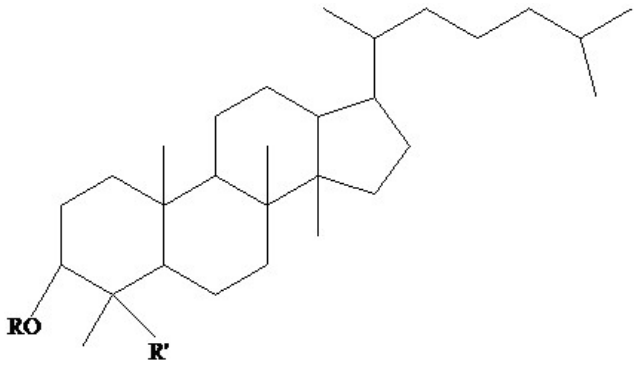
2.23.4 Saponins

Saponins are a group of natural compounds with triterpenoidal or steroidal aglycone structures, designated as genin or sapogenin, covalently linked to one or more sugar moieties (Augustin *et al.,* 2011). The saponins are naturally occurring surface-active glycosides. They are mainly produced by plants, but also by lower marine animals and some bacteria (Yoshiki *et al.,* 1998). They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid **(15)** or steroid **(16)** in nature (Yoshiki *et al.,* 1998).



Triterpenoid

(15)



Steroid

(16)

2.23.5 Tannins

Tannins are a group of complex oligomeric chains substances characterized by the presence of polyphenolic compounds. They are widely distributed in the plant kingdom in different parts (bark, needles, heartwood, grasses, seeds and flowers) of vascular plants, and can accumulate in large amounts in particular organs or tissues of the plant (Haslam, 1989). They have molecular weight higher than 500 kDa, reaching values above 20000 kDa. One of the major characteristic of tannins is its ability to form strong complexes with protein and to a lesser extent with other macromolecules such as starch, cellulose and minerals (Aguilar and Gutiérrez-Sánchez, 2001).

Tannins can be divided into hydrolysable and nonhydrolysable tannins or condensed tannins based on their structure and properties. Tannins do not function solely as primary antioxidants (*i.e.*, they donate hydrogen atom or electrons), they also function as secondary antioxidants. Tannins have the ability to chelate metal ions such as Fe (II), interfere with one of the reaction steps in the Fenton reaction, and thereby retard oxidation. The inhibition of lipid peroxidation by tannin constituents can act *via* the inhibition of cyclooxygenase.

2.23.6 Steroids

Steroids are (oxidized) derivatives of sterols; they have the sterol nucleus but lack the alkyl chain attached to the cholesterol ring, and are more polar than cholesterol (Nelson and Cox, 2005). Steroids are modified triterpenoids, lacking the three methyl groups at

C-4 and C-14. Hundreds of steroids are found in plants, animals and fungi. Skeletal modifications, especially to the side-chain, originate a wide range of biologically important natural products, e.g. sterols, steroidal saponins, cardioactive glycosides, bile acids, corticosteroids, and mammalian sex hormones. Sex steroids play important roles in the growth and differentiation of reproductive tissues and in the maintenance of fertility (Nelson and Cox, 2005).

2.23.7 Glycosides

Glycosides are complex organic substances, which hydrolyze to give sugar (glycone), and non-sugar (aglycone) compounds. They are known to exert pronounced physiological action, even though they may be poisonous to animals and man (Abdulshiri, 2004). Despite its toxicity, cardiac glycosides are the drug of choice for the treatment of congestive heart failure. In addition, glycosides with laxative, diuretic and antiseptic properties are used in therapy (Abdulshiri, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Consumable materials and reagents

Hand gloves, Disposable syringes, Absolute ethanol, Diethyl ether, Ethyl acetate, Methanol, n-hexane, Chloroform, Dichloromethane (DCM), Acetone, Acetonitrile, Water, DMSO, Hydrochloric acid, Phosphate buffer, 0.9% Normal saline, 10% Trichloroacetic acid (TCA), 0.75% Thiobarbituric acid (TBA), Biuret reagent, Distilled water, 10% formalin, Filter paper, Silica gel, Iodine crystals, Prep and analytical TLC plates,

3.1.2 Apparatus and equipment

Beakers, Measuring cylinders, Spatulas, Funnels, Dissecting set, Dissecting board, Homogenizer, Plain tubes, Test tubes, Test tubes rack, Conical flasks, Columns, Freezer, Spectrophotometer (Unispec SM7504UV, Surgified Medicals, England), Water bath (Uniscope SM801A, Surgified Medicals, England), Blender (Binatone , BLG-402), Plant Milling Machine (Classic YC 112m2-4, 5HP), Rotary Evaporator (Buchi Rotavapor R-200 – BUCHI Labortechnik AG, Switzerland), Bench centrifuge (Uniscope SM112, Surgified medicals, England), IR spectrophotometer (Bruker Tensor 27 FT-IR spectrophotometer), NMR spectrometer (Bruker AMX 400 MHz spectrometer and LC-MS spectrometer (Electrospray ionization Mass Spectrometry (ESI-MS) Waters Synapt G2, S.A).

3.2 Methods

3.2.1 Ethnobotanical survey

3.2.1.1 Study area

The study area Ado-Odo/Ota Local Government Area (LGA) of Ogun State comprises of eight locations, namely: Sango- Ota, Ota, Ota Market, Igbesa, Lusada Market, Igboloye, Iju, and Atan (Figure 4.1). The Ado –Odo/Ota LGA is one of the 20 LGA of Ogun State. It is the most industrialized portion of the state. It also borders the state with metropolitan Lagos State and the Republic of Benin. The capital of the LGA is Ota at 6°41′00″N 3°41′ 00″E to the north of the area. Other towns and cities include Ado-Odo, Igbesa, Agbara, Sango-Ota, and Itele. It has an area of 878 km² and a population of 526,565 by the 2006 census (Wikipedia, 2012). The area still have many villages with a large segment of the population without access to modern health care services and relying mostly on herbal medicine and traditional medical practitioners (TMP) for solutions for their health challenges.

3.2.1.2 Informed consent

Several people interviewed included Traditional Medicine Practitioner (TMP) (those who practiced traditional medicine popularly known as herbalist, Herb Sellers (those who sell herbs in shops or stalls, Herb Hawkers (those who hawk herbs and already prepared herbal mixture for dispensing) and the elderly who had knowledge of medicinal plant use. Informed consent was obtained verbally from all participants being interviewed. These informants were selected based on their vast knowledge of medicinal plants, many years of experience in treating diseases (gastric ulcer disease inclusive) using medicinal plants found in and around their areas.

3.2.1.3 Administration of questionnaire

Semi-structured questionnaires and oral interview were adopted to obtain the relevant ethno medicinal data and these were administered by one of the investigators.

A sample of the semi- structured questionnaire is given in appendix 1. The interviewees were 16 males and 14 females and their ages ranged from 28 to 70 years. Furthermore, the use-mentions
index was calculated for all plants (Andrade-Cetto 2009). The use-mentions index (-UMi-) was taken as the number of uses mentioned for a particular plant divided by the total number of informants interviewed.

3.2.1.4 Plant identification and authentication of plants from ethnobotanical survey

The curator of the Botanical Garden, University of Ibadan, and the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI), Ibadan, Nigeria identified the medicinal plants obtained from the ethnobotanical survey. Botanical names were established by comparing specimens with those at the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI) where vouchers specimens were also deposited (Table 4.1).

3.2.2 Plant material

3.2.2.1 Collection, identification and authentication of research plant materials

Fresh leaves of *Securidaca longepedunculata* were collected in January, 2013 from Adeshiyan village, Eruwa, Oyo state. It was identified by Mr. Oluwaseun Osiyemi of the Forest Herbarium Ibadan (FHI) where a herbarium specimen was deposited with Voucher number FHI 109974. Similarly, fresh leaves of *Luffa cylindrica* were collected within the premises of University of Ibadan in the month of January, 2013. The curator of the Botanical garden of the University of Ibadan, Ibadan, Oyo State, Nigeria identified it. The plant was authenticated at the Forest Herbarium Ibadan (FHI) where a herbarium

specimen was deposited and a Voucher number FHI 109973 was obtained.

3.2.2.2 Plant extraction

The leaves of *Securidaca longepedunculata* were dried at room temperature for several days, milled using a plant milling machine and a blender, and macerated. A total of 7.22 kg leaves (dried and milled) of *Securidaca longepedunculata* (S.L) was successively extracted with n-hexane, ethyl acetate and methanol. Briefly, the 7.22 kg of SL was initially soaked in 50 L of n-hexane for 72 h with intermittent shaking, decanted and filtered, and thereafter and the dried residue macerated with ethyl acetate, the resulting solution decanted and filtered, and finally the residue from the latter macerated with methanol. Afterwards the decanted respective solutions were concentrated using a rotary evaporator and stored in a refrigerator at 4^0C until when need for analysis.

The leaves of *Luffa cylindrica* were dried at room temperature for several days, milled using a plant milling machine and a blender, and macerated. A total of 6.0 kg leaves (dried and milled) of *Luffa cylindrica* (L.C) was successively extracted with n-hexane, ethyl acetate and methanol. The 6.0 kg of LC was initially soaked in 50 L of n-hexane for 72 h with intermittent shaking, decanted and filtered, and thereafter and the dried residue macerated with ethyl acetate, the resulting solution decanted and finally the residue from the latter macerated with methanol. Afterwards the decanted respective solutions were concentrated using a rotary evaporator and stored in a refrigerator at 4^0C until when need for analysis.

The concentration and percentage yield of the extracts were determined thus:

$$\% \text{ yield of the extract} = \frac{\text{Weight of extract}}{\text{Weight of (powdered) leaves}} \times 100$$

3.2.3 Phytochemical screening of secondary metabolites

Portions of the powdered leaves of S.L and L.C were subjected to standard chemical tests for qualitative analysis of alkaloids, steroids, anthraquinones, cardenolides, saponins, phenolics and flavonoids, cardiac glycoside, phlobatannins, tannins and triterpenes as described by (Odebiyi and Sofowora, 1990; Trease and Evans, 1996; Harborne, 1998; Awe and Sodipo, 2001; Sofowora, 2006 and Harborne, 2008).

3.2.3.1 Alkaloids

A known volume (1.0 mL) of 1% (v/v) HCl was added to 3.0 mL of the aqueous extract of each of (SL and LC) in a test tube. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was then used for the following tests:

- 2 drops of Mayer's reagent was added to 1.0 mL of the aqueous extract of each of (SL and LC). The appearance of a creamy precipitate indicated the presence of alkaloids in the extract.
- 2 drops of Wagner's reagent was added to 1.0 cm^3 of the aqueous extract of each of (SL and LC). The appearance of a reddish brown precipitate indicated the presence of alkaloids.

3.2.3.2 Saponins

(Frothing Test)- 2.0 mL of the extract of each of (SL and LC) in a test tube was vigorously shaken with water for 2 minutes and warmed. Frothing which persisted on warming indicated the presence of saponins.

3.2.3.3 Tannins

A known volume (1.0 mL) of freshly prepared 10% (w/v) ethanolic KOH was added to 1.0 cm^3 of the extract of each of (SL and LC). A white precipate indicated the presence of tannins

3.2.3.4 Phlobatannins

Deposition of a red precipitate when 3 mL of the aqueous extract of each of (SL and LC) was boiled with 2 mL of 1% aqueous hydrochloric acid indicated the presence of phlobatannins.

3.2.3.5 Glycosides

Liebermann's Test. 2.0 mL of acetic acid and 2 mL of chloroform was added to aqueous extract of each of (SL and LC). The mixture was then cooled and concentrated H_2SO_4 was added. Green color showed the entity of aglycone, steroidal part of glycosides.

Keller-Kiliani Test. A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% $FeCl_3$ mixture was mixed with the 10 mL aqueous extract of each of (SL and LC) and 1 mL concentrated H_2SO_4. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Salkowski's Test. 2 mL concentrated H_2SO_4 was added to the aqueous extract of each of (SL and LC). A reddish brown color formed which indicated the presence of steroidal aglycone part of the glycoside.

3.2.3.6 Phenolics

Two drops of 5% (w/v) of $FeCl_3$ was added to 1.0 mL of the extract of each of (SL and LC). A greenish precipitate indicates the presence of phenolics.

3.2.3.7 Anthraquinones

A known volume (3.0 mL) of the extract of each of (SL and LC) was shaken with 10.0 mL of benzene, filtered and 5.0 mL of 10% (v/v) NH_3 solution was added to the filtrate. A pink colour in the ammonical (lower) phase indicates the presence of free hydroxyl anthraquinones.

3.2.3.8 Cardenolides

A known volume (1.0 mL) of the extract of each of (SL and LC) was added to 2.0 mL of glacial acetic acid containing one drop of 5% w/v $FeCl_3$ solution. This was then underplayed with 1.0 mL of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides.

3.2.3.9 Steroids

About 0.5 g of the extract of each of (SL and LC) was dissolved in 2 mL of acetic anhydride mixed with 2 drops of $CHCl_3$. Two drops of concentrated H_2SO_4 was then carefully added. A greenish colour was indicative of steroids.

3.2.3.10 Terpenes

A known volume (1.0 mL) of the extract of each of (SL and LC) was added to 5 drops of acetic acid anhydride followed by a drop of concentrated H_2SO_4. The mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. A bluish-green colour indicated the presence of terpenes.

3.2.3.11 Flavonoids

A known volume (1.0 mL) of 10% w/v NaOH was added to 3.0 mL of the extract of each of (SL and LC). A yellow colouration indicates the presence of flavonoids.

3.2.4 Acute toxicity test

The acute oral toxicity test of S.L and L.C leaf extracts were carried out on healthy male Wistar rats weighing between 130 and 150 g using the method described by Lorke (1983).

Briefly, the animals were treated with the extracts of S.L and L.C (10, 100, 1000, 1,600, 2,900 and 5,000 mg/kg b. w), respectively. Only the methanol extracts of the plants were used for the acute toxicity. The rats were housed in cages. Randomly selected ones were marked on the head, neck, trunk and tail for individual identification. All rats were maintained at relevant environmental conditions (temperature: 23°C -31°C; photoperiod: 12 hrs natural light and 12 hrs darkness). The rats were allowed to acclimatize in the animal house for a week before starting the experiment. Drinking water and food were provided ad libitum throughout the experiment. They were observed frequently on the day of treatment during normal working hours and for the next 14 days to note the nature and time of all adverse effects and death. Three (3) animals were used for all the doses administered.

If mortality was observed in more than half of the test animals, then the following higher dose was not administered. The crude extract was suspended in a vehicle (distilled water). Another group of three rats was given distilled water as a placebo and regarded as the control group. Food was provided to the rats approximately an hour after treatment. They were observed in detail for any indications of toxicity effect within the first six hours after the treatment period and daily further for a period of 14 days. Surviving animals were weighed and visual observations for mortality, behavioural pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period. Observations started immediately after administration and then observed continuously for, 30 minutes, one hour after the treatment; intermittently for four hours, and thereafter over a period of 24 hours. The rats were observed for changes such as feeding habits, weight loss, mortality, loss of righting reflex, urination, increased or reduced activity. The dose(s) of the extract(s) of SL and LC that would be lethal to 50% of the population of the animals was determined as the LD_{50}.

3.2.5 *In vitro* antioxidant assays

The word “sample” in all of the *in vitro* antioxidant assays refers to extract of each of (SL and LC) used for the analysis.

3.2.5.1 Determination of total phenol content

The method of determining the total phenolic content by Singleton and Rossi, 1965 and as described by Gulcin *et al* (2003) using the folin-ciocalteu phenol reagent which is an oxidizing reagent was used. Briefly, a mixture of 0.1 mL of the sample and 0.9 mL of water was added to 0.2 mL of folin-ciocalteu phenol reagent and the resulting mixture vortexed. After 5 minutes, 1.0 mL of 7% (w/w) Na_2CO_3 solution was added and the resulting solution was distilled to 2.5 mL before incubating for 90 minutes at room temperature. The absorbance was read at 750 nm in a spectrophotometer. The standard used was the Gallic acid at 0.1 mg/mL in order to determine Gallic Acid Equivalent (GAE) of sample, after preparing a calibration curve. Distilled water was used as blank.

3.2.5.2 Determination of total flavonoid content

Standard quercetin with varying concentration 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL was used as standard in comparison to the sample. This was carried out based on the aluminum chloride colorimetric assay method according to Zhishen *et al.,* (1999) as described by Miliauskas *et al.,* (2004). To 0.1 mL of extract/standard was added 0.4 mL of distilled water. This was followed by 0.1 mL of 5% sodium nitrite. After 5 minutes, 0.1 mL of 10% aluminum chloride and 0.2 mL of sodium hydroxide was added and the volume was made up to 2.5 mL with distilled water. The absorbance at 510 nm was measured against the blank. The total flavonoid content of the plant, expressed as mg quercetin equivalents per gram of the plant extract is calculated as:

$$X = q * \frac{V}{w}$$

X = Total content of flavonoid compound in quercetin equivalent
q= concentration of quercetin established from the standard curve
V= volume of extract (mL)
w= weight of the crude methanolic extract obtained.

3.2.5.3 2, 2-Diphenyl-1-picrylhydrazyl hydrate (DPPH) assay

The radical scavenging ability of the oil was determined using the stable radical 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) as described by Brand-Williams *et al.* (1995). The reaction of DPPH with an antioxidant compound, which can donate hydrogen, leads to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. To 1 mL of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) of the sample or standard (vitamin C) in a test tube was added 1 mL of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30 minutes after which the absorbance was read at 517 nm against a DPPH control containing only 1 mL methanol in place of the sample.
The percent of inhibition was calculated in following way:

$$I\% = [(A_{blank}-A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against sample concentration.

3.2.5.4 Ferrous on-chelating ability assay

The ferrous ion-chelating (FIC) assay was carried out according to the method of Singh and Rajini, 2004 with some modifications. Solutions of 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine were diluted 20 times. Briefly, an aliquot (1 mL) of different concentrations of sample was mixed with 1 mL FeCl2·4H2O. After 5 minutes of incubation, the reaction

was initiated by the addition of ferrozine (1 mL). The mixture was shaken vigorously and after a further 10 minutes incubation period the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine–Fe^{+2} complex formation was calculated by using the formula:

$$\text{Chelating effect \%} = [(A_{control}-A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ = absorbance of control sample (the control contains FeCl2 and ferrozine, complex formation molecules) and A_{sample} = absorbance of a tested samples.

3.2.5.5 Inhibition of nitric oxide radical

The inhibition of nitric oxide radical activity of the sample was carried out according to the method of Green *et al.* (1982) as described by Marcocci *et al*. (1994). Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which was measured by Griess reaction. The reaction mixture, containing 0.1 mL of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) of the sample and 0.9 mL of sodium nitroprusside (2.5 mM) in phosphate buffer saline was incubated under illumination for 150 minutes. After incubation, 0.5 mL of 1% sulphanilamide in 5% phosphoric acid was added and incubated in the dark for 10 minutes, followed by addition of 0.5 mL 0.1% NED (N-1-napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was measured at 546 nm (Marcocci *et al*., 1994). The percentage inhibition of nitric oxide radical formation was calculated as expressed above in DPPH radical scavenging assay.

3.2.5.6 Reductive potential

The reductive potential of the sample was determined according to the method of Oyaizu (1986) and as described by Gulcin *et al*., (2004). To 1 mL of the sample was added 1 mL of phosphate buffer (pH 6.6, 0.2 M) and vortexed after which 2.5 mL of 1% potassium ferric cyanide was added. The reacting mixture was then incubated in a water bath at 50^{o}C for 20 minutes. 2.5 mL of 10% trichloroacetic acid was then added to the mixture and then centrifuged for 10 minutes. To 2.5 mL (100 µl) of the supernatant was added 2.5

mL (100 µl) of distilled water and 0.5 mL (20 µl) of 0.1% ferric chloride and the resulting mixture was vortexed. The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates greater reductive potential. The reductive potential was expressed as reductive potential index (REI)

$$\text{REI} = \frac{\text{absorbance of extract}}{\text{Absorbance of 10µg/ml vitamin C standard}}$$

3.2.6 Antiulcer bioassays of the plant extracts

3.2.6.1 Experimental animals for bioassays

One hundred and five (105) albino Wistar rats of both sexes with average weight 151 g were used for the experiments. These animals were purchased from the animal house of the University of Agriculture, Abeokuta, Ogun State, Nigeria. The rats were kept in wooden cages with wooden shaven beddings to prevent coprophagy. The male rats were separated from the female rats to prevent copulation. The rats were supplied water *ad libitum* and fed standard feed obtained from Graceland feeds, Ota, Ogun-state, Nigeria. They were kept under standard conditions of temperature and humidity. The animals were acclimatized for a period of one week in the animal house of the Department of Chemical Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.

3.2.6.2 Antiulcer screening of the crude extracts of plants using animal model

A total of 75 rats were divided into 15 groups of 5 rats each such that the average weight of each group was approximately the same. The treatment groups as shown in Table 3.1

3.2.6.3 Ethanol-induced mucosal damage in rats

Wistar rats, with average weight 151 g were fasted for 24 h but given water ad libitum. The test drug was given to the animals orally. Fifty minutes later, 1 mL of absolute

ethanol was

Table 3.1 Study design for antiulcer screening of the crude extracts of plants

Groups	Treatment
Control group	Normal saline
Ulcer group	Absolute ethanol (only)
Reference drug	50 mg/kg *b.w.* cimetidine
A1SL	150 mg/kg *b.w* Methanol extract of SL
A2SL	300 mg/kg *b.w* Methanol extract of SL
B1SL	150 mg/kg *b.w* n-Hexane extract of SL
B2SL	300 mg/kg *b.w* n-Hexane extract of SL
C1SL	150 mg/kg *b.w* Ethyl acetate extract of SL
C2SL	300 mg/kg *b.w* Ethyl acetate extract of SL
A1LC	150 mg/kg *b.w* Methanol extract of LC
A2LC	300 mg/kg *b.w* Methanol extract of LC
B1LC	150 mg/kg *b.w* n-Hexane extract of LC
B2LC	300 mg/kg *b.w* n-Hexane extract of LC
C1LC	150 mg/kg *b.w* Ethyl acetate extract of LC
C2LC	300 mg/kg *b.w* Ethyl acetate extract of LC

administered orally. The animals were sacrificed after 1 h and their stomachs excised and opened along the greater curvature, rinsed with 1.15% KCl and ulcer scoring/severity grading was done (Robert *et al.*, 1979; Witt *et al.*, 1985).

3.2.6.4 Quantification of ulceration

Ulceration index, which is the extent of ulceration in the rat, was carried out: stomach mucosa was rinsed with saline and lesions in glandular portion were then exposed and examined under a X10 magnifying glass. Ulcer index of each animal was calculated by adding the values and their mean values were determined by following the scoring system according to Lee *et al* (2005) with slight modifications (measurement of lesions) (Kayode *et al*., 2015): ((i) No lesion – 0 (ii) Bleeding and slight lesions (0.5 -1.0) mm = 1 (iii) Moderate lesions (1.0 – 1.5) mm = 2 (iv) Severe lesions (1.5 – 2.5) mm = 3 (v) Perforated ulcers (2.5 – 3.5) mm = 4.

Percentage inhibition was calculated using the following formula;

$$\% \text{ Inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{treated}}}{UI_{\text{ulcer control}}} \times 100$$

Kayode *et al*., 2009.

3.2.6.5 Collection and preparation of tissue homogenate

The rats were sacrificed by ether anesthesia and thereafter cut open to excise the stomach and the gastric content was poured into a graded sample bottle. The stomach was washed in ice cold 1.15% KCl solution, blotted, weighed, cut into two halves and one half was put into sample bottles containing 10% formalin and the other half was put into sample

bottles containing phosphate buffer. The stomach in the phosphate buffer were homogenized in 4 volumes of the homogenizing buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 11,900rpm for 20 minutes in a Beckman L5-50B ultracentrifuge at 0˚C. The supernatant was decanted and the post mitochondrial fraction were collected and stored in a freezer at -4˚C until assays where carried out. All the above procedures were carried out at temperatures between 0-4˚C.

3.2.6.6 Determination of total protein concentrations

Protein concentrations of post mitochondrial fractions of the brain and liver tissues were estimated by the procedure of Lowry *et al*., 1951 using bovine serum albumin (BSA) as standard.

3.2.6.7 Determination of lipid peroxidation

Lipid peroxidation in post mitochondrial fraction was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method as described by Varshey and Kale (1990). Briefly, 0.4 mL of the post mitochondrial fraction was mixed with 1.6 mL of phosphate buffer and 0.5 mL of 30% TCA was added, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 minutes at 80^0C, cooled in ice and centrifuged at room temperature for 10 minutes at 3, 000 rpm. The absorbance of the clear supernatant was measured against reference blank (distilled water) at 532 nm using spectrophotometer

3.2.6.8 Determination of superoxide dismutase (SOD)

The level of the SOD enzyme was determined using the method of Misra and Fridovich (1972). An aliquot of 0.2 mL of the diluted tissue supernatant was added to 2.5 mL of 0.05 M Carbonate buffer, (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3 mL of freshly prepared 0.3 mM epinephrine to the mixture, which was quickly mixed by inversion. The reference cuvette contained 2.5ml of carbonate buffer, 0.3 mL of epinephrine and 0.2ml of water. The increase in

absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

3.2.6.9 Determination of catalase (CAT)

This was done according to the method of Beers and Sizer (1952). This method is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as unstable intermediate. The chromic acetate so produced is measured colorimetrically at 570-610 nm. It must be noted that dichromate has no absorbance at the wavelength and its presence in the assay mixture does not interfere with the determination. Catalase preparation (in samples) is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/ acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction mixture. Different amounts of H_2O_2 ranging from 10 to 100 micromoles were taken into small test tubes and 2 mL of dichromate/ acetic acid solution was added. Addition of the reagent instantaneously produced an unstable blue precipitate of perchromic acid. Subsequent heating for about 10 minutes in a boiling water bath changed the color of the solution to stable green due to formation of chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made up to3 mL and the absorbance read at 570 nm using Beckman DU 70 spectrophotometer. The concentrations of the standard (H_2O_2) were plotted against absorbance.

3.2.6.10 Determination of reduced glutathione (GSH)

Reduced glutathione levels were assessed by the method of Kapetanovic and Mieyal, 1979.

Briefly, 100 µL of supernatant of tissue homogenate was added to an equal volume of the metaphosphoric acid and then centrifuged at 2,000xg for 2 min to remove protein. Then 50 µL of 4 M triethanolamine per mL of the supernatant was added to increase the pH of the sample. For total GSH assay, 50 µL of sample was added to 150 µL of a reaction mixture containing 0.4 M 2-(N-morpholino) ethanesulphonic acid, 0.1 M phosphate (pH

6.0), 2 mM EDTA, 0.24 mM NADPH, 0.1 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) and 0.1 unit GR. The absorbance was measured using a microplate reader at 5 min intervals for 30 min. Reduced glutathione (GSH) concentration were expressed as μmol g-1 of wet tissue.

3.2.6.11 Histopathology

Tissue sample from the stomach of the rats in each group of the experiments were fixed in 10% formalin solution for a minimum of 24 h and then dehydrated by washing in ascending grades of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned with a microtrome, stained with hematoxyline and Eosin (H and E) and mounted on Canada Balsam. All sections were examined under light microscope (x10, x20 and x40) magnification. Photographs of the lesions were taken with an Olympus photo microscope for observation and documentation of histopathologic lesions (Ode, 2011).

3.2.7 Chromatographic methods

Different chromatographic methods were employed for isolation of compounds from the active crude extracts of SL and LC.

3.2.7.1 Vacuum liquid chromatography (VLC)

3.2.7.1.1 Vacuum liquid chromatography of ethyl acetate leaf extract of *Securidaca longepedunculata*

The ethyl acetate leaf extract (91.37 g) of *Securidaca longepedunculata* was made into slurry using ethyl acetate and adsorbed onto silica gel. This was allowed to dry. A vacuum column chromatography cup and a vacuum pump was used for the chromatographic set up. The column chromatography was performed on silica gel (TLC, grade without the

binder) (Merck; Germany) and successively eluted with increasing polarities of solvents starting from 100% n-hexane, n-hexane/DCM, DCM/EtoAC to 100% MeOH. Fractions were collected and pooled based on their TLC profiles and the pooled fractions were concentrated. Antiulcer bioassay was thereafter carried out on each of the pooled fraction.

3.2.7.1.2 Vacuum liquid chromatography of ethyl acetate leaf extract of *Luffa cylindrica*

The ethyl acetate leaf extract (79.87 g) *Luffa cylindrica* was made into slurry using ethyl acetate and adsorbed onto silica gel. This was allowed to dry. A vacuum column chromatography cup and a vacuum pump was used for the chromatographic set up. The column chromatography was performed on silica gel (TLC, grade without the binder) (Merck; Germany) and successively eluted with increasing polarities of solvents starting from 100% n-hexane, n-hexane/DCM, DCM/EtoAC to 100% MeOH. Fractions were collected and pooled based on their TLC profiles and the pooled fractions were concentrated. Antiulcer bioassay was thereafter carried out on each of the pooled fraction.

3.2.7.2 Column chromatography of the most active VLC fractions

Open column chromatography was performed on silica gel 120 – 200 mesh (Merck; Germany) and successively eluted with solvents of increasing polarities starting from 100% n-hexane, n-hexane/DCM, DCM/EtoAC to 100% MeOH. Solvent mixture and ratios were modified in some cases for the subfractions obtained and further fractionation as situation demands.

Column 1: length 108 cm, internal diameter 3.2 mm.

Column 2: length 112 cm, internal diameter 2.0 mm.

3.2.7.2.1 Column chromatography of VLC fraction D_{SL}

Column chromatography was carried out on 20 g of the VLC fraction **D_{SL}**. The solvent ration used were n-hexane/ethyl acetate (40:60), (65:35), (25:75), (15:85), and (10:90); followed by ethyl acetate/methanol (95:5), (90:10), (87.5:12.5), (70:30), (55:45) and (90:10).

3.2.7.2.2 Column chromatography of VLC fraction B_{SL}

Column chromatography was performed on 8 g of the VLC fraction **B_{SL}**. The solvent ration used were n-hexane/ethyl acetate (80:20), (60:40), (50:50), (40:60), (30:70) and (20:80).

3.2.7.2.3 Column chromatography of VLC fraction B_{LC}

Column chromatography was done on 6.8 g of the VLC fraction BSL. The solvent ration used were n-hexane 100%, n-hexane/ethyl acetate (65:35), (25:75), (15:85), (10:90) and (85:15).

3.2.7.2.4 Column chromatography of column fraction LCA1 (from B_{LC})

Column chromatography was performed on 500 mg of the VLC fraction B_{LC}. The solvent ration used were n-hexane 100%, n-hexane/ethyl acetate (90:10).

3.2.7.2.5 Column chromatography of LCC3 fraction from B_{LC}

Column chromatography was carried out on 1.63 g of LCC3 from the VLC fraction B_{LC}. The solvent mixture used were n-hexane 100%, n-hexane/DCM, DCM, DCM/ethyl acetate at varying degree of solvent ratios.

3.2.7.2.6 Column chromatography of the column sub-fraction of (B11M) (*Securidaca longepedunculata*)

Column chromatography was performed on 0.53 g of the sub-fraction of B11M. The solvent ration used were n-hexane/DCM (60:40), DCM (100%), DCM/ethyl acetate (98:2) and (90:10).

3.2.7.3 Thin layer chromatography

Prep-thin layer chromatography was carried out on Si gel 60 plates (20 x 20 cm). Also, analytical TLC was conducted with Si gel 60 F254 plates (10 x 5 and 5 x 1 Merch, Germany). The solvent systems are of varying ratios of mixture of solvents until a clear separation was achieved.

3.2.7.4 Infrared (IR) spectroscopy

The isolates or compounds were dried in the fume cupboard. The isolates (1 or 2 mg) were then dissolved in Ethyl acetate and were run on ATR in the mid-range (4000 cm^{-1} to 400 cm^{-1}) and the recorded data were analyzed using the OPUS 6.5 software.

3.2.7.5 Nuclear magnetic resonance (NMR) spectroscopy

The solvents from the isolates or compounds were removed by placing the vials in a fume cupboard. The compound or isolate was dissolved in deteurated chloroform and NMR spectroscopy carried out. All peaks are reported relative to tetramethylsilane (d 0.00)

3.2.8 Cytotoxicity screening of isolated compounds

3.2.8.1 Preparation of cells

Vero or C3A cells were counted and then seeded into half of a 96 well plate. Cells were seeded into the wells with 6000 cells/well in 100 µL aliquots. Plates were incubated at 37°C overnight.

Two different cells lines were used in this experiment, Vero to measure genotoxicity and C3A to measure cytotoxicity. Vero lineage was isolated from kidney epithelial cells extracted from an African green monkey in 1962 by Yasumara and Kawakita at the Chiba

University in Japan. Vero is a common mammalian continuous cell line. This cell line is adherent and exhibits some degree of contact inhibition after forming a monolayer and is useful to grow slow replicating viruses (ATCC, 2016a). NucRed staining is used to visualize genotoxicity of Vero cells. NucRed binds to nucleotides and is a cell-permeable dye that causes viable cells to fluoresce.

C3A was isolated from liver epithelial cells extracted from a human male that had a hepatocellular carcinoma in the late 1970's. C3A is a clonal derivative of Hep G2 that was selected for its strong contact inhibition of growth, high production of alpha fetoprotein, ability to grow in glucose deficient medium and high albumin production. Hoechst 33342 and propidium iodide (PI) staining is used to visualize cytotoxicity of C3A cells (ATCC, 2016b). Hoechst stains viable cells blue and PI stains apoptotic or necrotic cells red, annexin V can be used to detect early stages of apoptosis by forming a green fluorescence. The annexin V binds to phosphatidylserine residues, which are normally present on the surface of the cell during early apoptosis.

3.2.8.2 Treatment of cells

Compounds 17 to 21 corresponding to A1 from *Luffa cylindrica*, PPB from *Luffa cylindrica* B11MF from *Securidaca longepedunculata*, B9JN2 from *Securidaca longepedunculata* and PBT from *Securidaca longepedunculata* respectively, was used to treat the C3A cell line after incubation. The compounds as well as the controls (melphalan) were diluted to obtain the following concentrations; 50, 25 and 12.5 µg/mL from a 100 µg/mL working stock solution. The working stock solution was made by taking 1 µL of the Melphalan 100 mg/mL stock into 10 mL EMEM complete medium. The 50 µg/mL of each treatment was made by taking 500 µL of the 100 µg/mL working stock into 500 µL of EMEM medium. For the 25 µg/mL, 250 µL of 100 µg/mL working stock into 750 µL of EMEM medium, and for the 12.5 µg/mL concentration 125 µL of the 100 µg/mL working stock was added to 875 µL of EMEM medium.

3.2.8.3 The hoechst/annexin V-FITC/PI staining

The 96 well plate containing the C3A cell line was aspirated to remove the medium. 5

µg/mL of Hoechst 33342 and 2 µg/mL Annexin V-FITC was added in 50 µL aliquots to each well. The 96well plate was incubated for 15 minutes and then 50 µL aliquots of 1x PI was added per well. (Pringle *et al*., 2018)

3.2.9 Statistical analysis

Data are expressed as Mean±SD or Mean±SEM. Data were analysed using student t-test and one-way ANOVA at $p=0.05$. Values of $p<0.05$ were considered significant (Bamgboye, 2002).

