CHAPTER ONE

Gastrointestinal disorders in diarrhoea diseases mechanisms and medicinal plants potentiality as therapeutic agents.

1. Introduction

The gastrointestinal tract (GIT) is dedicated to processing and absorbing nutrients and fluids essential for the maintenance of good health (Martinez-Augustin *et al.*, 2009). For the GIT to function optimally, a balance is maintained between intestinal motility and intestinal fluid volume. The latter process is finely regulated through the control of fluid absorption via intestinal villous epithelial cells and secretion across the intestine via intestinal crypt cells (Martinez-Augustin *et al.*, 2009). Net fluid absorption driven by osmotic gradients controlling the movement of electrolytes (sodium ions [Na^+] and chloride ions [Cl^-]), sugars and amino acids across the epithelial lining of the lumen, predominate in these opposing processes (Pash *et al*, 2009). In contrast, motility is controlled by the activation of enteric nervous system (ENS) by either neurotransmitters, inflammatory mediators or epithelium membrane lipid peroxidation by-products (Wood, 2004). Any upset of this delicate intestinal fluid balance (decrease fluid absorption and increase fluid secretion), and/or changes in GIT motility usually causes intestinal disorders clinically evident as diarrhoea (Vitali *et al.*, 2006).

Diarrhoea is loosely defined as an alteration in the normal bowel movement characterized by an increase in the volume, frequency and water content of stool (Baldi *et al.*, 2009). The pathophysiology of diarrhoea include microbial and parasitic infections (Hodges and Gill, 2010), stress (oxidative or physical) (Soderholm and Perdue, 2006), dysfunctional immunity (Schulzke *et al*., 2009), disrupt GIT integrity and neurohumoral mechanisms (Vitali *et al.*, 2006; Spiller, 2004). Diarrhoea can also be a symptom of other diseases such as cholera, irritable bowel syndrome (IBS), gastroenteritis (intestinal inflammation and ulcerative colitis) (Schiller, L. R., 1999; Baldi *et al.*, 2009), malaria (Gale *et al*., 2007) and diabetes mellitus (Forgacs and Patel, 2011).

The mechanism causing diarrhoea can be secretory (resulting from osmotic load within the intestine), hyper motility (resulting from rapid intestinal transitions) or hypo motility (resulting in decreased intestinal fluid re-absorption) or combination of these mechanisms (Vitali *et al.*, 2006). The symptoms are either caused by an increase in fluid and electrolyte secretion predominantly in the small intestine or a decrease in absorption which can involve both small and large intestine (Pash *et al*, 2009; Spiller and Garsed, 2009). Physiologically, diarrhoea is considered beneficial to the GIT as it provides an important mechanism of flushing away harmful luminal substances (Valeur *et al.*, 2009). However, diarrhoea becomes pathological when the loss of fluids and electrolytes exceeds the body's ability to replace the losses.

As a disease, diarrhoea is considered one of the most dangerous GIT disorders as death can result in severe cases due to dehydration and loss of electrolytes (WHO and UNICEF, 2004). According to the World Health Organization (WHO)/United Nation Children Fund (UNICEF) report, more than 1 billion diarrhoeal episodes occurred in human across the world yearly, with about 5 million deaths especially in infants (Thapar and

Sanderson, 2004). In addition to causing acute disease and mortality, diarrhoea associated malnutrition could result in stunted growth, non-optimal immune functionality and increase susceptibility to infections. Diarrhoea therefore poses a major health challenge to human, as it could lead to premature mortality, disability and/or increase health-care costs (Guerrant *et al.*, 2005).

In animal production, diarrhoea is presumed to impose heavy productivity losses on affected farms, although true effects in monetary terms cannot be easily appreciated. The apparent on-farm losses are reduction in productivity (milk, wool, egg, meat and meat quality), increased mortality and morbidity, weight loss and abortion (Chi *et al.*, 2002). Episodes of diarrhoeal diseases can also affect the export market and hurt consumer's confidence in the products (Yarnell, 2007).

The most common modern method of managing diarrhoea is the replacement of lost fluids and electrolytes with either oral or intravenous electrolyte preparation (Thapar and Sanderson, 2004). While fluid replacement is usually effective, severe fluid losses requires additional pharmacological treatment to mitigate the on-going fluid loss. For this, drugs with antispasmodic, antimotility, antioxidative, anti-secretory/pro-absorptive and/or anti-inflammatory properties (depending on the causative agents) may be used to treat diarrhoeal (Wynn and Fougere, 2007). The issue of antimicrobial therapy for self-limiting and non-infectious diarrhoea is usually not encouraged to avoid development of drug resistance microbes. However, in cases of established infectious diarrhoea with known pathogenic agents, specific therapeutic intervention using antimicrobial drugs targeting the causative microbes may be applied. At present, the current standard therapeutic options are insufficient because of limited available modalities with broad based activities against the large number of diarrhoeal disease mechanisms and apparent side effects. The problems associated with some of the standard therapies include antimicrobial resistance, drug toxicity, constipation and addiction.

As a result there is an urgent need for new therapeutic drugs with lower cost, high efficacy, little or no side effects and wider availability especially in rural areas where diarrhoea causes large scale infant mortality. Plants, which servs as dietary source to animals and people, may also provide a good source of new therapeutic drugs.

1.2. Plant metabolites as potential therapeutic agent

Plant serves as dietary source to animals and humans providing sufficient nutrients to meet metabolic requirements for their well being, growth and productivity. However, it can also contribute to achieving optimal health and development as well as serving an essential role in reducing the risk or delaying the onset of diseases and disorders (Kosar *et al*, 2006; Halliwell, 1997). Medicinal plants have therapeutic properties due to biosynthesis of various complex phytochemical substances grouped broadly as phenolics, alkaloids and terpenoids. Synergistic interaction among the multiple phytochemicals may be responsible for the overall bioactivity of a given medicinal plant. Pharmacological and clinical studies of phytochemical in plants have shown that they exhibit various medicinal uses and serve as the major backbone of traditional medicine (Van Wyk and Wink, 2004). Medicinal plants have played some key roles in the health care needs of rural and urban

settlements for human, livestock and animals. Plant extracts, formulations, or pure natural compounds are used in controlling diverse diseases ranging from coughs, inflammation, and diarrhoea to parasitic infection in human and veterinary medicine. A large number of these medicinal plants have been screened and validated for their ethnopharmacological use as antidiarrhoeal agents of varied mechanisms (Gutierrez et al., 2007). However, the literatures available on the pharmacological evaluation of medicinal plants used traditionally in treating diarrhoea in South Africa are mainly on antimicrobial screening models. Little literature information is available on other antidiarrhoeal mechanisms and *in vivo* study

For this study, 27 South African medicinal plants used as diarrhoeal remedies with ethnopharmacological background identified as requiring further biological evaluation. Thereafter, *Bauhinia galpinii* and *Combretum vendae* were choosing for further investigation based on the results from preliminary screening. *Bauhinia galpinii* was previously investigated for its antioxidant activities and three compounds (two active and one inactive) were isolated from the acetone leave extract (Aderogba et al., 2007). Methanol and dichloromethane leaf extracts of *B. galpinii* are reported to have antimutagenic property (Reid et al., 2006). The acetone root extract of *B. galpinii* has also been found to be highly cytotoxic (LD_{50} 2.70 µg/ml) against Vero cell lines (Samie et al., 2009). Antimicrobial activity of *Combretum vendae* against four bacterial pathogens (Ahmed et al., 2009) and apigenin has been isolated from the acetone leaf extract (Eloff et al., 2008).

In many previous studies relatively non-polar extractants were used despite the fact that traditionally aqueous extracts are used. This is probably due to difficulties in analyzing complex molecules extracted by polar extractants, because phenolics may play an important role in managing diarrhoea the focus of this study will be on more polar extractant.

1.3. Aims

To investigate the biological activities of the phenolic-enriched extracts and fractions of 27 medicinal plants against some diarrhoea pathoaetiologies and evaluating the antidiarrhoeal mechanisms of *Bauhinia galpinii* and *Combretum vendae* extracts using in vitro isolated organ methods, as means of validating their ethnopharmacological used in South African traditional medicine to treat diarrhoea.

1.4. Specific objectives

❖ To evaluate the effect of the extracts, fractions and isolated compound(s) against pathogenic microbes that are known to induce diarrhoea.

❖ To determine the antioxidative properties of the extracts, fractions and isolated compound(s) using the DPPH radical scavenging, the ABTS radical scavenging, the hydroxyl radical scavenging, the linoleic acid peroxidation inhibition and the ferric reducing antioxidant power (FRAP).

❖ To determine the effects of the most promising extracts on the contractility process of the isolated rat ileum induced by spasmogens, receptor agonists, antagonists and ion channels activators.

- ❖ To fractionate the extracts and elucidate the component(s) that exhibit antimicrobial and antioxidant properties.
- ❖ To evaluate the safety, efficacy and toxicity of the crude extracts and the pure active component(s).

1.5. Hypothesis

The phytochemical constituents of medicinal plants used in traditional medicine have antioxidant, anti-inflammatory, antimicrobial and /or anti-spasmodic activities that could help in alleviating diarrhoeal diseases in human and animals.

CHAPTER TWO

2.0. Literature review

2.1. Diarrhoea as a disease

Diarrhoea is a common clinical sign following on altered bowel movement, decreased intestinal absorption of fluids and increased intestinal electrolyte secretion resulting in loose and watery stool (Baldi *et al.*, 2009). The mechanisms of diarrhoea diseases can be secretory due to impaired electrolyte absorption and osmotic load within the intestine, hyper motility resulting from rapid intestinal transitions of material or hypo motility resulting in decreased intestinal fluid re-absorption or combination of these mechanisms (Vitali *et al.*, 2006). The symptoms are either caused by an increase in fluid and electrolyte secretion predominantly in the small intestine or a decrease in absorption which can involve both small and large intestine (Pash *et al*, 2009; Spiller and Garsed, 2009).

Diarrhoeal disease can be either infectious or non-infectious in nature with infection pathogenesis responsible for the major total episode worldwide. In infectious diarrhoea, the potential causative pathogens include bacterial agents (Mathabe *et al.*, 2006), rarely fungal (Robert *et al.*, 2001), viral and parasite pathogens (Brijesh *et al*., 2006). Non-infectious diarrhoea can be caused by adverse reactions to drugs, toxins, allergy to food, poisons and acute inflammation which promote the release of secretagogues and some enteric nervous system (ENS) receptors (prostaglandin, serotonin, substance P, vasoactive intestinal peptides, and hormone) in the GIT (Wynn and Fougere, 2007). Diarrhoea is usually classified according to the duration of the symptoms:

- Acute diarrhoea: mostly caused by enteric pathogenic infections, intoxicants or food allergy. This type of diarrhoea is self-limiting without pharmacological intervention and usually resolves within two week from onset or,
- Persistent diarrhoea: mostly result from a secondary cause such as enteric infections or malnutrition, and usually last for more than 14 days, or
- Chronic diarrhoea: mostly result from congenital defects of digestion and absorption. This usually last for more than 30 days (Thapar and Sanderson, 2004; Baldi *et al.*, 2009).

Other methods of classifying diarrhoea include stool characteristics or pathological mechanisms such as watery, osmotic, altered motility or inflammatory diarrhoea (Ravikumara, 2008) as shown in Fig. 2.1.

- Watery diarrhoea typically referred to as secretory diarrhoea results from increased chlorine secretion, decreased sodium absorption and increased mucosal permeability.
- Osmotic diarrhoea, also a watery form of diarrhoea, is caused by the ingestion of non-absorbable indigestible material (Baldi *et al.*, 2009) or absence of brush border enzymes required for the digestion of dietary carbohydrates (Podewils *et al*, 2004).

- Inflammatory diarrhoea is characterized by the presence of mucus, blood, and leukocytes in the stool, and is usually induced by an infectious process, allergic colitis or inflammatory bowel disease (IBD) (Ravikumara, 2008).
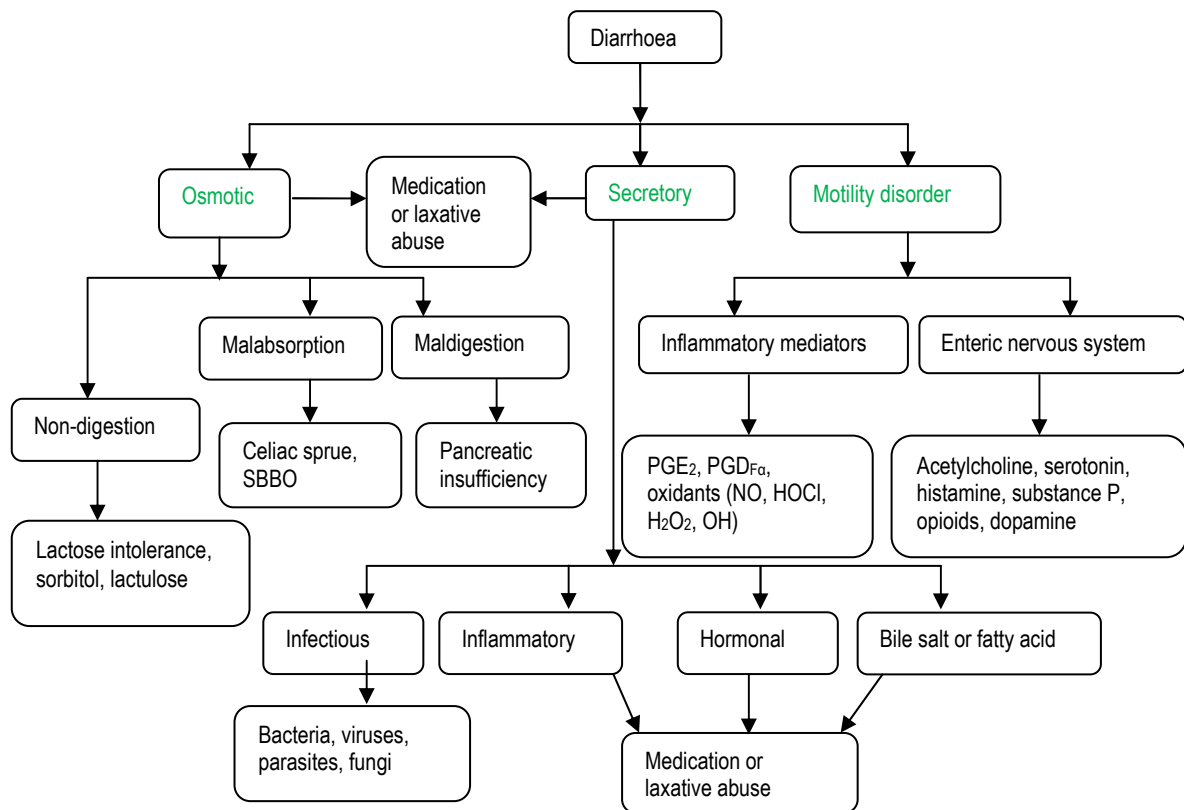

Fig.2.1: Classification of diarrhoea and the stimulants (modified from Ebert, 2005)

PGE_2= prostaglandin E_2, $PGD_{F\alpha}$= prostaglandin $D_{F\alpha}$, NO=nitric oxide, HOCl=hydrogen chlorate, H_2O_2=hydrogen peroxide, OH=hydroxyl radical, SBBO = small-bowel bacterial overgrowth

2.2. Pathophysiology of Diarrhoea

A "healthy gastrointestinal tract (GIT)" can be defined as one where a balance is reached between the bacteria colonising the environment and the immune system. Any disturbance in this homoeostasis will result in GIT disorders like diarrhoea. The general causes of diarrhoea are: microbial infection (bacteria, viruses and parasites), intestinal inflammation, altered GIT motility as a result of damage to enteric nervous system (ENS) and immune dysfunctions. The mechanisms of infectious diarrhoea include:

- Microbial attachment and localized effacement of the intestinal epithelium: Some enteric pathogens have the ability to attach and alter the surface of the invaded cell characterized as attaching and effacing lesion. Attachments of the infectious pathogens to the apical surface of the enterocyte-like cells create favourable conditions for bacterial growth and multiplication (Ramaroa and Lereclus, 2006). The mechanism of effacing lesion involves the localized destruction of the adjacent epithelial microvilli and

the formation of a pedestal-like structure from the accumulation of cytoskeletal proteins, such as actin, beneath the site of attachment (Thapar and Sanderson, 2004; Guerrant *et al,* 1999).

- Production of enterotoxins that subvert mucosal transport systems: In the case of intoxication, the causative organisms may or may not be present in the transmitting medium, but act through preformed enterotoxins (examples of such organisms are *S. aureus* (α-haemolysin) *and* emetic type *Bacillus cereus* (cereulide) (Granum, 2006). The toxins may be cytotoxic or haemolytic, thus causing damage to intestinal epithelial cells. The mechanism of actions used by the enterotoxins to cause diarrhoea include (Laohachai *et al*, 2003):

(1) Decrease in intestinal surface area and, hence a decreased fluid absorption rate.

(2) Changes in mucosal osmotic permeability, resulting from mucosal destruction.

(3) Changes in fluid and electrolytes homeostasis through the toxin's action on ion channels.

- Direct epithelial cell invasion: Epithelial cells are the first major cell type encountered by infectious pathogens in the intestinal mucosa and the main site of host-pathogens interaction (Ramaroa and Lereclus, 2006). Intestinal mucosal epithelial cells are essential for initiation of the immune response to different microorganisms (Hodges and Gill, 2010). In addition to forming a physical barrier that protects the host's internal organs from the external environment, epithelial cells produce a variety of cytokines and chemokines in response to microbial infection (MacNaughton, 2006). The survival strategy of some pathogens is the invasion of epithelial mucosa cells through the activation or inhibition of different signal transduction pathways and induces cytoskeletal rearrangement within the host cell.

- Production of cytotoxins: Microbial cytotoxins degrades of the epithelial cell surface membrane and consequently results in loss of host epithelial cell layer (Ramaroa and Lereclus, 2006; Thapar and Sanderson, 2004). Diarrhoea occurs through the destruction of the epithelial cells due to loss of absorptive surface area and impaired secretion mechanisms.

- Immune activation: Some pathogens may induce diarrhoea indirectly via excessively stimulation of the immune system. The inflammatory mediators such as cytokines (interferon-γ, tumour necrosis factor (TNF-α), interleukin-6 (IL-6), and IL-1β) (Johnson *et al*., 2010), reactive oxygen species (ROS), reactive nitrogen species (RNS) (Sprague and Khalil, 2009) can all interfere with the epithelial tight junctions (TJs), thereby resulting in diarrhoea.

- Immune inhibition: With the direct involvement of the immune system in the protection of the body against both pathogenic and enteric flora, any descent in functionality in the immune could allow the opportunity for pathogenic and/or enteric microbes to establish themselves in abundance with resultant decrease in GIT function and diarrhoea.

2.3. Detailed pathophysiology of diarrhoea

2.3.1. Inflammation in diarrhoea

Inflammation is the body's first line of defence against infection and hazardous stimuli in people and animals (Iwalewa *et al.*, 2007) with injury or infection in the GIT, resulting in the activation of neutrophils and macrophages. Once activated, the immune cell (e.g. macrophage) assist with the killing of pathogenic microorganisms and/ or the removal of harmful and cell debris (Stables and Gilroy, 2010). This task is achieved through the release of numerous pro-inflammatory cytokines (tumour necrosis factor [TNF]-α and interleukin [IL]-1β, IL-3, IL-6); chemokines and chemoattractants (IL-8 and monocyte chemoattractant protein [MCP]-1) (Conforti *et al.*, 2008) (Fig 2.2).
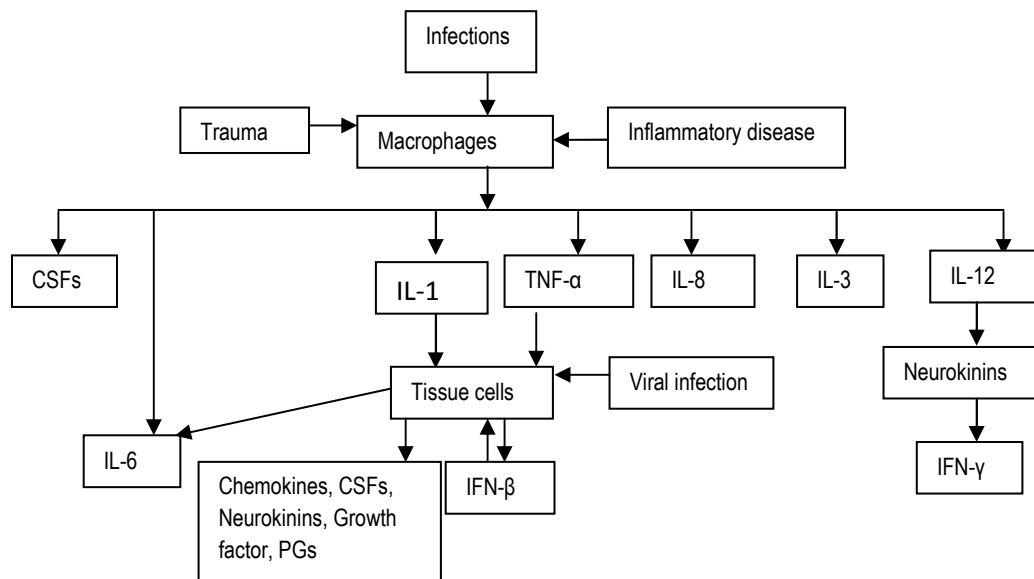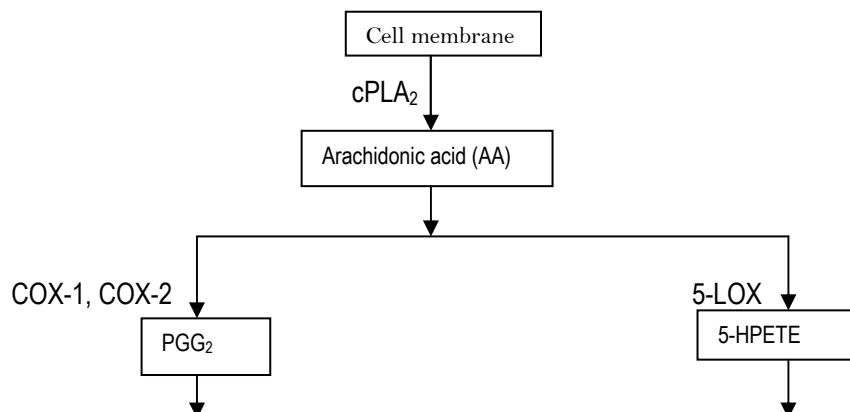

Fig. 2.2. Cytokines production network in the tissues (modified from Hopkins, 2003)

(IL=interleukin, TNF=tumour necrosis factor, CSF=colony stimulating factor, PGs=prostaglandins, IFN=interferon).

Other inflammatory nediators include ROS and RNS, eicosanoids such as cyclooxygenase products (prostaglandin E_2 (PGE_2) or lipoxygenase products (leukotrienes (LTB_4) (Nardi *et al.*, 2007) (Fig 2.3), pain provoking mediators (histamine and bradykinin) (Matu and van Staden, 2003), and/ or cationic antimicrobial peptides (CAMP). Another antimicrobial properties of inflammation is disruption of the epithelial lining which limit microbial survival and colonization of the GIT in inflamed intestine due to loss of replication niche
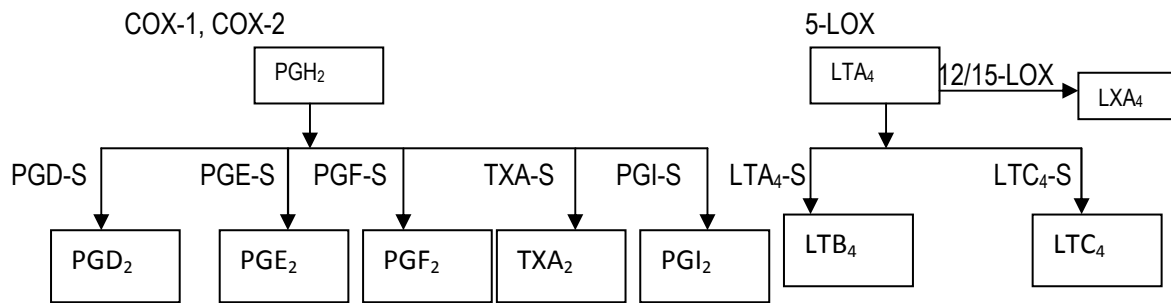
COX-1, COX-2

5-LOX

PGH$_2$

LTA$_4$ → 12/15-LOX → LXA$_4$

PGD-S | PGE-S | PGF-S | TXA-S | PGI-S | LTA$_4$-S | LTC$_4$-S

PGD$_2$ | PGE$_2$ | PGF$_2$ | TXA$_2$ | PGI$_2$ | LTB$_4$ | LTC$_4$

Fig.2.3. Biosynthetic pathways for the eicosanoids (modified from Haeggstrom *et al*., 2010)

(COX=cyclooxygenase, LOX=lipoxygenase, S=synthase, PG=prostaglandin, LT= leukotriene, TXA=thromboxane, PGI=prostacyclin, cPLA$_2$=phospholipase A$_2$)

While inflammation process is beneficial to the body as it removes the insulting cause (Lee *et al.*, 2007a; Pharaoh *et al*., 2006) the large recruitment and activation of neutrophil and macrophages can induced changes in gut motility, neuronal functionality, and hydroelectrolyte movement with resultant diarrhoea (Gelberg, 2007). Some infectious enteric pathogens elicit inflammatory cascade and mediators to manifest diarrhoea (Guttman and Finlay, 2009).The mechanisms involved in the inflammatory modulated diarrhoea may include several factors listed below and shown in Fig 2.1.