CHAPTER FOUR

RESULTS

4.1 Ethnobotanical survey

A total of 41 plant species (Table 4.1) belonging to 27 families were identified from the survey conducted at Ado/Odo Ota Local Government Area, Ogun State (Figure 4.1). Table 4.1 shows the list of identified plant species, families, local names and plant parts used. The most prominent among these plant families are the Asteraceae and the Euphorbiaceae families with five and four species, respectively. Other plant families include Malvaceae (3), Apocynaceae (1), Cucurbitaceae, Fabaceae and Musaceae with 2 species each among others. The most frequently mentioned species are *Carica papaya* (0.400) and *Musa paradisiaca* (0.400) each of which were mentioned 12 times while *Zingiber officinale* (0.267) and *Allium sativum* (0.200) were mentioned 8 and 6 times respectively, by the respondents. The species distribution according to the ethnobotanical survey with use mention index is also given in Table 4.1. The species distributions according to families of plant used for the treatment of gastric ulcer are illustrated in Figure 4.2. Percentage occurrence of plant parts used is shown in Figure 4.3. The most common plant parts used are the leaves. Some of the herbs are prepared in combination with other herbs. Plate 1 shows some of these plants used in the treatment of gastric ulcer

in Ado/Odo Ota local government area of Ogun State, Nigeria. During the interactions with the respondents, some claimed that use of single plants was sufficient to cure the disease (e. g *Musa paradisiaca, Talinum triangulare*, *Momordica charantia* etc) while others insisted that only a mixture of certain plants (e .g *Garcinia kola, Carica papaya* and *Plukenetia conophora*) would be efficacious.

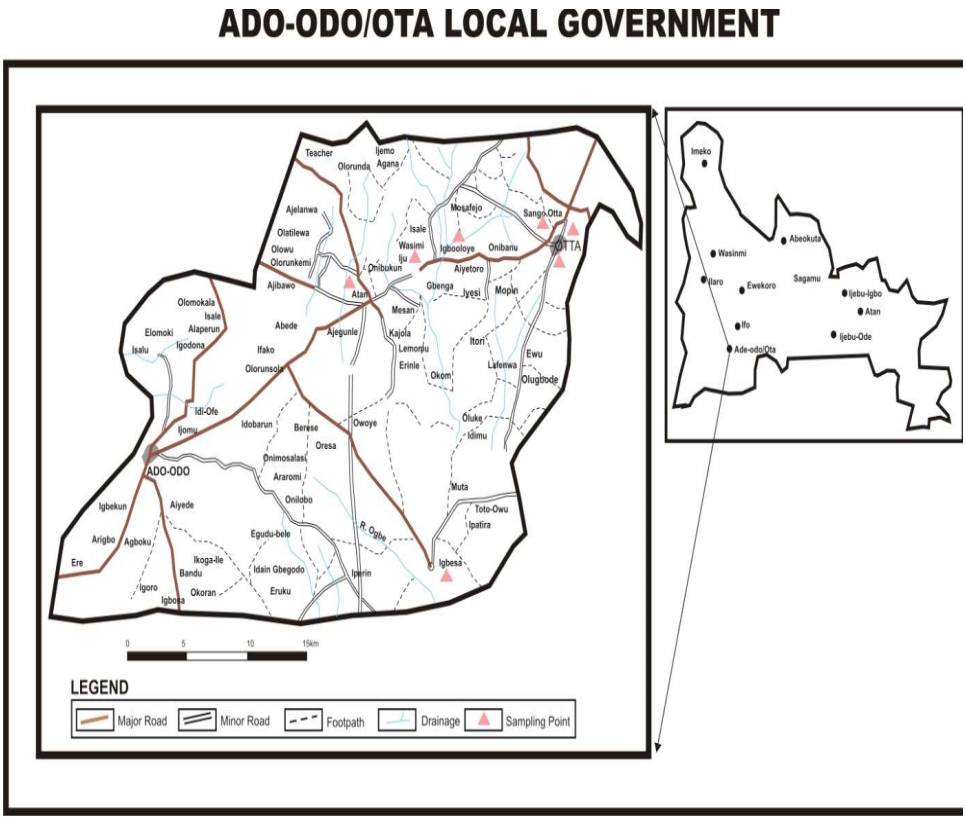


Figure 4.1. Map of study area (Ado/Odo Ota LGA, Ogun State)

Figure 4.2: Species distribution according to families of plants used for treatment of gastric ulcer

KEY: 1= Fabaceae, 2= Malvaceae, 3= Zingiberaceae, 4= Asteraceae, 5= Apocynaceae, 6= Amaryllidaceae, 7= Xanthorrhoeaceae, 8= Convolvulaceae, 9= Basellaceae, 10= Caricaceae, 11= Menispermaceae, 12= Vitaceae, 13= Boraginaceae, 14= Annonaceae, 15= Clusiaceae, 16= Icacinaceae, 17= Euphorbiaceae, 18=Meliaceae, 19= Cucurbitaceae, 20= Musaceae, 21= Lamiaceae, 22= Chrysobalanaceae, 22=Lauraceae, 23=Phyllanthaceae, 24=Rosaceae, 25=Polygalaceae, 26 =Myrtaceae, 27=Talinaceae

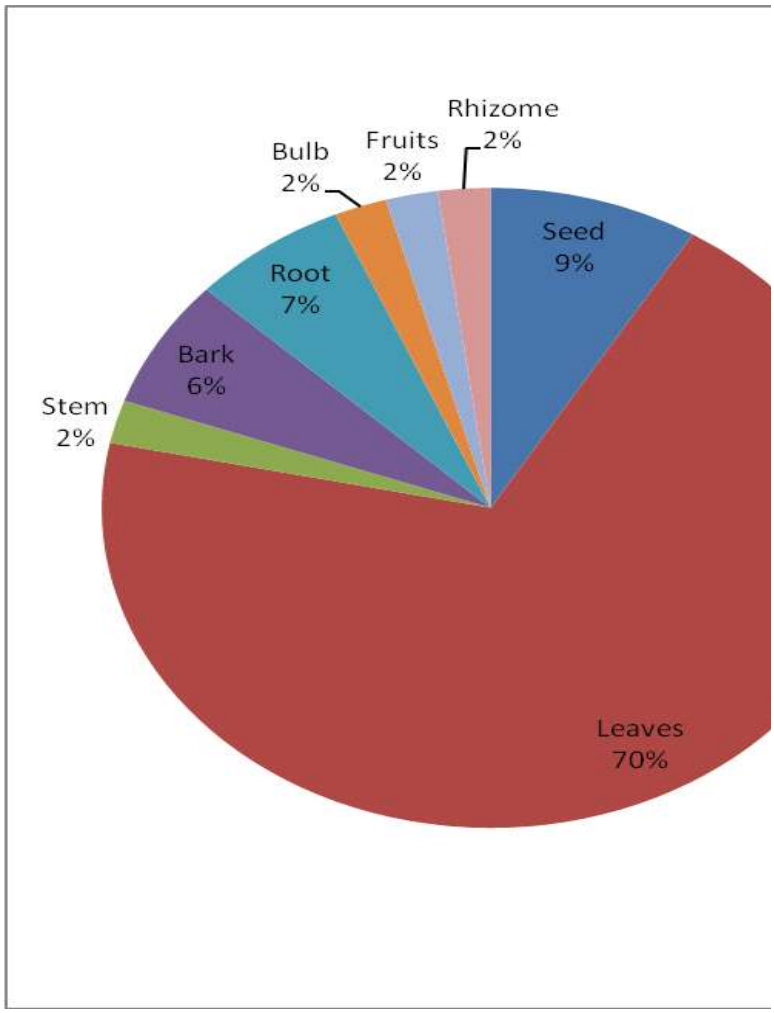

Rhizome
2%
Fruits
2%
Bulb
2%
Seed
9%
Root
7%
Bark
6%
Stem
2%
Leaves
70%

Figure 4.3: Percentage occurrence of parts of plant used in the treatment of gastric ulcer

Table 4.1: Medicinal plants used in the treatment of gastric ulcer

Botanical name	**Family**	**Voucher /Specimen No.**	**Local name**	**Part(s) Used**	**NTM**	**UMI**
Acacia nilotica (Guill. &Perr.) Kuntze	Fabaceae	AAAK 1	Boni-booni	Seeds, leaves	1	0.033
Aframomun melegueta (Roscoe) K. Schum.	Zingiberaceae	AAAK 3	Atare	Seed	2	0.067
Ageratum conyzoides L.	Asteraceae	DPHUI 1080	Imi-esu	Leaves	1	0.033
Alafia barteri Oliv.	Apocynaceae	DPHUI 1129	Agbari etu	Stem, leaves	1	0.033
Allium sativum L.	Amaryllidaceae	DPHUI 1116	Ayuu	Bulb	6	0.200
Aloe vera (L.) Burm. f.	Xanthorrhoeaceae	AAAK 6	Eti eerin oyinbo	Leaves	2	0.067
Aspilia africana (Pers.) C.D. Adams	Asteraceae	DPHUI 1083	Yunriyun	Leaves	1	0.033
Basella alba L.	Basellaceae	AAAK 4	Amunu tutu	Leaves	4	0.133

Carica papaya L.	Caricaceae	DPHUI 1175	Ibepe	Leaves, bark	12	0.400
Cissampelos owariensis P. Beauv. Ex DC.	Menispermaceae	AAAK 9	Ewejokoje	Leaves	1	0.033
Cissus populnea Guill. &Perr.	Vitaceae	AAAK 10	Ogbolo	Root	1	0.033

Table 4.1: Table 4.1: (Cont.)

Botanical name	**Family**	**Voucher/ Specimen No.**	**Local name**	**Part(s) Used**	**NTM**	**UMI**
Corchorus olitorius L.	Malvaceae	DPHUI 1068	Ewedu	Leaves	2	0.067
Crassocephalum crepidioides (Benth.) S. Moore	Asteraceae	AAAK 7	Efo ebolo	Leaves	3	0.100
Ehretia cymosa Thonn.	Boraginaceae	AAAK 11	Jaoke	Leaves	1	0.033
Enantia chlorantha Oliv.	Annonaceae	DPHUI 0306	Awopa	Bark	2	0.067
Garcinia kola Heckel	Clusiaceae	DPHUI 1128	Orogbo	Seed	4	0.133
Icacina trichantha Oliv.	Icacinaceae	DPHUI 0679	Gbegbe	Leaves	1	0.033
Jatropha curcas L.	Euphorbiaceae	DPHUI 0901	Botuje	Leaves	3	0.100
Jatropha gossypiifolia L.	Euphorbiaceae	DPHUI 1001	Botuje pupa	Leaves	3	0.100
Khaya senegalensis (Desr.) A. Juss.	Meliaceae	DPHUI 0717	Ogano/Oganwo	Leaves	1	0.033

Luffa cylindrica (L.) M. Roem.	Cucurbitaceae	DPHUI 1074	Kankan ayaba	Leaves	1	0.033
Manihot esculenta Crantz.	Euphorbiaceae	DPHUI 0608	Paki/Gbaguda	Leaves	2	0.067

Table 4.1: (Cont.)

Botanical name	**Family**	**Voucher/Specimen No.**	**Local name**	**Part(s) Used**	**NTM**	**UMI**
Momordica charantia L.	Cucurbitaceae	DPHUI 1066	Ejirin	Leaves	2	0.067
Musa paradisiaca L.	Musaceae	AAAK 2	Ogede agbagba	leaves	12	0.400
Musa sapientum L.	Musaceae	DPHUI 1110	Ogede	Leaves	1	0.033
Ocimum gratissimum L.	Lamiaceae	DPHUI 1052	Efinrin	Leaves	2	0.067
Parinari laxiflora Ducke	Chrysobalanaceae	AAAK 14	Abere	Seed	1	0.033
Persea americana Mill.	Lauraceae	DPHUI 1382	Piya	Leaves	1	0.033
Phyllanthus amarus Schumach. &Thonn.	Phyllanthaceae	DPHUI 1064	Iyin olobe Eyin olobe	Root, Leaves	1	0.033
Plukenetia conophora Mull. Arg.	Euphorbiaceae	AAAK 13	Awusa	Root	4	0.133
Pseudocedrela kotschyi (Schweinf.) Harms.	Meliaceae	DPHUI 0056	Emi-gbegiri	Leaves	1	0.033
Pyrus communis L.	Rosaceae	AAAK 15	Pear	Leaves, Fruits	1	0.033

Securidaca longepedunculata Fresen.	Polygalaceae	DPHUI 1131	Ipeta	Leaves, Bark	1	0.033

Table 4.1: (Cont.)

Botanical name	**Family**	**Voucher /Specimen No.**	**Local name**	**Part(s) Used**	**NTM**	**UMI**
Senna podocarpa (Guill. &Perr.) Lock	Fabaceae	DPHUI 0079	Asunrin	Leaves	1	0.133
Sida carpinifolia L. f.	Malvaceae	AAAK 8	Oso kotu	Leaves	1	0.133
Syzygium guineense (Willd.) DC.	Myrtaceae	AAAK 12	Ewe ori	Leaves	1	0.033
Talinum triangulare (Jacq.) Willd.	Talinaceae	AAAK 5	Gbure	Leaves	4	0.133
Tridax procumbens L.	Asteraceae	DPHUI 1056	Igbalode	L eaves	1	0.033
Urena lobata L.	Malvaceae	DPHUI 1130	Esinsin agborin	Leaves	2	0.067
Vernonia amygdalina Delile	Asteraceae	DPHUI 0669	Ewuro	Leaves	12	0.400
Zingiber officinale Roscoe	Zingiberaceae	DPHUI 0374	Atale	Rhizome	8	0.267

KEY:

NTM= Number of Time Mention

UMI= Use Mention Index

4.1.1 Methods of preparation

Herbal remedies can either be prepared from dry plants purchased from the markets or from freshly collected samples around homes or gardens. However, respondents affirmed that both sources of plant materials are efficient in herbal preparation except in some cases where freshly collected samples were preferred. The main methods of preparations are decoction (boiling in water) and juice extraction. Others are mashing and roasting, infusion (extracted in hot water) and concoction (mixing of different plants). Decoction was the most preferred method. The time required for boiling was variable and dependent on nature of plant material. In all cases the preparation was to be taken orally.

4.1.2 Herbal remedy recipes

The dried leaves of *Alafia barteri, Pseudocedrela kotschyi and* the bark of *Securidaca longepedunculata* are boiled together in water and made into a decoction. A cupful is to be taken three times daily. Barks of *Garcinia kola, Carica papaya* and *Plukenetia conophora* are mashed and roasted. The mixture is taken with cold pap, once or twice daily for four weeks. The leaves of *Manihot esculenta, Jatropha curcas, Jatropha gossypiifolia and Syzygium guineense* are boiled in water and decoction taken twice daily for one month or until symptoms disappear. The barks of *Plukenetia conophora*, *Garcinia kola and Carica papaya are* soaked in alcohol and the infusion taken twice daily. The fresh leaves of *Ocimum gratissimum* and *Vernonia amygdalina* are squeezed to extract the juice which is taken three times daily after meals. Unripe *Musa sapientum* is boiled in water. The infusion is taken three times daily. Bark of unripe *Musa paradisiaca* is mashed and roasted. This is taken with water or cold pap, three times daily after meals. Fresh leaves of *Talinum triangulare* are squeezed to extract the juice which is taken three times daily after meals. Fresh leaves of *Talinum triangulare* are squeezed to extract the juice which is taken with honey three times daily after meals. Fresh leaves of *Ehretia cymosa* are squeezed to extract the juice and taken three times daily after meals. Fresh leaves of

Urena sp. are squeezed to extract the juice which is taken three times daily after meals. Fresh leaves of *Momordica charantia are* squeezed to extract the juice. The extracted juice is mixed with red palm oil and saccharin. The mixture is taken three times daily. A mixture of the fruits of *Carica papaya* and *Aframomun melegueta* is to be taken with cold pap daily. Fresh leaves of *Carica papaya* is boiled in water. The decoction is taken regularly. Leaves of *Jatropha gossypiifolia* are washed and soaked in alcohol. The infusion is taken twice daily. The seeds of *Acacia nilotica* is mashed and roasted, and mixed with honey or palm oil. The mixture is taken regularly.

Percentage yield of leaf extracts, phytochemical screening and acute toxicity

The methanol crude extract of LC gave the highest yield of 281.75 while the n-hexane crude extract of LC had the lowest yield (0.75) (Table 4.2). All the animals used for the acute toxicity gained weight normally for 14 days period of observation and shows no signs of abnormality even beyond. The summary of the acute oral toxicity testing of the methanol crude extracts of the two are plants are depicted in Table 4.3. Screening of aqueous extract of *Securidaca longepedunculata* and *Luffa cylindrica* leaves for secondary metabolites revealed ten and nine different chemical compounds for *Securidaca longepedunculata* and *Luffa cylindrica* respectively, which were present in high or moderate appreciable amounts. Anthraquinones were not detected at all in both plants while saponin was absent only in *Luffa cylindrica*. Alkaloids, tannins, phlobatannins, phenolics, cardenolides, steroids, terpenes and flavonoids were present in varying degree of intensity (Table 4.4).

4.3 *In vitro* antioxidants assays

The methanol leaf extract of SL (MSL) gave the highest total phenolics content (TPC) value of 79.93 ± 4.62 mg GAE/ g extract whereas; the MLC has the lowest TPC value of 11.21 ± 0.65 mg GAE/g extract. The ELC and the ESL gave TPC values of 19.04 ± 0.61 and 17.36 ± 0.81 mg GAE/g extract, respectively (Table 4.5). Table 4.5 also shows the results obtained for the total flavonoid content (TFC). ELC gave the highest TFC value of

678.41 ± 76.30 mg QE/g extract. Coincidentally, MSL and ESL gave 340.68 mg QE/g extract while the lowest of all was MLC with TFC value of 20.56 ± 5.09 mgQE/ g extract.

Table 4.2 Yield of n-hexane, ethyl acetate and methanol crude extracts of the leaves

of *Securidaca longepedunculata* (SL) and *Luffa cylindrica* (LC)

Extracts	**Weight of extract (g)**	**% Yield**
n-hexane S.L	134.61	1.9
Ethyl acetate S.L	204.45	2.8
Methanol S.L	168.2	2.3
n-hexane L.C	42.72	0.7
Ethyl acetate L.C	98.3	1.6
Methanol L.C	281.75	4.7

Table 4.3: Phytochemical screening of *S. longepedunculata* and *L. cylindrica* leaves

Secondary Metabolites	***S. longepedunculata***	***L. cylindrica***
Alkaloids	+	++
Saponins	++	–
Tannins	+	+
Phlobatanins	+	+
Cardiac glycosides	++	++
Phenolics	++	++
Anthraquinones	–	–
Cardenolides	++	++
Steroids	+	+
Terpenes	+	+
Flavonoids	++	++

Key: ++ = High, + = Moderate, - = Absent

Table 4.4: Acute toxicity of the methanol extracts of leaves of *Securidaca longepedunculata* and *Luffa cylindrica* leaves in albino rats.

(SL) Doses (mg/kg b. w)	**Outcomes**	**(LC) Doses (mg/kg b. w)**	**Outcomes**	
10*	No abnormal or unusual behavior, signs or symptoms observed. No death recorded	10*	No abnormal or unusual behavior, signs or symptoms observed. No death recorded	
100*	Same as above	100*	Same as above	
1000*	Same as above	1000*	Same as above	
2,900#	Same as above	2,900#	Same as above	
5,000#	Same as above	5,000#	Same as above	

*number of animals (n=3), #number of animals (n=1)

The DPPH radical scavenging activity of the crude extracts of SL and LC are depicted in (Table 4.5). The MSL showed the highest DPPH radical scavenging activity with IC_{50} values of 0.47 ± 0.01 µg/mL. The IC_{50} values of ESL, MLC and ELC are (1.41 ± 0.06, 5.75 ± 0.71 and 1.04 ± 0.002) µg/mL, respectively. Metal chelating activity (MCA) are shown in (Table 4.6). MLC gave the highest MCA with IC_{50} of 3.30 ± 0.00 µg/mL. The MSL showed the lowest MCA value with IC_{50} of 11.65 ± 0.13 µg/mL. The results of inhibition of nitric oxide radical activity are illustrated in (Table 4.6). ELC showed the highest inhibition of nitric oxide radical activity with IC_{50} value of 0.10 ± 0.21 µg/mL. In addition, MLC gave the highest reductive potential index (REI) value of 0.66 ± 0.09 µg/Ml. ESL showed the lowest REI value of 2.36 ± 0.08. MSL and ELC showed REI values of 1.64 ± 0.09 and 1.18 ± 0.05 µg/mL, respectively (Table 4.6).

4.4 Effect of *Securidaca longepedunculata* and *Luffa cylindrica* leaf extracts (crude) on ethanol-induced lesions, protein content and lipid peroxidation in rats

The methanol, ethyl acetate and hexane extracts of *Securidaca longepedunculata* were tested in ethanol-induced gastric lesions. As shown in Table 4.7, the animals pretreated with150 mg and 300 mg MESL, HESL, EtESL respectively, showed significant reduction ($p<0.05$) in the extent of lesions when compared to the untreated group (ulcer group (UG). The group pretreated with cimetidine (CG) also showed a significant decrease

($p<0.05$) in the extent of lesions as compared to the UG. However, the 150mg/kg EtSL treated group showed a greater decrease in the extent of lesion than the CG group and therefore appears to be more potent than the cimetidine treated group.

Percentage inhibition of ethanol-induced gastric mucosal damage obtained for all the groups pre-treated with *Securidaca longepedunculata* and *Luffa cylindrica* are shown in Table 4.7. Cimetidine has percentage protection of (76.0 and 91.3 %) and the graded doses of the MESL (150 and 300 mg/kg) shows percentage protection of 95.7 % and 80.4 % respectively; while HESL (150 and 300 mg/kg) shows percentage protection of 91.3 % and 97.8 % respectively; and EESL (150 and 300 mg/kg) shows percentage protection of 91.3% and 97.8% respectively. These extracts of *Securidaca longepedunculata* have the highest percentage inhibition and seems to have a more potent gastro protective activity against gastric mucosal damage induced by absolute ethanol.

Table 4.5: *In vitro* antioxidants assays (TPC, TFC and DPPH) of the methanol and ethyl

acetate extracts of S.L and L.C

Extracts	**TPC (mg GAE/g extract)**	**TFC (mgQE/g extract)**	**DPPH radical scavenging activity (IC_{50}, µg/mL)**
MSL	79.93 ± 4.62	340.68 ± 35.61	0.47 ± 0.01
ESL	17.36 ± 0.81	340.68 ± 30.94	1.41 ± 0.06
MLC	11.21 ± 0.65	20.56 ± 5.09	5.75 ± 0.71
ELC	19.04 ± 0.61	678.41 ± 76.30	1.04 ± 0.02
STANDARD			0.01 ± 0.00*

KEY:

TPC= Total Phenol Content, TFC= Total Flavonoid Content, MSL= Methanol extract of SL, ESL= Ethyl acetate extract of SL, MLC= Methanol extract of LC, ELC= Ethyl acetate extract of LC, *Ascorbic acid

Table 4.6: *In vitro* antioxidants assays (metal chelating activity, inhibition of nitric oxide radical activity and reductive potential index) of the methanol and ethyl acetate extracts of S.L and L.C

Extracts	Metal Chelating Activity (IC50, µg/mL)	Inhibition of Nitric Oxide Radical Activity (IC_{50}, µg/mL)	Reductive Potential Index (REI) (mg/mL)
MSL	11.65 ± 0.13	3.34 ± 0.64	1.64 ± 0.09
ESL	6.96 ± 0.49	1.94 ± 0.14	2.36 ± 0.08
MLC	3.30 ± 0.00	4.02 ± 0.63	0.67 ± 0.09
ELC	3.91 ± 0.09	0.10 ± 0.21	1.18 ± 0.05
STANDARD	0.01 ± 0.002**	19.33 ± 0.37*	0.26 ± 0.00

KEY:

MSL= Methanol extract of SL, ESL= Ethyl acetate extract of SL, MLC= Methanol extract of LC, ELC= Ethyl acetate extract of LC, *Ascorbic acid, **EDTA

Similarly, the results obtained for the level of lesions inhibition and percentage protection

by methanol, ethyl acetate and hexane extracts of *Luffa cylindrica* revealed that all the extracts have protection against ethanol-induced gastric ulcer in a dose dependent manner.

The leaf extracts of LC and SL decreased lipid concentrations and increased protein contents as illustrated in Table 4.8 and 4.9 respectively

4.5 *In vivo* antioxidants assays

The results obtained for the *in vivo* antioxidant activity (SOD, CAT and GSH) are illustrated in Figures 4.4, 4.5, 4.6, 4.7, 4.8, and 4.9 respectively. The higher SOD levels in the ethanol treated groups as compared to the normal indicated increased production of O_2^- (superoxide anion) within the tissue as elevated O_2^- level leads to increase in concentration of cellular radical level. This effect was significantly reversed by prior administration of leaf extracts of SL and LC (Figures 4.4 and 4.5). A similar result was obtained for obtained the catalase of LC (Figure 4.6). However, there was no significant difference between the ulcer group and those pretreated with the leaf extract of SL. The results obtained for GSH for both plants are similar to those of SOD (Figure 4.9).

4.6 Histopathological evaluation of experimental animals

The normal rats treated only with distilled water and (no ulcer induction by ethanol) showed normal gland architecture without erosion. No inflammation of cells, no haemorrahagic damage. There was no epithelial loss and no mucosa oedema. There was no evidence of gastric injury (Plate 1). The ethanol treated animals (without pretreatment with plant extracts) showed loss of gland architecture with erosion of the epithelial layer and evident oedama. There was also inflammation of cells and haemorrhagic damage. It gave a maximal index of gastric injury as depicted in plate 2. Plate 3 shows the representative of the histology of the stomach of the rat treated with 150 mg/kg *b. w* n-Hexane extract of *Luffa cylindrica* (pretreated group). The architecture of the walls of the stomach showed the following indices of gastric injury: Epithelial loss= 1; Mucosa oedema

=2; haemorrhagic damage =1; Inflammatory cells =2. Total = 6

Figure 4.4: Effect of *Luffa cylindrica* leaf extract on mucosal gastric superoxide dismutase activity (SOD) in absolute ethanol-induced gastric ulcer

Values are mean ± S.E.M (n=5). * = significantly different from control ($p<0.05$)

= significantly differen\t from ulcer group ($p<0.05$), (One-Way ANOVA Test).

Figure 4.5: Effect of *Securidaca longepedunculata* leaf extract on mucosal gastric superoxide dismutase activity (SOD) in absolute ethanol-induced gastric ulcer

Values are mean ± S.E.M (n=5). * = significantly different from control ($p<0.05$)

= significantly different from ulcer group ($p<0.05$), (One-Way ANOVA Test).

Figure 4.6: Effect of *Luffa cylindrica* leaf extract on mucosal gastric catalase (CAT) activity in absolute ethanol-induced gastric ulcer

Values are mean ± S.E.M (n=5). * = significantly different from control ($p<0.05$)

= significantly different from ulcer group ($p<0.05$), (One-Way ANOVA Test).

Figure 4.7: Effect of *Securidaca longepedunculata* leaf extract on mucosal gastric Catalase (CAT) activity in absolute ethanol-induced gastric ulcer

Values are mean ± S.E.M (n=5). * = significantly different from control ($p<0.05$)

= significantly different from ulcer group ($p<0.05$), (One-Way ANOVA Test).

Figure 4.8: Effect of *Luffa cylindrica* leaf extract on mucosal gastric reduced glutathione (GSH) activity in absolute ethanol-induced gastric ulcer

Values are mean ± S.E.M (n=5). * = significantly different from control ($p<0.05$)

= significantly different from ulcer group ($p<0.05$), (One-Way ANOVA Test).

Figure 4.9: Effect of *Securidaca longepedunculata* leaf extract on mucosal gastric reduced glutathione (GSH) activity in absolute ethanol-induced gastric ulcer

Values are mean ± S.E.M (n=5). * = significantly different from control ($p<0.05$)

= significantly different from ulcer group ($p<0.05$), (One-Way ANOVA Test).

Table 4.7: Effect of *Securidaca longepedunculata* (SL) *Luffa cylindrica* (LC) Crude leaf extracts on absolute ethanol-induced gastric ulcer

Extracts	(mg/kg)	% Inhibition	
		SL	LC
HE	(150)	91.3	84.7
	(300)	97.8	97.8
EE	(150)	91.3	97.8
	(300)	97.8	100
ME	(150)	95.7	93.4
	(300)	80.4	97.4
Cimetidine (CG)	(50)	76.0	
	(100)	91.3	
Ethanol (1 mL) (UG)		0.0	
Control (Normal Saline)		-	

HE = n-Hexane Extract, E E = Ethyl acetate Extract, ME = Methanol Extract

Table 4.8: Effect of leaf extracts of *Luffa cylindrica* on protein content and lipid peroxidation in ethanol-induced ulcerations in rats

Groups	Dose (mg/kg b. w)	Total Protein (mg/g) (Mean ± S.E.M)	MDA Concentra ($\times 10^{-8}$µmol/g^{-1} pr (Mean ± S.E.M
L C ME	(150)	80.66 ± 1.06^{ab}	1.36 ± 0.01^{abc}
L C ME	(300)	69.28 ± 1.10^{abc}	2.91 ± 0.02^{abc}
LC EE	(150)	62.54 ± 1.81^{abc}	2.45 ± 0.05^{abc}
LC EE	(300)	86.20 ± 1.96^{abc}	2.29 ± 0.03^{abc}
L C HE	(150)	90.73 ± 2.37^{abc}	2.10 ± 0.05^{abc}
L C HE	(300)	74.02 ± 1.91^{abc}	1.60 ± 0.02^{abc}
Ethanol	(1 mL/130 g)	42.60 ± 1.12^{ac}	4.68 ± 0.10^{ac}
Cimetidine	(50)	80.10 ± 1.32^{ab}	2.56 ± 0.09^{ab}
Cimetidine	(100)	95.20 ± 2.54^{ab}	1.12 ± 0.01^{ab}
Normal control (1 mL Normal Saline)		144.00 ± 9.43^{b}	0.14 ± 0.01^{bc}

Student t-test was used in the analysis of this data. The results are expressed as Mean±S.E.M (n=5); $^{a}p<0.05$ significantly different from the control group; $^{b}p<0.05$ significantly different from the ethanol group; $^{c}p<0.05$ significantly different from the cimetidine group.

Table 4.9: Effect of leaf extracts of *Securidaca longepedunculata* on protein content and lipid peroxidation in ethanol-induced ulcerations in rats

Groups Dose (mg/kg b. w)	Total Protein (mg/g) (Mean ± S.E.M)	MDA Concentration ($\times 10^{-8}$ µmol/g^{-1} protein) (Mean ± S.E.M)
S L ME (150)	86.38 ± 1.89^{abc}	2.41 ± 0.04^{abc}
S L ME (300)	81.98 ± 3.23^{abc}	2.36 ± 0.09^{abc}
S L EE (150)	83.70 ± 4.13^{abc}	1.88 ± 0.01^{abc}
S L EE (300)	81.62 ± 0.72^{abc}	1.97 ± 0.03^{abc}
S L HE (150)	73.38 ± 1.17^{abc}	1.47 ± 0.05^{abc}
S L HE (300)	76.22 ± 4.25^{abc}	1.96 ± 0.01^{abc}
Ethanol (1 mL/130 g)	42.60 ± 1.12^{ac}	4.68 ± 0.10^{ac}
Cimetidine (50)	80.10 ± 1.32^{ab}	2.56 ± 0.09^{ab}
Cimetidine (100)	95.20 ± 2.54^{ab}	1.12 ± 0.01^{ab}
Normal control (1 mL Normal Saline)	144.00 ± 9.43^{bc}	0.14 ± 0.01^{bc}

Student t-test was used in the analysis of this data. The results are expressed as Mean±S.E.M (n=5); $^{a}p<0.05$ significantly different from the control group; $^{b}p<0.05$ significantly different from the ethanol group; $^{c}p<0.05$ significantly different from the cimetidine group.

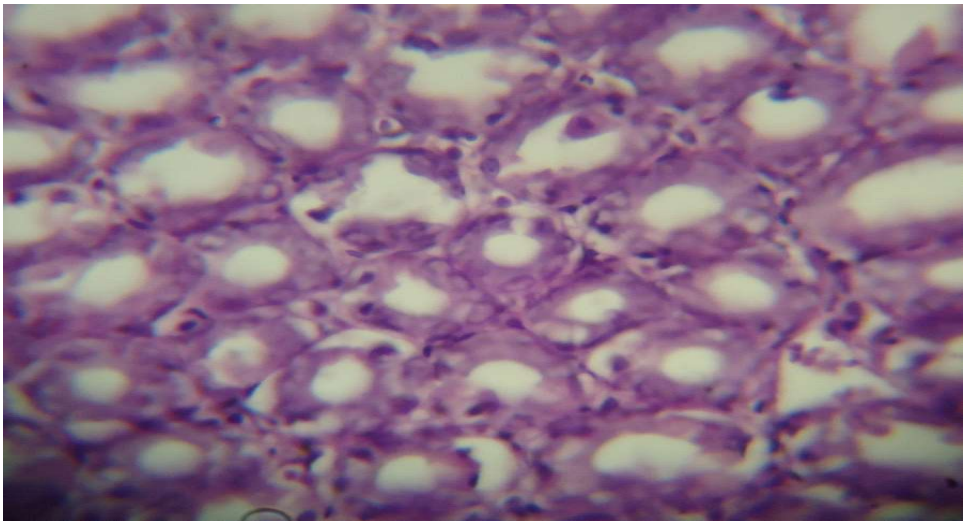


Plate 1: The histology of the stomach of the control rat (untreated animal). The architecture of the walls of the stomach showed the following indices of gastric injury: Epithelial cell loss=0, Mucosal edema=0, Haemorrhagic damage=0, inflammatory cells=0. No Evidence of gastric injury

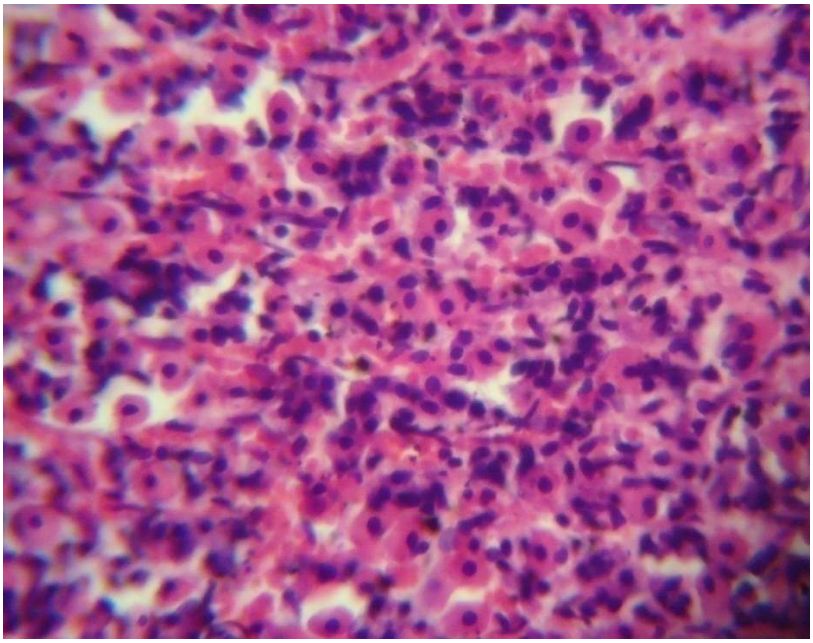


Plate 2: The histology of the stomach of the ethanol treated animal. The architecture of the walls of the stomach showed the following indices of gastric injury: (Epithelial loss= 3; Mucosa oedema =4; Haemorrhagic damage = 4; inflammatory cells = 3). Total = 14, Maximal index of gastric injury

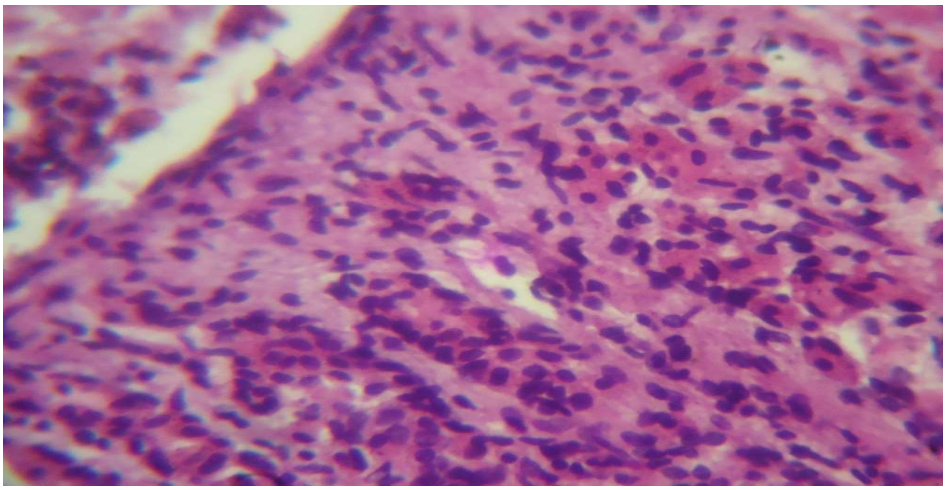


Plate 3: The histology of the stomach of the rat treated with 150 mg/kg *b. w* n-Hexane extract of *Luffa cylindrica* (pretreated group). The architecture of the walls of the stomach showed the following indices of gastric injury:

Epithelial loss= 1; Mucosa oedema =2; haemorrhagic damage =1; Inflammatory cells =2. Total = 6

4.7 Vacuum liquid chromatographic fractions and the antiulcer screening of the fractions.

The pooled VLC fractions are obtained from the VLC perfomed on the ethyl acetate extracts from S.L and L.C and the subsequent antiulcer screening of all the pooled VLC fractions are given in Tables 4.10, 4.11, 4.12 and 4.13.