- Epithelial barrier disruption: Gastrointestinal epithelium barrier provide a physical defence against hostile environment within the intestine lumen (Blikslager, 2010). The intestinal barrier is determined by interactions among several barrier components including the adhesive mucous gel layer, the mucosal immune system and the tight junctions (TJs) (Schenk and Mueller, 2008). The intercellular TJs are the most essential component of the intestinal physical barrier. TJs are multiple protein complexes located around the apical end of the lateral membrane of the epithelial cells. It performs dual functions as a selective/semipermeable paracellular barriers allowing movement of ion, solutes and water through the intestinal epithelium while also preventing the translocation of luminal antigens, microorganisms and their toxins into the mucosa (Groschwitz and Hogan, 2009; Guttman and Finlay, 2009. Disruption of the intestinal TJ barrier by inflammatory cytokines, reactive oxygen species and pathogens (Guttman *et al*., 2006) impair intestinal TJ function cause an increase in intestinal permeability resulting diarrhoea (Schenk and Mueller, 2008) as shown in Fig. 2.4.
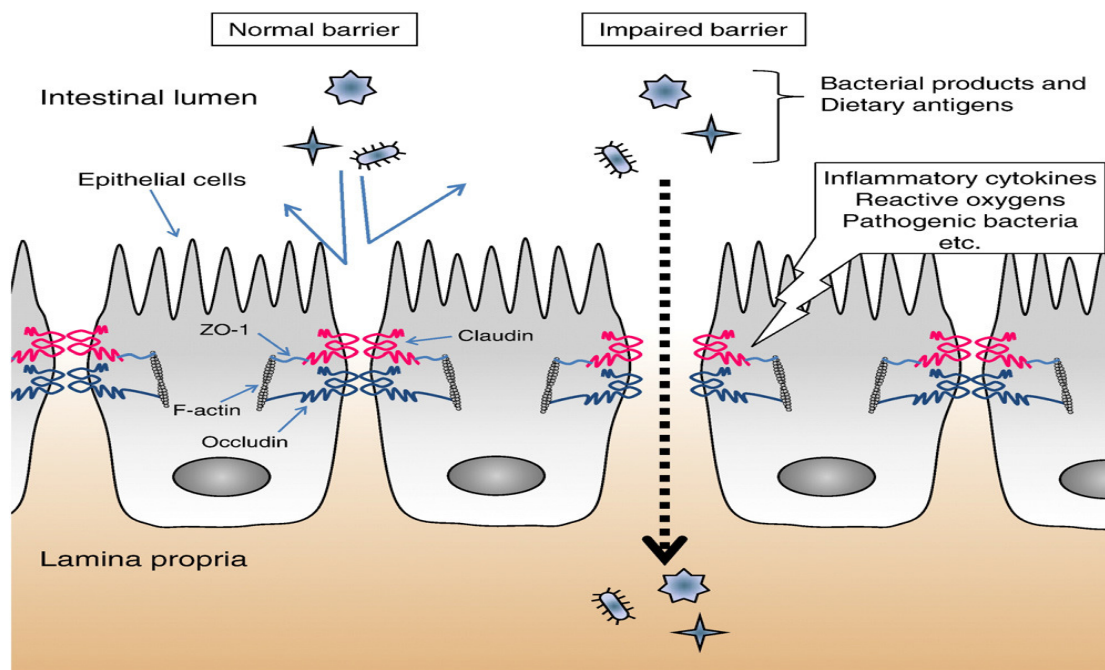
Fig. 2.4. The mechanisms of intestinal epithelial tight junctions as a physical barrier to movement of selected solute materials across the GIT. The intestinal TJs tightly regulate intestinal paracellular permeability. The barrier impairment induced by extracellular stimuli, such as inflammatory cytokines and reactive oxygens, allows the lumina bacterial products and dietary antigens to cross the epithelium and enter circulation (Suzuki and Hara, 2010).

- Reduced absorption capacity: Nutrient-coupled absorption of electrolytes takes place in the brush border microvilli (Dudeja and Ramaswamy, 2006). In an inflamed or infected intestinal tract, the total absorptive surface area is decreased due to brush border shortening resulting in malabsorption (Cotton et al., 2011). Small intestinal malabsorption occurs due to impaired absorption of water, glucose and electrolytes creating an osmotic gradient that draws water into the small intestinal lumen resulting small intestinal distension and rapid peristalsis, consequently diarrhoea (Schulke et al., 2009; Gelberg, 2007; Beavis and Weymouth, 1996).

- Chloride ion hypersecretion: Diarrhoeal agents such as inflammatory mediators, microbial toxins, neurotransmitters and endogenous hormones can activate inappropriate chloride ion (Cl^-) secretion from the colonic crypt epithelial cells. Excessive secretion of chloride ion (Cl^-) from the intestinal crypt is the driving force for many diarrhoea aetiologies. The underlying mechanism is the increase in intracellular levels of cyclic nucleotides (cAMP and cGMP) and/or cytosolic calcium. This process, in turn, drives the secretion of fluid and electrolytes into the intestinal lumen, which may overwhelm the intestinal absorptive mechanism, thereby resulting in secretory diarrhoea with potential effect of severe dehydration (Petri Jr. et al., 2008).

- Interference with ability to digest: Inflammatory response in the intestine may negatively affect the ability of the enterocyte to digest nutritional material. The process causes maldigestion occurs due to a deficiency in various brush border digestive enzymes, especially for carbohydrate and lipids (Schulke et

al., 2009). The high level of undigested carbohydrate and lipids are conversion to short chain fatty acids by the colonic microbiota and the amount may exceed colonic capacity for their absorption. Excess short chain fatty acids induced osmotic gradient pulling water and secondarily, ions into the intestinal lumen resulting in osmotic diarrhoea of colonic origin (Field, 2003). Maldigestion of ingested food coupled with osmotic diarrhoea ultimately lead to long-term malnutrition in affected host (Ogoina and Onyemelukwe, 2009).

- Inflammatory mediators as secretagogue: The release of cytokines such as interleukin-8 (IL-8) and eicosanoids into the gastrointestinal tract activated the macrophage of the immune system. The activated macrophages release inflammatory mediators such as PGE_2, LTE_4, platelet activating factor (PAF), histamine, serotonin, adenosine resulting in cell damage mediated by T lymphocytes or proteases and oxidants generated (H_2O_2, $O_2^{\cdot-}$, $OH^{\cdot}$, NO) by mast cells. Some of the inflammatory mediators (PGE_2, LTB_4, histamine) also serve as secretagogue causing secretory diarrhoea (Field, 2003).

- Stimulate enteric nervous system (ENS): Inflammation causes structural changes to the ENS that ranges from axonal damage to neuronal death (Stanzel *et al.*, 2008). The changes include altered neurotransmitters synthesis, storage and release, therefore contributing to the altered intestinal motility during the onset and progression of many GIT disorder (Stanzel *et al.*, 2008) (See section 2.3.3 for more detailed).

2.3.2. Oxidative damage in diarrhoea

Excessive generation of reactive oxygen species (ROS) or reactive nitrogen species (RNS) by the intestinal immunological system as a result of intestinal infection, irritation, inflammation, and depleted endogenous antioxidant defence causes oxidative stress (Granot and Kohen, 2003). This condition has been implicated as one of the causes of diarrhoea (Peluso *et al.*, 2002; Granot and Kohen, 2003).

The pathophysiology of oxidative stress (production) is complex and results from the normal immune response in conditions of disease (infectious and non-infectious), and is initiated by activated mitochondrial of the leukocyte. The free radicals produced are unstable and highly reactive charged function to destroy invading organism (Dwyer *et al.*, 2009). The mechanisms of ROS and RNS production involved an incomplete reduction of oxygen and nitrogen in the electron transfer chain of respiratory process in the mitochondria. In addition, immune reactions during infection or autoimmune responses through inflammation activation of a variety of inflammatory cells, which in turn activate the oxidant-generating enzymes including NADPH oxidase, inducible nitric oxide synthase (iNOS), myeloperoxidase, and eosinophil peroxidase. The ROS generated in the body are superoxide anions, hydroxyl radical, singlet oxygen and hydrogen peroxide (from leukocyte respiratory burst). The RNS included nitric oxide (NO) (produced by inducible nitric oxide synthase (iNOS). Other miscellaneous reactive species are reactive halogen and pseudohalogen species (produced by myeloperoxidase, eosinophil peroxidise,

lactoperoxidase). It is well-established in vitro that free radicals may also be generated via transition metal-mediated oxidation, the so-called Fenton type chemistry, but due to the limited availability of unbound transition metals, these reactions are probably unlikely to play a major role as a source of oxidants in vivo (Chen et al., 2000).

However, since their effect is usually non-specific and aimed at the lipid membrane, the chain reaction initiated by the immune system will destroy the body's macromolecules unless scavenged (terminated). At normal physiological conditions a balance is maintained between amounts of free radicals generate and endogenous antioxidant defence system that scavenged/quenched the radicals preventing their harmful effects. Cellular antioxidant endogenous defence mechanisms are divided into three parts depending on their function:

- o Quenching antioxidants: The tissue have inherent antioxidant network capable of donating electrons to oxidants, thus quenching their reactivity under controlled conditions and the derivatives become harmless to cellular macromolecules. The antioxidants however become radicals themselves, but far more stable incapable of inducing cellular damage. The oxidised antioxidants are subsequently recycled to their active reduced state by a number of efficient cellular processes fuelled by energy from NADPH. This recycling process is the main key to the efficiency of the antioxidant network.

- o Repairing/removing oxidative damage: This level of antioxidant defence involves the ability to detect and repair or remove oxidised and damaged molecules before it become a threat to normal body.physiological process.

- o Encapsulating non-repairable damage: Finally, the body is also equipped with controlled cell suicide or apoptosis, if the extent of the oxidative damage exceeds the capacity of repair and removal.

However, a shift in favour of the radical generation, increase the burden in the body (oxidative stress) which causes tissue injury and subsequently diseases. The proposed mechanisms through which these products induced diarrhoea are presented in Fig 2.5 and discussed below:

- ❖ Lipid peroxidation are primary mechanisms for intestinal cellular malfunction, and can destroy the capacity of membranes to maintain ionic gradients resulting in an aberration in ion transport, particularly affecting potassium efflux and sodium/calcium influx (Dudeja and Ramaswamy, 2006). The production of arachidonic acid metabolites in the lipid peroxidation process can also contribute to intestinal dysfunction including diarrhoea. The ROS and RNS-induced lipid peroxidation process involves three major stages (Catala, 2009): the initiation stage, where the oxidant abstracts hydrogen from polyunsaturated fatty acids of the cell membrane, forming a radical lipid. The propagation stage may involve the rearrangement of the lipid radical to form conjugated dienes and can interact with oxygen to form lipid peroxide radicals.
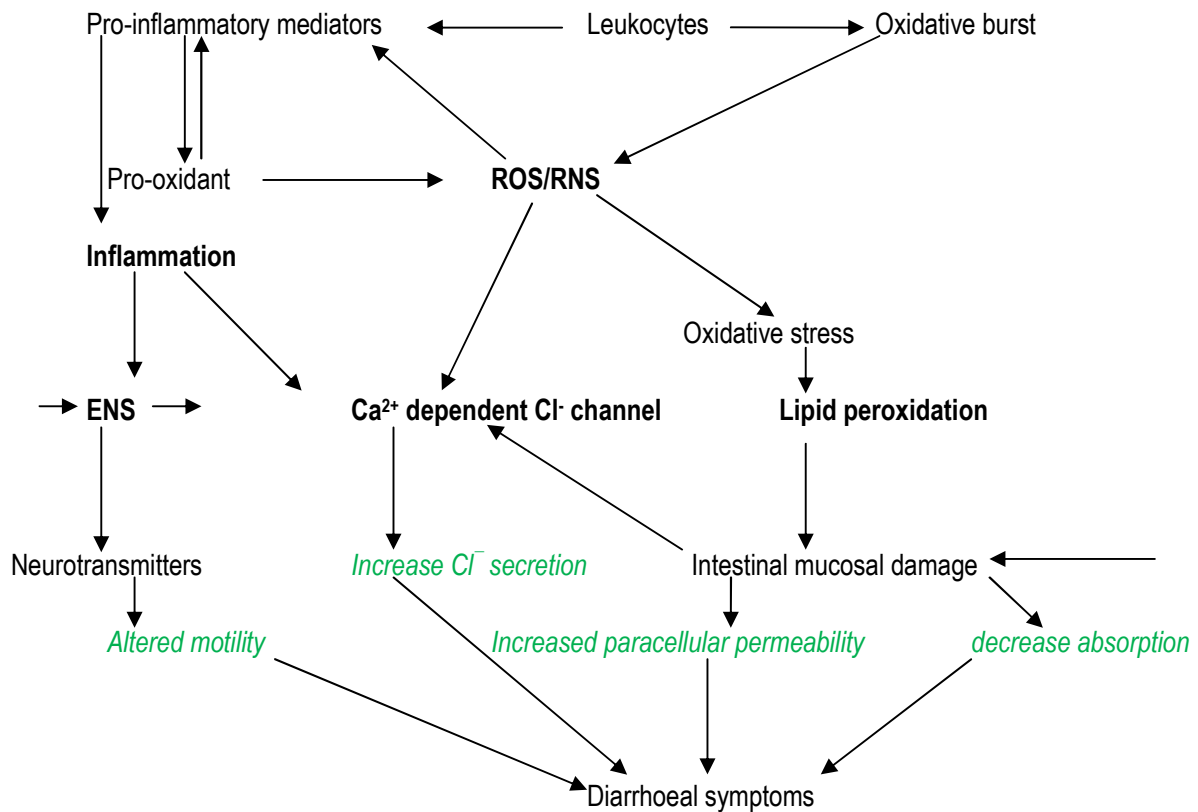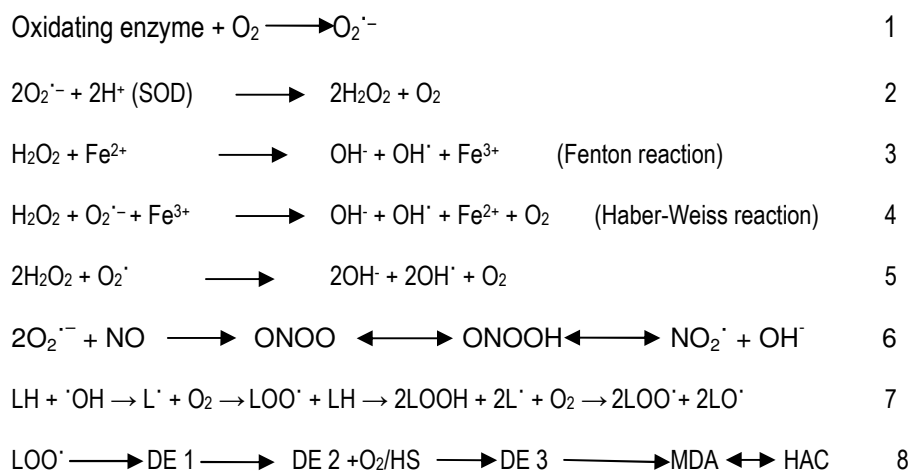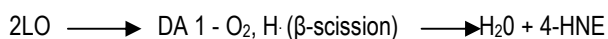
Enterotoxins ◄───────── **Enteric infection/irritant** ──────────┐
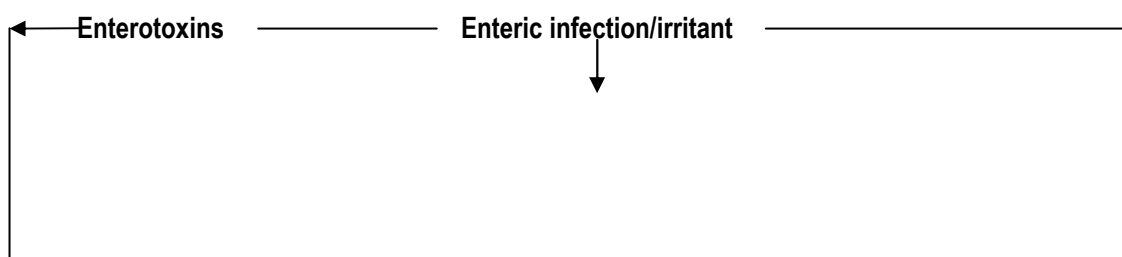
Fig.2.5 The integrative pathophysiology and mechanism of diarrhoeal disease

Bold (pathogenesis and areas of possible pharmacological intervention in diarrhoea), italic (mechanisms through which diarrhoea manifest)

The peroxide radicals can in turn abstract hydrogen from lipids to produce lipid hydroperoxide and a new radical. Lipid hydroperoxides can be oxidized, via reaction with reduced iron (Fe^{2+}) to lipid alkoxy radical and lipid peroxide, thus continuing the chain reaction of lipid peroxidation. In the final stage, the lipid peroxide radicals in the presence of reduced metals can be degraded to form highly reactive and potent toxic aldehydes such as malondialdehyde (MDA) (Fig 2.6 and 2.7). The chain reaction can be terminated by endogenous antioxidant enzymes and exogenous antioxidant molecules by forming non-reactive substances (Catala, 2009).

$$\text{Oxidating enzyme} + O_2 \longrightarrow O_2^{\cdot -} \qquad\qquad 1$$

$$2O_2^{\cdot -} + 2H^+ \text{ (SOD)} \longrightarrow 2H_2O_2 + O_2 \qquad\qquad 2$$

$$H_2O_2 + Fe^{2+} \longrightarrow OH^- + OH^{\cdot} + Fe^{3+} \quad \text{(Fenton reaction)} \qquad 3$$

$$H_2O_2 + O_2^{\cdot -} + Fe^{3+} \longrightarrow OH^- + OH^{\cdot} + Fe^{2+} + O_2 \quad \text{(Haber-Weiss reaction)} \qquad 4$$

$$2H_2O_2 + O_2^{\cdot} \longrightarrow 2OH^- + 2OH^{\cdot} + O_2 \qquad\qquad 5$$

$$2O_2^{\cdot -} + NO \longrightarrow ONOO \longleftrightarrow ONOOH \longleftrightarrow NO_2^{\cdot} + OH^- \qquad 6$$

$$LH + {}^{\cdot}OH \rightarrow L^{\cdot} + O_2 \rightarrow LOO^{\cdot} + LH \rightarrow 2LOOH + 2L^{\cdot} + O_2 \rightarrow 2LOO^{\cdot} + 2LO^{\cdot} \qquad 7$$

$$LOO^{\cdot} \longrightarrow DE\ 1 \longrightarrow DE\ 2 + O_2/HS \longrightarrow DE\ 3 \longrightarrow MDA \longleftrightarrow HAC \qquad 8$$

$$2LO \longrightarrow DA\ 1 - O_2,\ H \cdot (\beta\text{-scission}) \longrightarrow H_2O + 4\text{-HNE} \qquad\qquad 9$$

Figure 2.6: Lipid peroxidation chain reaction (Valko *et al*., 2007).

(Equations 1 is generation of superoxide by enzymes such as NAD(P)H oxidase, xanthine oxidase and mitochondria, 2) Superoxide radical is dismutated by the superoxide dismutase (SOD) to hydrogen peroxide, 3 and 4) hydroxyl radical and hydroxyl ion hydrogen peroxide in the presence of transitional metal, 5 and 6 chain reaction to generate more radicals, 7 lipid peroxidation of phospholipids, 8 cyclization and scission of the lipid peroxide radical to generate cytotoxic malonydialdehyde (MDA), hydroxyacrolein (HAC), 4-Hydroxy nonenal (4-HNE) (Fig.2.7).
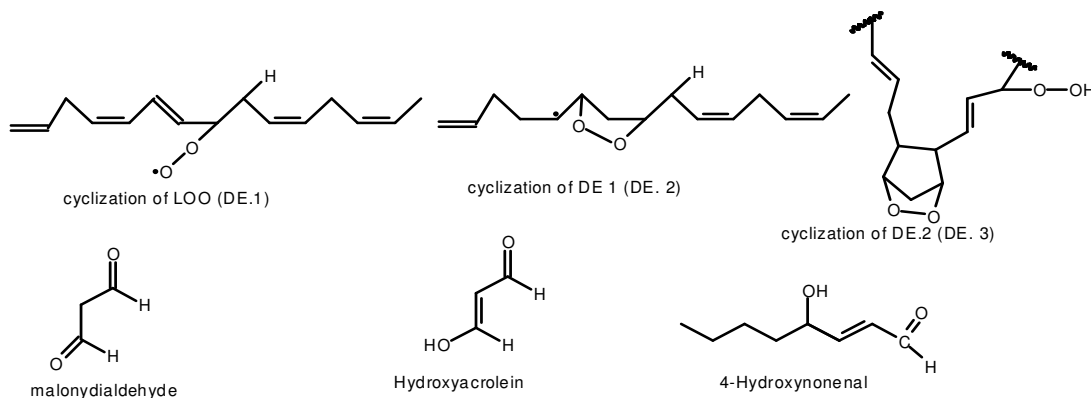

cyclization of LOO (DE.1)

cyclization of DE 1 (DE. 2)

cyclization of DE.2 (DE. 3)

malonydialdehyde

Hydroxyacrolein

4-Hydroxynonenal

Fig.2.7. Chemical structures of lipid peroxidation intermediates outlined in Fig 2.6

❖ Some of the reactive species such as HOCl and NH_2Cl can also act as secretagogues on their own or can evoke the release of acetylcholine or other neurotransmitters, thus stimulating the enteric nervous system (ENS) to cause increased contractility or motility of intestinal tract (Gaginella *et al.*, 1992). The reactive species also induce gene expression by stimulating signal transduction such as Ca^{2+}-signalling and protein phosphorylation.

❖ Increased production of inflammatory mediators: The onset of lipid peroxidation process leads to changes in the physiological integrity of the cell membrane. The body responds to the process by the release of pro-inflammatory eicosanoids such as (prostaglandins, prostacyclins and leukotrienes) and pro-inflammatory cytokines (Nardi *et al*, 2007) such as interleukins (IL-1B, IL-3,IL-6), interferons (IFN), tumor nuclear factor (TNF-α) and platelet-activating factor (PAF) (Conforti *et al*, 2008; Kunkel *et al*, 1996).

2.3.3. Enteric nervous system in diarrhoea

The enteric neural network is responsible for the control of propulsive transport and segmental peristalsis in the GIT, as well as secretion and absorption across the intestinal lumen (Wood, 2004; Bohn and Raehal, 2006). While enteric nervous system (ENS) functions independently of the central nervous system (CNS), it is modulated by the parasympathetic and sympathetic autonomic nervous system (Farthing, 2003). As a unit, the ENS is a complicated physiological with autoregulation being mediated by a number of neurotransmitters such as acetylcholine, serotonin, substance P, histamine and endorphin (Farthing, 2002). Diarrhoea can result from the alteration of these systems:

❖ Smooth muscle contractility: Many agonists and/or antagonists elicit contractility in GIT smooth muscle (longitudinal or circular) through activation of various receptors located within the muscle (Holzer, 2004). In some cases the activation of the smooth muscle receptors by neurotransmitters and inflammatory mediators include reactive oxygen species causes relaxation (spasmolytic). While in other cases, the process lead to increase in spontaneous or induced contraction (spasmogenic). Ionic channel (Ca^{2+} and Cl^-) are also known to play important roles in smooth muscle contraction (Giorgio et al., 2007). Anion and fluid secretion into the intestine lumen are stimulated through activation of the receptors on enteric secretomotor pathways and epithelial cells, consequently causing secretory diarrhoea.

❖ Motility: Intestinal motility dysfunctions include situations in which movement of material along the GIT is repetitive and rapid (diarrhoea) and/or too slow (pseudo-obstruction, slow transit constipation) (Talley, 2006; Giorgio et al., 2007) are controlled by activities of neurotransmitters on the ENS. Pathogenic bacterial overgrowth is common as a result of intestinal hypomotility or low transit time which may lead to mucosal inflammation, increased accumulation and absorption of toxins which are known pathophysiology of diarrhoea. The mechanisms may include impaired digestion as in the deconjugation of bile salts with subsequent fat malabsorption, leading to fatty acid diarrhoea or osmotic effects of malabsorption of sugars resulting in osmotic diarrhoea. Diarrhoea also results from an increase in the gut motility (hypermotility) inducing an accelerated transit of food intake. The net fluid absorption from the food intake is reduced due to less adequate contact time with the GIT epithelial lining for the absorption of fluids before excretion.