4.8 Column chromatographic fractions

The results of the column chromatography carried out on the fractions obtained from the vacuum liquid chromatography are shown in Tables 4.14, 4.15 and 4.16.

4.9 Spectra data for compound 17 (isolated as A1 from *Luffa cylindrica*) (Table 4.17) (Appendix IIIA - IIIJ)

Weight: 5 mg

^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 2.33 (1H, s), 2.18 (12H, s), 2.00 (1H, s), 1.77 (1H, d), 1.26 (3H, m), 0.85 (1H, m) (Appendix IIIA).

^{13}C-NMR ($CDCl_3$, 400 MHz): δ ppm 206.92, 31.91, 30.89, 30.02, 29.68, 29.34, 22.67, 14.09 (Appendix IIIB).

IR data (cm^{-1}): 3426.15, 2922.59, 2852.99, 1721.51, 1460.89 (Appendix IIIJ).

4.10 Preparative thin layer chromatography of LCC3B

Prep TLC was carried out on LCC3B sub fraction obtained from LCC3 (Table 4.18) and the isolated compound coded PB, which was further purified using prep TLC and the purified compound given a new code PPB

Weight: 6 mg

Table 4.10: Vacuum liquid chromatography of ethyl acetate extract (*Luffa cylindrica*)

VLC POOLED Fractions		**(Weight g)**	**Solvent Mixture**	**Ratio Per 500 mL**
A_{LC}	1 -5	(7.92)	Hexane/ DCM	70:30
B_{LC}	6-12	(10.88)	DCM/Hexane	90:10
C_{LC}	13-18	(15.60)	DCM/Ethyl acetate	60:40
D_{LC}	19-34	(40.87)	Ethyl acetate/ Methanol	70:30

Table 4.11: Vacuum liquid chromatography of ethyl acetate extract (*Securidaca longepedunculata*)

VLC POOLED Fractions (Weight g)	**Solvent Mixture**	**Ratio Per 500 mL**
A_{SL} 1 -4 (7.78)	Hexane	80:20
B_{SL} 5-12 (8.73)	DCM/Hexane	90:10
C_{SL} 13-18 (21.29)	Ethyl acetate/DCM	60:40
D_{SL} 19-32 (29.53)	Ethyl acetate/Methanol	70:30

Table 4.12: Effect of VLC fractions of *Luffa cylindrica* on absolute ethanol-induced gastric ulcer

Groups	(weight mg/kg *b. w*)	Inhibition (%)
$A1_{LC}$	**(50)**	**61.5**
$A2_{LC}$	**(100)**	**92.3**
$B1_{LC}$	**(50)**	**92.3**
$B2_{LC}$	**(100)**	**94.9**
$C1_{LC}$	**(50)**	**38.5**
$C2_{LC}$	**(100)**	**46.2**
$D1_{LC}$	**(50)**	**46.2**
$D2_{LC}$	**(100)**	**61.5**
Ethanol	**(1 mL/130 g)**	**0.0**
Cimetidine	**(50)**	**64.1**
Cimetidine	**(100)**	**71.8**
Normal control	**(1 mL Normal Saline)**	**-**

Table 4.13: Effect of VLC fractions *Securidaca longepedunculata* on absolute

ethanol-induced gastric ulcer

Groups	**(weight mg/kg *b.w)***	**Inhibition (%)**
A1SL	50	64.8
A2SL	100	71.8
B1SL	50	82.1
B2SL	100	92.3
C1SL	50	71.8
C2SL	100	76.9
D1SL	50	87.2
D2SL	100	92.3
Ethanol	(1 mL/130 g)	0.0
Cimetidine	50	64.1
Cimetidine	100	71.8
Normal control	(1 mL Normal saline)	-

Table 4.14: Column Chromatography of VLC Fraction D_{SL} (*Securidaca longepedunculata*)

Column Pooled Fractions	(Yield g)	Solvent Mixture	Ratio Per 500 mL	Colour (daylight)
(1 -7)	0.15	Hexane/Ethyl acetate	40 : 60	Brownish-yellow
(8-19)	0.53	Hexane/Ethyl acetate	65 : 35	Brownish
(20-29)	0.87	Hexane/Ethyl acetate	25 : 75	Black
(30-49)	2.21	Hexane/Ethyl acetate	15 : 85	Greenish-black
(50-66)	2.57	Hexane/Ethyl acetate	10 : 90	Greenish
(67-76)	1.81	Ethyl acetate/Methanol	95 : 5	Greenish-black
(77 -90)	3.41	Ethyl acetate/Methanol	90 : 10	Black
(91-104)	3.45	Ethyl acetate/Methanol	87.5 : 12.5	Brownish
(105-124)	2.47	Ethyl acetate/Methanol	70 : 30	Blackish-yellow
(125-149)	2.13	Ethyl acetate/Methanol	55 : 45	Blackish-yellow
(150-200)	0.68	Ethyl acetate/Methanol	90 : 10	Blackish-yellow

Table 4.15: Column chromatography of VLC fraction B_{SL} (*Securidaca longepedunculata*)

Column Pooled Fractions	(Yield mg)	Codes Assigned	Solvent Mixture	Ratio Per 100 mL	Colour (daylight)
(2 -6)	660	B26	Hexane/ Ethyl acetate	80 : 20	Brown (oily)
(7-8)	170	B78	Hexane/ Ethyl acetate	80 : 20	Creamy (oily)
(9-10)	1,080	**B9J**	Hexane/ Ethyl acetate	80 : 20	Grey
(11-13)	1,000	**B11M**	Hexane/ Ethyl acetate	80 : 20	Dark-brown
(14-16)	650	B1416	Hexane/ Ethyl acetate	80 : 20	Black
(17-20)	560	**B17T**	Hexane/ Ethyl acetate	60 : 40	Green
(21-29)	570	BU29	Hexane/ Ethyl acetate	50 : 50	Greenish
(30-33)	130	B3033	Hexane/ Ethyl acetate	40 : 60	Greenish
(34-35)	130	B3435	Hexane/ Ethyl acetate	30 : 70	Black
(36-40)	ND	B3640	Hexane/ Ethyl acetate	20 80	Black
(41-55)	ND	B4155	Hexane/ Ethyl acetate	20 : 80	Black

ND = NOT DETERMINED

Table 4.16: Column chromatography of VLC fraction B_{LC} (*Luffa cylindrica*)

Column Pooled Fractions		**(Yield mg)**	**Solvent Mixture**	**Ratio Per 500 mL**	**Colour (daylight)**
(1)	(LCA1)	500	Hexane	100	Yellow (oily)
(2-7)	(LCB2)	ND	Hexane/Ethyl acetate	65 : 135	Brownish
(8-10)	(LCC3)	4360	Hexane/Ethyl acetate	25 : 75	Black
(11-13)	(LCD4)	800	Hexane/Ethyl acetate	15 : 85	Greenish-black
(14)	(LDE5)	130	Hexane/Ethyl acetate	10 : 90	Greenish
(15-21)	(LDF6)	730	Hexane/Ethyl acetate	85 : 15	Greenish-black
(22 -33)	(LDG7)	115	Hexane/Ethyl acetate	85 : 15	Black

ND = NOT DETERMINED

Table 4.17: Column chromatography of column fraction LCA1 (from B_{LC}

(*Luffa cylindrica*))

Column Pooled Fractions	**Solvent Mixture**
1. (1 - 10) A1	**Hexane (100%) (colourless)**
2. (11 - 20) BC	Hexane : Ethyl acetate, 90 : 10 (yellow, oily

Table 4.18: Column chromatography of LCC3 fraction from B_{LC} (*Luffa cylindrica*)

Column Pooled Fractions	**Solvent Mixture**
1. (1 - 10) **LCC3**A (50 mg)	Hexane/DCM
2. (11 - 13) LCC3B (70 mg)	**DCM**
3. (14-20) **LCC3**C (810 mg)	DCM/Ethyl acetate

4.10.1 Spectra data of compound 18 (isolated as PPB from *Luffa cylindrica*) (Appendix IVA - IVJ)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 4.08 (1H, t), 3.57 (1H, d), 1.51 (4H, s), 1.18 (4H, s), 0.81 (2H, d) (Appendix VIA).

^{13}C-NMR ($CDCl_3$, 400 MHz): δ ppm 129.93 (C-7), 122.47 (C-6), 63.05 (C-1), 38.97 (C-2), 30.79 (C-4), 29.74 (C-3), 27.88 (C-3), 25.81 (C-5), 22.62 (C-10), 14.11 (C-8) (Appendix IVB)

IR data (cm^{-1}): 3337.95, 3054.47, 1624.45, 1264.42 (Appendix IVJ)

4.11 Spectra data for compound 19 (isolated as B11MF from *Securidaca longepedunculata*) (Table 4.19) (Appendix VA - VJ)

Weight: 7 mg

^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 5.27 (2H, m, H-6), 5.07 (1H, d, H-22), 4.98 (2H, d, H-23), 3.53 (3H, m, H-3), 1.18 (3H, s, H-19), 0.85 (s, H-26), 0.81 (m, H-27), 0.73 (13H, d, H-18). (Appendix VA)

^{13}C-NMR ($CDCl_3$, 400 MHz): δ ppm 154.59 (C-5), 139.61 (C-22), 129.78 (C-23), 127.97 (C-6), 71.10 (C-3), 56.04 (C-14), 55.08 (C-17), 49.46 (C-24), 45.85 (C-4), 40.27 (C-12), 40.19 (C-13), 37.14 (C-1), 36.59 (C-20), 33.69 (C-22), 32.12 (C-8), 31.92 (C-7), 31.46 (C-2), 29.76 (C-16), 29.70 (C-25), 26.21 (C-23), 21.55 (C-26), 19.82 (C-11),19.20 (C-27/19), 18.91 (C-21), 11.98 (C-18), 11.85 (C-29). (Appendix VB)

IR data (cm^{-1}): 3337.85, 2925.14 and 2854. (Appendix VJ)

Table 4.19: Column chromatography of the column sub-fraction of B11M (*Securidaca longepedunculata)*

Column Pooled Fractions	**Codes Assigned**	**Solvent Mixture**	**Ratio Per 50 mL**	**Colour (daylight)**
1. (1 -14)	B11MA	Hexane/DCM	40 : 60	Brown (oily)
2. (15-33)	**B11MCF**	DCM	100	Creamy (oily)
3. (34 &37)	B11MC	DCM/Ethyl acetate	98 : 2	Grey
4. (35, 36, 38-54)	B11MCC	Hexane/Ethyl acetate	90 : 10	Dark-brown

4.12 Spectra data for compound 20 (isolated as B9JN2 from *Securidaca longepedunculata*) (Appendix VIA - VIJ)

Weight: 8 mg

^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 5.28 (1H, m, 4.05 (H, q), 2.28 (2H, t), 2.10 (1H, s) 1.98 (1H, td), 1.55 (2H, dd), 1.24 (19H, m), 0.81 (4H, dd) (Appendix VIA).

^{13}C-NMR ($CDCl_3$, 400 MHz): δ ppm 178.87, 130.23, 130.03, 129.73, 128.07, 127.91, 77.32, 77.21, 77.07, 76.69, 33.86, 31.92, 30.91, 29.76, 29.69, 29.58, 29.52, 29.43, 29.36, 29.32, 29.24, 29.06, 29.03, 27.21, 27.15, 25.63, 24.70, 22.68, 22.57, 14.10 (Appendix VIB).

IR data (cm^{-1}): 3464.22, 2984.49, 2941.33, 2876.95, 1735.82 (Appendix VIJ).

4.13 Spectra data for compound 21 (isolated as PBT from *Securidaca longepedunculata*) (Appendix VIIA - VIII)

Weight: 6 mg

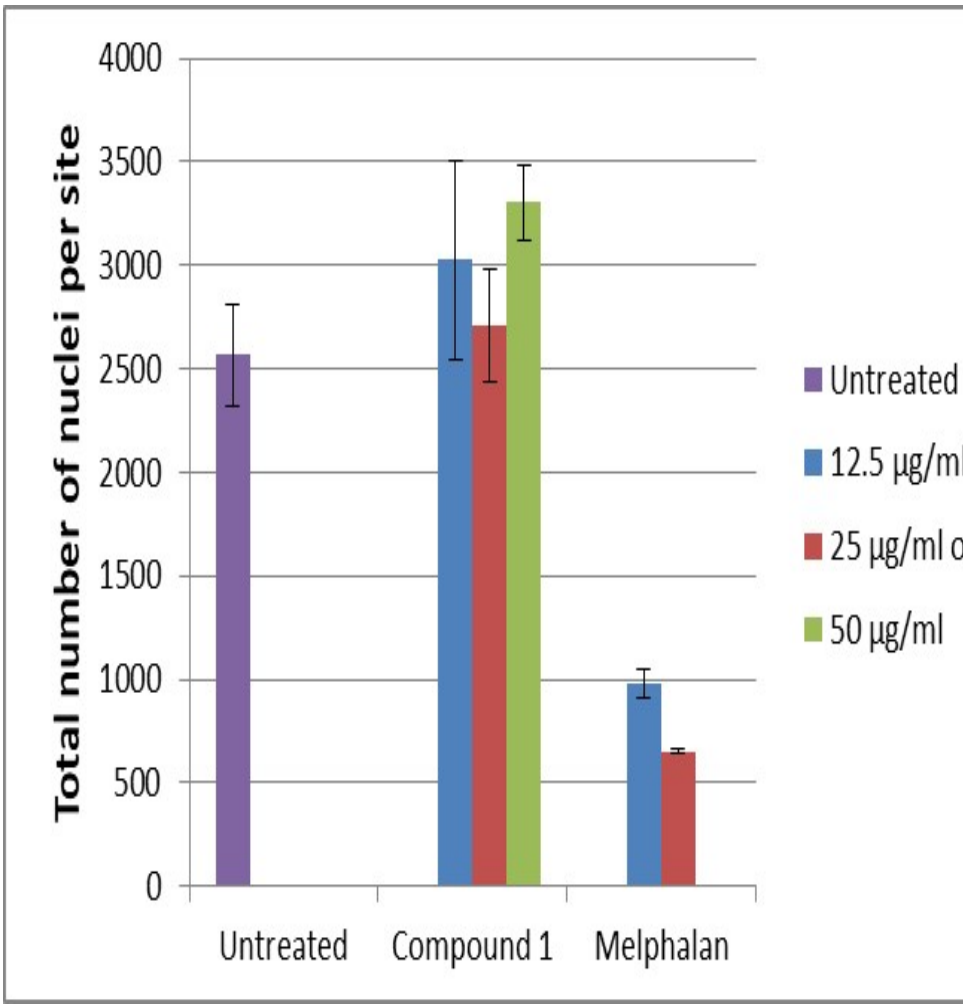
^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 5.31 (?H, d), 4.04 (?H, d), 3.95 (?H, m), 3.49 (?H, m), 2.18 (1H, d), 1.54 (2H, dd), 1.35 (3H, d), 1.27 (5H, m), 1.18 (1H, d), 1.10 (1H, s), 0.88 (4H, m) (Appendix VIIA).

^{13}C-NMR ($CDCl_3$, 400 MHz): δ ppm 39.37, 37.45, 37.40, 37.29, 37.21, 37.10, 32.77, 31.92, 30.92, 29.70, 29.36, 27.97, 27.43, 24.79, 24.46, 24.02, 22.71, 22.62, 21.41, 19.76, 19.70, 14.11 (Appendix VIIB).

IR data (cm^{-1}): 3000, 2904, 1725, 1450 (Appendix VIII).

4.14 Effects of isolated compounds on cell proliferation

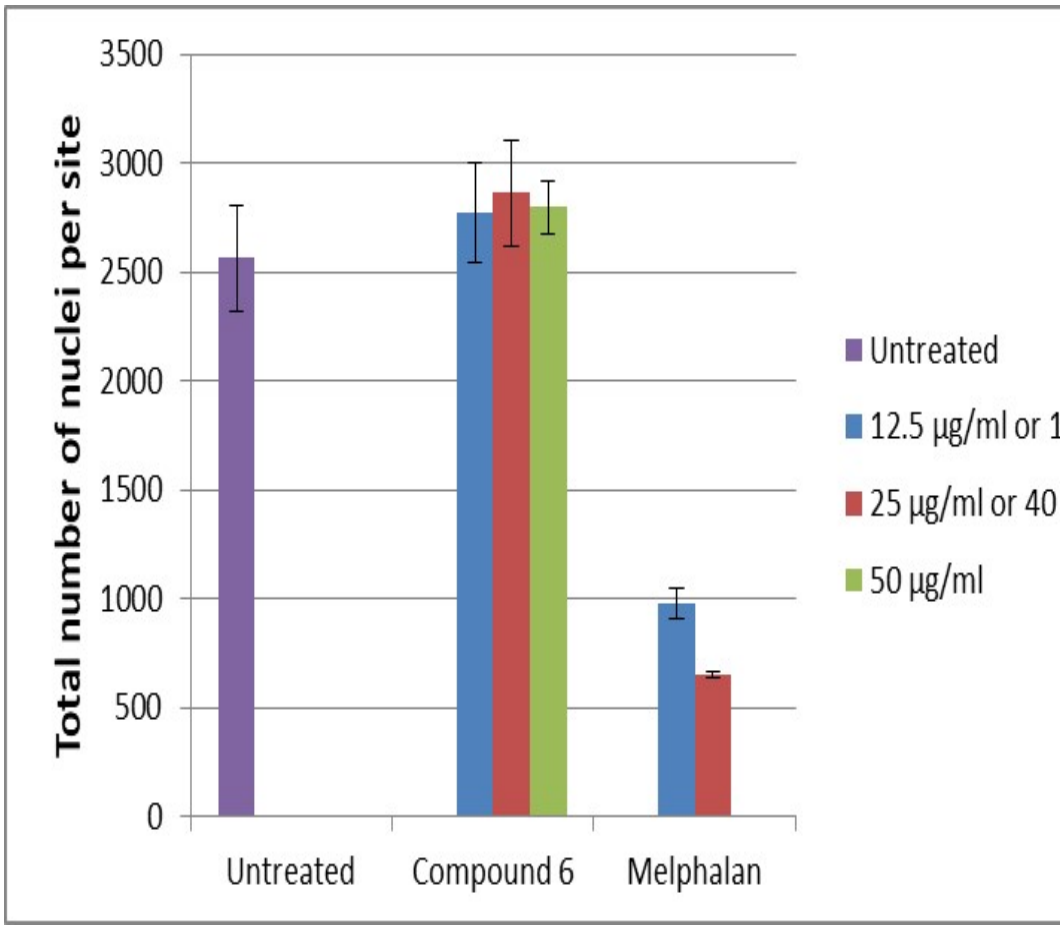
Compound 17 (isolated as A1 from *Luffa cylindrica)* showed significant increases in the total number of cells at the doses used (Figure 4.10). All the doses of Compound 18 (isolated as PPB from *Luffa cylindrica)* used stimulated cell proliferation as shown in figures 4.11 and no evidence of toxicity was observed. Compound 19 (isolated as B11MF from *Securidaca longepedunculata*) exhibited anti-apoptotic and cell proliferative activities except the highest dose that showed decrease in the total number of cells (Figure 4.12). Compound 20 (isolated as B9JN2 from *Securidaca longepedunculata*) also did not exhibit cell proliferative activity; moreover, the highest dose showed anti-proliferative tendency as illustrated in Figure 4.13. Compound 21 (isolated as PBT from *Securidaca longepedunculata*) was highly toxic to the cells as depicted in Figure 4.14.



Untreated **Compound 17** **Melphalan**

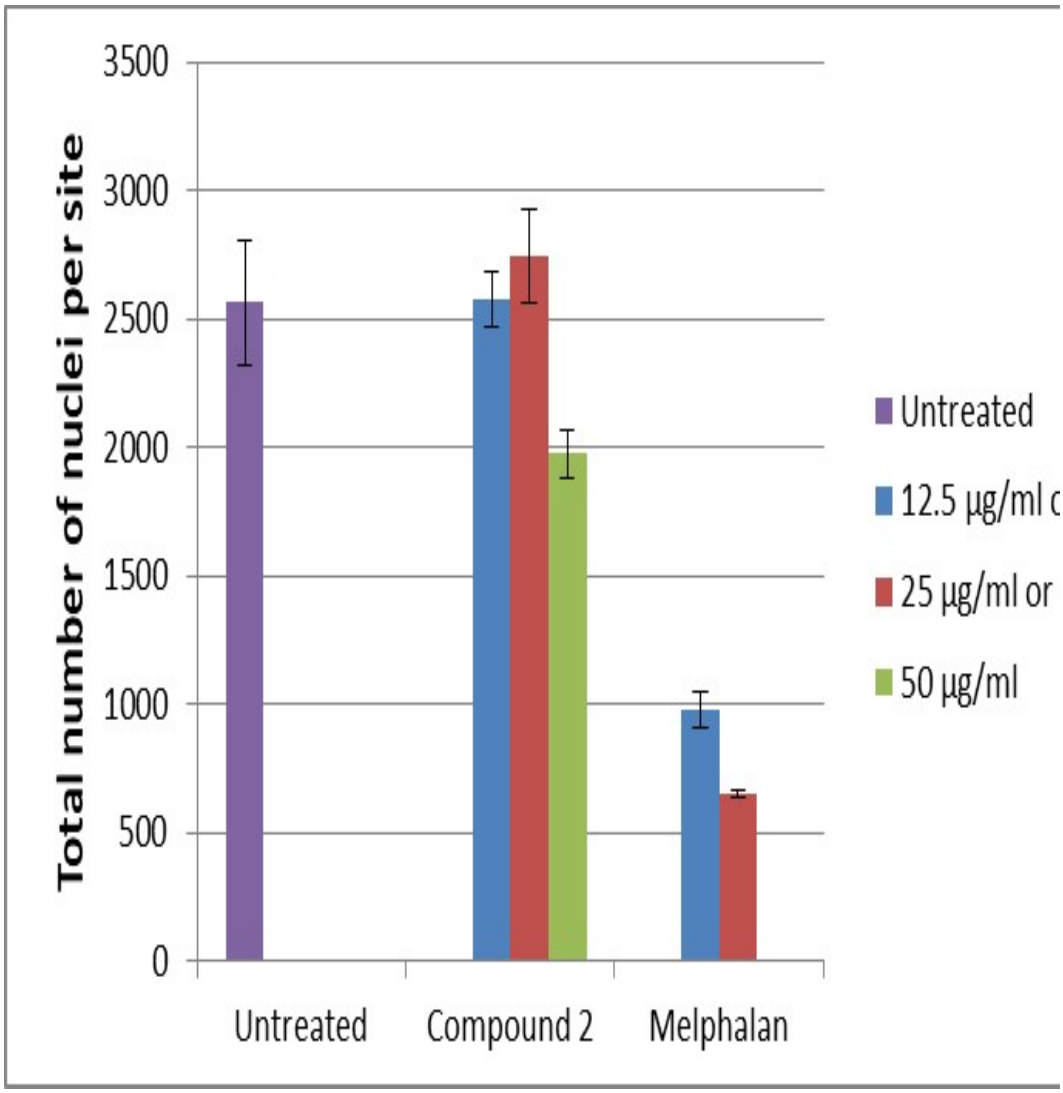
Figure 4.10: The effect of compound 17 (from *Luffa cylindrica*) on cell

growth.



Untreated **Compound 18** **Melphalan**

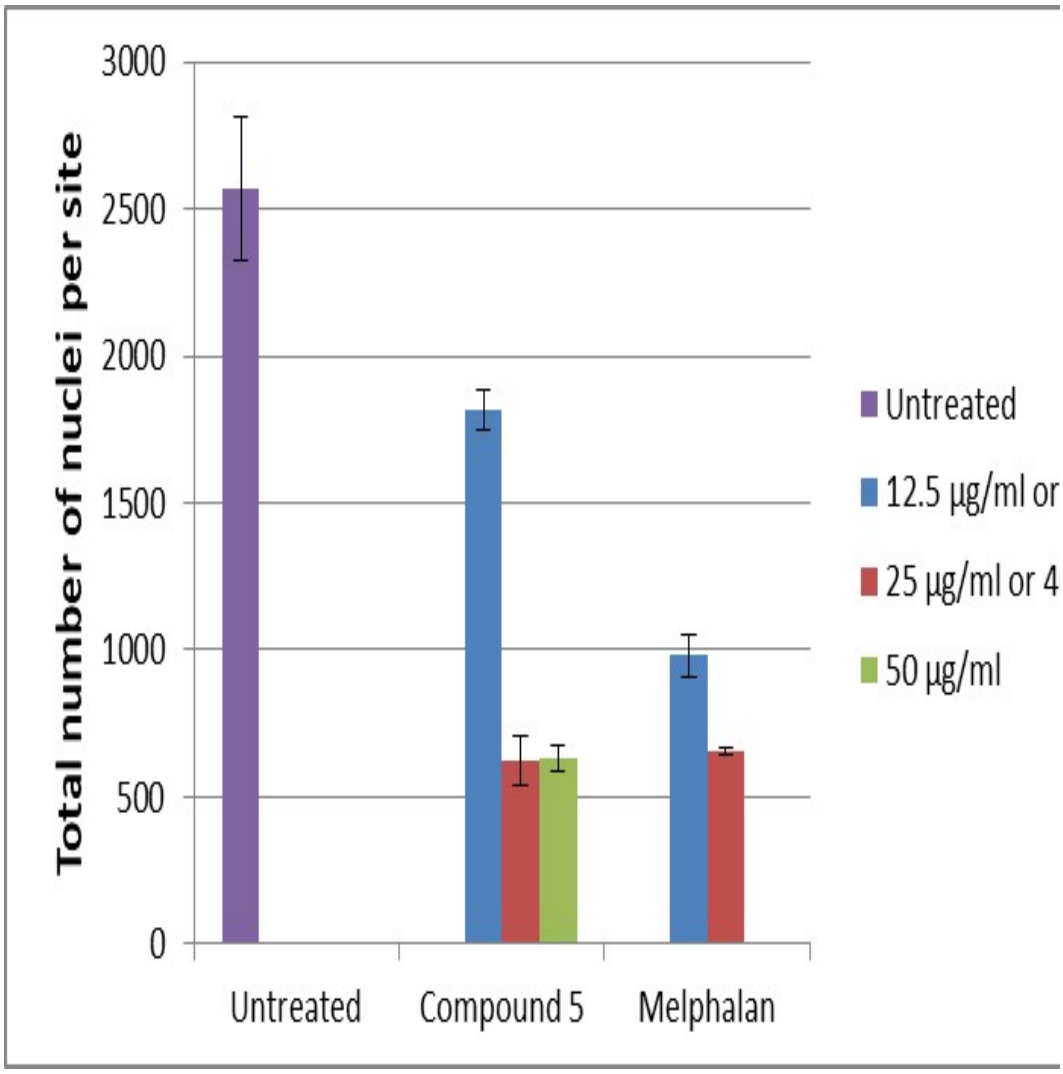
Figure 4.11: The effect of compound 18 (from *Luffa cylindrica*) on cell growth


Total number of nuclei per site
3500
3000
2500
2000
1500
1000
500
0
Untreated
Compound 2
Melphalan
Untreated
12.5 µg/ml c
25 µg/ml or
50 µg/ml

Untreated **Compound 19** **Melphalan**

Figure 4.12: The effect of compound 19 (from *Securidaca longepedunculata*) on cell growth

Figure 4.13: The effect of compound 20 (from *Securidaca longepedunculata*) on cell proliferation


Total number of nuclei per site
3000
2500
2000
1500
1000
500
0
Untreated
Compound 5
Melphalan
Untreated
12.5 µg/ml or
25 µg/ml or 4
50 µg/ml

Untreated Compound 21 Melphalan

Figure 4.14: The effect of compound 21 (from *Securidaca longepedunculata*) on cell growth

CHAPTER FIVE

DISCUSSION

5.1 Ethnobotanical survey

The plant families with the highest number of species used in the treatment of peptic ulcer from the ethnobotanical survey carried out in Ado/Odo Ota LGA area of Ogun State, Southwest, Nigeria includes Asteraceae and the Euphorbiaceae families with five species each followed by Malvaceae, Liliaceae, Rosaceae and Musaceae with 2 species each which is indicative of their importance in the treatment of peptic ulcer in this region. Although, Apocynaceae, Leguminosae, Caricaceae, Portulacaceae, Zingiberaceae, Lamiaceae, Clusiaceae, Tiliaceae are only represented by one species each, there is also a need to explore them scientifically for antiulcer drug development. Results revealed that quite a number of plants parts, the respondents have found; especially the leaves, roots, rhizomes, fruits, seeds and very rarely the whole plants efficient in the treatment of gastric ulcer. The most prominent plant species in the medicinal recipes according to the use-mentions index were *Carica papaya* (Caricaceae), *Zingiber officinale* (Zingiberaceae), *Musa paradisiaca* (Musaceae) and *Allium sativum* (Liliaceae) showing that they may possess important anti-ulcer properties.

Investigations on the plant parts used and the mode of preparation and administration indicated that irrespective of the plant part(s) or combinations used, water was the main medium for all the medicinal preparations. In addition to pure herbal preparations, in some cases the drug was administered along with honey, sugar, cold pap or palm oil.

These ingredients may be used to enhance the effect of the herbal preparations by serving as vehicle for some chemical compounds or are simply added to make the preparations palatable.

Many researchers have reported antiulcer activity of some of the plants species mentioned in this survey (*Aspilia africana*, *Parquetina nigrescens*, *Excoecaria agallocha* and *Cassia nigricans*) or other species of the same families identified in this survey (Asteraceae, Apocynaceae, Euphorbiaceae and Leguminosae) (Kayode *et al*., 2009; Ubaka *et al.*, 2010). Some species of the family Asteraceae are used in wound healing, wound cleansing, anti-inflammatory and for curing stomach aches and pains (Heinrich *et al*., 1998) and this may explain why some of the plants identified in this survey are from the family Asteraceae. Plants of the family Lamiaceae are used for treating stomach pain and as relaxant (Pardo de Santayana *et al*., 2005), which may be connected to stomach ulcer. Ekpendu (2003) reported the antiulcer property of the following plant families: Leguminosae, Liliaceae, Malvaceae, Myritaceae and Tiliaceae among others in the Benue area of Nigeria. These five plant families have also been identified in this present work as part of plant families used by the people of Ado-Odo area of Ogun State, Nigeria.

The result of this survey showed that the majority of the herb sellers/traditional medical practitioners (TMPs)/herbalists claimed no occurrence of side effects following patient's use of herbal preparations for the treatment of peptic ulcer. However, some of the traditional healers said they usually advice their patients to avoid eating spicy food or irregular eating habit, alcohol and sweetened substances. They are also encouraged to take milk but not in excess.

Although there are many products in the market for the treatment of gastric ulcers, including antacids, proton pump inhibitors, anticholinergics and histamine H_2-antagonists, most of these drugs produce several adverse reactions, such as gynecomastia, hematopoietic changes, acute interstitial nephritis (Ra and Tobe, 2004), nephrotoxicity and hepatoxicity (Fisher and Le Couteur, 2001). Previous studies have shown that various natural products of plant origin-*Xylocarpus grantum (*Lakshmi *et al.*, 2010), *Tectona grandis* (Singh *et al.,* 2010), *Desmodium gangeticum* (Dharmani *et al*., 2005), *Ocimum sanctum* (Dharmani *et al*., 2004)*, Parquetina nigrescens* (Kayode *et al.,* 2009),

Asparagus racemosus (Sairam *et al*., 2003) among others have been reported to possess anti-ulcer activity. Compounds from natural products like plant products are some of the most attractive sources of new drugs, and have shown promising results in the evaluation of anti-ulcer drugs for the treatment of gastric ulcers, induced in the laboratory animals in various experimental models (Borrelli and Izzo, 2000). Many plants are known to have a long history of use for soothing inflamed and injurious mucus membranes in the digestive tract. For example, *Licorice* may protect the stomach and duodenum by increasing production of mucin, (Goso *et al*., 1996). Moreover, some active components of plants have been shown to confer antiulcer properties on certain plants. Consequently, reports of laboratory research have shown that compounds like flavonoids may inhibit growth of *H. pylori*, in addition to their direct cytoprotective effects (Beil *et al*., 1995).

5.2 Phytochemical screening

Phytochemical screening of the plants revealed the presence of antioxidant compounds such alkaloids, flavonoids, terpene, steroids, tannins, cardiac glycosides and phenolics in both plants. These phytochemicals have been previously identified in both plants by other researchers and their co-workers (Ndamitso *et al*., 2013 and Rajasree *et al*., 2016).

5.3 Acute toxicity

Acute oral toxicity of *Securidaca longepedunculata* and *Luffa cylindrica* did not show any abnormal sign or symptoms or any behavioral changes in the doses administered. No death was also recorded. These observations suggest that the oral exposure to the leaves of these plants are relatively safe. The result of the acute toxicity agrees with those reported in literature (Agbaje *et al*., 2012)

5.4 *In vitro* antioxidant

Ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity. Ethanol is metabolized in the body and release superoxide anion and hydroperoxy free radicals. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa

(Gulcin *et al*., 2004) and scavenging these free radicals can play an appreciable role in healing these ulcers (Halliwell and Gutteridge, 2001). The antioxidant properties of the leaf extracts of *Securidaca longepedunculata* and *Luffa cylindrica* may have scavenged the free radicals produced by the metabolism of ethanol and thereby inhibit ulceration.

Phenols and flavonoids are potent antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989). According to previous reports, a highly positive relationship between total phenols and antioxidants activity was found in many plant species. (Oktay *et al*., 2003). The phenolic compounds may contribute directly to the antioxidative action of the leaf extracts *Securidaca longepedunculata* and *Luffa cylindrica*. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play important role in stabilizing lipid peroxidation (Yen *et al*., 1993). Several phenolics compound have antiulcer properties in rats (Sairam *et al*., 2002). Caffeic, ferulic, p-coumaric and cinnamic acids have demonstrated significant antiulcer activity (Barros *et al*., 2008).

Flavonoids and other polyphenols belong to the recently popular phytochemicals, chemicals derived from plant material with potentially beneficial effects on human health. The antioxidant activity of phenols and flavonoids is efficient in trapping superoxide anion (O_2^{-}), hydroxyl ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$) and alcohoxyl ($RO^{\cdot}$) radicals (Repetto and Llesuy, 2002). Several *in vitro* studies have demonstrated that flavonoids can scavenge superoxide, hydroxyl and peroxyl radicals, affecting various steps in the arachidonate cascade via cyclooxygenase-2 or lipoxygenase. In addition to these important effects, they have membrane-stabilizing properties and also affect some processes of intermediary metabolism and inhibit lipid peroxidation in different systems. Some of them have been shown to increase the mucosal content of prostaglandins and mucus in gastric mucosa, showing cytoprotective effects. Several of them prevent gastric mucosal lesions produced by various experimental ulcer models and protect the gastric mucosa against different necrotic agent (Alanko *et al*., 1999). The antiulcerogenic pharmacological effect of the leaf extracts and chromatographic fractions of *Securidaca longepedunculata* and *Luffa cylindrica* are partly related to their flavonoid and phenol contents (Alanko *et al*., 1999;

(Repetto and Llesuy, 2002).

Numerous antioxidants methods and modifications have been proposed to evaluate antioxidant activity and to explain antioxidant function. Of these, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, O_2^- and OH· Quenching assays are most commonly used for evaluation of antioxidants activities of extracts (Gulcin *et al*., 2004).

The ability of the leaf extracts of *Securidaca longepedunculata* and *Luffa cylindrica* to scavenge DPPH radicals indicates their antioxidant activity. DPPH is a free radical donor and is capable of attracting electron of hydrogen to form a stable molecule (Gyamfi *et al*., 1999).

Metal chelating capacity is important since it reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh *et al*., 1999; Gulcin *et al*., 2004)). It was reported that chelating agents, which forms bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Gordon, 1990; Gulcin *et al*., 2004).

Reactive nitrogen species (RNS) has been suggested to be involved in gastric mucosal damage (Halliwell and Gutteridge, 2001). The leaf extracts *Securidaca longepedunculata* and *Luffa cylindrica* showed inhibition of nitric oxide free radicals. The RNS scavenging activity of the leaf extracts of *Securidaca longepedunculata* and *Luffa cylindrica* (probably due to the presence of phenolic compounds in the plants) may have contributed to their gastroprotective ability against ethanol-induced gastric ulcer.

5.5 Antiulcer screening

Oral administration of absolute ethanol solution to the control group clearly produced the expected characteristic zonal necrotizing mucosal lesions. Absolute ethanol solution (1 ml/ 151g b.wt) induced gastric ulcer in all treated animals and this agrees with reports in

other previous studies (Lee *et al*., 2005, Kayode *et al*., 2009 and Kayode *et al*., 2015). Pretreatment with 150 mg/kg and 300 mg/kg of MESL, HESL, EtESL, MELC, HELC and EtELC induced a significant gastroprotective effect in varying degrees. The significant percentage inhibition of ulceration shown by the groups pretreated with extracts of *Luffa cylindrica* and *Securidaca longepedunculata* suggests that these treatment agents have gastro protective properties (Lee *et al*., 2005, Hiruma-Lima *et al*., 2006, Bhattacharya *et al*., 2007).

5.6 Protein concentration, lipid peroxidation and histopathology

Also, pretreatment with crude extracts of *Luffa cylindrica* and *Securidaca longepedunculata* (150 mg/kg and 300 mg/kg) increased the protein concentration in the experimental animals which will help in the repair of worn out tissues in the body. Increased protein levels have been linked to improved healing rates. The significantly increased ($p<0.05$) levels of protein concentration observed in the pretreated groups conforms to previous work of Pompeo, (2007). Furthermore, lipid peroxidation indicates oxidative stress in the body. Free radicals have been implicated in the pathogenesis of tissue damage caused by physical agents (e.g ionizing radiation), chemicals (e.g., carbon tetrachloride), paraquat, and ischemia followed by perfusion (Freeman and Carpo, 1982). Lipid peroxidation is considered one of the biochemically measureable processes by which free radicals cause membrane damage, cell damage, and tissue injury (Freeman and Carpo, 1982). Lipid peroxidation was determined by the amount of malondialdehyde (MDA) produced. All the experimental animals including the normal control group showed the occurrence of lipid peroxidation which could be as result of the animals being fasted for 24 hours. However, all the extracts of *Luffa cylindrica* caused depletion in the levels of lipid peroxidation. In the same vein, 300 mg/kg methanol, 150 mg/kg and 300 mg/kg n-hexane extracts of *S. longepedunculata* revealed reduced lipid peroxidation levels. The significantly decreased ($p<0.05$) levels of lipid peroxidation observed in most of the extracts treated groups before acute exposure to ethanol conforms to previous works of (Bhattacharya *et al*., 2007). The ability of medicinal plants to deplete lipid peroxidation levels is usually attributed to their antioxidant properties. Furthermore, the

results of the histopathology study corroborate or support the observations obtained in the other results thereby suggesting that the leaf extracts of these selected pants have antiulcer potentials.

5.7 *In vivo* antioxidant

Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury *in vivo* (Pihan *et al*., 1987). Ethanol increases superoxide anion and hydroxyl radical production and lipid peroxidation in the gastric mucosa (Gonzalez *et al*., 2001). Results in the present study also indicate similar alterations in the antioxidant status after ethanol-induced ulcer. Preventive antioxidants, such as superoxide dismutase and catalase enzymes are the first line of defense against reactive oxygen species. Reduced glutathione is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation (Halliwell, 1995; Bafna and Balaraman, 2004).

Administration of the leaf extracts of *Luffa cylindrica* and *Securidaca longepedunculata* resulted in significant modulations in the SOD, catalase, and reduced glutathione levels as compared to the control animals, which suggests their efficacy in preventing free radical-induced damage.

The higher SOD levels in the ethanol treated groups as compared to the normal indicated increased production of O_2^- (superoxide anion) within the tissue as elevated O_2^- level leads to increase in concentration of cellular radical level. This effect was significantly reversed by prior administration of leaf extracts of SL and LC providing a close relationship between free radical scavenging activity and the antioxidant capacity of the plants. Intragastric administration of superoxide dismutase has been shown to be able to protect the gastric mucosa against the damaging effect of ethanol, which suggest the involvement of superoxide free radicals in the pathogenesis of ethanol-induced gastric mucosal damage (Hernandez *et al*., 2000; Repetto and Llesuy, 2002).

Ethanol treatment induces intracellular oxidative stress and produces mitochondrial permeability transition and mitochondria depolarization, which precedes cell death in

gastric mucosal cells. Intracellar antioxidants such as glutathione may have a significant protective action against ethanol in gastric mucosal cells (Hirokawa *et al*., 1998; Repetto and Llesuy, 2002). However, there was no significant difference in the catalase levels between the ulcer group and those pretreated with the leaf extract of SL. Therefore, this suggests that catalase may not play a role in the gastroprotective and antioxidant mechanisms of this plant.

5.8 Isolated compounds from *Luffa cylindrica*

5.8.1 Compound 17 (isolated as A1)

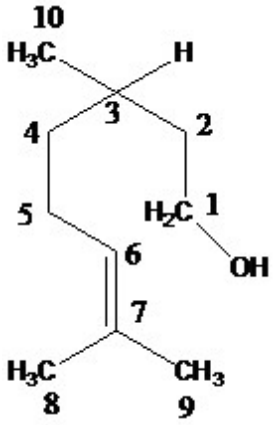
Compound 17 has a carbonyl functional group at δ ppm 206.92 in the ^{13}C NMR which was absent in the DEPT135 NMR spectra (Appendix LIII). The presence of a carbonyl functional group in the compound was further buttressed by the IR data (Appendix LXI). It has a CH_2 and a CH at δ ppm 29.34 and 30.89 ppm, respectively. The rest of the carbons are CH_3. The compound is not aromatic since both the proton and carbon NMR showed no signal in the aromatic regions. However, it appears that compound 17 might have been contaminated with acetone, which is probably responsible for the highly enhanced peaks at 206.92 ppm in the ^{13}C NMR spectrum and 2.18 ppm in the proton NMR spectrum. The signal at 30.02 is also likely another peak from acetone contamination. When the peaks for acetone are removed, what is left suggests a straight chain alkane related hydrocarbon. It shows only three types of hydrogens. Many hydrocarbons (including alkanes) are found in plants, animals and their fossils (Aja *et al*., 2014).

5.8.2 Compound 18 (isolated as PPB)

The IR data obtained for compound 18 revealed absorbance at 3337.95, which indicates an O-H stretch, 3054.47 and 1624.45, refers to an alkene stretch while the band at 1264.42 indicates a primary and secondary alcohol. Compound 18 is not an aromatic compound because no aromatic signals detected in the NMR. It has four CH_2 signals at δ ppm 63.05, 38.97, 30.79 and 27.88 while it has two CH signals at δ ppm 122.47 and 29.74. Compound 18 was identified as citronellol and conforms to that previously

isolated and identified by Masahiro, 1985 (Table 5.1 and 5.2)

Citronellol is a monoterpene alcohol (terpenoid), soluble in alcohol but insoluble in water (Wany *et al*., 2013). Citronellol has been isolated from the leaves of medicinal plants such as Cymbopogon citratus and this essential oil had been reported to possess effective antioxidant against oxidative stress and free radicals (Wany *et al*., 2013).



(18)

Table 5.1: The 1H –NMR spectra data of compound 18

S/N	δ_H, (ppm)	Multiplicity	Reported Data (Masahiro *et al*., 1985)	Reported Data (ChemBook)	Reported Data (FooDB Database)
1	4.08	t	5.10 (1H, br t)	5.10	5.10
2	3.57	D	3.68 (2H, t)	3.67	3.66
3			1.96 (2H, br q)	1.98	1.99
4			1.69 (3H, d)	1.68	1.68
5	1.51	S	1.61 (3H, s)	1.60	1.60
6	1.18	S	1.17 -1.75 (6H, m)	1.83 – 1.05	1.58 -1.18
7	0.81	D	0.92 (3H, d)	0.98	0.91

Table 5.2: The ^{13}C –NMR spectra data of compound 18

Position	**δ_C, (ppm)**	**DEPT**	**Reported Data (Advanced NMR Techniques)**	**Reported Data (Advanced NMR Techniques) DEPT**
1	63.05	CH_2	61	CH_2
2	38.97	CH_2	40	CH_2
3	29.74	CH	30	CH
4	30.79	CH_2	37	CH_2
5	27.88	CH_2	25	CH_2
6	122.47	CH	125	CH
7	129.93	C	131	C
8	14.11	CH_3	17	CH_3
9 10	25.81 22.62	CH_3 CH_3	25 19	CH_3 CH_3

5.9 Isolated compounds from *Securidaca longepedunculata*

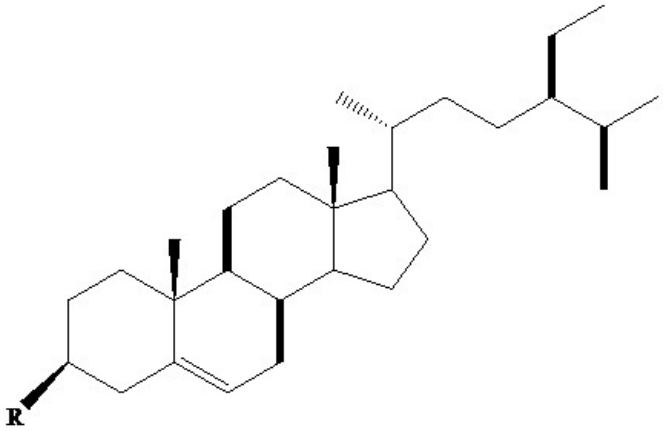
5.9.1 Compound 19 (isolated as B11MF)

Compound 19 was isolated and purified using a prep TLC technique from the column chromatography of fraction B11M. B11M was originally obtained from the VLC fraction 4 of the ethyl acetate extract of *Securidaca longepedunculata* (SL). B11M was a dark brown fraction (Column fraction) of Hexane/Ethyl acetate 80:20. Dichloromethane 100% was used to isolate BIIMF using prep. TLC. Compound 19 (isolated as BIIMF), a white powder (7 mg) was a mixture of two compounds. Therefore, the first part was assigned compound 19 -1 and the second part designated as compound 19 -2. A signal at 5.27 ppm in the ^{1}H NMR can be attributed to an olefinic proton at carbon 6. The other multiplet at 3.53 ppm is assignable to the proton at carbon 3. This low field signal is due to the attachment of β-OH to Carbon-3. There are six methyl protons between 0.47 and 1.30 ppm. The signals at 127.97 and 154.59 ppm in the ^{13}C NMR indicate a double bond between carbon 5 and carbon 6. These observations are those of the initial part of compound 19, named Compound 19 -1 and they are similar to those of a phytosterol known as sitosterol and are also comparable to reported data from literature (Tables 5.3 and 5.4) in which sitosterol was isolated and identified (Ram *et al*., 2008/2009; Yahya *et al*., 2011; Nowshir, 2012 and Consolacion *et al*., 2015) Therefore, compound 19 -1 is identified as sitosterol.

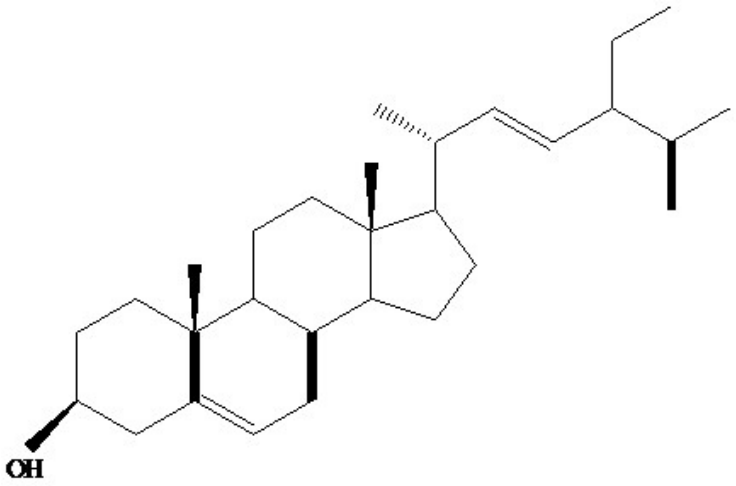
The latter part of compound 19, designated compound 19 -2 has similar signals with compound 19 -1. In addition, it has two protons at 5.07 and 4.98 ppm in the ^{1}H NMR which is due to double bond between carbon 22 and 23. These spectra data of compound 19 -2 are similar to those of stigmasterol and comparable to published data (Tables 5.3 and 5.4) of stigmasterol in literature (Consolacion *et al*., 2015; Kojima *et al*., 1990) compound 19 -2 is therefore identified as stigmasterol.

These two phytosterols are sometimes isolated as a mixture (Ria *et al*., 2006; Carvalho *et al*., 2008 and Consolacion *et al*., 2015). Also, the intensities of the peaks show that it is a mixture of two sterols, the methyl proton peaks in the ^{1}H-NMR is another indication that compound 3' is a mixture of two compounds (phytosterol) (Ria *et al*., 2006). The ^{13}C

NMR spectrum of β-sitosterol and stigmasterol are very similar with both having 29 carbons skeleton with some overlapping carbon atoms signals. Other signals not accounted for might be due to common and trace impurities such as acetone (Gregory *et al*., 2010). Sitosterol and stigmasterol have been isolated previously from *Securidaca longepedunculata* (Mongalo *et al*., 2015). The IR data showed bands at 3337.85 indicating an O-H broad, 2925.14 and 2854 revealed C-H of aliphatic



(19-1)



(19-2)

Table 5.3: The ^{13}C –NMR spectra data of compound 19

S/N	δ_C (ppm)	DEPT	POSITION	Reported Data (Consolacion *et al*., 2015)	Reported Data (Kojima *et al*., 1990)
1.	11.85	CH_3	**C-29**	11.85, 12.25	12.0, 12.3
2.	11.98	CH_3	**C-18**	11.97	11.9, 12.0
3.	18.91	CH_3	**C-21**		18.8, 21.2
4.	19.82	CH_2	**C-11**	21.07	21.1

5.	19.20	CH_3	**C-27** **C-19**	19.81 19.39	19.0 19.4
6.	21.55	CH_3	**C-26**	19.02, 21.20	19.8, 21.1
7.	26.21	CH_3	**C-23**		26.0
8.	29.70	CH	**C-25**	29.13, 31.89	29.1, 31.9
9.	29.76	CH_2	**C-16**	28.24	28.2, 28.9
10.	31.46	CH_2	**C-2**	31.65	31.6
11.	31.92	CH_2/CH_3	**C-7**	31.89	31.9
12.	32.12	CH	**C-8**	31.90	31.9
13.	33.69	CH_3	**C-22**	33.93	33.9
14.	36.56	CH_2	**C-10**	36.49	36.5
15.	36.59	CH	**C-20**	36	36.1, 40.5
16.	37.14	CH_2	**C-1**	37.24	37.2
17.	40.19	CH_3	**C-13**	42.20	42.3, 42.2
18.	40.27	CH_2	**C-12**	39.76	39.8, 39.7
19.	45.85	CH_2	**C-4**	42.29	42.3

Table 5.3: (Cont.)

S/N	**δ_C (ppm)**	**DEPT**	**POSITION**	**Reported Data (Consolacion *et al.*, 2015)**	**Reported Data (Kojima *et al.*, 1990)**
20.	49.46	CH	**C-24**	45.82, 51.23	45.8, 51.2
21.	51.01	CH	**C-9**	50.14	50.1
22.	55.08	CH_2	**C-17**	55.93	56.0, 55.9
23.	56.04	CH_3	**C-14**	56.75	56.8
24.	71.10	CH_2/CH	**C-3**	71.81	71.8

25.	127.97	CH	**C-6**	121.72	
26.	129.78	CH	**C-23**	129.25	129.2
27.	139.61	CH	**C-22**		138.86
28.	154.59	C	**C-5**	140.74	140.7

Table 5.4: The ^{1}H –NMR spectra data of compound 19

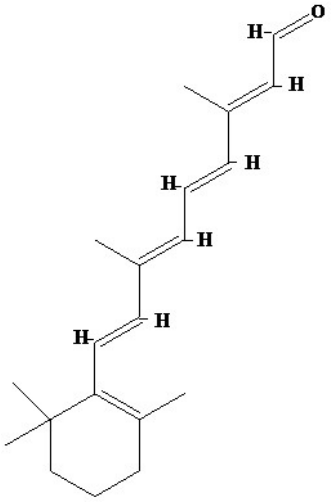
S/N	**δ_H, (ppm)**	**Multiplicity**	**Position**	**Reported Data (Consolacion *et al*., 2015)**	**Reported Data (Yahya *et al*., 2011)**	**Reported Data (Kojima *et al*., 1990)**
1.	0.73	d, 13H	**H-18**		0.69	0.68, 0.69
2.	0.81	m,	**H-27**		0.81	0.81, 0.79
3.	0.85	s,	**H-26**		0.85	0.83, 0.83

4.	1.18	s, 3H	**H-19**		1.02,	1.01, 1.01
5.	3.53	m,	**H-3**		3.53, 3.53	3.52, 3.52
6.	4.98	d,	**H-23**	5.00	5.02	
7.	5.07	d, 1H	**H-22**	5.13	5.16	
8.	5.27	m, 2H	**H-6**	5.33, 5.33	5.36, 5.36	5.35, 5.35

5.9.2 Compound 20 (isolated as B9JN2)

The IR data obtained for compound 20 revealed absorbance at (2984.49 and 2941.33) cm^{-1}, which, refers to an alkene stretch while the band at 1735.82 cm^{-1} indicates a carbonyl (aldehyde). The peak at 178.87 ppm present in the ^{13}C –NMR spectrum, further confirms the carbonyl functional group seen in the IR data. This signal is missing in the depth 135 spectrum as expected. This compound has signals at the olefinic regions, which is one of the characteristics of terpenes (Leal *et al*., 2003, Pacheco *et al*., 2009, Wu *et al*., 2009, Aminimoghadamfarouj and Nematollahi, 2017). The ^{13}C –NMR spectrum shows signals corresponding to 20 carbon atoms, which are similar to those of diterpenes (Block *et al*., 2004, Abdissa *et al*., 2017). It is a probably a terpene, a diterpene to be specific.

The olefinic carbons are the peaks at (130.23, 130.03, 129.73, 128.07 and 127.91) ppm and these peaks are indicative of a diterpene (Seca *et al.,* 2008, Abdissa et al., 2017). These peaks are similar to those reported in literature (Wang *et al*., 2013). We propose that this compound is a retinal (Vitamin A aldehyde) related compound. The comparison of the ^{13}C –NMR data with those of literature also suggest that compound 20 is a retinal related compound (Wang *et al*., 2013).



(20)

Table 5.5: The ^{13}C –NMR spectra data of compound 20

S/N	δ_C (ppm)		Reported Data (Wang *et al*., 2013)
1.	31.92		34.91
2.	33.86		40.28
3.	22.57		20.00
4.	29.76		33.71
5.	130.03		130.66
6.	-		138.45
7.	127.91		129.68
8.	-		138.29
9.	130.23		131.91
10.	129.73		130.70
11.	-		140.50
12.	128.07		135.89
13.	-		153.36
14.	130.03		130.11
15.	178.87		190.16
16.	29.58		29.5
17.	29.52		29.5
18.	22.68		22.28
19.	14.10		13.15
20.	-		12.87

Table 5.6: The ^{1}H –NMR spectra data of compound 20

S/N	δ_H, (ppm)	Multiplicity	Reported Data (Wang *et al*., 2013)	
1.	0.81	Dd, 4H		
2.	1.24	m, 19H	1.12	
3.	1.55	dd, 2H	1.73	
4.	1.98	td, 1H	1.97	
5.	2.10	s, 1H		
6.	2.28	t, 2H		
7.	4.05	q, ?H	5.97	
8.	5.28	m, 1H	6.04	

5.9.3 Compound 21 (isolated as PBT)

The IR data obtained for compound 21 showed absorbance at 3000 cm^{-1}, which indicates

an alkene stretch. Another band was seen at 1725 cm^{-1}, which refers to a carbonyl function group that is probably ketonic and not aldehydic because there was no corresponding peak found in the carbon-13 NMR spectrum, may be because there was no hydrogen atom to enhance the signal to be visible. Compound 21 is not an aromatic compound because no aromatic signals detected in the NMR spectra. The peaks in the carbon-13 NMR of compound 21 resembles those of compounds containing repeating units of an isoprenoid with 20 carbon atoms (Block *et al*., 2004, Seca *et al.,* 2008, Abdissa *et al*., 2017). It is likely a diterpene related compound. The lack of sufficient data especially those of mass spectroscopy is a major limitation to predicting the structure of this compound.

5.10 Cytotoxicity

The ability of some of the isolated compounds from the leaf extracts of *Securidaca longepedunculata* and *Luffa cylindrica* to stimulate cell proliferation and their protective capacity against apoptosis and necrosis induced cell death, indicate a contributing property that can enhance gastric ulcer (wound) healing (Schafer and Wemer, 2008; Pringle *et al*., 2018).

5.11 Conclusion

The study on ethnobotanical survey is ethnopharmacologically relevant because despite the number of literature on the ethnobotanical studies in Nigeria, there are no published work on the antiulcer plants used among the people of Ado-Odo/Ota Ogun State where traditional healers claim to have been managing and curing the peptic ulcer disease in their patients with success. This documentation provides the basis for further research in developing new, effective, safe and affordable plant-derived antiulcer drugs from our rich resources of indigenous medicinal plants. The study also plays a part in documenting and conserving traditional knowledge of antiulcer plants for future use.

The present study demonstrates that the leaf extracts of *Securidaca longepedunculata* and *Luffa cylindrica* are possible potent gastroprotective and antiulcer agents against ethanol-induced gastric ulcer. The obtained results show that the crude extracts of leaves of

Securidaca longepedunculata and *Luffa cylindrica* induce a significant antiulcer effect in ethanol-induced gastric lesions. In summary, our data suggest that the leaf extracts of *Luffa cylindrical and Securidaca longepedunculata* appear to be effective against gastric ulcer induced by ethanol. We suggest that the gastroprotective and antiulcer effects could be partly attributed to free radical scavenging property of the plant extract, inhibition of gastric acidity and strengthening of the gastric mucosal barrier through antioxidant enzyme induction. The antiulcer and gastroprotective activities of *Securidaca longepedunculata* and *Luffa cylindrica* are reported. Phytosterols and citronellol exhibited cell proliferative and anti-apoptotic activities, which could be partly responsible for their antiulcerogenic property.

5.12 Contribution to Knowledge

To the best of our knowledge there had been no documentation or report on ethnobotanical survey of medicinal plants used in the treatment/management of ulcers among the people of Ado/Odo Ota LGA of Ogun State, Southwest, Nigeria. Today we have a report on it that can provide the platform for further scientific research in that area.

Our study showed for the first time that the crude extracts of leaves of *Securidaca longepedunculata* possess gastro protective and antiulcer tendencies

On the basis of the present results and available reports, we were able to provide appropriate scientific information on the antiulcer property of *Luffa cylindrical* and *Securidaca longepedunculata*.

The gastroprotective and antiulcer effects is predictably attributed to free radical scavenging property of the plants extracts, inhibition of gastric acidity and strengthening of the gastric mucosal barrier through antioxidant enzymes induction.

The isolated compounds from SL though have been previously isolated from the

plant are being identified as the antiulcer constituent of the plant.

Citronellol, might be the first time its isolation from LC is being reported and identified as the antiulcer constituent.

5.13 Recommendation

The mechanism of actions of the extracts/or compounds isolated from the selected plants should be investigated.

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APPENDICES

Appendix I

Semi-structured questionnaire

Demographic Information

Name (Optional)

Age

Sex

Religion

Date

Village/L.G.A.

Tribe

Nationality

Occupation/Function/Specialty

How long have you been in this business?

Training

Education

No. of Staff

Treatment of Gastric Ulcers, Plants and Recipes used for Treatment

Where do you get medicinal plants from?

Why do people come to buy medicinal plant from you?

Do you have herbs for ulcers?

Is it a single plant or mixture?

Please give the name(s) of the plant(s)

Which part of the plant do you use?

Methods of preparation

(a) Do you have ulcer patients? (b) How many?

Frequency of treatment

(a) Duration of treatment (b) Dosage

Mode of Administration

Any accompanied side effects

Any accompanied verbal instructions

Sources of knowlegde of treatment (e.g. Ancestral, Training)

Any other technique used?

Prevalence of ulcer in your area: High, Moderate, Low

Do you know/have plants that prevent ulcer?

Results/Response from your patients after treatment

Any other information you wish to give

Comments (by interviewer)

Appendix II

Preparation of reagents

Phosphate buffer (pH 7.4)

Dipotassium hydrogen orthophosphate, K_2HP0_4 (6.97)g and 1.36 g of potassium dihydrogen orthophosphate KH_2P0_4 were dissolved in little amount of distilled water and made up to 1000 mL mark in a litre standard volumetric flask. The solution was adjusted to pH 7.4.

Potassium chloride (1.15% KCl)

Potassium chloride KCl (23.0g) was dissolved in distilled water and made up to 2 litres with distilled water and stored at $4^{O}C$.

Formalin (10% Formalin from 40% formalin)

25mls of 40% formalin was transferred into 100mls volumetric flask and made up to mark with distilled water.

Sodium chloride (0.9% Normal saline (NaCl))

Sodium chloride NaCl (2.7g) was dissolved in distilled water and made up to 300ml. This was stored at 4^0C.

Trichloroacetic acid (10% TCA)

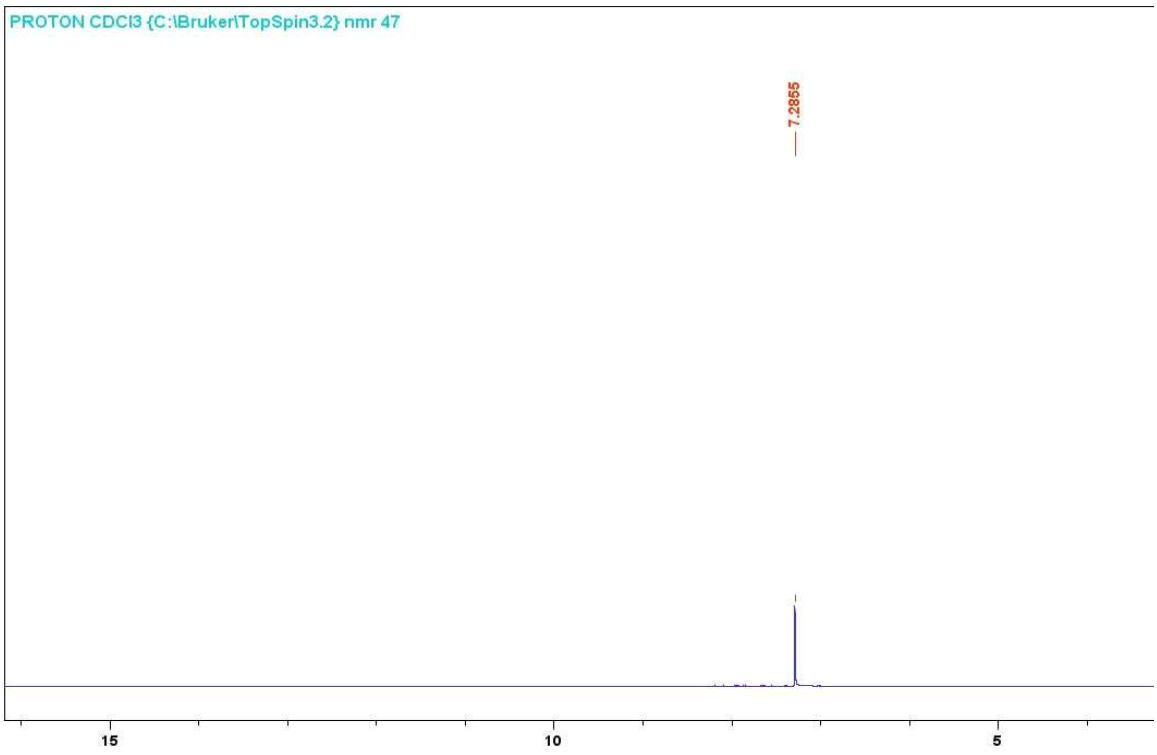
Trichloroacetic acid (TCA) (30g) (Sigma, London) was dissolved in distilled water and made up to 300mls by the same and stored at 4^0C

Thiobarbituric acid (0.75% Thiobarbituric acid (TBA))

Thiobarbituric acid (TBA) (0.075g) (Sigma, London) was dissolved in 10mls of 0.1M HCL. Dissolution was aided by shaking in a boiling water bath. It was prepared fresh.