2.3.4. Cystic fibrosis transmembrane conductance regulator (CFTR) regulation

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cyclic adenylate monophosphate (cAMP)-activated Cl^- channel expressed in epithelial cells in the intestine and other fluid-transporting tissues (Thiagarajah and Verkman, 2003). Diarrhoeal pathogens and their toxins can induce secretory diarrhoea by simultaneously stimulation of the active secretion of Cl^- and inhibition of Na^+ absorption across the apical membrane of enterocyte with resulting massive fluid and electrolyte loss into GIT (Schuier et al., 2005). The cellular signalling mechanisms include an increase in cellular cAMP and cyclic guanylate monophosphate (cGMP), which may result in activation of the CFTR Cl^- channel. Pharmacological blocking of CFTR with drugs such as glibenclamide and $CFTR_{inh}$-172 inhibits salt and water loss in diarrhoea (Schuier et al., 2005).

2.4. Specific Agents of Diarrhoea

2.4.1. Bacterial causes of diarrhoea

2.4.1.1. *Escherichia coli*

E. *coli* is a gram-negative rod shaped bacteria that shares a symbiotic relationship with animal host as part of normal digestive intestinal flora. Under certain define conditions these organisms or pathogenic strains of these organisms are known to induce diarrhoea (Clarke, 2001; Le Bouguenec, 2005). There are six main types of

pathogenic *E. coli* associated with diarrhoea, namely enterotoxigenic *E. coli* (ETEC), enteroinvasive *E .coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffusively adherent *E. coli* (DAEC) (Clarke, 2001). While the exact process by which each type of these *E. coli* induces diarrhoea symptoms varies significantly, the basic pathophysiology involves their inherent ability to adhere to epithelial cells and colonize the host tissues (Le Bouguenec, 2005). The characteristics and mode of actions of each type of the pathological strains in diarrhoea diseases are listed in Table 2.1. Infections from some of the strains of *E. coli* are self-limiting and can resolve without pharmacological intervention. However, symptomatic, supportive and antibiotic, or a combination of the therapies may be beneficial in the chemotherapeutic management of some cases involving ETEC, EIEC and EPEC infection (Elsinghorst, 2002). The use of antibiotics recommended, antimicrobial chemotherapeutic agents such as tetracycline, doxycycline, and ciprofloxacin may be used (Casburn-Jones and Farthing, 2004; Elsinghorst, 2002) in infectious diarrhoea.

Table 2.1: The mechanism of actions and symptoms of enteric pathogenic *E. coli* (Thapar and Sanderson, 2004; Clarke, 2001).

Strain	Mechanism of action	Symptoms	Treatments
Enterotoxigenic *E. coli* (ETEC)	Colonization of the small bowel mucosa, followed by elaboration of heat-labile (LT) and heat stable (ST) enterotoxins. The ST enterotoxins are classified as STa and STb. The binding of STa to guanylate cyclase C receptor results in increased intracellular cyclic guanylate monophosphate (cGMP) level. The resultant effect is the stimulation of chloride secretion or inhibition of sodium chloride absorption causing intestinal fluid secretion. LT enterotoxins consist of two serotypes (LT-I and LT-II). LT activate adenylate cyclase causing intracellular increase in cyclic adenosine monophosphate (cAMP) levels resulting in decrease sodium absorption by villous cells and subsequent active chloride secretion by crypt cells thus leading to osmotic diarrhoea.	Watery diarrhoea ranging from mild, self-limiting disease to severe purging.	Supportive therapy with antibiotic in cases of severe infection.
Enteroinvasive *E. coli* (EIEC)	Invasion of the epithelium and mucosal destruction eliciting inflammatory response accompanied by necrosis and ulceration of the large bowel with resultant release of blood and mucosa into the stool.	Bloody, mucoid diarrhoea and dysentery	Antibiotic in cases of bloody diarrhoea
Enterohaemorrhagic *E. coli* (EHEC)	Adhesion followed by liberation of a potent toxin which is cytotoxic to Vero cells (referred to as shiga-like cytotoxin I and II). Other mechanism attributed to the EHEC virulence includes adhesin	Bloody diarrhoea, fever, vomiting, haemorrhagic colitis, haemolytic uremic syndrome (HUB), acute renal failure, haemolytic anaemia,	Symptomatic therapy
Enteropathogenic *E. coli* (EPEC)	Adherence of the bacterium to the gut epithelium causing attachment and effacement lesion on intestinal epithelial cells, alteration of intracellular calcium and cytoskeleton.	Self-limiting watery diarrhoea with fever and vomiting	Antibiotic in severe cases
Enteroaggregative *E. coli* (EAEC)	Aggregating pattern of adherence to intestinal mucosa produces enteroaggregative heat-stable (EAST) enterotoxins causing cellular damage and function similar to, but distinct from ST enterotoxins.	Watery mucoid diarrhoea	Antibiotic in severe cases
Diffusively adherent *E. coli* (DAEC)	Elaboration of α-haemolysin and cytotoxic necrotising factor 1 (CNF-1).	Watery diarrhoea	Supportive therapy

2.4.1.2. *Staphylococcus aureus*

S. aureus is a gram-positive coccus present in normal intestinal and skin flora of human and homoeothermic animal. Under define conditions, the pathogenic strains produces heat stable staphylococcal enterotoxins (SETs) and toxic shock syndrome toxins (TSST-1) (de Oliveira, 2010) both of which are known to induce diarrhoea. Toxicity from SET results from the consumption of the preformed heat-stable enterotoxins (α-haemolysin) in contaminated food. Upon ingestion of the food contaminated with the SETs, the toxins results in signs of nausea, vomiting, fluid accumulation in ileal loops, and diarrhoea associated with fever (Rosengren *et al*, 2010; Perez-

Bosque and Moreto, 2009). The main sources of *S. aureus* toxin contaminants are raw material and food processing unit such as human handling, water and environment (Linscott, 2011).. Serotonin receptor antagonists have been reported to ameliorate the vomiting, diarrhoea and prostration induced by SETs . The mechanism behind toxicity results from the activation of autonomous nervous system with resultant hyperperistalsis as well as activation of central pathways which control vomiting and diarrhoea (Podewils *et al*, 2004).

In contrast, TSST-1 is characterized by sudden onset of fever, vomiting, diarrhoea, erythematous rash with skin peels, hypotensive shock, impairment of renal and hepatic functions, and sometime death. Toxicity results via the production of pro-inflammatory cytokines and chemokines. Toxicity is usually exacerbated by further interaction between the activated immune system and inflammatory mediators (Krakauer *et al*., 2001).

2.4.1.3. *Campylobacter jejuni*

C. jejuni is an invasive Gram-negative, spiral-shaped rod bacterium present in the GIT of mammals, birds and primates (Lengsfeld *et al*., 2007). The major source of *Campylobacter* infection in mammals is from poultry and poultry products (Podewils *et al*, 2004). The clinical signs of *campylobacter* infections include pyrexia, abdominal pains, watery diarrhoea and dysentery (Podewils *et al*, 2004). The characteristic mechanisms *Campylobacter* infection involves invasion and translocation of the epithelium with a concomitant induction of inflammation (Hu *et al*., 2008).

2.4.1.4. *Shigella* spp

Shigella (*Shigella flexneri, Shigella dysenteriae, Shigella sonnei and Shigella boydii*) is a Gram-negative rod, non motile and facultative anaerobic bacterium that invades the colon with resulting inflammation and diarrhoea (Podewils *et al*, 2004). *Shigella flexneri* is responsible for dysenteric symptoms and persistent illness while *Shigella dysenteriae* type-1 produces Shiga-toxin like EHEC causes bloody diarrhoea (Podewils *et al*, 2004). *Shigella sonnei* causes bacterial gastroenteritis and bacillary dysentery and *Shigella boydii* causes fever, chills, abdominal pain and diarrhoea.

2.4.1.5. *Vibrio cholerae*

V. cholerae is a motile, facultative anaerobic Gram-negative rod associated with potentially fatal diarrhoea (Granum, 2006) that results from the ingestion of the cholera enterotoxins (CT) from contaminated water and seafood (Podewils *et al*, 2004). Watery, colourless mucous- flecked stool and vomiting are the main clinical signs associated with cholera which in severe cases can result in a life-threatening fluid and electrolyte imbalance (Podewils *et al*, 2004). Pathophysiologically, toxicity results from the CT induction of intestinal hypersecretion through the activation of the mucosal epithelium cAMP-adenylate cyclase system in the mucosal epithelium (Casburn-Jones and Farthing, 2004).

In addition, it has been speculated that ROS/RNS production in *V. cholerae* infection could also contribute to intestinal damage through lipid peroxidation of the cellular and mitochondrial membrane thereby further increasing membrane permeability and fluid loss (Gorowara *et al*., 1998). Other species of *Vibrio* such as V. *parahaemolyticus* and *V. vulnificus* also caused watery diarrhoea, abdominal cramps, nausea, vomiting. These organisms infect host from raw or undercooked seafood or cooked seafood contaminated with seawater (Linscott, 2011).

2.4.1.6. *Bacillus cereus*

B. cereus is a sporulating bacterium that causes both food poisoning and non-gastrointestinal infection (Al-khatib *et al*., 2007; Ramarao and Lereclus, 2006). In food poisoning, two main types of diseases namely diarrhoeal and emetic food poisoning are common. The diarrhoeal type of *B. cereus* food poisoning is caused by enterotoxins such as haemolysin BL (HBL), non-haemolytic enterotoxin (NHE) and cytotoxin K (CytK) (Lund *et al*., 2000).with clinical signs of abdominal pain with diarrhea. Causes of the diarrhoeal forms always occurs from accidental contamination of food like meat, vegetables, pasta, deserts cakes, sauces and milk (Linscott, 2011).

In constrast the emetic form is induced by a small preformed heat and acid stable cyclic peptide (cereulide) (Agata *et al*., 1995; Ehling-Schulz *et al*., 2004) with clinical symptoms of sudden onset of nausea and vomiting, with or without diarrhoea (Linscott, 2011). The major sources include cooked foods, like meat or fried rice that have not been properly refrigerated. While the other species of *Bacillus* such as *B. subtilis*, *B. licheniformis*, *B pumilus* and *B. megaterium* are usually considered relatively safe, but they can also produce enterotoxins and emetic toxins involved in foodborne illness (From *et al*, 2007)

2.4.1.7. *Yersinia* species

Yersinia species are Gram-negative facultative anaerobic nonsporing rods or coccobacilli bacteria belonging to the Enterobacteriaceae family. Three human pathogenic species namely: *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis* are recognized (Fallman and Gustavsson, 2005). Y. *pestis* is the causative agent of bubonic plague characterized with the onset of fever, chills, headache, and weakness, followed by swelling and tenderness of lymph nodes while *Y. enterocolitica and* Y. *pseudotuberculosis* cause an enteric infection in humans called yersiniosis with clinical signs such as diarrhoea, vomiting, fever and abdominal pain (may mimic appendicitis) following ingestion from undercooked pork, unpasteurized milk, tofu, contaminated water, chitterlings (Linscott, 2011, Damme *et al*., 2010).

2.4.1.8. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive bacterium which causes life-threatening invasive diseases referred to listeriosis in human and animals (Chaturongakul *et al*., 2008; Todd and Notermans, 2011). Upon ingestion of the bacteria from contaminated foods such as unpasteurized milk, soft, cheese made with unpasteurized milk

Linscott, 2011), the organism may, colonize the intestinal tract with resultant diarrhoea (Chaturongakul *et al.*, 2008).

2.4.1.9. *Clostridium* spp

C. difficile is an anaerobic, spore-forming, Gram-positive bacillus widely distributed in the environment and present in the colon flora of less than 3% of healthy adults (Beaugerie *et al.*, 2003). *C. difficile* causes a spectrum of diseases ranging from benign diarrhoea to fatal colitis and most often as a consequent of antibiotics treatment Most antibiotics predispose *C. difficile* overgrowth leading to the production and accumulation of and diarrhoea are Toxins A (enterotoxin) and B (cytotoxin) in the intestine. Both toxins A and B inactivate intracellular Rho-proteins by glycosylation, leading to desorption of the cytoskeleton, production of inflammatory cytokines and damage to tight junctions The most commonly antibiotics associated with *C. difficile* overgrowth include cephalosporins, clindamycin and broad-spectrum penicillins (Wistrom *et al.*, 2001).