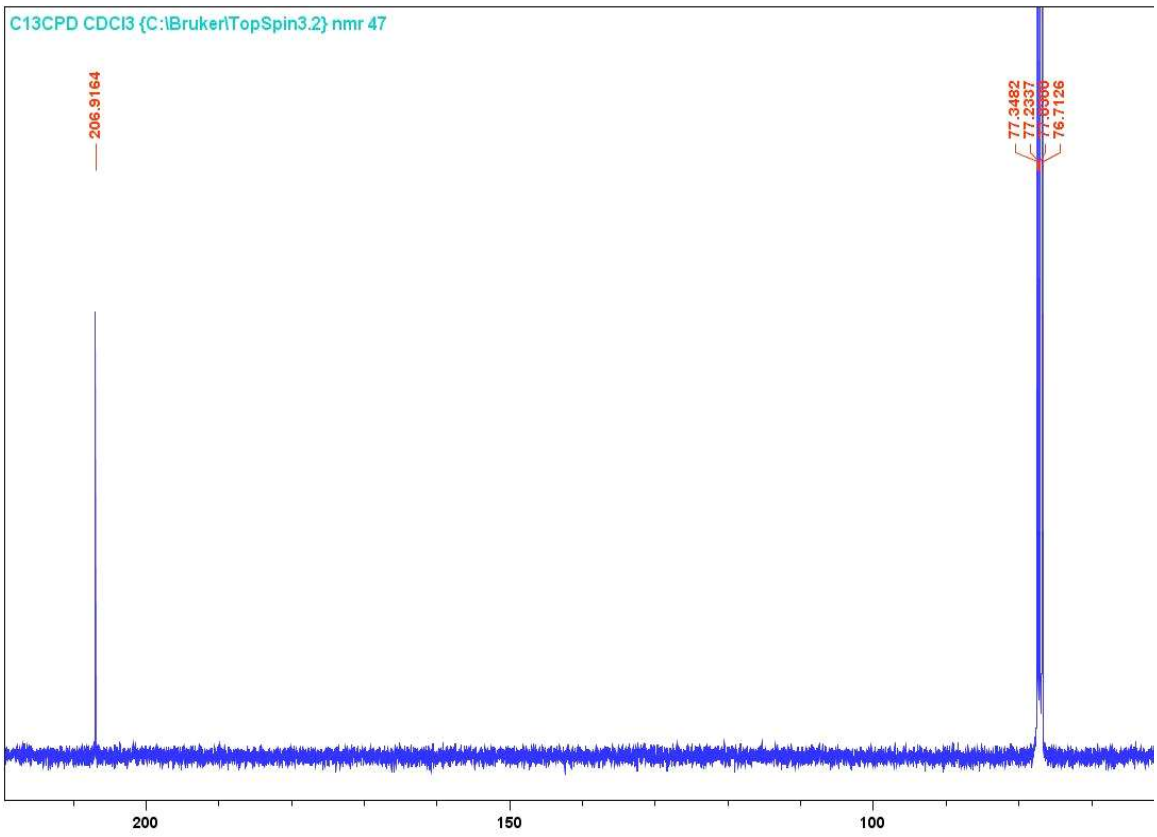
Appendix IIIA

The ^{1}H –NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



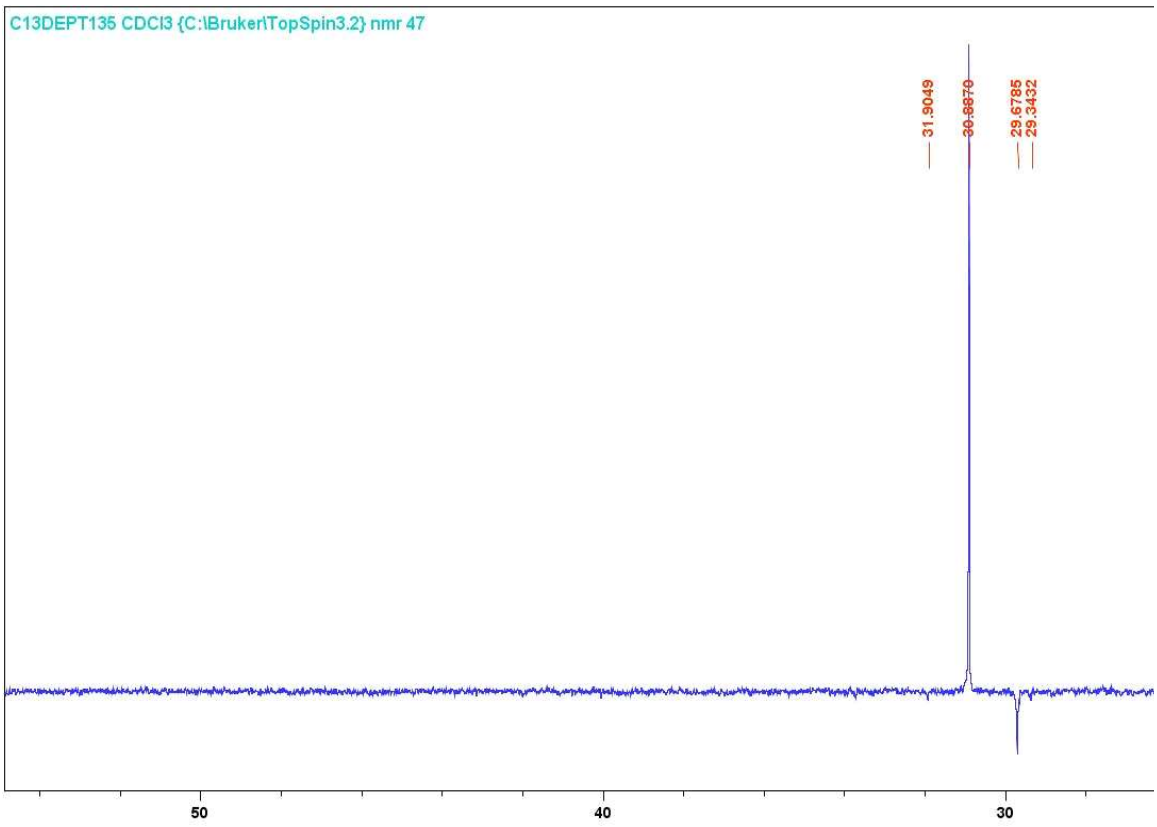
Appendix IIIB

The ^{13}C–NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



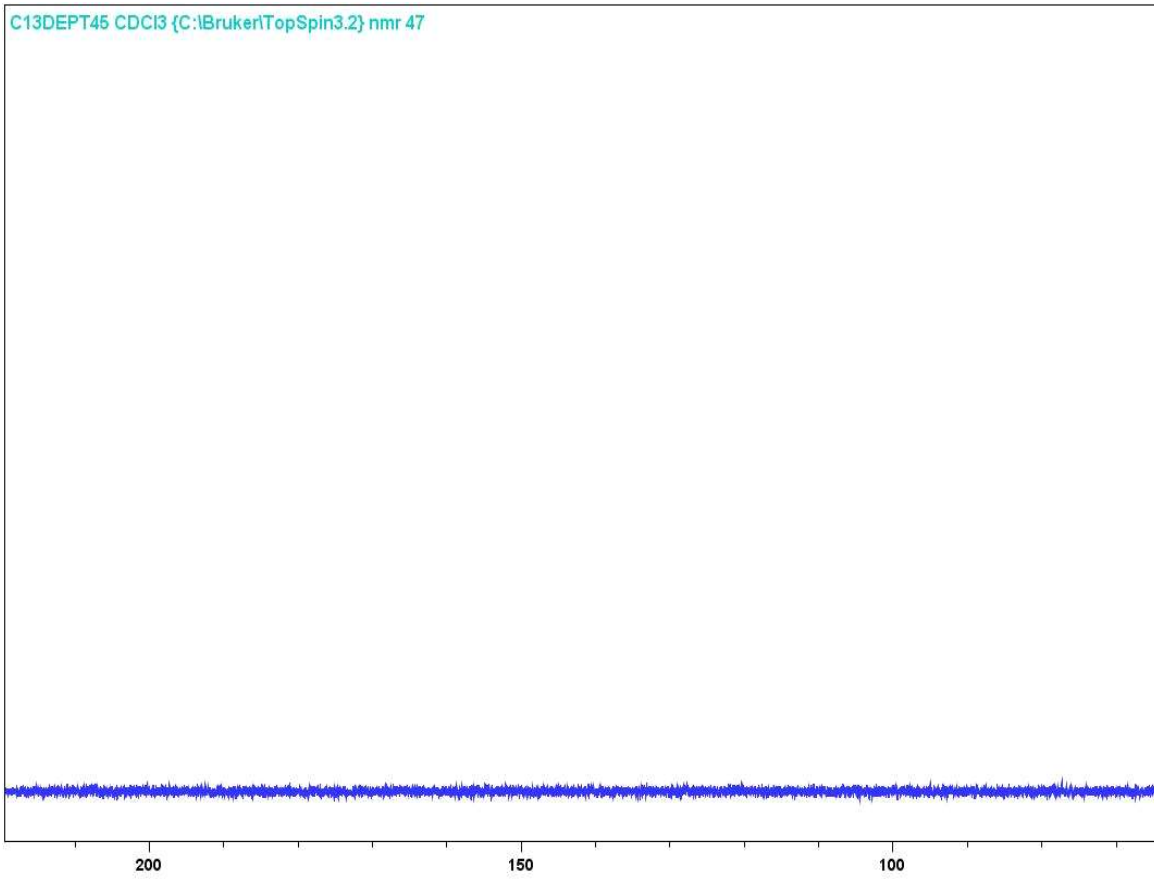
Appendix IIIC

The C13DEPT135–NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



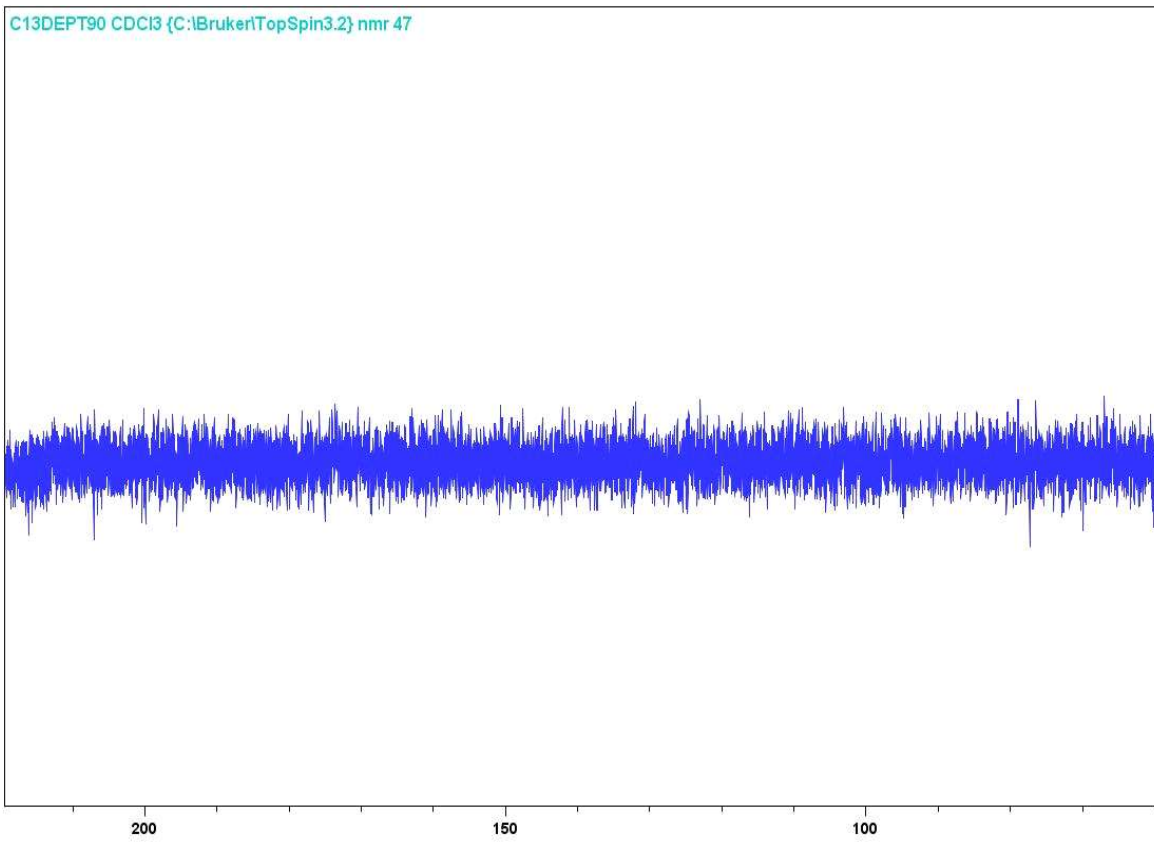
Appendix IIID

C13DEPT45–NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



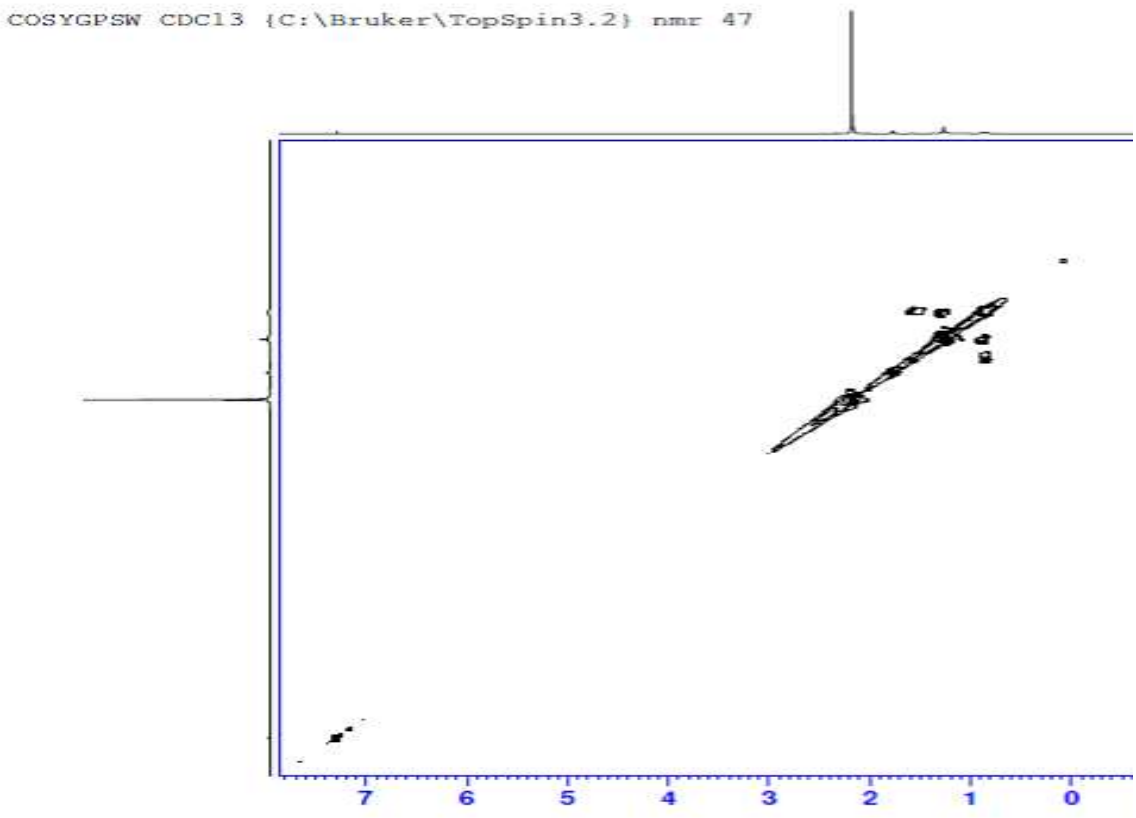
Appendix IIIE

The C13DEPT90–NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



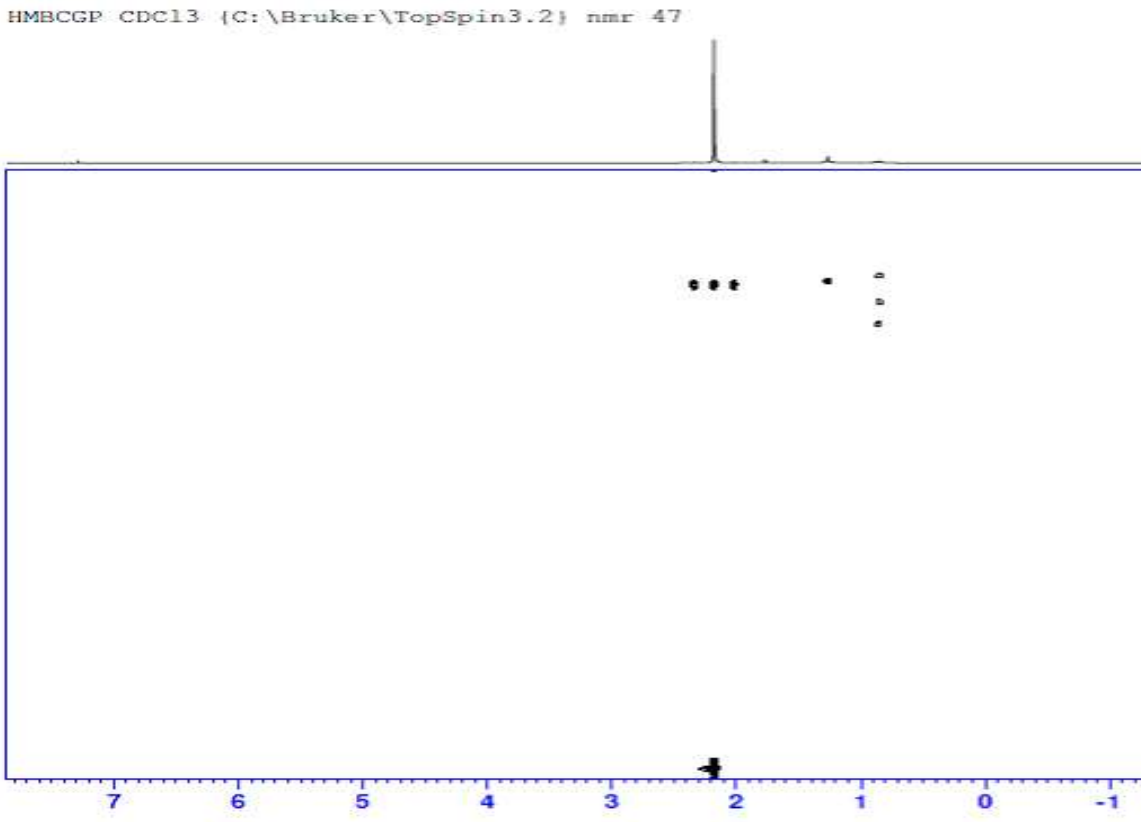
Appendix IIIF

The COSY NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



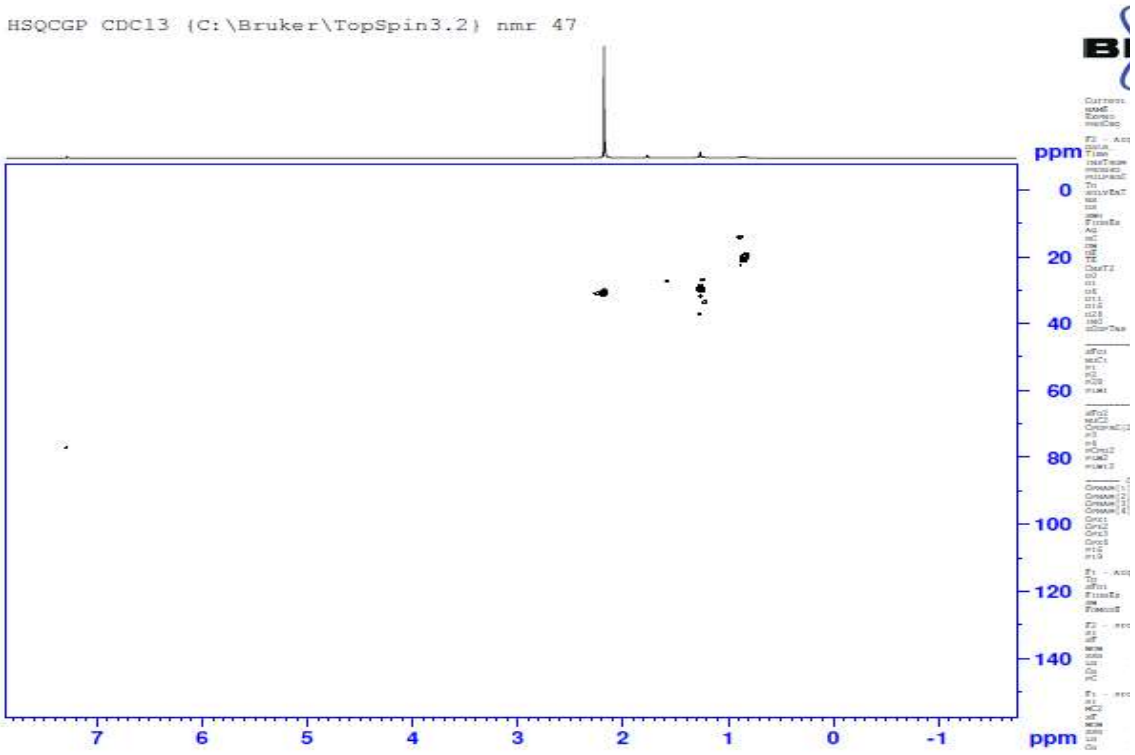
Appendix IIIG

The HMBC NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



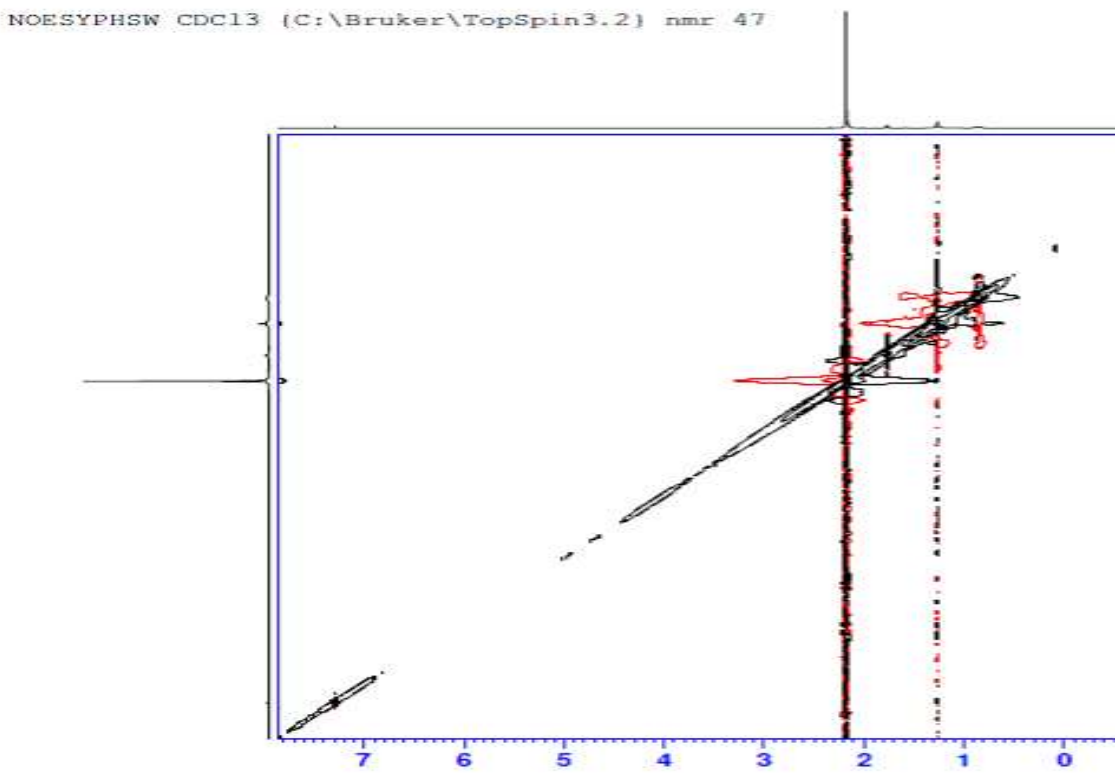
Appendix IIIH

The HSQC NR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



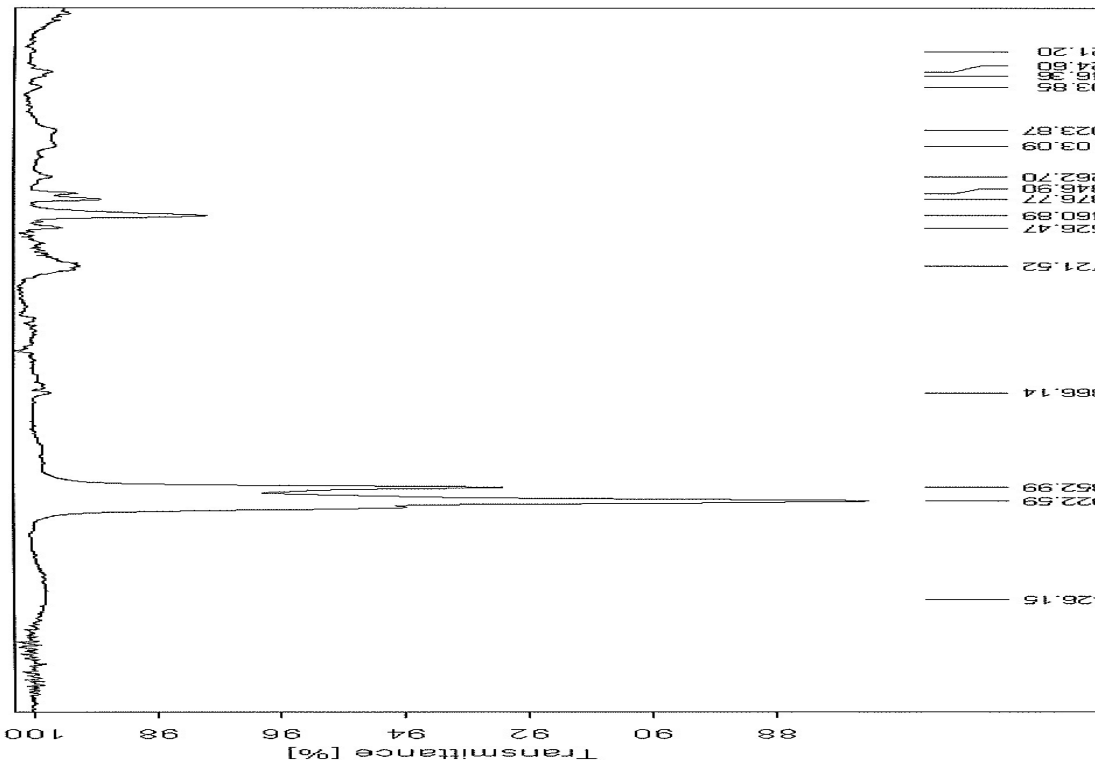
Appendix IIII

The NOESY 2D–NMR spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



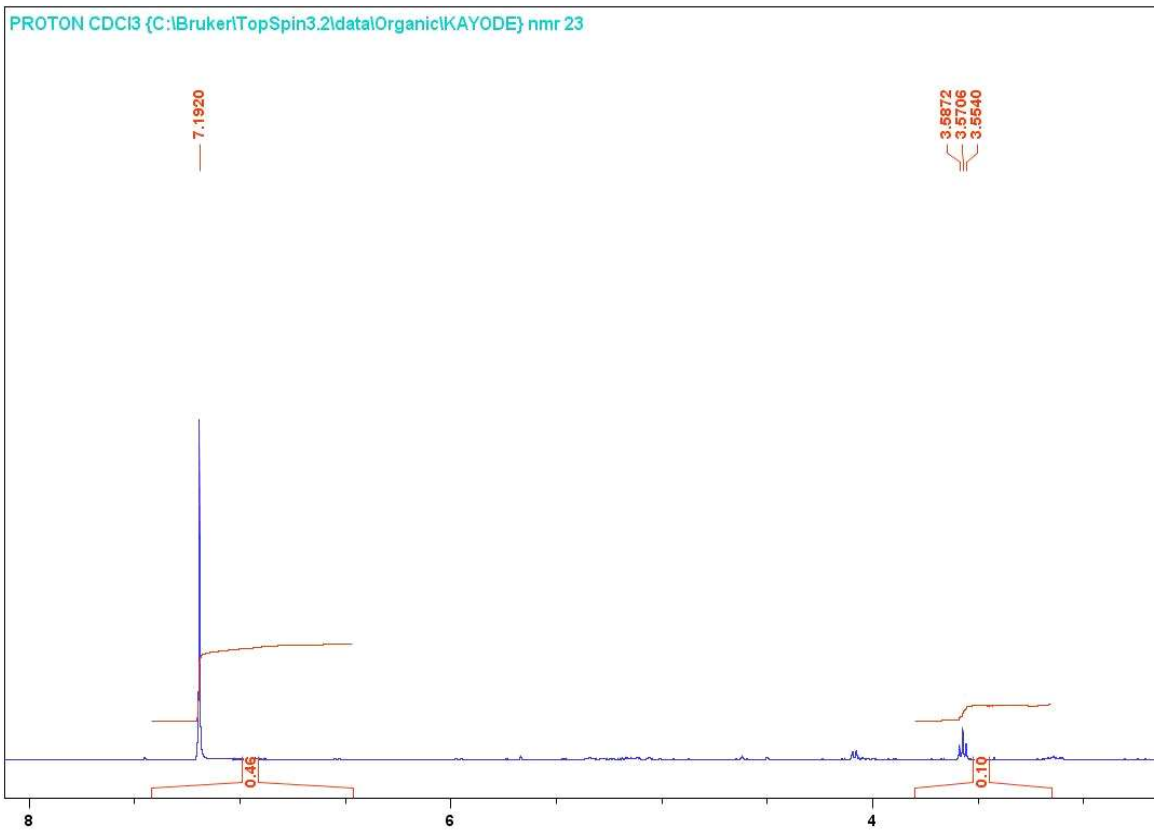
Appendix IIIJ

The IR DATA spectrum of compound 17 isolated from the leaf extract of *Luffa cylindrica*



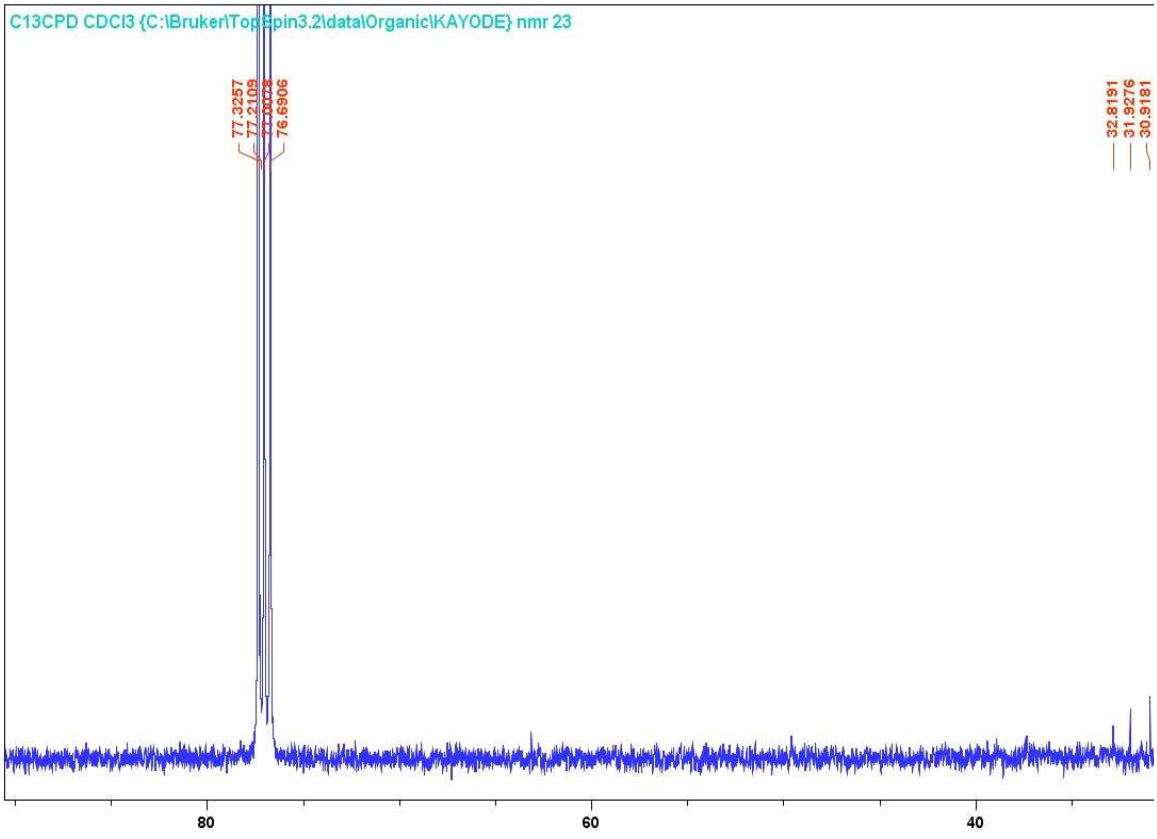
Appendix VIA

The ^{1}H –NMR spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



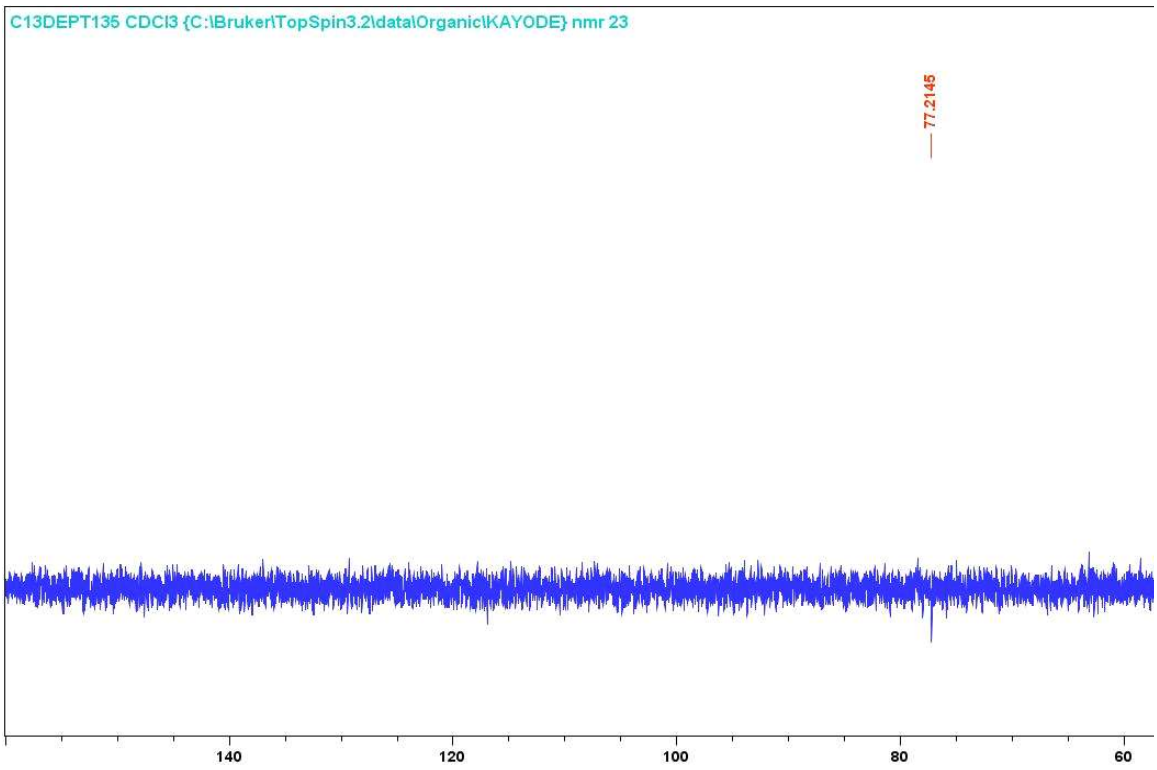
Appendix IVB

The ^{13}C–NMR spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



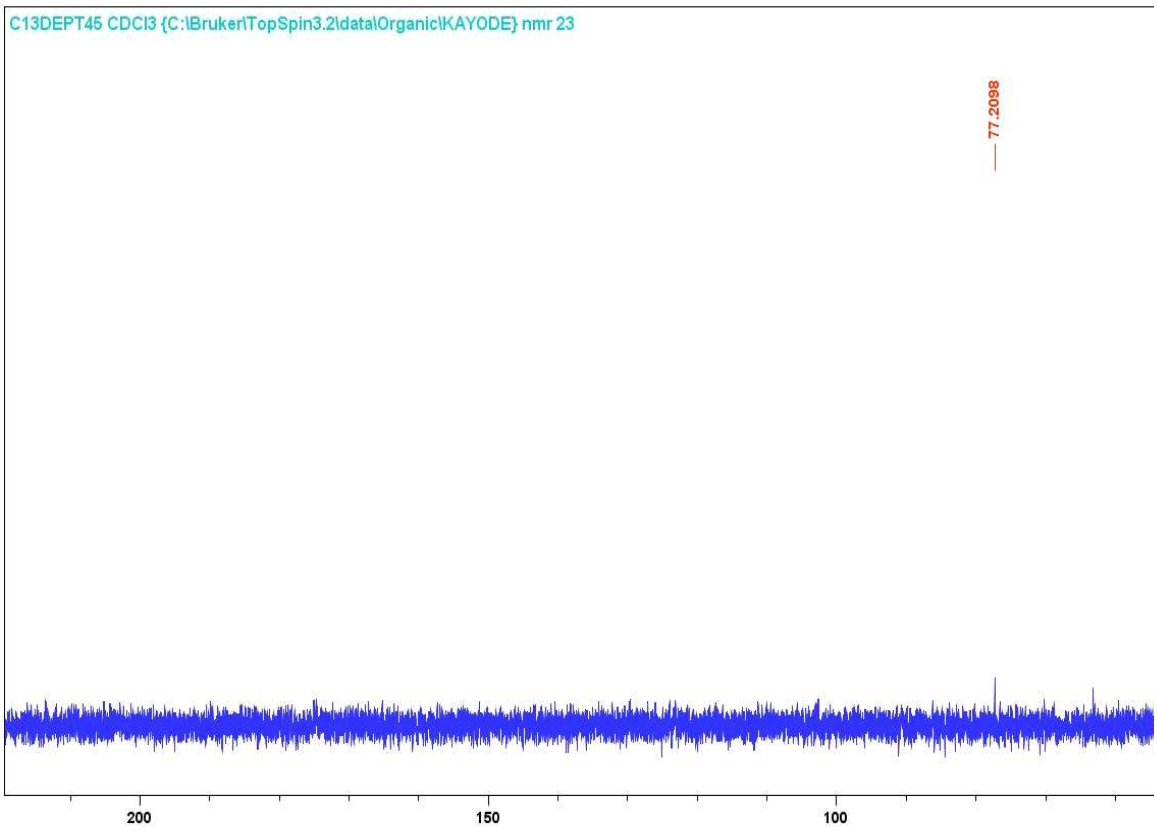
Appendix IVC

The C13DEPT135–NMR spectrum (0-40) of compound 18 isolated from the leaf extract of *Luffa cylindrica*



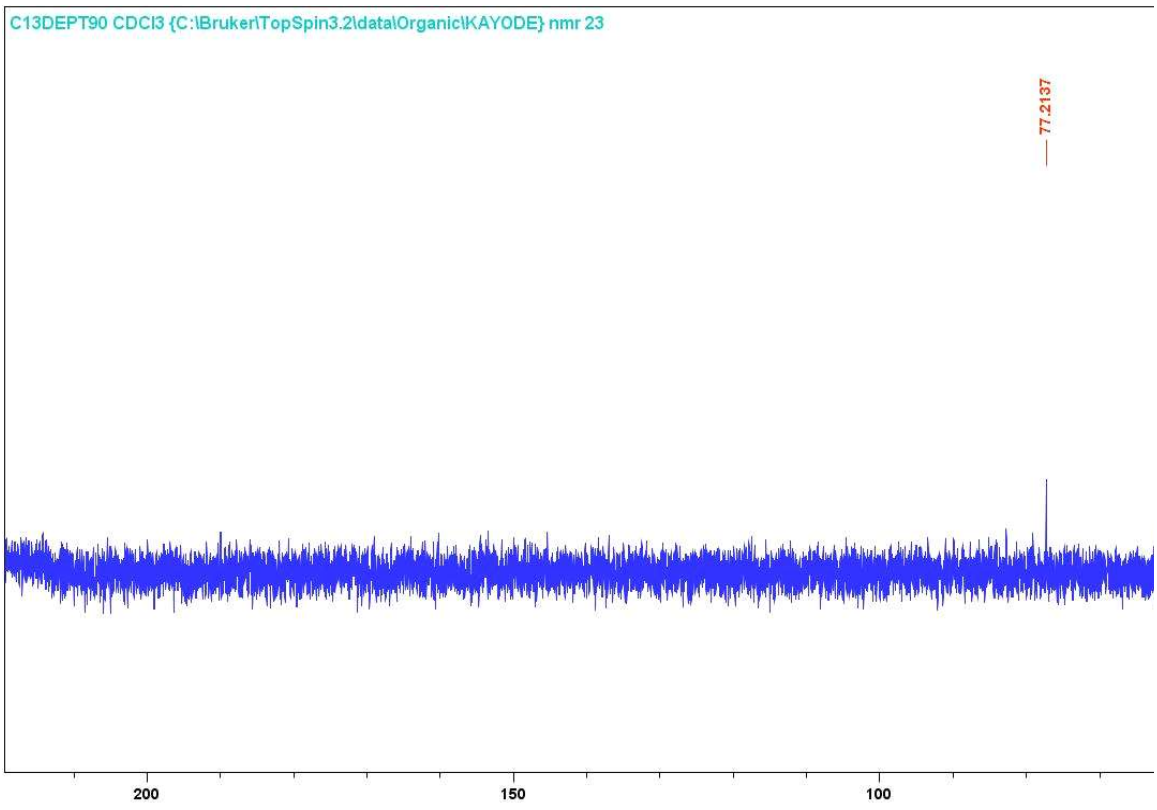
Appendix IVD

The C13DEPT45–NMR spectrum (0-40 ppm) of compound 18 isolated from the leaf extract of *Luffa cylindrica*



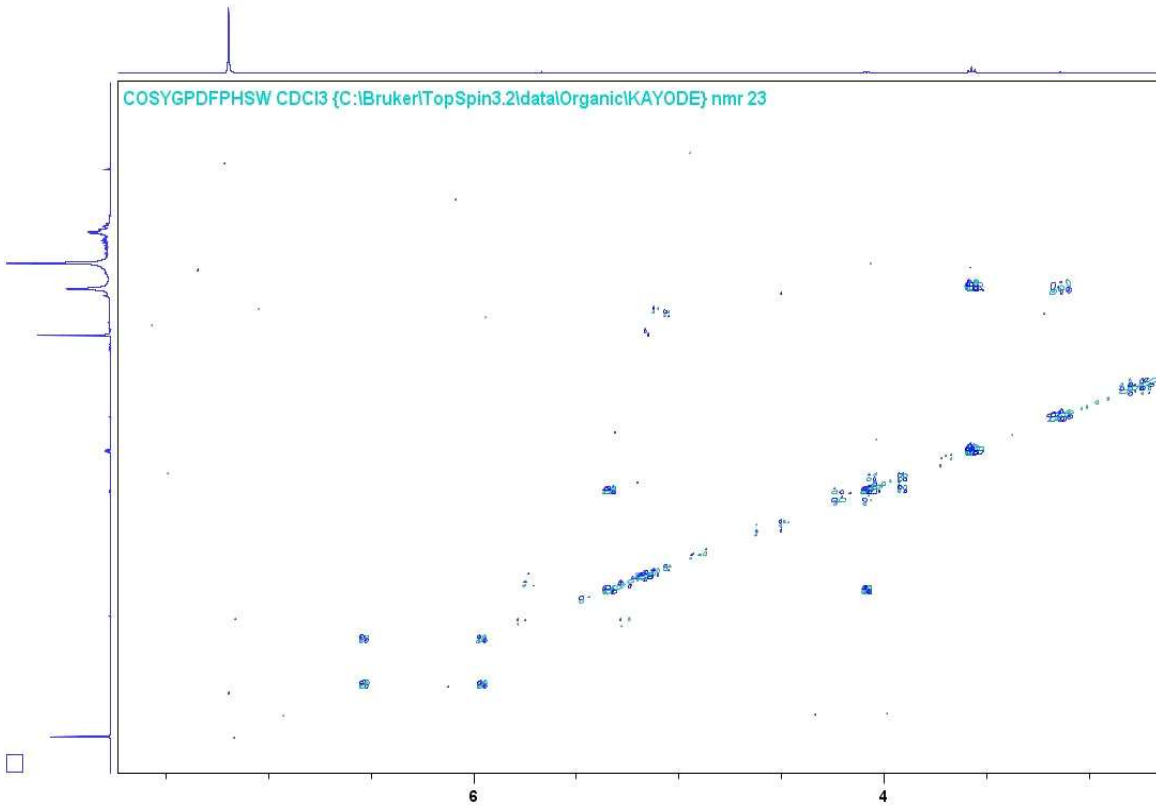
Appendix IVE

The C13DEPT90–NMR spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



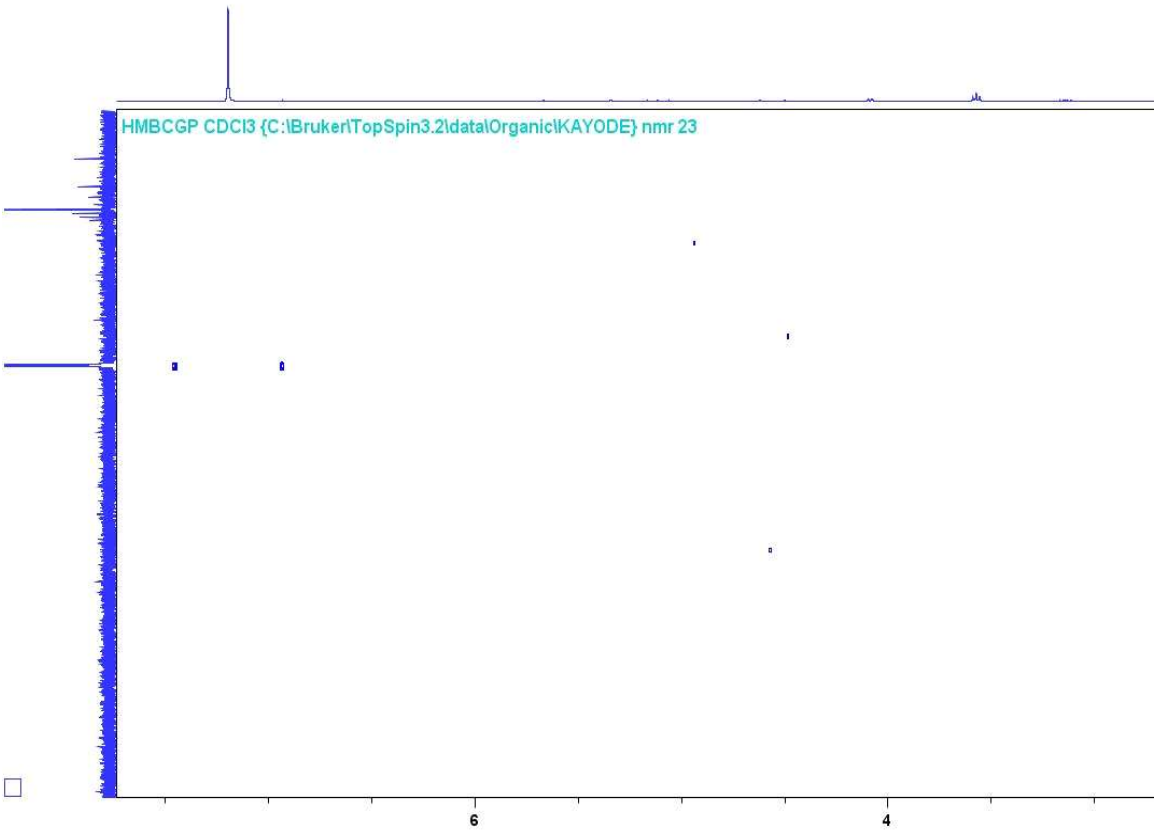
Appendix IVF

The COSY NMR spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



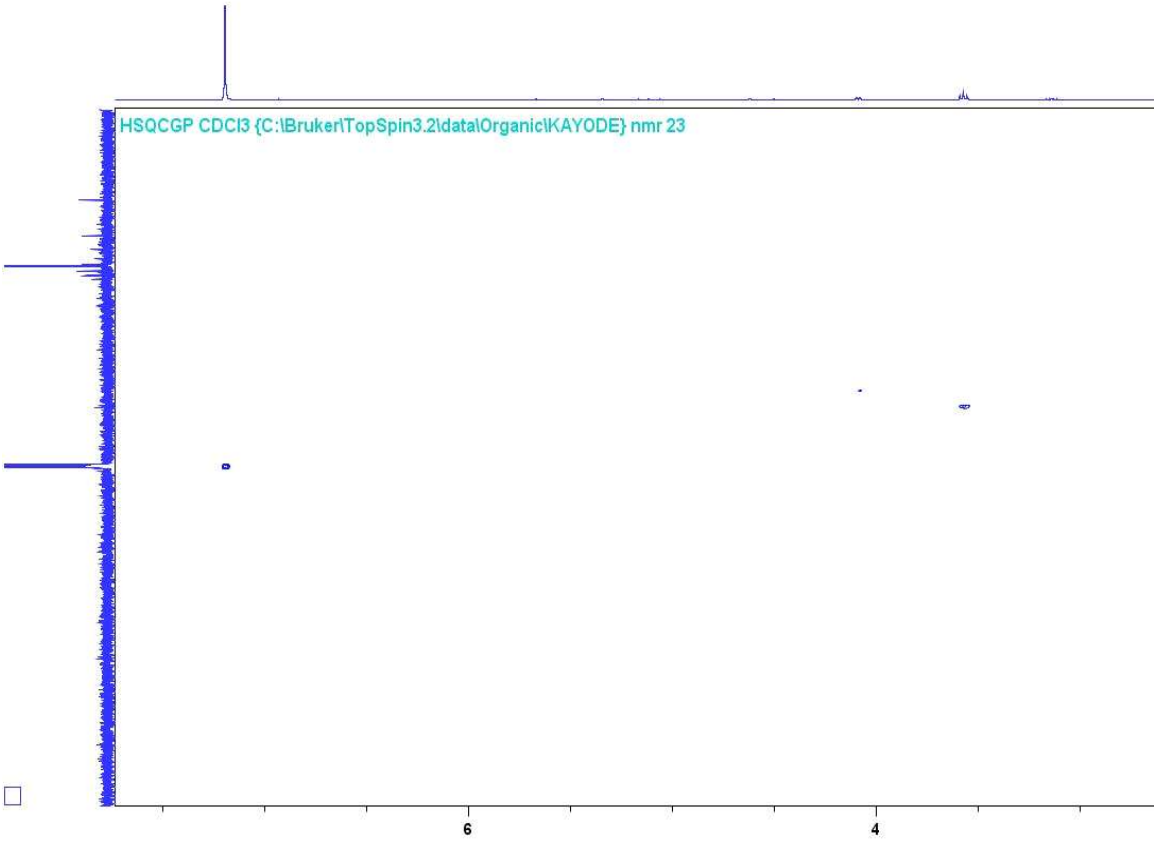
Appendix IVG

The HMBC NMR spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



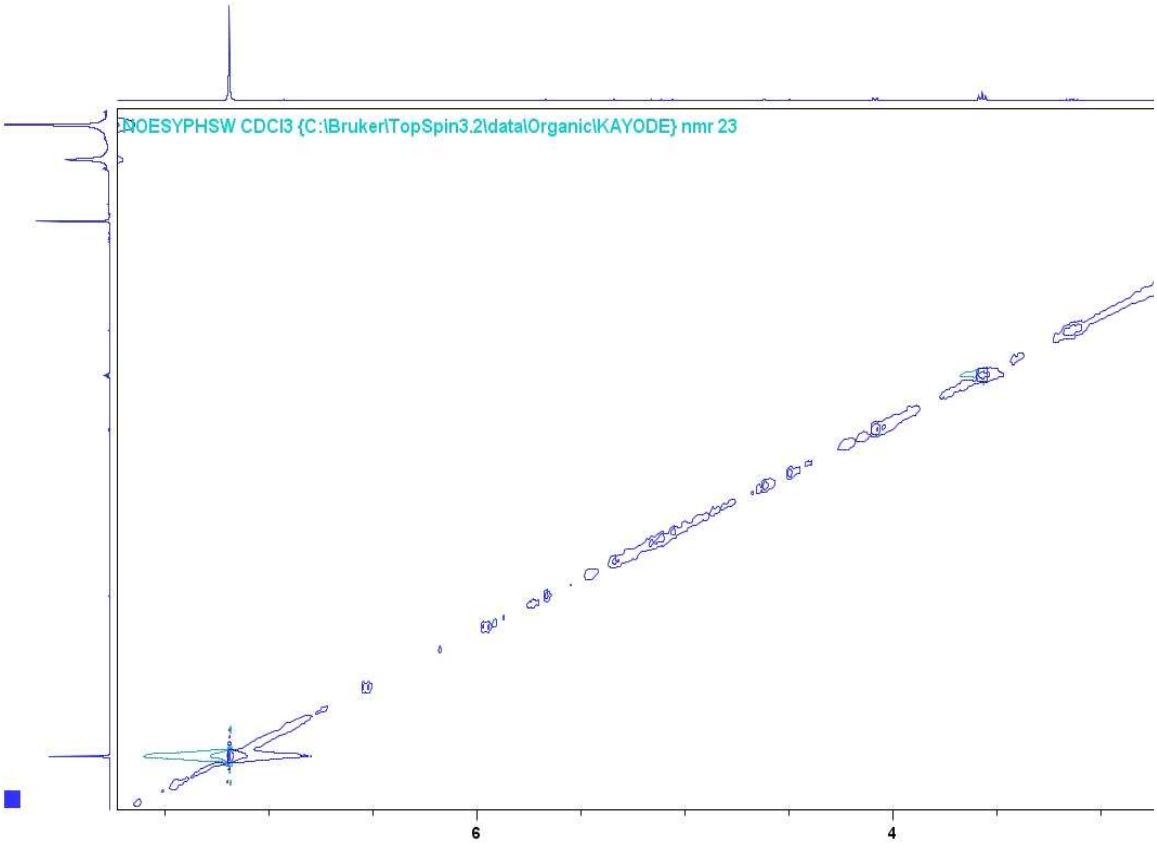
Appendix IVH

The HSQC NMR spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



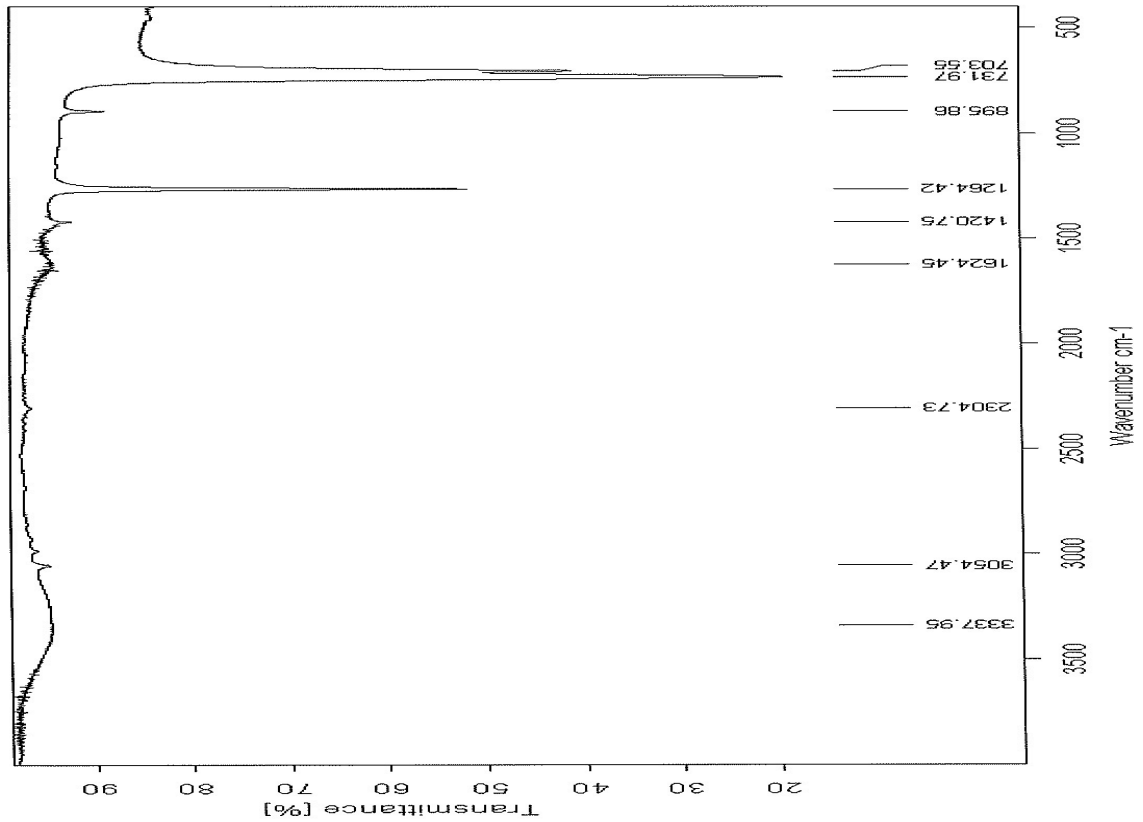
Appendix IVI

The NOESY NMR spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



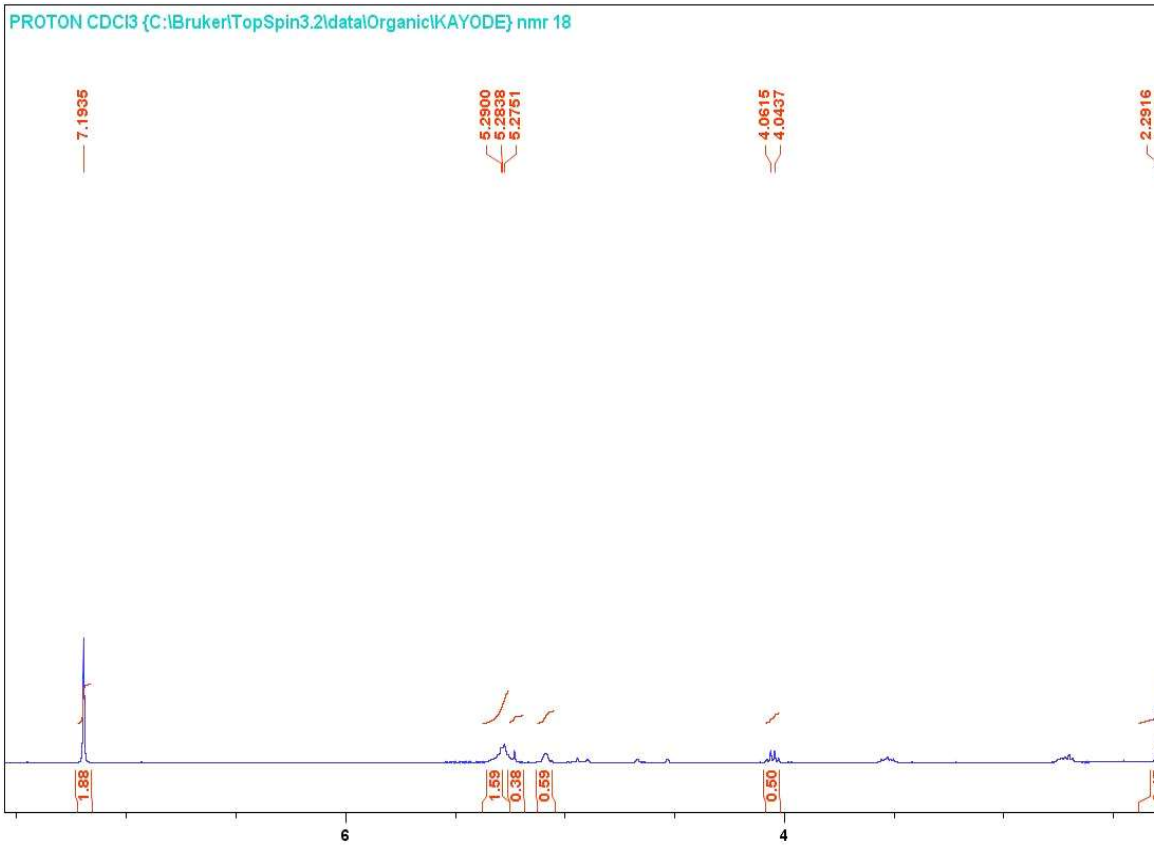
Appendix IVJ

The IR DATA spectrum of compound 18 isolated from the leaf extract of *Luffa cylindrica*



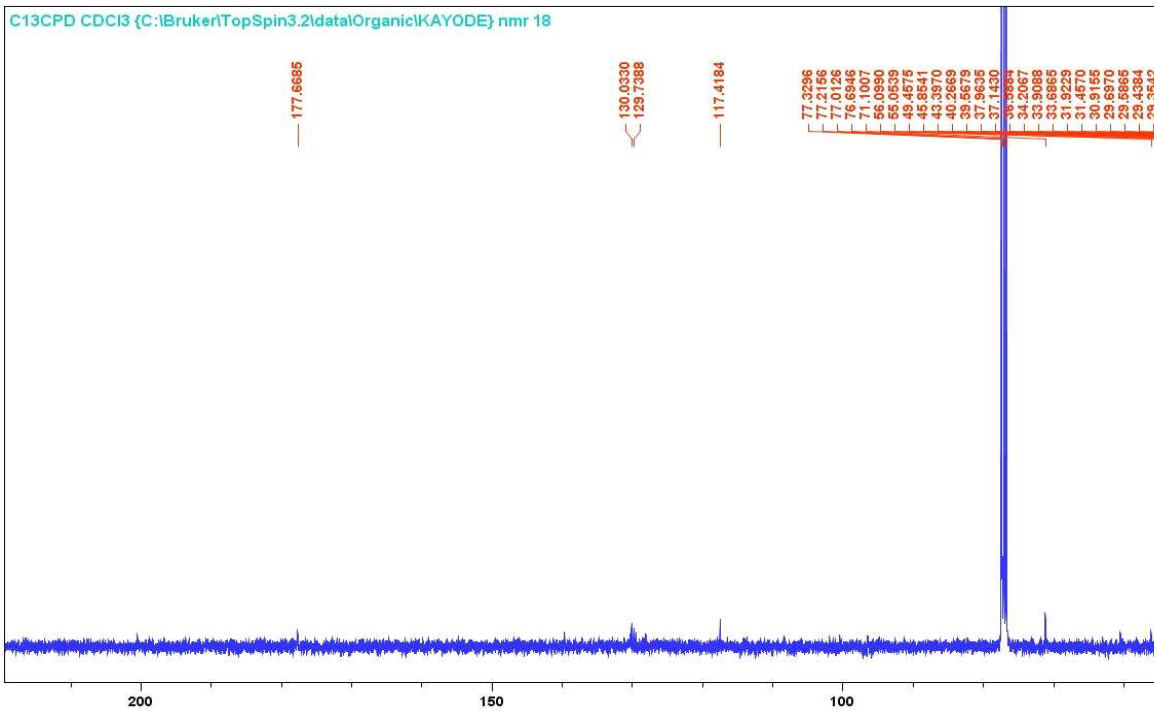
Appendix VA

The ^{1}H –NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*



Appendix VB

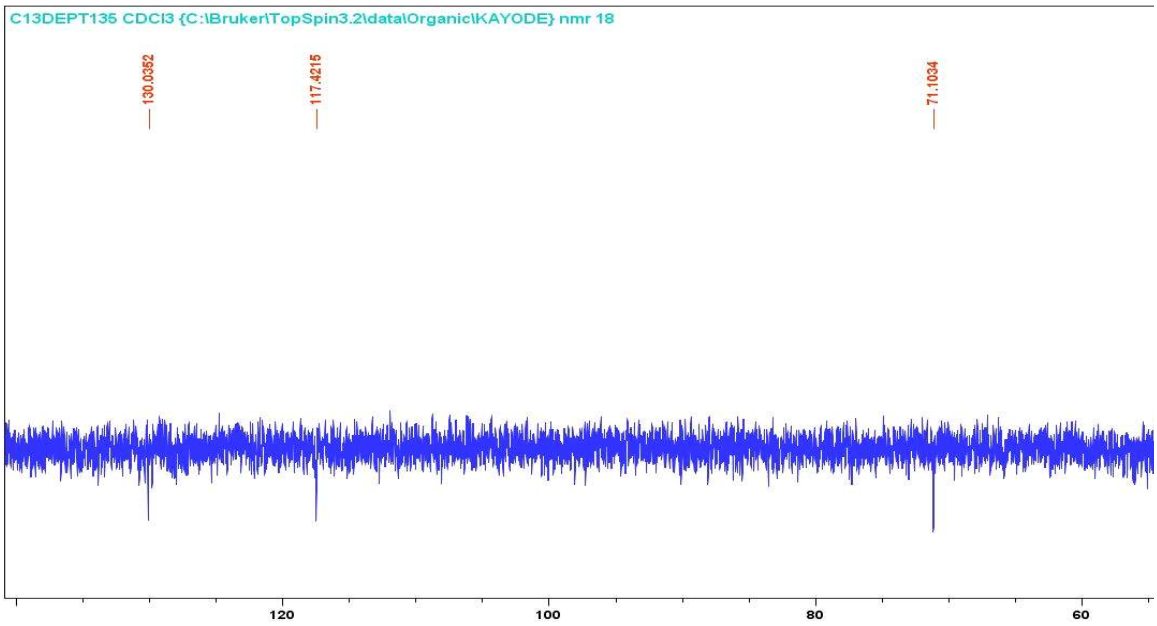
The ^{13}C–NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*



Appendix VC

The C13DEPT135–NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*

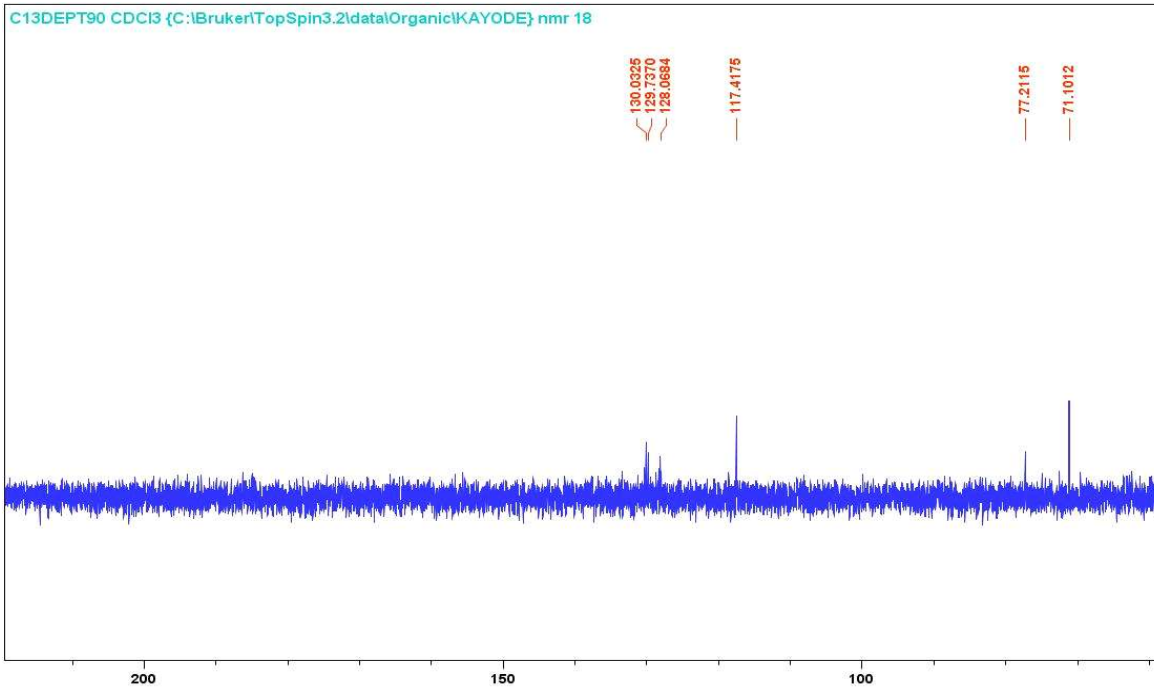
C13DEPT135 reveals signals for all carbons except the quaternary carbons (without protons) CH_2 groups (all will be negative) while CH and CH_3 are positive.