In contrast, *C. perfringens* is an important food poisoning bacterium with clinical sign as diarrhoea, abdominal cramping and nausea. The main sources of infection include contaminated meat, poultry, gravy and inadequately reheated food (Linscott, 2011). *C. botulinum* may also play a role in diarrhoeal diseases when the preformed botulinum toxin is consumed from improperly canned foods, herb-infused oils, baked and potatoes in aluminium foil. Symptoms of infection include abdominal cramping, nausea, vomiting, diarrhea, double vision, long term nerve damage and possible even death from paralysis (Linscott, 2011).

2.4.1.10. *Salmonella typhimurium*

S. typhimurium is a bacterium that may be associated with mild gastroenteritis to enteric (typhoid) fever, bacteraemia and septicaemia commonly referred to as salmonellosis (Mastroeni and Maskell, 2006). The clinical signs of salmonellosis include diarrhoea, fever and abdominal cramps. In people with typhoid fever, *Salmonella* spreads systemically from the gut to blood stream and other parts of the body resulting in mortality if not treated adequately with antibiotics (Castillo *et al.*, 2011). The virulence of the *Salmonella* bacterium differs among the different animal species depending on *Salmonella* serovar involved, strain, infective dose, host animal species, age and immune status of the host (Castillo *et al.*, 2011). The pathogenesis *of Salmonella* involves adhesion/invasion to specific intestinal epithelial cells, mainly in the ileum (Guttman and Finlay, 2009).

2.4.1.11. *Enterococcus faecalis*

E. faecalis is a gram-positive bacterium that survives symbiotically in the human or animal's intestinal tract. However, under conditions such as the disruption delicate host-commensal relationship following antibiotic use, abdominal surgery or changes in host immunity, the enterococci becomes invaders of the intestinal wall (Butler, 2006) through the production of adhesin, aggregating and binding substances (Butler, 2006). *E. faecalis* is known to produce superoxide (O_2^-) that can results in hydroxyl radical formation which contributes to oxidative

stress in the intestine and membrane lipid peroxidation (Huycke and Moore, 2002; Sun *et al*, 2010; Peluso *et al.*, 2002; Granot and Kohen, 2003) (Fig 2.5).

2.5. Fungal induced diarrhoea symptoms

2.5.1. *Candida albicans*

C. albicans is a yeast fungus and exist as a member of normal flora in the GIT and mucocutaneous membrane (Forbes *et al.*, 2001). However, following the use of antibiotic therapy that result in sterilization of the GIT flora, *C. albicans* can overgrowth to take the place of removed organisms with end result of diarrhoeal symptoms(Henry-Stanley *et al.*, 2003). Other predisposing factors include altered intestinal permeability and diminished host immunity response. It has been postulated that this organism produces virulence factors which increases fungal adherence to host cells, fungal secretion of proteolytic enzymes and fungal morphological switching (ability to change and grow in several distinct morphological forms: yeast, hyphae, and pseudohyphae, according to environmental conditions) (Henry-Stanley *et al.*, 2003). Clinical signs associated with enteric candidiasis are abdominal pain, cramping, rectal irritation and absence of nausea, vomiting, bloody and mucus stool, and pyrexia (Levine *et al.*, 1995).

2.6. Viral induced diarrhoea

2.6.1. Rotavirus

Rotavirus is a major cause of severe diarrhoea and account for 30% and 80% of all cases of acute gastroenteritis (Savi *et al*, 2010). The diarrhoeal mechanism of the organism includes the production of enterotoxin NSP4 which inducesd Na^+-glucose dependent malabsorption and destruction of enterocytes (cytotoxicity). The toxin also has a direct effect on the intestinal barriers by blocking TJs formation with resultant diarrhoea through a 'leak flux' mechanism in which water is secreted into the lumen of the intestine (Dickman *et al.*, 2000).

2.6.2. Norovirus

Norovirus is considered one of the major global causes of gastroenteritis (Mattison, 2010) The diseases is opportunistic and is usually transmitted through faecal contamination of food, water or contact with an infected host following poor hygiene. The organism has the ability to infect small intestine and induce intestinal TJ dysfunction, malabsorption through villus surface area reduction and an increased number of cytotoxic intraepithelial lymphocytes (Troeger *et al.*, 2009). Clinical signs associated with infection are nausea, vomiting, diarrhoea and abdominal pains (Koopmans, 2008).

2.6.3. Hepatitis A virus

Hepatitis A is a small, non-enveloped spherical with cubic symmetry, thermostable and acid resistant virus. While the primary target organ of infection is the liver, the resultant hepatitis (Koff, 1998) result in clinical signs dark

urine, jaundice, malaise, weakness, fever, anorexia, nausea and vomiting, abdominal pains, and diarrhoea (Koff, 1992). Sources of infection usually result from contaminated water on raw produce, food, and shellfish or exposure to the water itself (Linscott, 2011).

2.6.4. Human immunodeficiency virus (HIV)

Chronic diarrhoea is one of the complications associated with HIV infection and acquired immune deficiency syndrome (AIDS) due to multiple enteric opportunistic microbes (DuPont and Marshall, 1995). While HIV is important in secondary enteric diseases as a result of immune suppression (CD4+ T-lymphocytes destruction), the virus can result in diarrhoea directly by altering the mucosa structural arrangement viz referred to as HIV-enteropathy (Epple *et al*., 2009). The diarrhoea resulting from HIV appears to be caused by the releases of cytokines from the infected immune cells (Schmitz *et al*., 2002).

2.7. Protozoa induced diarrhoea

2.7.1. *Giardia intestinalis*

G. intestinalis (syn. *Giardia doudenalis, Giardia lamblia*) is a flagellate protozoa parasite of the upper small intestine that exists as a motile trophozoite (Cotton *et al*., 2011). The organism colonizes the small intestinal lumen and induces non-inflammatory and malabsorptive diarrhoea (Schulzke *et al*., 2009). The pathophysiology of Giardiasis involves Na+-dependent D-glucose absorption impairment, active electrogenic anion secretion activation, mucosal inflammation and leak flux (Buret, 2007; Troeger *et al*., 2007). Clinical signs of *Giardia* infection include bloating, steatorrhea and nausea. Chronic infection cause weight loss, growth retardation and development in young children

2.7.2. *Entamoeba histolytica*

E. histolytica is a protozoa parasite which infects the large intestine with resultant intestinal dysfunction characterized by invasive illness and severe dehydration commonly referred to as amoebiasis (Ralston and Petri, 2011). The pathophysiology of amoebiasis involves villus structural destruction, increased epithelial permeability and alteration of TJs (Lauwaet *et al*., 2004). The organism also causes invasive disease such as colitis and abscess through massive host tissue destruction. The clinical signs are usually similar to *S.dysenteriae* or enteroinvasive *E. coli* with blood and pus contaminated stool. Other related infectious species include *E. dispar* and *E. moshkovskii* (Ralston and Petri, 2011).

2.7.3. *Cryptosporidium parvum*

C. parvum is an intracellular protozoa parasite that infects epithelia causing cryptosporidiosis (Kenny and Kelly, 2009) which manifest clinically as profuse watery diarrhea, containing mucus, but rarely blood or leukocytes

(O'Hara and Chen, 2011). Some other clinical signs of cryptosporidiosis include nausea, vomiting, cramp-like abdominal pain and mild fever (Linscott, 2011). The period and severity of clinical symptoms of intestinal cryptosporidiosis depends on the immune status of the infected individual (Linscott, 2011). Cryptosporidiosis in the healthy individuals is usually a self-limiting illness with approximate duration of 9-15 days while in immunocompromised patient the infection in severe and life-threatening (O'Hara and Chen, 2011).

2.7.4. *Cyclospora cayetanensis*

C. cayetanensis is a protozoan parasite which invades the epithelial cells of the small intestine upon ingestion of sporulated oocysts in contaminated food or water (Chacin-Bonilla, 2010; Manfield and Gajadhar, 2004). Pathomechanisms of *C. cayetanensis* infection are intestinal inflammation associated with pathological lesions such as villus blunting, and malabsorption. The clinical signs of the infection include watery diarrhoea, loss of appetite, weight loss, abdominal bloating and cramping, increased flatulence, nausea, fatigue, and low-grade fever (Linscott, 2011).

2.8. Parasitic induced diarrhoea

2.8.1. *Trichinella spiralis*

T. spiralis is a food-borne zoonotic parasite responsible an enteral phase and a muscular phase (Cui *et al*., 2011). The adult worms live in the duodenal and jejunal mucosa of flesh-eating animals and humans, while the larvae live in skeletal muscles of the same hosts (Cui *et al*., 2011). The source of infection is raw or undercooked contaminated meat (pork, bear, walrus and moose), cross-contaminated ground beef and lamb (Linscott, 2011). The clinical intestinal symptoms manifest one or 2 days after ingesting the contaminated meat due to the matured adults penetrating the intestinal mucosa, resulting in nausea, abdominal pain, vomiting, and diarrhoea (Linscott, 2011). *T. Spiralis* induced changes in intestinal function by hypersensitivity mechanism resulting in an increased intestinal chloride and fluid secretion (Cui *et al*., 2011).

2.9. Immune disordered induced diarrhoea

2.9.1. Compromised immune system

The main function of the immune system is to protect against disease through the recognition and removal of pathogens or their sequelae (Gertsch *et al*., 2010). To fulfil this role, the body make use of an innate immune system that defends it in a non-specific manner via molecular interaction and inflammation (Gertsch *et al*., 2010) and an adaptive system comprised of specialized effector cells (T and B cells) which not only recognize antigens but play a role in their active removal (Gertsch *et al*., 2010). In the GIT, the important protective system which prevent the normal flora from becoming pathogenic are gut-associated lymphoids tissue (GALT), epithelial-derived antimicrobial peptides (AMPs) (such as defensins, cathelicidinand lysozyme present in the secretion which constantly washes the mucosal surfaces), the mesenteric lymph nodes, the liver's Kupfer cells, mast cells, within the intestinal walls and the reticuloendothelial system of the intestinal walls. In diseases characterized by immune suppression such as HIV, the immune system is destroyed which makes a person more susceptible to

infectious diseases (Gertsch *et al*., 2010). One of the clinical signs that may result is diarrhoea as the above-mentioned micro-organisms colonize the compromised GIT.

2.9.2. Hyperactive immune system

The normal response of the immune system during conditions of antigen stimulation is generally an inflammatory response with the release of numerous inflammatory mediators (interferon-γ, tumour necrosis factors (TNF-α), interleukin-6 (IL-6), and IL-1β) which in conjunction plays a role in the removal of the causative agents (Sprague and Khalil, 2009). On the removal of inciting cause, the inflammatory response is usually terminated. However, on occasion when the body failed to terminate the inflammatory response, the inflammatory agents can be equally as destructive to the host's own tissues. The latter usually result from the damage of the epithelial mucosa cells, from the generated ROS/RNS and subsequent lipid peroxidation. With the destruction of the epithelial cells, the body loses absorptive capacity with resultant diarrhoea. The produced cytokines also has the ability to directly increase intestinal mucosa permeability and fluid loss. Inflammatory bowel disease is one of the diseases caused by up-regulated immune system (Gertsch *et al*., 2010).

2.10. Antibiotic therapy induced diarrhoea

Diarrhoea develops in some patient following antibiotic chemotherapy with one of the following mechanisms:

➢ Antibiotic toxicity: Some antibiotic (levofloxacin, azithromycin and amoxicillin-clavulanate) may have a direct negative effect on the GIT with resultant poor absorption or enteropathy characterized by infiltration of the lamina propria by macrophage (Dobbins, 1968, Hartmut, 2010). In addition, antibiotic such as erythromycin has prokinetic action on the GIT, mediated through motilin receptor stimulating potential (Catnach and Fairclough, 1992, Annese *et al*., 1992).

➢ Alteration of digestive function: The removal of some commensal organisms by antibiotic (Hofmann, 1977) could result in decreased carbohydrate digestion (Saunders and Wiggins, 1981) which leads to accumulation of these osmotically active substances in the intestinal lumen. The net result is the accumulation of electrolytes and water from osmotic pull in the intestinal lumen which is evidence as diarrhoea (Beaugerie and Pettit, 2004).

➢ Overgrowth of pathogenic microorganisms: The use of antibiotic may result in the removal of beneficial GIT flora. As a result of the disruption of this balance ecosystem, various pathogenic organisms (as listed above) can overgrow and colonise the GIT (Beaugerie and Pettit, 2004). The predisposing antibiotic for various diarrhoeal pathogens include cephalosporins, clindamycin and broad-spectrum penicillins for *C. difficile* (Wistrom, 2001; Stoddart and Wilcox, 2002); β-lactams or pristinamycin for *Klebsiella oxytoca* (Wu *et al*., 1999) or tetracycline for *S. aureus*. S*almonella* and *Candida* overgrowth can be a consequence of wide range of antibiotic with broad base actions (Danna, 1991; Gupta and Ehrinpreis, 1990).
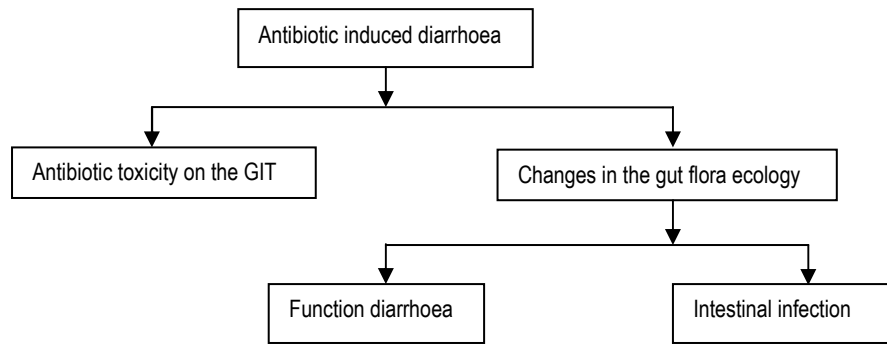
Fig. 2.8. Mechanism of antibiotic induced diarrhoea

2.11. Diabetic complications induced diarrhoea

Gastrointestinal disorders manifesting as diarrhoea (watery stool) or constipation (dry and hard stool) is a common symptom in the diabetic patient (Gould and Sellin, 2009) with a prevalence of approximately 12.5 to 32.4% and 60% respectively. In addition the oral hypoglycaemia medications used for the management of diabetes viz metformin, acarbose, miglitol (Forgacs and Patel, 2011), exenatide and orlistat (Gould and Sellin, 2009) may also induced diarrhoea as side effect while the recommended dietary material such as the non-digestible sweeteners (sorbitol, mannitol and D-xylose) induce an osmotic diarrhoea Forgacs and Patel, 2011). Bacterial overgrowth may also result in diabetic patient from decreased functioning of the immune system as described above (Forgacs and Patel, 2011).

2.12. Food allergy induced diarrhoea

Diarrhoea is one of the clinical manifestations of food allergy (Wang et al., 2010). The mechanisms of action include active ion secretion, altered epithelial barrier function (Groschwitz and Hogan, 2009), and mucosal damage resulting in malabsorption and osmotic diarrhoea (Wang et al., 2010). The initiation of food induced intestinal allergy is regulated by numerous inflammatory cells and mediators, including mast cells and T_H2 cytokines (IL-4, IL-5, and IL-13) (Wang et al., 2010). The release of neurotransmitters (serotonin, histamine) and inflammatory mediator (COX-2 and LOX) by mast cell induced ion secretion in the presence of allergen (Schenk and Mueller, 2008). These neurotransmitters and inflammatory mediators also stimulate intestinal contractions (altered intestinal motility) which act synergistically with ion secretion to cause diarrhoea.

2.13. Potential mechanisms in the control of diarrhoea
2.13.1. Oxidative damage and antioxidants in diarrhoeal management

Several endogenous strategies are available in human and animal body to combat oxidative damage. These provide ways for normal oxidative metabolism to occur in the body without damaging the cells and also allow for normal ROS/RNS-mediated cellular response such as phagocytosis and intracellular signalling (Valko et al, 2007). Therefore, the possibility exists that returning the animal to a more neutral oxidative balance, may promote repair of damaged membranes (Nose, 2000). As a result antioxidants and/or radical scavengers may be

beneficial in the attenuation of diarrhoea. The best known antioxidants as treatment are selenium, vitamin E, vitamin C and the proanthocyanidins in red wine and resverastrol in commercial grape seed extract.

2.13.2. Inflammation and anti-inflammatory agents in diarrhoea management

As a result of the negative impact the inflammatory cascade can have on the functionality of the GIT, modulation of these processes through the use of drugs may be of benefit. Possible mechanisms include attenuation of inflammatory process through the use of anti-inflammatory, antioxidative and radical scavenging mechanisms. Potential target include drugs with cyclooxygenase and lipoxygenase enzyme inhibitory activity. The drugs that are used commonly for this are non- steroidal anti-inflammatory drugs (NSAIDs) like indomethacin, aspirin, ibuprofen, diclofenac and coxibs.

2.13.3. Enteric nervous system in diarrhoea symptoms and treatment

The ENS is an important target for pharmacological intervention in diarrhoea through the use of agonists and antagonists that target these ENS endogenous receptors. Numerous pharmaceutical agents are currently available for alleviating many of the clinical signs of diarrhoea. The effects and possible sites of pharmacological intervention against the activities of neurotransmitters in diarrhoeal symptoms are presented in Table 2.2.

Table 2.2: Neurotransmitters and modulators of ENS causing intestinal secretion in diarrhoea

Neurotransmitters	Effects on GIT	Receptors	Potential pharmacological intervention	modulator
Acetylcholine	Main endogenous excitatory neurotransmitter in the GIT.	Nicotinic and muscarinic receptor subtypes M_1 and M_3.	Non selective nicotinic acetylcholine receptor antagonist or specific muscarinic acetylcholine receptor antagonist	atropine
Serotonin	Modulate muscular contraction and relaxation, intestinal fliud secretion and enhanced colonic transit.	$5\text{-}HT_3$, $5\text{-}HT_4$	$5\text{-}HT_3$ receptor antagonist (diarrhoea) and $5\text{-}HT_4$ receptor agonist (constipation)	Metoclopramide, granisetron, ondansetro, tropisetron, palonsetron, alosetron, cisapride
Substance P	Transmitter of enteric neurones and extrinsic afferent fibre, control of GI motility, secretion, vascular permeability, immune function and pain sensitivity	NK_1, NK_2 and NK_3	NK_1 and NK_2 receptor antagonist	
Histamine	Modulation of GIT motility, enhancement of gastric acid secretion, increases mucosal Cl⁻ ion secretion, and	H_1, H_2, H_3	H_1, H_2 antagonist	Cimetidine and ranitidine

	modulator of immune functions.			
Opioid peptide	decrease motility, increase transit time, increase fluid absorption from the intestine	Mu (μ),delta (δ) and kappa (κ)	μ, δ agonist	loperamide racecadotril, diphenoxylate
Nitric oxide	ENS neurotransmitter, pro-absorptive and secretory agent		iNOS inhibitor, NO modulator	N^G-nitro-l-arginine methyl ester (L-NAME
Dopamine	Prokinetic and antiemetic, enhance antral contractility and inhibit fundus receptive relaxation	D_2	antagonist	Domperidone
Motilin	induced antral contractility			Erythromycin, motilides, motilactides

2.14. Plants as potential source of therapeutic agents in alleviating diarrhoeal symptoms

Due to the widespread occurrence of diarrhoea as a disease together with the prevalence coinciding with human development, plants and fungi have featured widely in the management of the disease. Their use has become so common in human and veterinary medicines that a number of compounds considered to be allopathic are of natural origin. A non exhaustive list includes:

- Antioxidant: The natural vitamins and red pigments present in plant.
- Anti-inflammatory: Salicylic acid from willow bark.
- ENS modifiers: Atropine from *Atropa belladonna*, tincture of opium from *Papaver somniferum*.
- Antibiotic: By definition all antibiotics are natural product produced by fungi. Almost all the available classes of antimicrobials are of fungal origin.

Alongside these naturally derived allopathic medicines, medicinal plant have been widely use in alleviating diarrhoeal symptoms in humans and animals (Brijesh *et al*., 2006; Gutierrez *et al*., 2007). Numerous species of these plants have been screened and validated for their use in treatment of diarrhoea (Gutierrez *et al*., 2007).

2.14.1. Anti-infectious mechanisms of plant secondary metabolites against diarrhoeal pathogens

- Antimicrobial: Many plant metabolites are known to exhibit some level of toxicity toward microorganisms. Numerous mechanisms of actions have been hypothesized to explain their antimicrobial activity such as microbial enzymes inhibition, deprivation of essential growth substrates, cell membrane disruption (Cowan, 1999) or direct interference with metabolic pathways.
- Antiadhesion: Adhesion of some enteric pathogen to the mucosa epithelium of the host cells is the first important step in intestinal infections that may lead to the development of diseases (Ofek and Sharon, 1990). Application of antiadhesives chemotherapy can be effective only against microorganisms that

depend on the surface contact with host cells as prerequisite for survival, multiplication and virulence (Lengsfeld *et al.*, 2007).

- Antitoxin: Since enteric pathogens may induce diarrhoea through the production of toxin (endotoxin or cytotoxin) the neutralization with plant antidiarrhoeal compounds may beneficial in the management of diarrhoea. Activated charcoals processed from plants are also used as toxin binders. Other binder includes pectin obtained from apples.

- Immunomodulatory: With immune suppression being a pre-disposing, drugs or medicinal plant preparations with immune stimulating activities may help in attenuating many infectious diseases.

2.14.2. Antioxidative mechanisms of plant phytochemical as potential antidiarrhoeal agents:

- Free radical scavenging: Many plant preparations and phytochemicals have strong antiradical activities which can antagonize the deleterious action of free radical. The mechanism may be electron transfer or hydrogen donating to stabilized the free radical molecules generating radicals that are relatively stable due to delocalization resulting from resonance and unavailability of site for further attack by molecular oxygen (Mello *et al*, 2005).

- Complexation of catalytic metallic ion: Metallic ions such as ferrous ion (Fe^{2+}), cuprous ion (Cu^{2+}), Manganese ion (Mg^{2+}) and Zinc ion (Zn^{2+}) can also generate free radicals (Kane, 1996). Many plant molecules moderate oxidation activity by complexing with the free transition metal thereby inactive their capacity to catalyze oxidative process.

- Pro-oxidation enzymatic pathways: With the generation of oxidant being enzyme driven, the antioxidant activities of plant phytochemical may be able to inhibit these enzymatic pathway or inactivation of the enzyme.

- Lipid peroxidation inhibition: Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation and reduction of the deleterious effect of the cytotoxic products.

- Inhibition of nitric oxide (NO): NO generated by inducible NOS (iNOS) can act synergistically with other inflammatory mediators in the development of diarrhoea. The inhibition or down-regulation of iNOS expression may be beneficial to reduce the inflammatory response.

- Immune system optimization: Over expression of immune system may cause damage to surrounding tissues and consequently results in a host of diseases and illness including diarrhoea. Many medicinal plants and phytochemical compounds protect against oxidative stress due to immunomodulatory activity (Wang *et al.*, 2002).

2.14.3. Anti-inflammatory mechanisms of plant phytochemical in diarrhoea management:

▪ Cyclooxygenase inhibition: Compounds with COX enzyme inhibitory potential could be use as anti-inflammatory agents. Some plant secondary metabolites such as alkaloids, phenols, , terpenoids and their derivatives have potential to inhibit the formation of pro-inflammatory signalling molecules such as prostaglandin (Polya, 2003)

▪ Lipoxygenase inhibition: Lipoxygenase metabolites are critical mediators of inflammation and thus important in the pathogenesis of abdominal distress and diarrhoea associated with intestinal inflammation. Plant phytochemicals with lipoxygenase inhibitory potential are candidate for anti-inflammation and the resulting diarrhoea.

▪ Modulation of cytokines activity: A number of diarrhoea pathogenesis causes severe intestinal inflammatory with hypersecretion of pro-inflammatory cytokines (MacNaughton, 2006). Inhibition of the pro-inflammatory cytokine mediator's can remove their negative activities associated with gastrointestinal disorders including diarrhoea.

2.14.4. Antidiarrhoeal mechanisms of plant phytochemicals

✓ Antispasmodic: Spasmolytic agents are used in the treatment hypermotility of the GIT while prokinetic agents are used in treatment of hypomotility (Gilani, 2005). Many phytochemicals demonstrated various range of spasmolytic or antispasmodic activities against spontaneous or agonist induced contraction on isolated parts of the GIT.

✓ Antisecretory: Microbial enterotoxins cause diarrhoea by disturbing the balance between intestinal absorption and secretion in favour of the later. Therefore, inhibition of the intestinal secretion is one therapeutic model for treating diarrhoea (Velazquez *et al*., 2009).