Appendix VD

The C13DEPT90–NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*

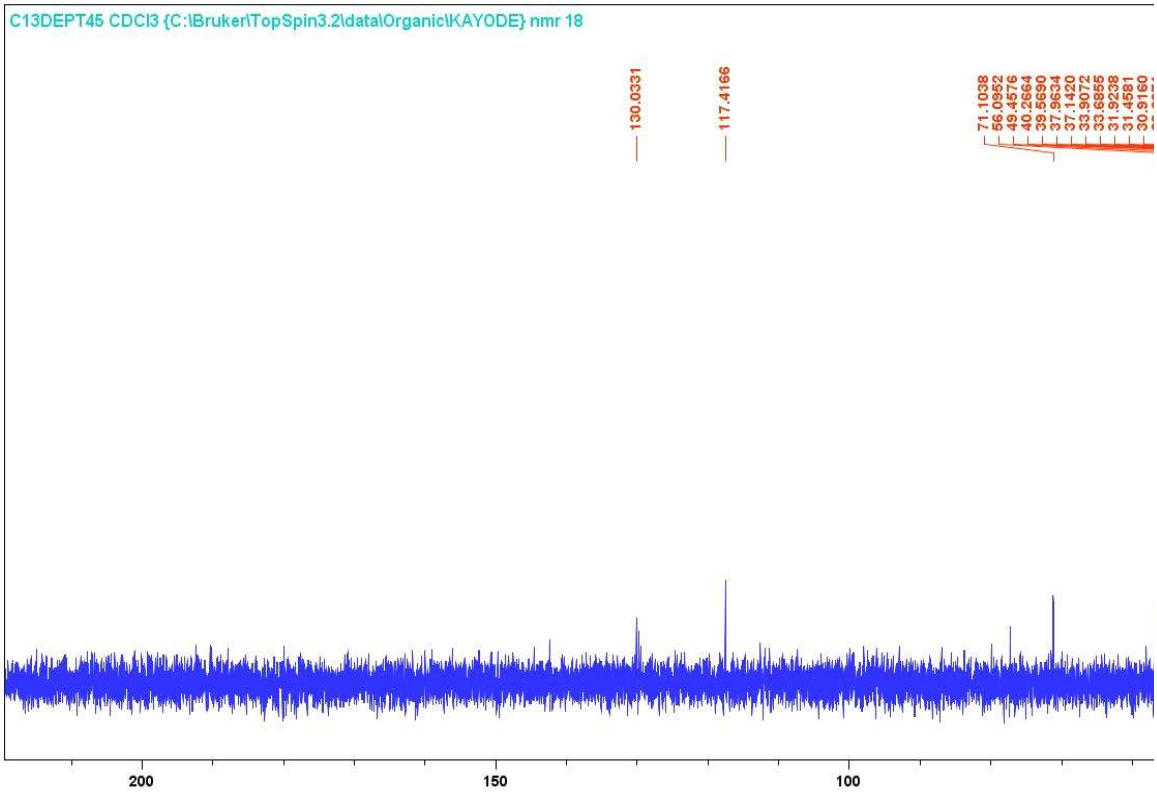
C13DEPT90 reveals only signals of CH groups



Appendix VE

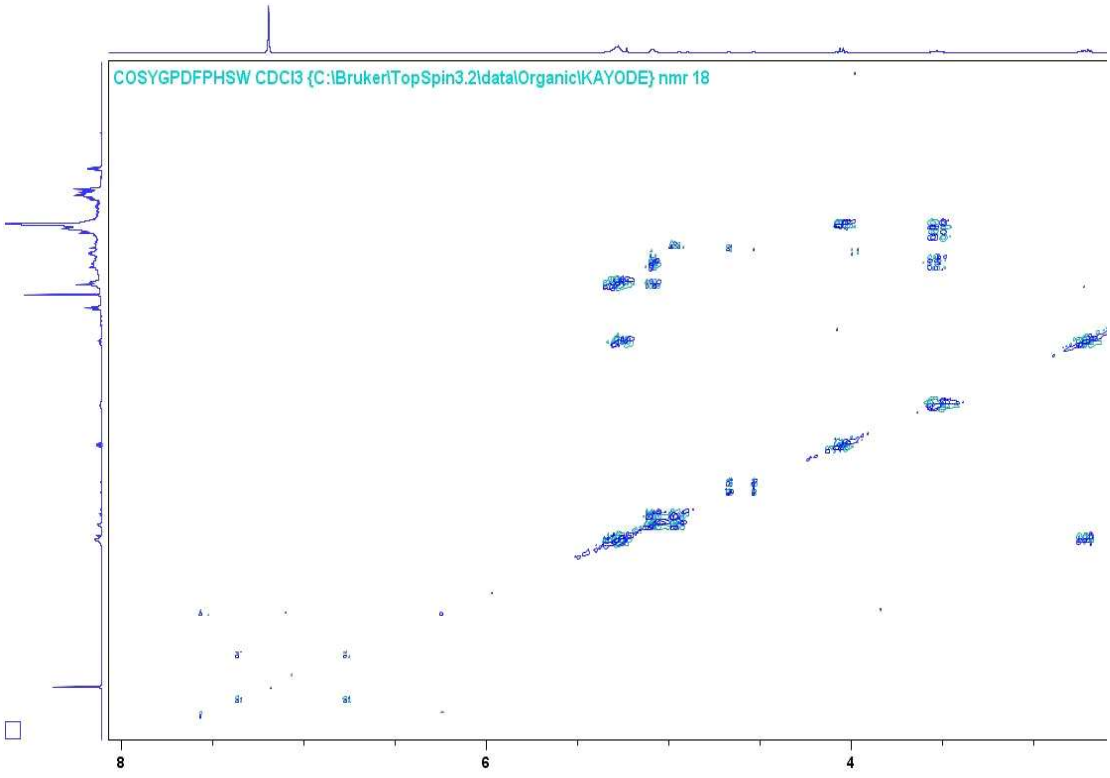
The C13DEPT45–NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*

C13DEPT45 shows all positive signals (of CH_3 and CH groups)



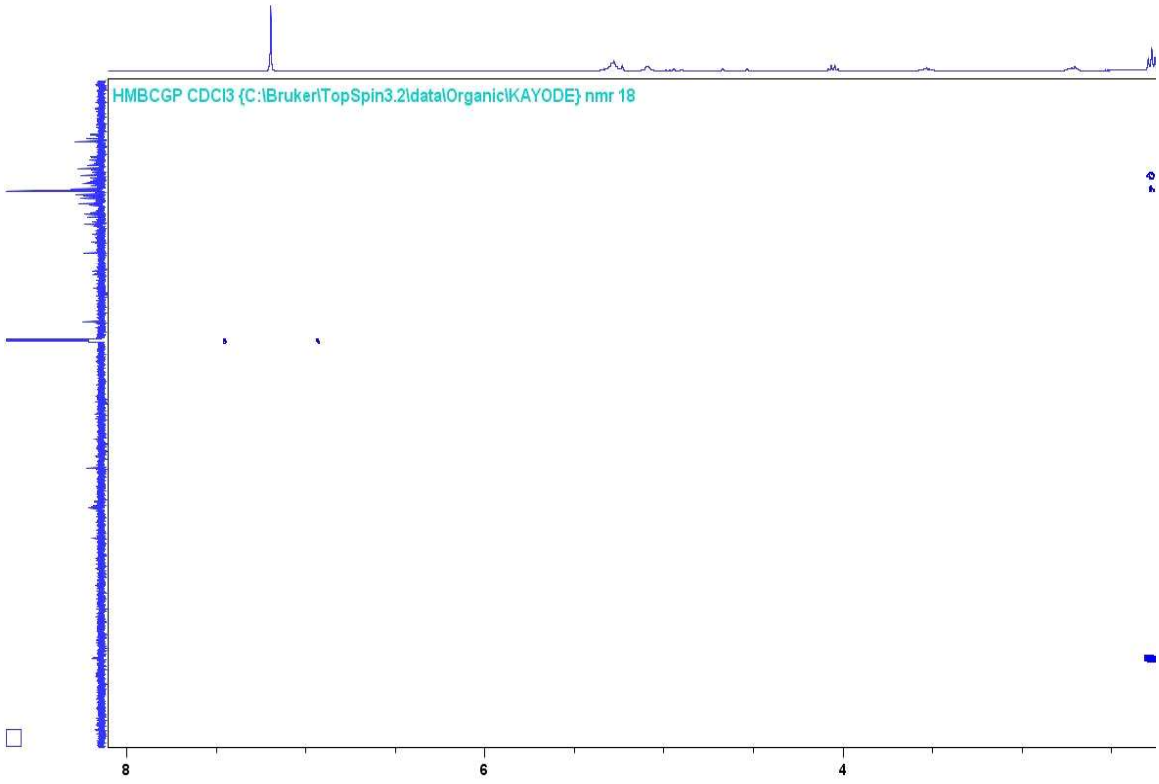
Appendix VF

The H-H Correlation Spectroscopy (COSY) NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*



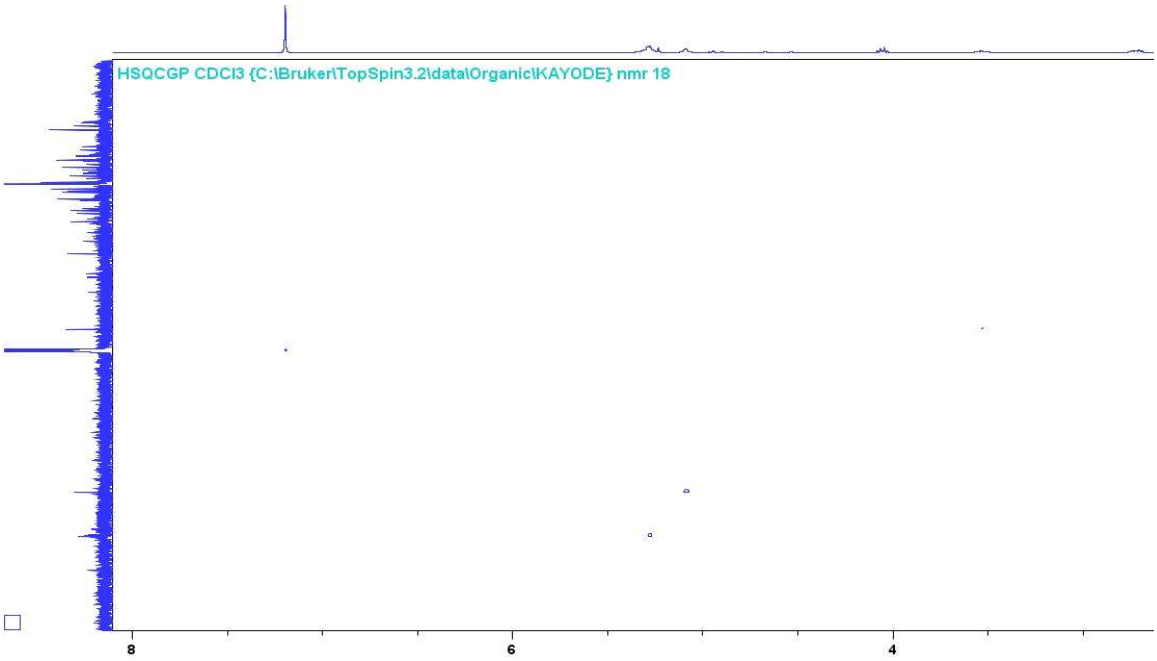
Appendix VG

The Heteronuclear Multiple Bond Correlation (HMBC) (H-C correlation) NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*



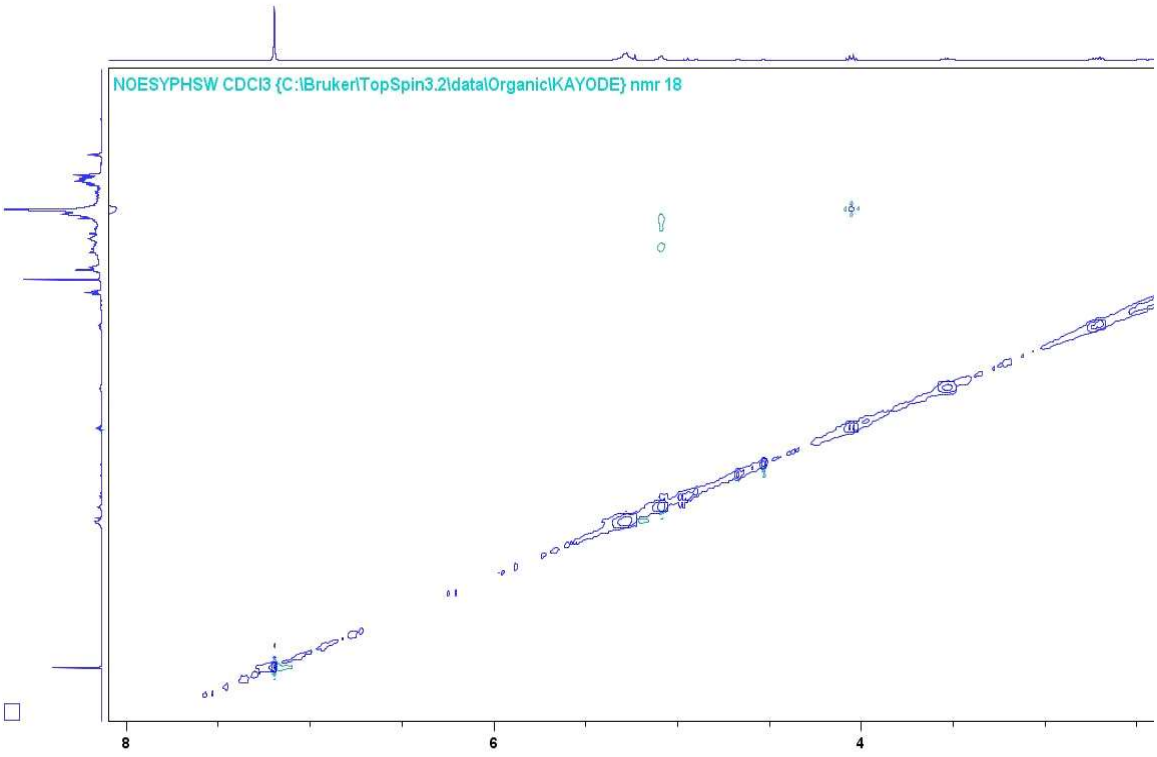
Appendix VH

The Heteronuclear Single Quantum Coherence or Heteronuclear Single Quantum Correlation (HSQC) NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*



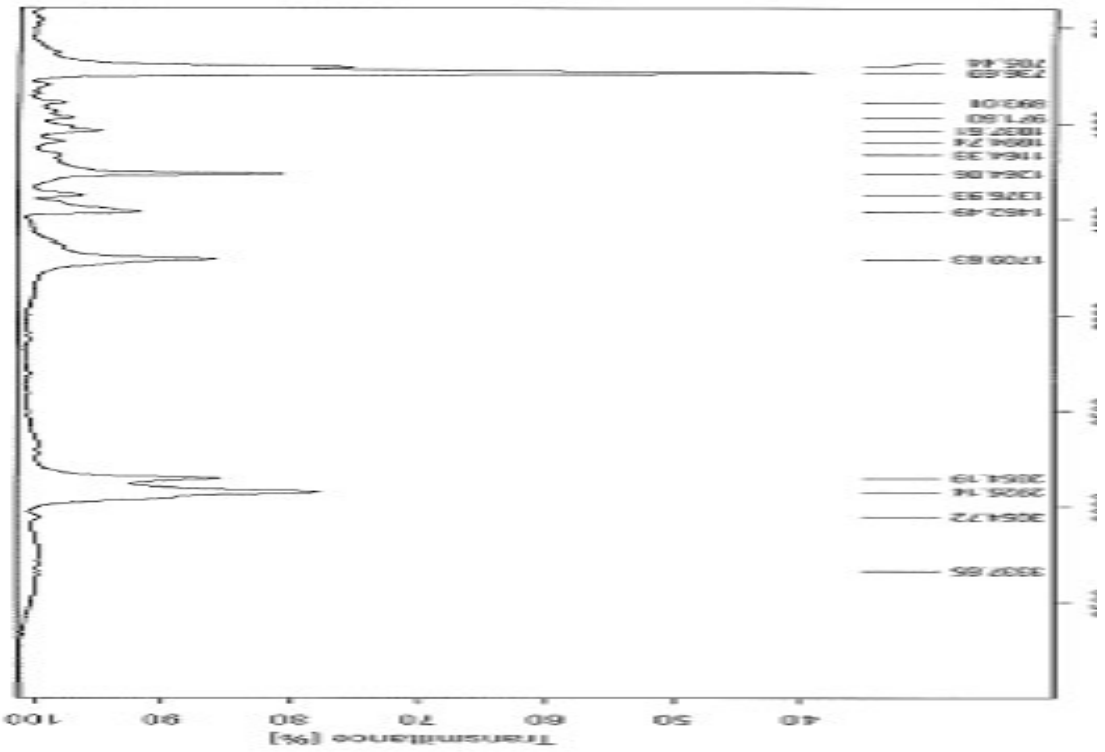
Appendix VI

TheNuclear Overhauser Effect Spectroscopy (NOESY) NMR spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*



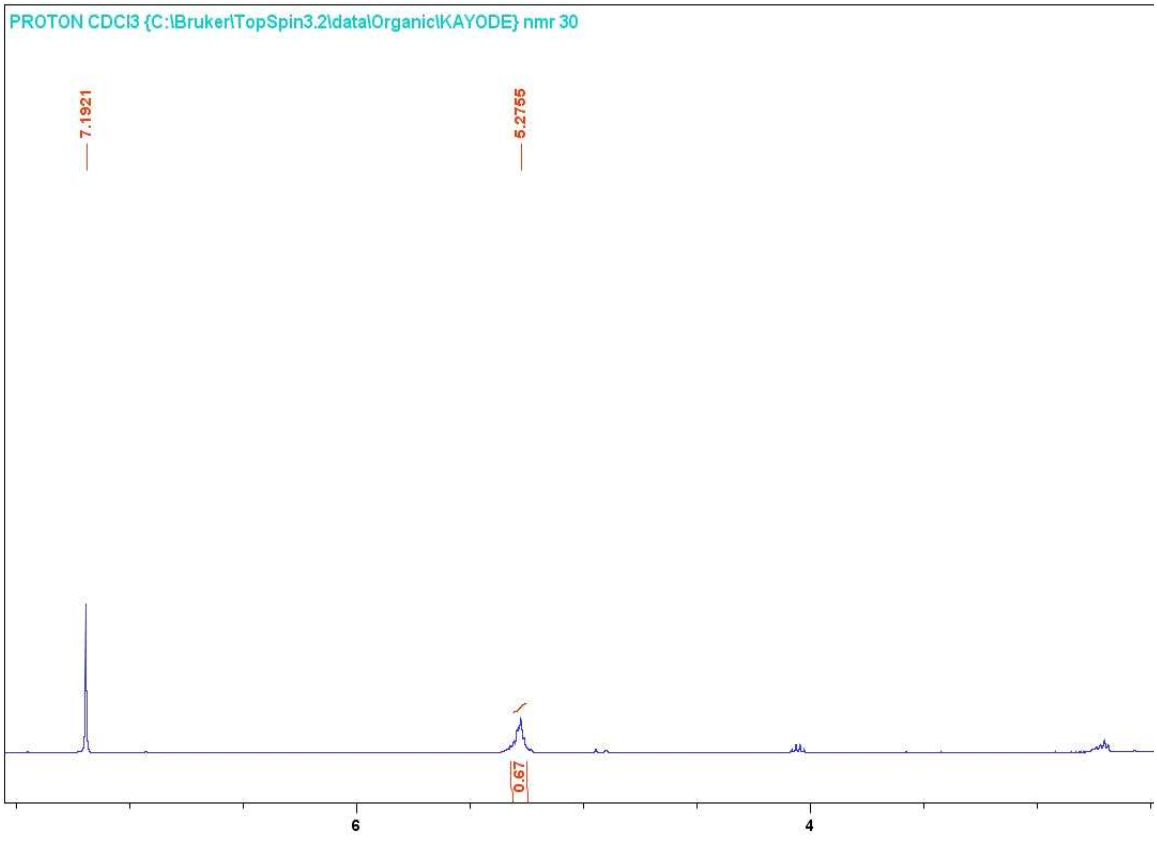
Appendix VJ

The IR DATA spectrum of compound 19 isolated from the leaf extract of *Securidaca longepedunculata*



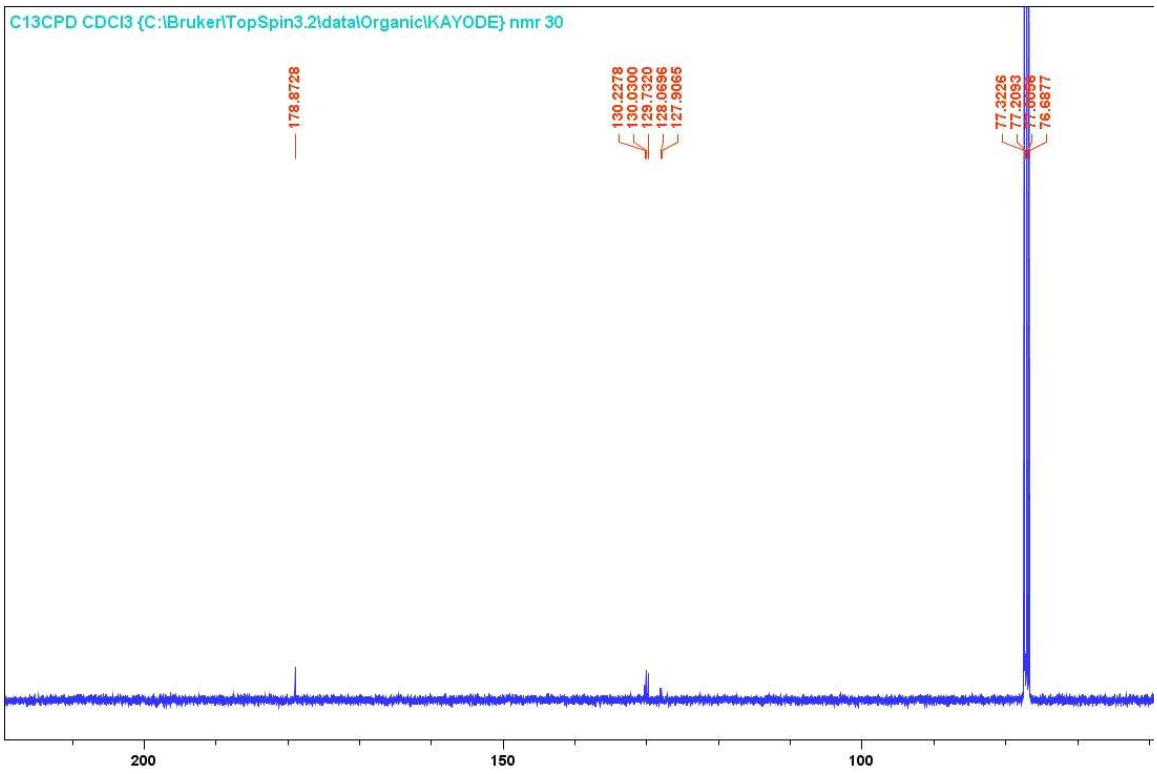
Appendix VIA

The ^{1}H –NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



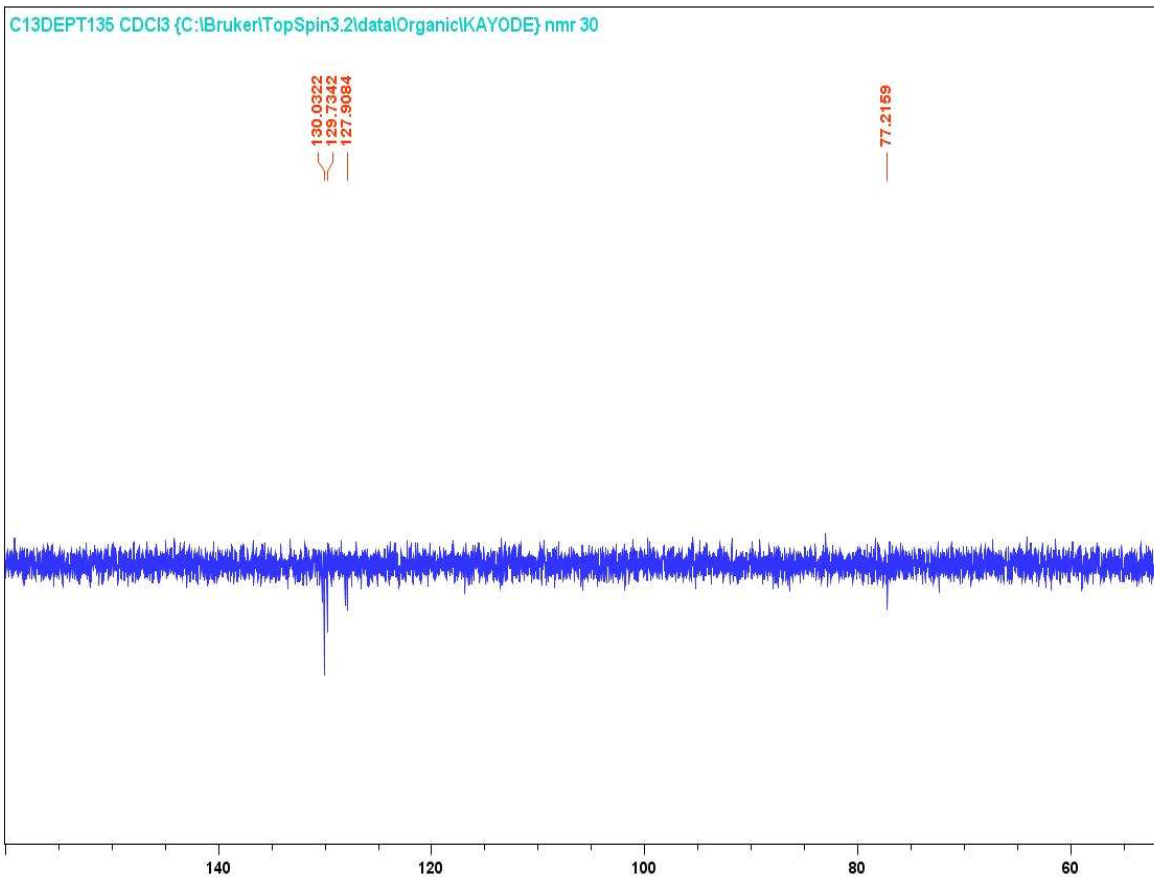
Appendix VIB

The ^{13}C–NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



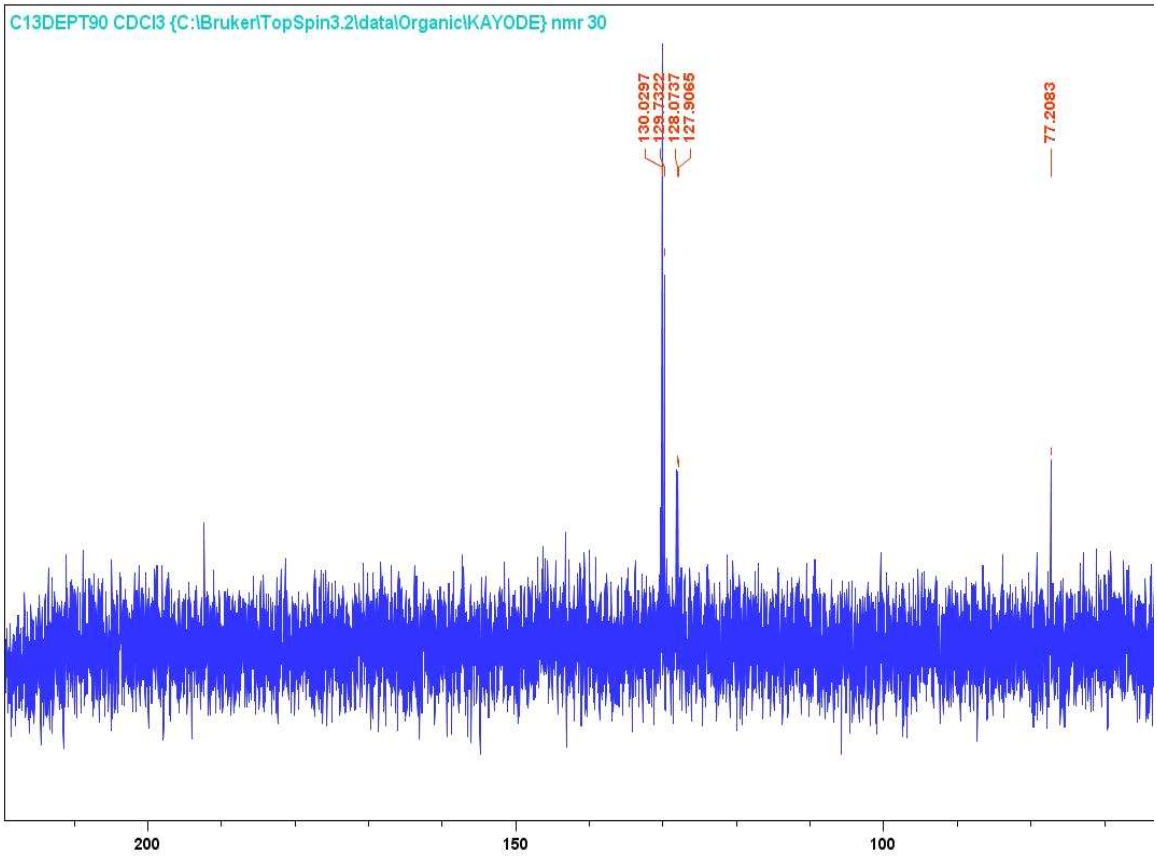
Appendix VIC

The C13DEPT135–NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



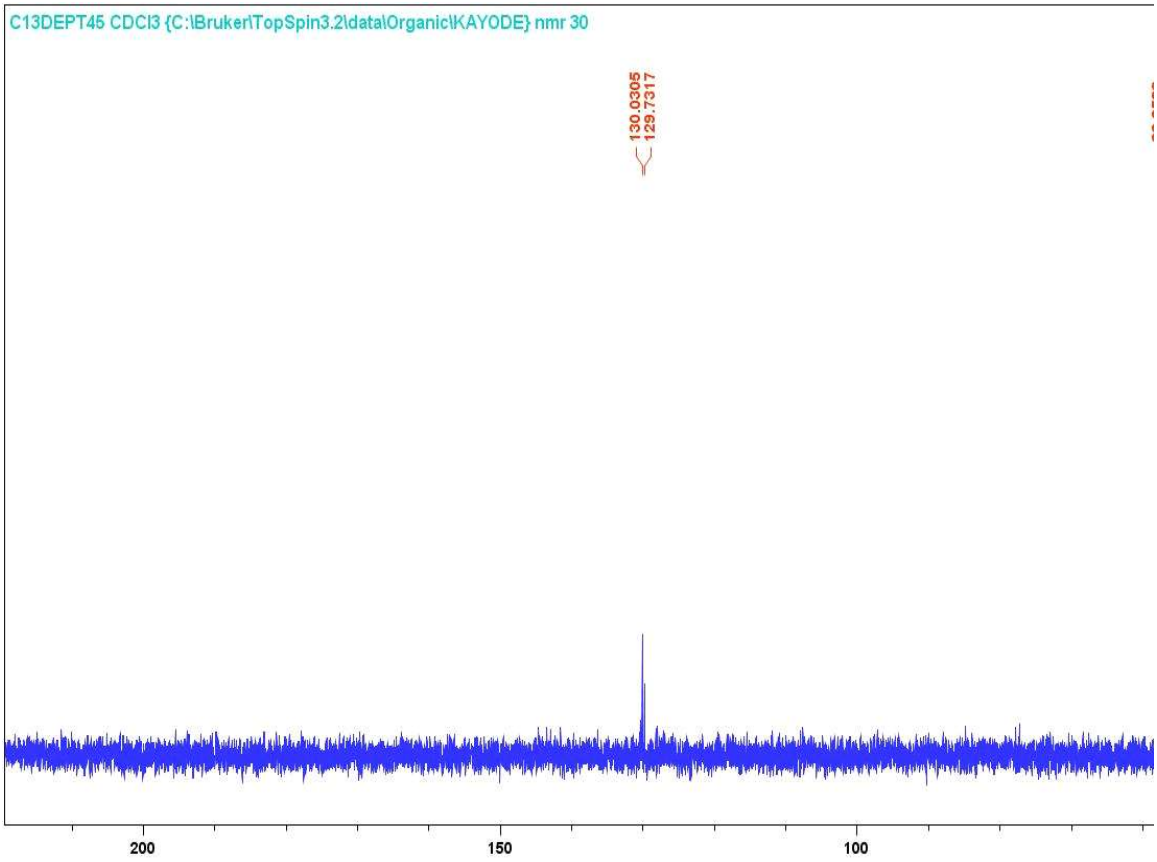
Appendix VID

The C13DEPT90–NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



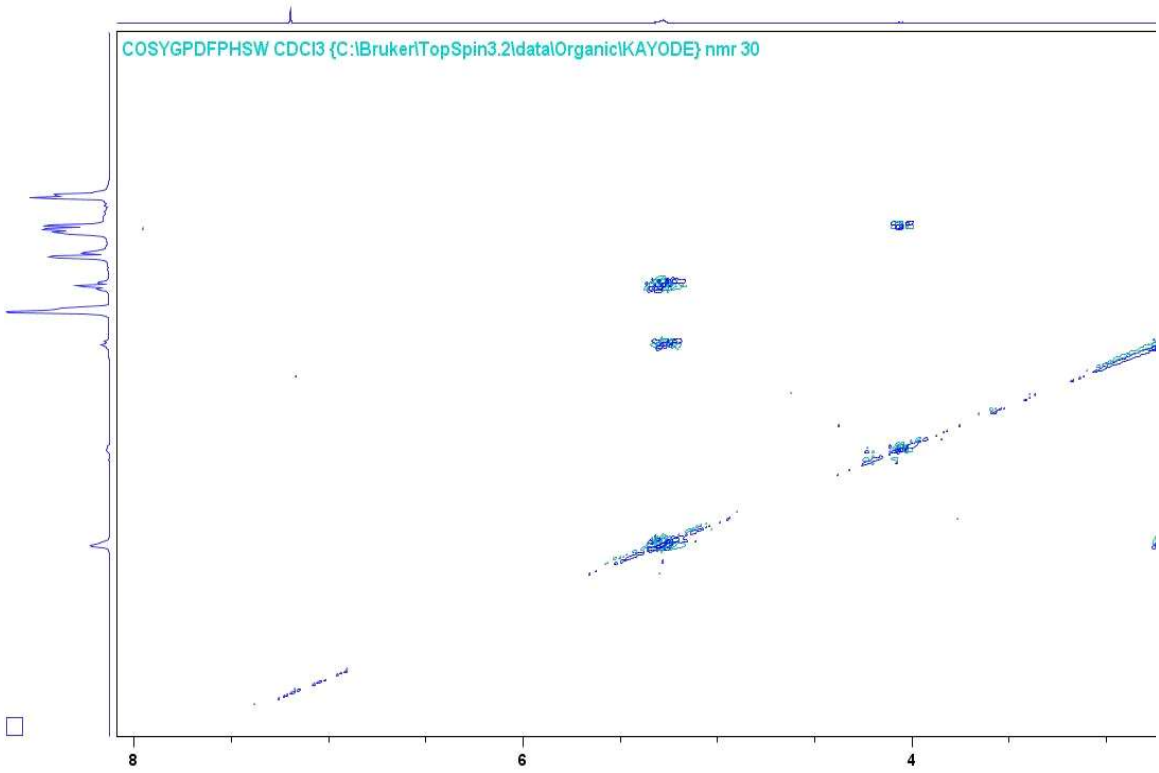
Appendix VIE

The C13DEPT45–NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



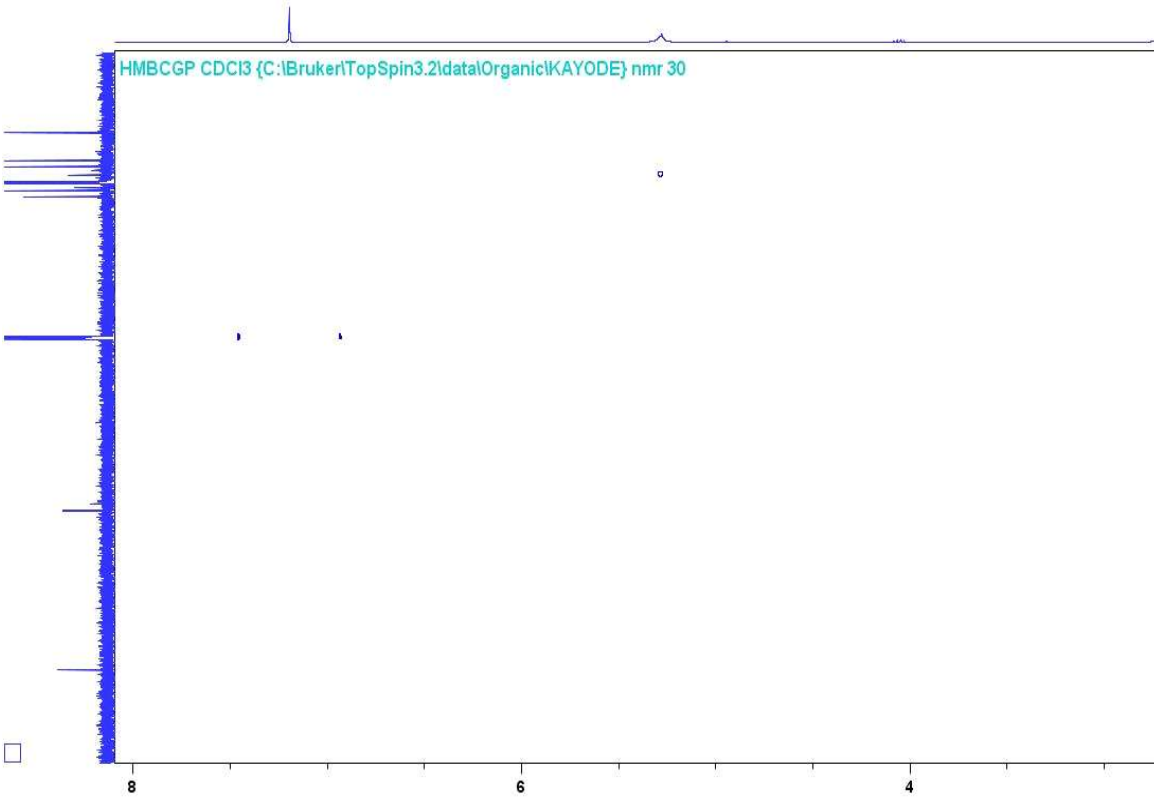
Appendix VIF

The COSY NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



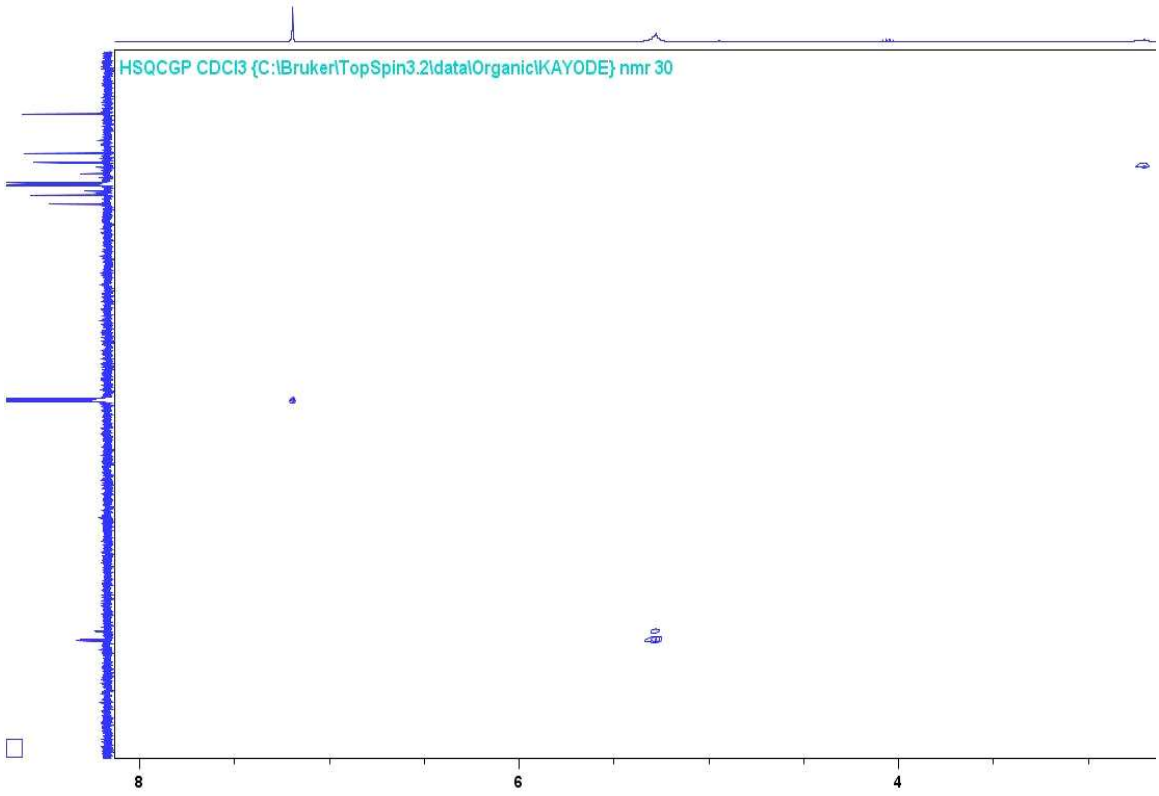
Appendix VIG

The HMBC NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



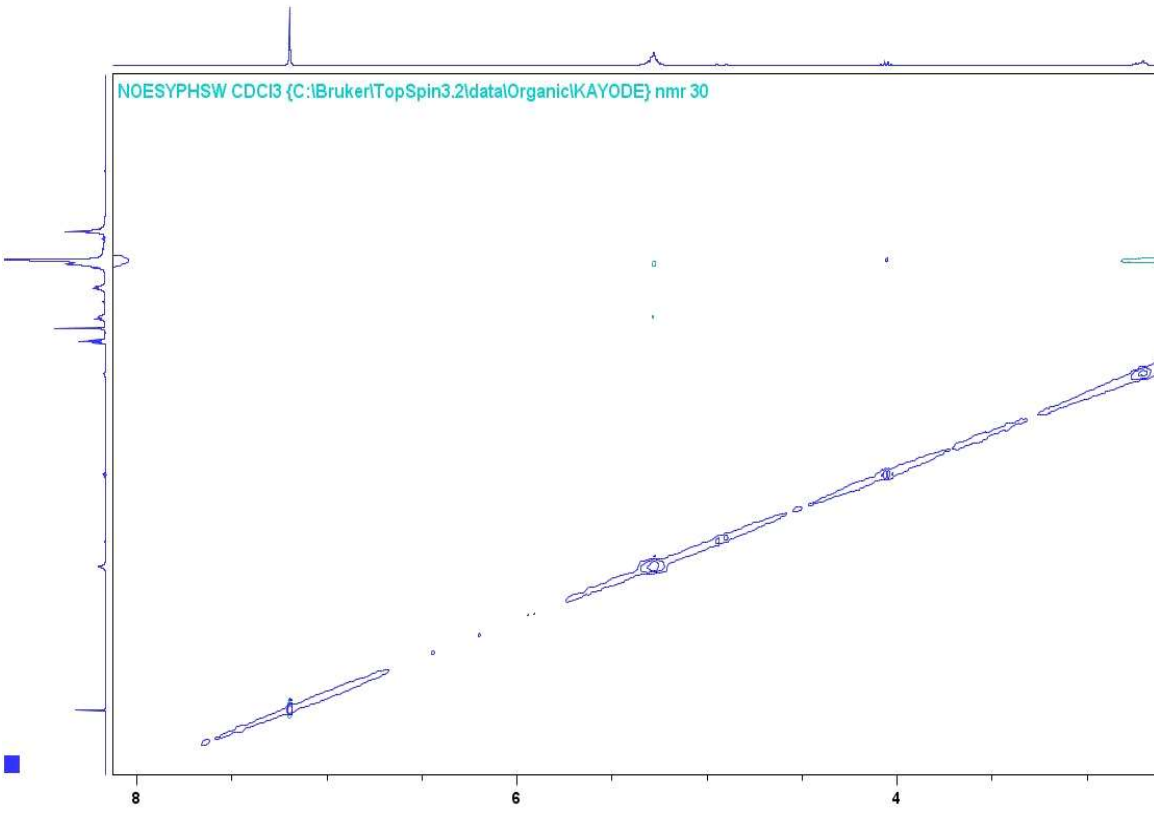
Appendix VIH

The HSQC NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



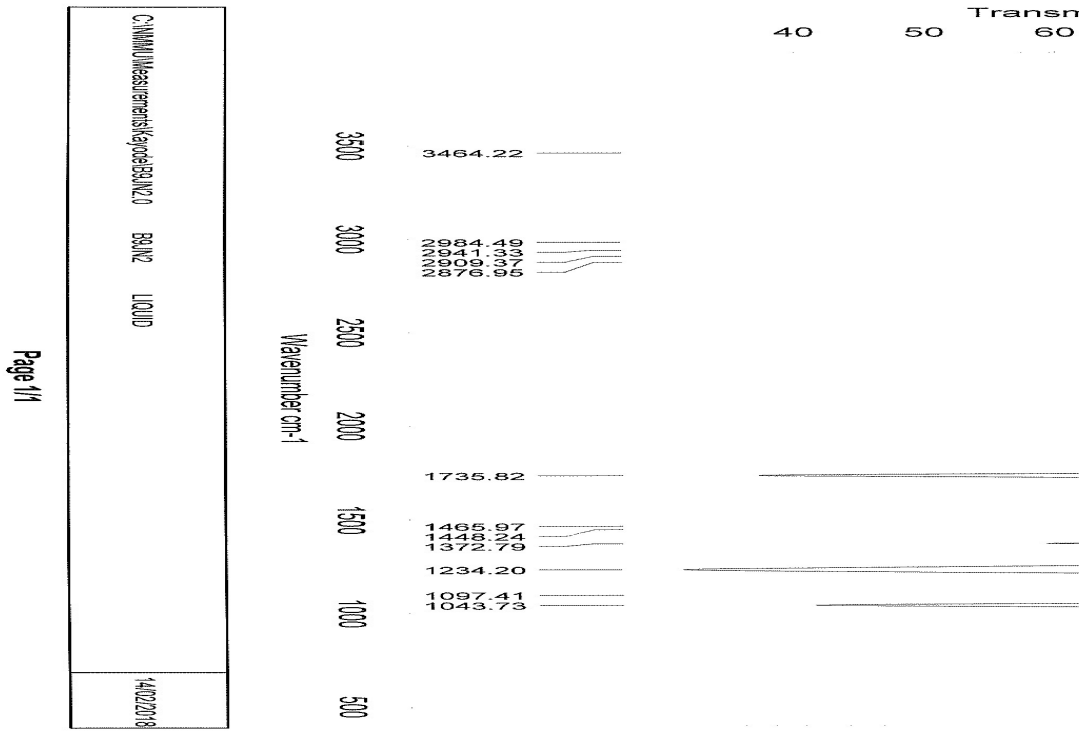
Appendix VII

The NOESY NMR spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



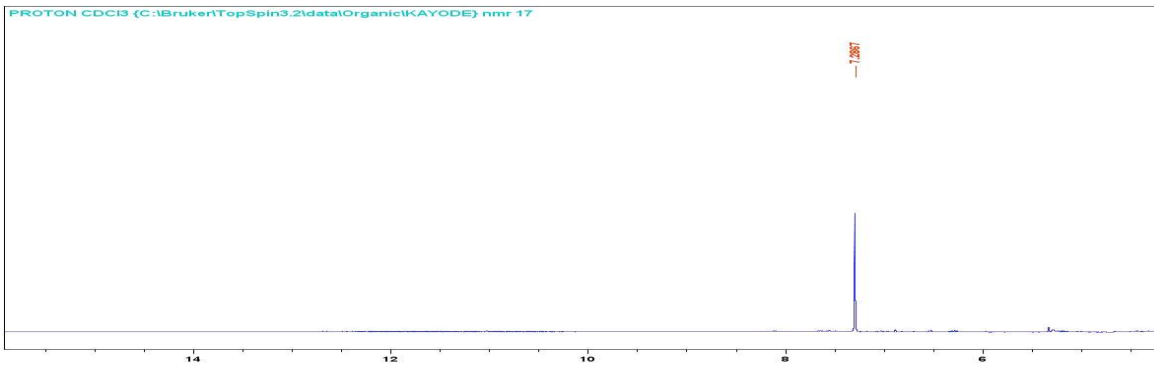
Appendix VIJ

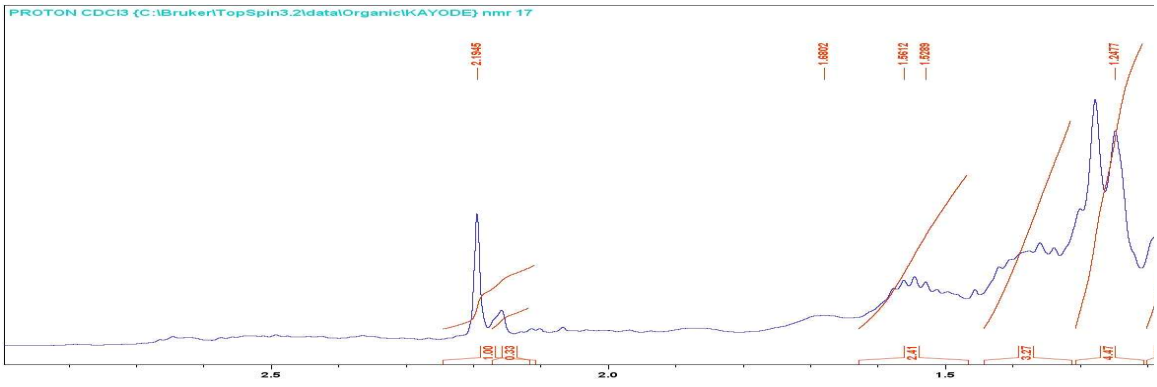
The IR DATA spectrum of compound 20 isolated from the leaf extract of *Securidaca longepedunculata*



Appendix VIIA

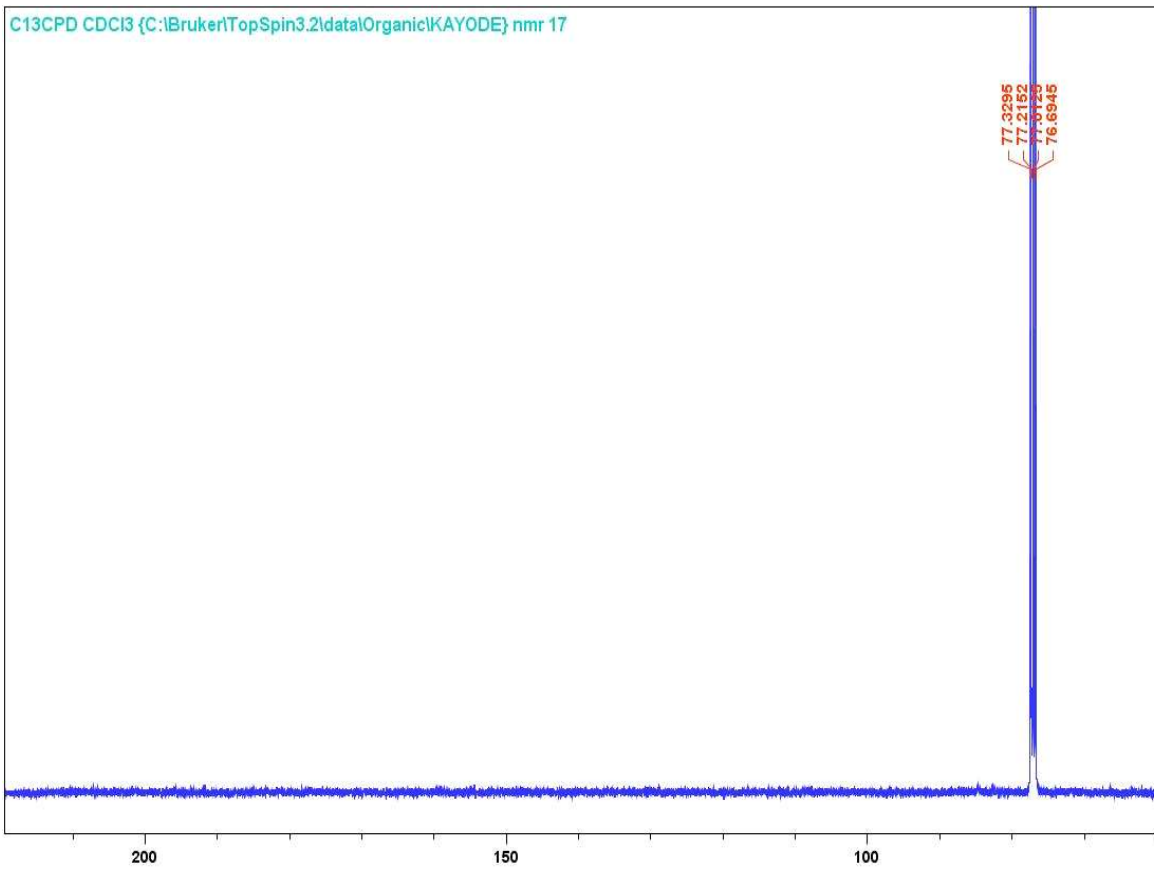
The ^{1}H –NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata* showing the enlarged area of detected signals.





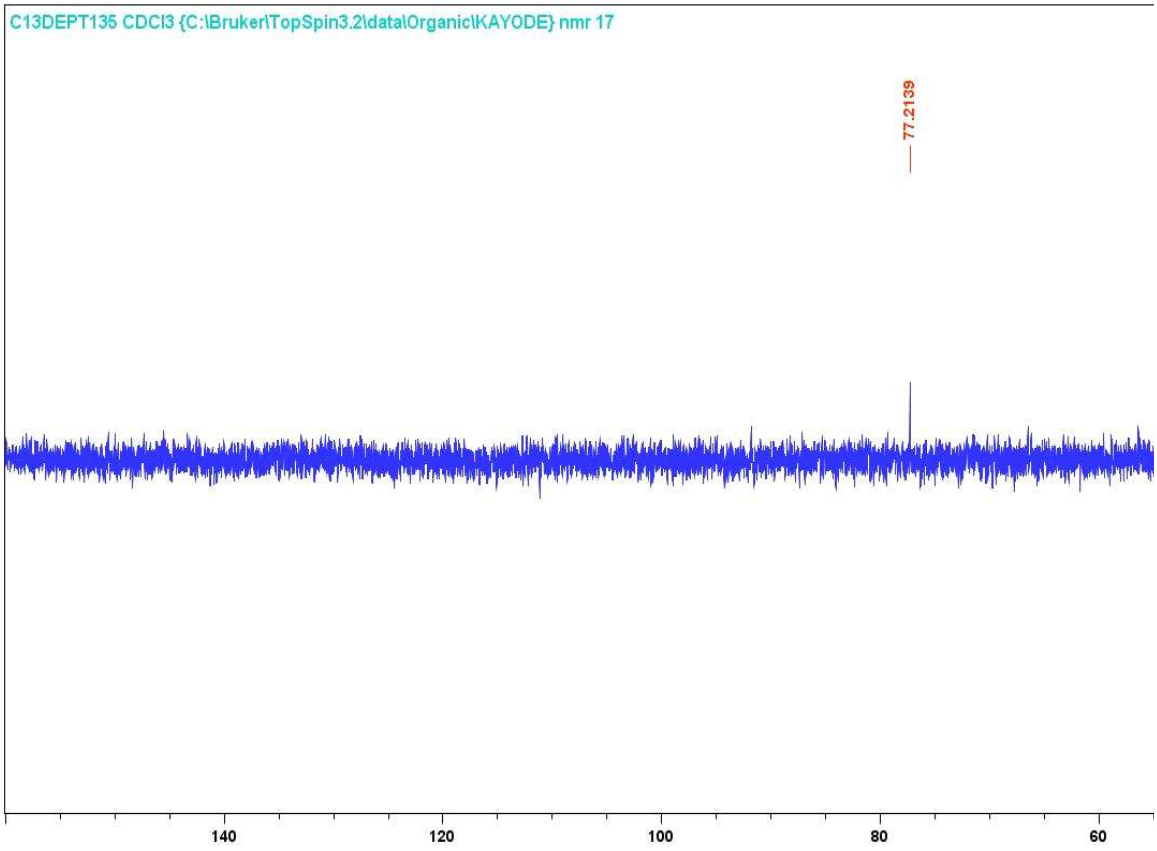
Appendix VIIB

The ^{13}C–NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



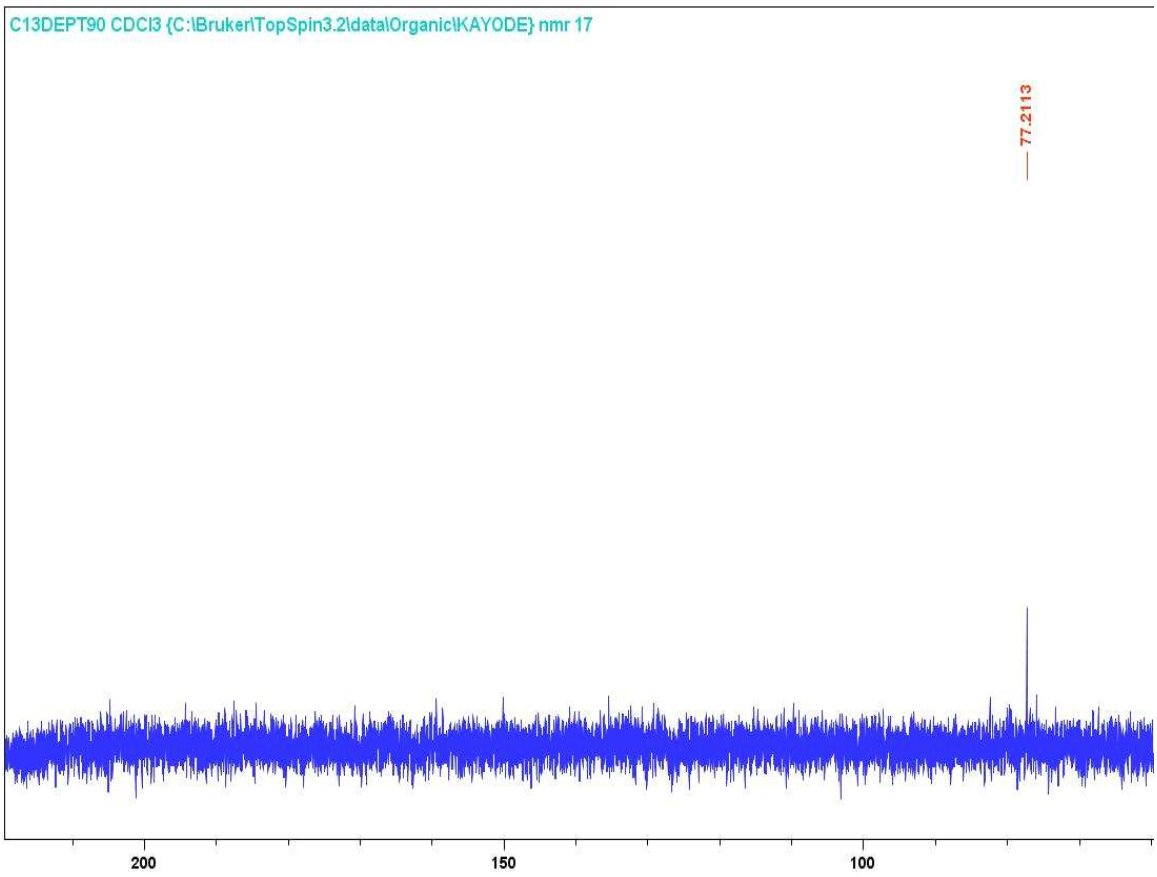
Appendix VIIC

The C13DEPT135–NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



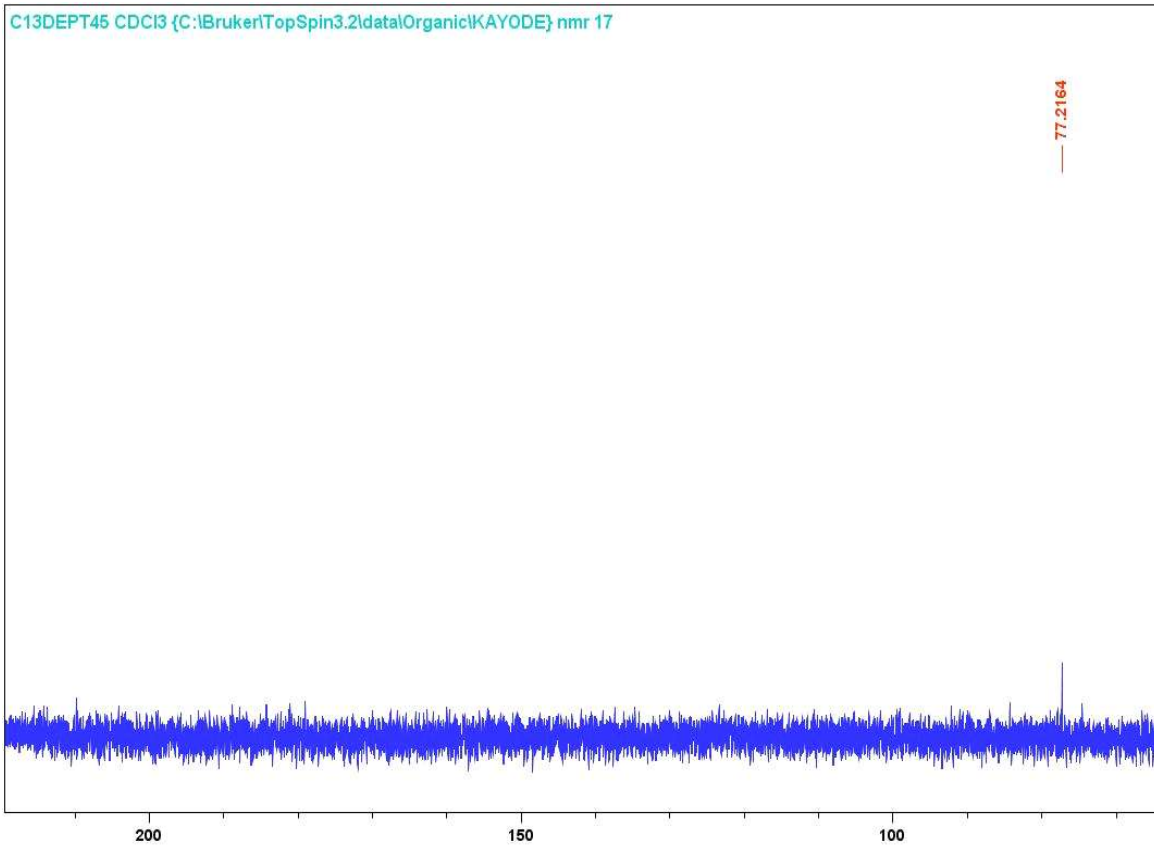
Appendix VIID

The C13DEPT90–NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



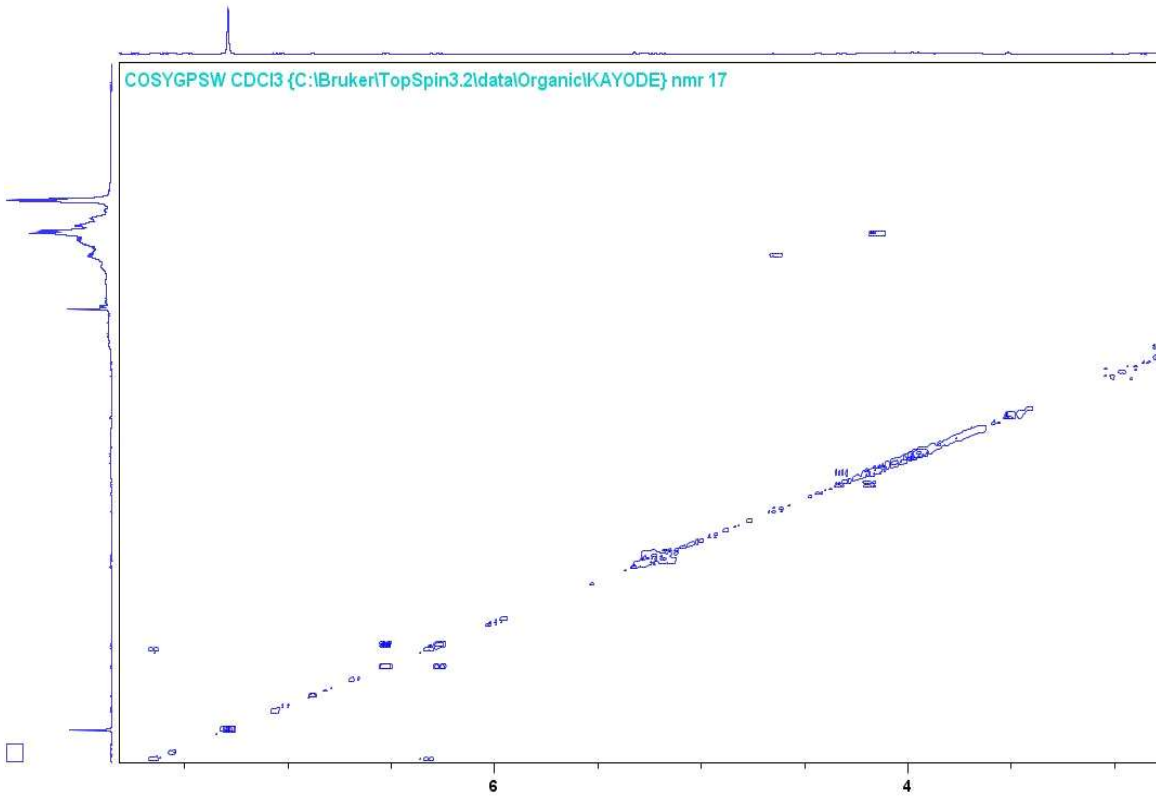
Appendix VIIE

The C13DEPT45–NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



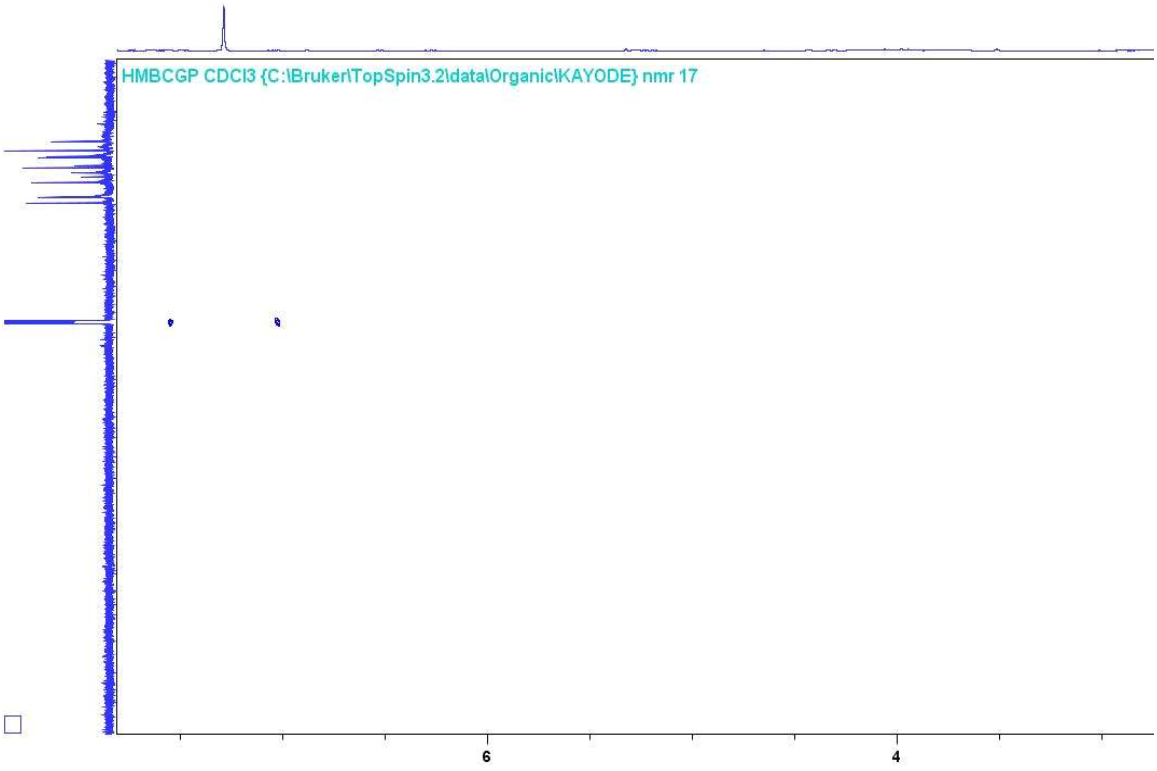
Appendix VIIF

: The COSY NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



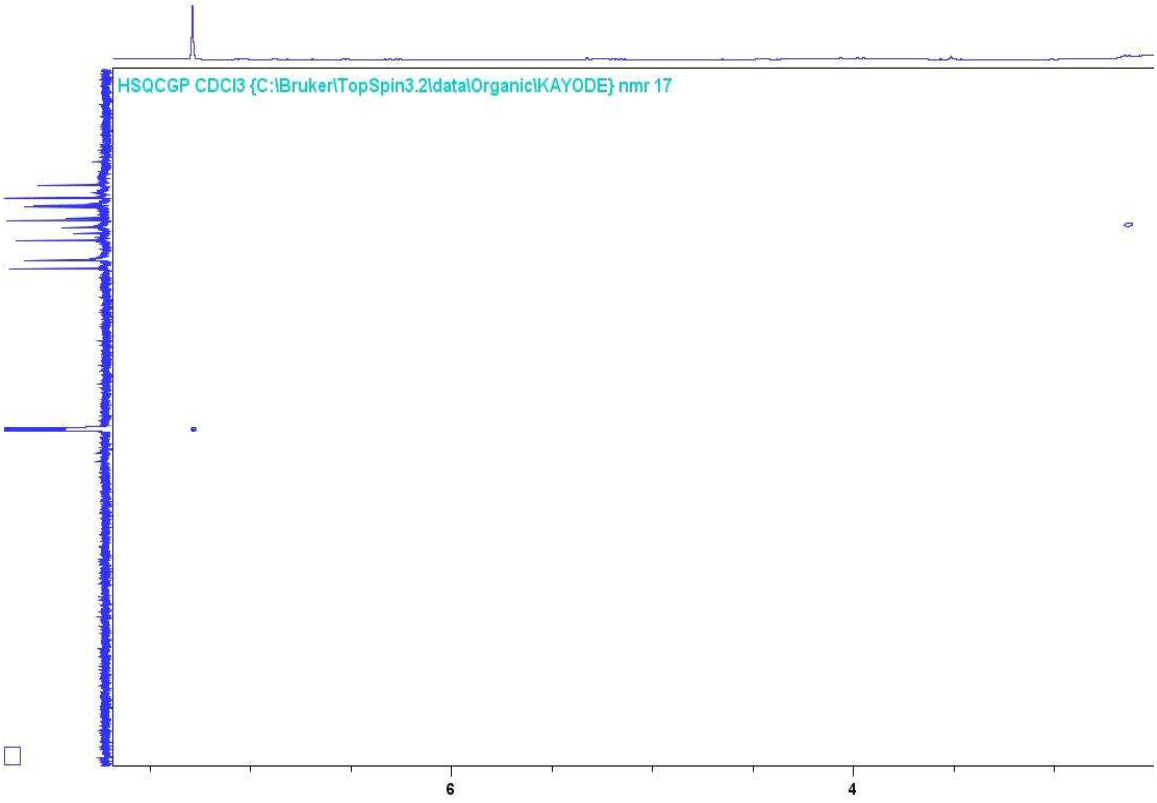
Appendix VIIG

The HMBC NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



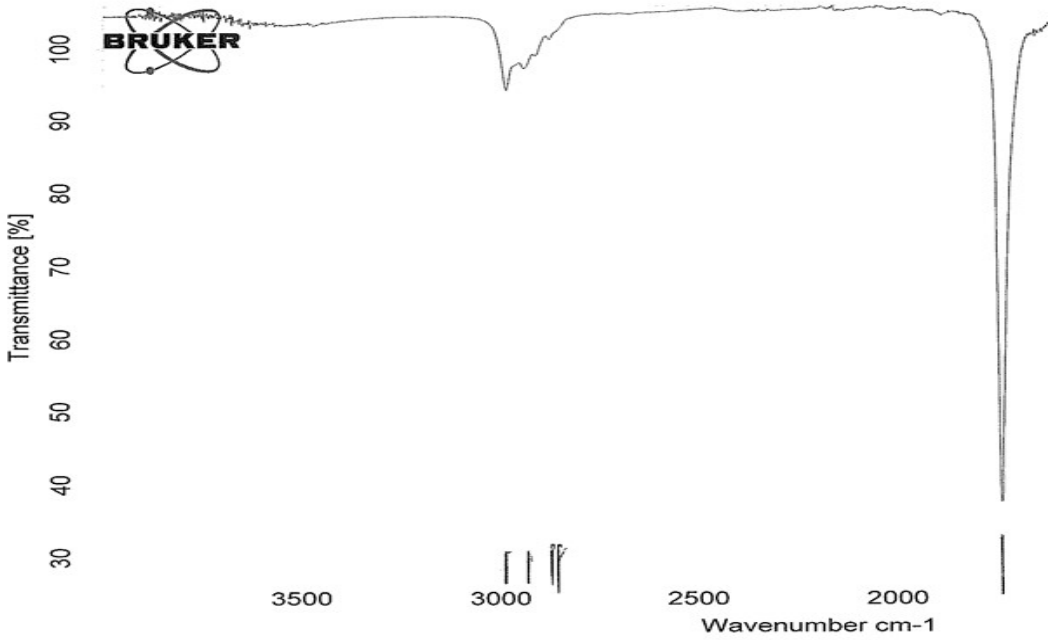
Appendix VIIH

The HSQC NMR spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



Appendix VIII

The IR DATA spectrum of compound 21 isolated from the leaf extract of *Securidaca longepedunculata*



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