2.15.0. Classification of phytochemicals with antidiarrhoea potential

2.15.1. Terpenoids

Terpenoids are the most structurally diverse groups of natural products formed by fusion of isoprene monomers in plants. This class of plant secondary metabolites are grouped according to the number isoprene units or numbers of carbon in their skeletal structure (Zwenger and Basu, 2008). The group include monoterpenes which contain two units of isoprene with C_{10} and are present as essential or volatile components of herbs, spices and flowers. Sesquiterpenes are derivatives of three isoprene units containing 15 carbon atoms in their structures and are present in essential oil. This group of compounds acts as phytoalexins, antimicrobial and antifeedant in plants. Diterpenes contains 20 carbon atoms derived from four units of isoprene and pharmacological activities such as taxol (anticancer) and forskolin (for treating glaucoma). Triterpenes are contains 30 carbon atoms skeleton formed by the head-to-head joining of two C_{15} chains, each of which contains three isoprene units joined head-to-tail. Tetraterpenes such as carotenoids are 40 carbon atoms made of 8 isoprene units.

Several terpenoids have been identified so far to have good activity in one or more of the antidiarrhoeal mechanisms described above. Some of the compounds include:

- Niloticane from *Acacia nilotica* (L.) Willd ex Del. subsp. *kraussiana* (Benth) Brenan (Fabaceae) had inhibitory effect on (*Bacillus subtillis*, *Staphylococcus aureus*, and *Escherichia coli* at 4.0, 8.0 and 33.0 µg/ml respectively) (Eldeen *et al*, 2010).

- 1,3-Dihydroxy-12-oleanen-29-oic acid; 3,30-Dihydroxyl-12-oleanen-22-one; 1,3,24-Trihydroxyl-12-olean-29-oic acid; 1,23-Dihydroxy-12-oleanen-29-oic acid-3-*O*-2,4-di-acetyl-1-rhamnopyranoside isolated from *Combretum imberbe* have been reported to have MIC of 16.0 µg/ml against *E. coli* (Angeh *et al*., 2007).

- Oleanolic acid, ursolic acid, and betulinic acid from *Chaenomeles speciosa* have the potential of blocking the binding of virulence heat labile unit B (LTuB) of *E. coli* enterotoxin to ganglioside receptor (GM 1). The IC_{50} values enterotoxin binding activity were 202.8±47.8, 493.6±100.0, and 480.5±56.9 µM respectively (Chen *et al*., 2007).

- Glycyrrhizin from *Glycyrrhiza uralensis* have been reported to have LTuB-binding inhibitory activity with IC_{50} of 3.26±0.17 mM, therefore, can suppress LT-induced intestinal fluid accumulation (Chen *et al*., 2009).

- Oleanolic acid and echinocystic acid both isolated from *Luffa cylindrica* (cucurbitaceae) were reported to increase phagocyte index, stimulate macrophage, increase humoral and cell-mediated immune responses (Khajuria *et al*., 2007).

Ursolic acid

Oleanolic acid

3,30-Dihydroxyl-12-oleanen-22-one

1,3,24-Trihydroxyl-12-olean-29-oic acid

Betulinic acid

R= O-2,4-di-acetyl-1-rhamnopyranoside

1,23-Dihydroxy-12-oleanen-29-oic acid-3-O-2,4-di-acetyl-1-rhamnopyranoside

Asiatic acid

1,3-Dihydroxy-12-oleanen-29-oic acid

Arjunolic acid

Niloticane

Betulonic acid

Sugiol

Maslinic acid

Erythrodiol
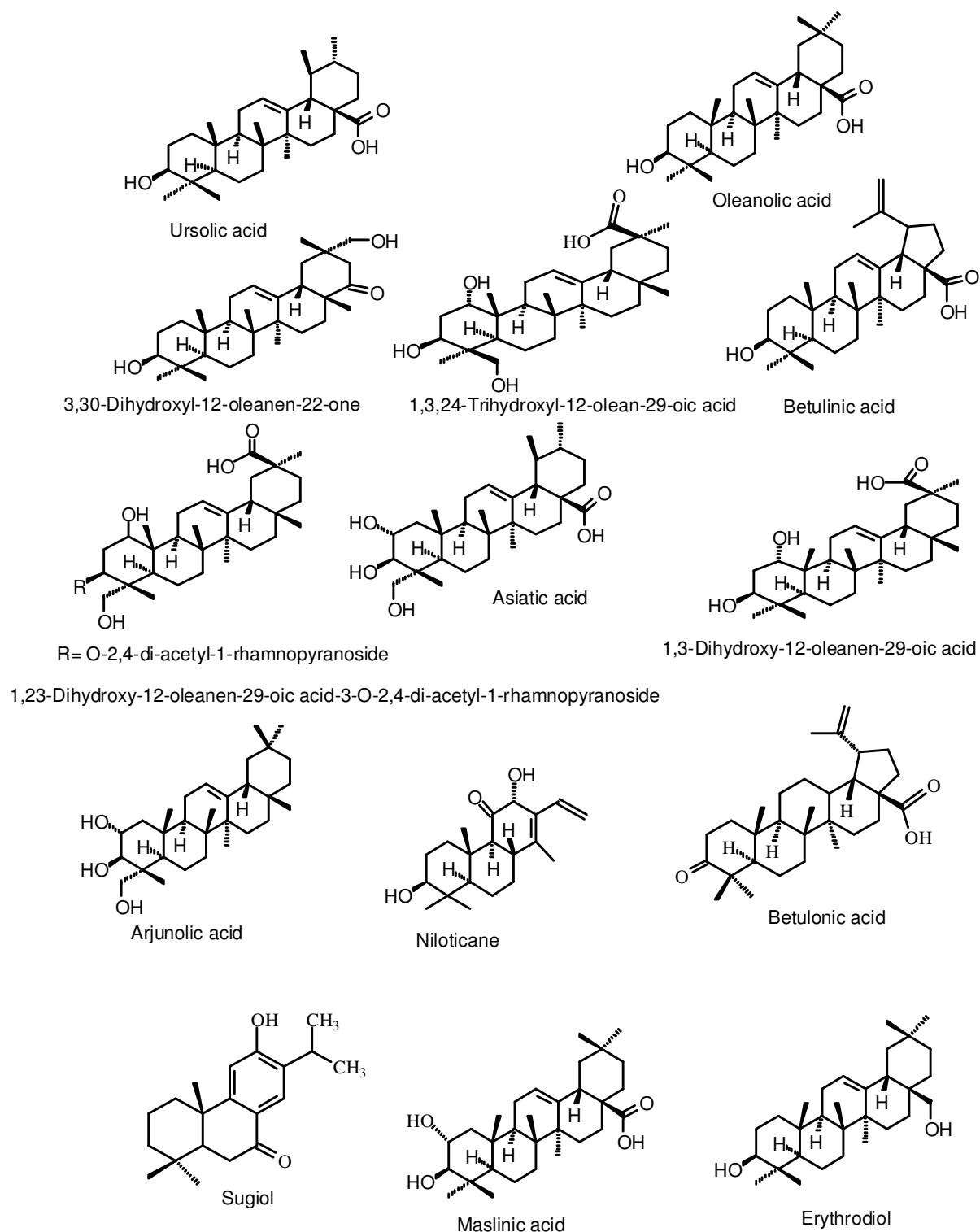
Fig. 2.9. Chemical structures of bioactive terpenoids against diarrhoeal mechanisms

- Sugiol (a diterpenoid) isolated from *Amentotaxus formosana* reported to exhibit good xanthine oxidase inhibitory activity with IC_{50} of 6.8±0.4 µM compared to standard allopurinol with IC_{50} of 2.0±0.7 µM (Lin *et al.*, 2010).

- *Epi*betulinic acid and betulonic acid isolated from *Maytenus cuzcoina* Loesener demonstrated nitric oxide inhibition of 89.1±4.4% (IC$_{50}$ of 0.7 μM) and 69.2±5.1% (IC$_{50}$ of 0.3 μM) respectively in vitro (Reyes *et al*., 2006).

- Betulonic acid isolated *Maytenus cuzcoina* Loesener to have demonstrated PGE inhibition activity of 58.4±3.9% (IC$_{50}$ of 2.7 μM) (Reyes *et al*., 2006) in vitro.

- Maslinic acid, oleanolic acid, erythrodiol and uvaol isolated from olive pomace oil was shown to have concentration dependent IL-6, TNF-α modulatory effects in a human mononuclear cells culture assay (Marquez-Martin *et al*., 2006).

- Ganoderic acids C and D isolated from *Ganoderma lucidum* have been reported to have anti-allergic properties through histamine release inhibitory mechanisms (Rios, 2008). Friedoolean-type triterpenoid (bryonolic acid) (Rios, 2005) and cucurbitacin structure, including dihydrocucurbitacin B (Escandell *et al*., 2007) and cucurbitacin R (Escandell *et al*., 2010) from *Cayaponia tayuya* (Cucurbitaceae) were reported to exhibit anti-allergic activities through inflammatory responses modulation.

2.15.2. Alkaloids

Alkaloid refers to a group of heterocyclic nitrogen compounds and many exhibit remarkable physiological and pharmacological activities (Samy and Gopalakrishnakone, 2008). Most alkaloids are derived from amino acid precursor and are classified based on their structure as pyridine, tropane or pyrrolizidine alkaloids. Though, alkaloids have many pharmacological mechanisms such as microbiocidal effect on diarrhoeagenic pathogens, the main antidiarrhoea effect is probably that of delayed intestinal transition of bowel materials (Cowan, 1999). Some of the pharmacological important antidiarrhoeal alkaloids include:

- Kurryam, koenimbine and koenine isolated from *Murraya koenigii* Spreng (Rutaceae) seed were reported to be active against various diarrhoeal mechanisms such as castor-induced diarrhoea, GIT motility, PGE$_2$ induced enterpooling (Mandal *et al*., 2010).

- 8-acetonyldihydronitidine and 8-acetonyldihydroavicine both isolated from *Zanthoxylum tetraspermum* had strong antistaphyloccocal activities with MICs of 1.56 and 3.12 μg/ml respectively. However, the two compounds were reported to have no inhibitory activity against *E. coli* (Nissanka *et al*., 2001).

- Boldine isolated from *Peumus boldus* Molina and *Corydalis ternate* Nakai had good antioxidant properties, indicating the effectiveness of the compound in preventing various oxidative-stress related illnesses like inflammatory cascades, immune dysfunctions. However, at high concentration, it causes cellular damage and potentiates lipoperoxidation, which is a pro-oxidant property.
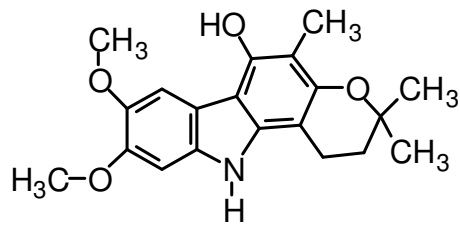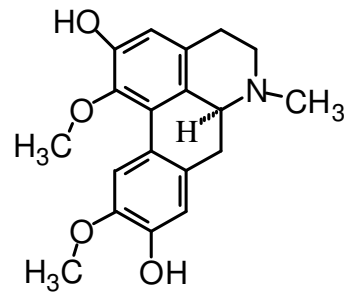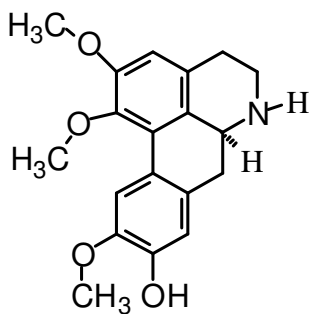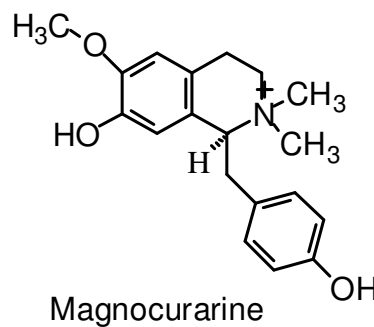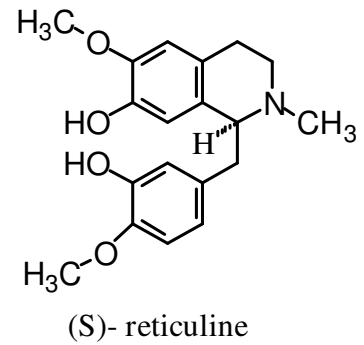
Kurryam
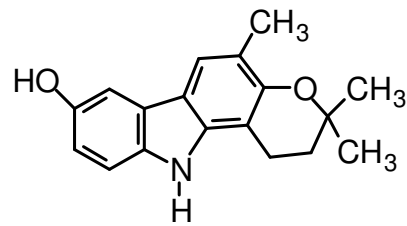
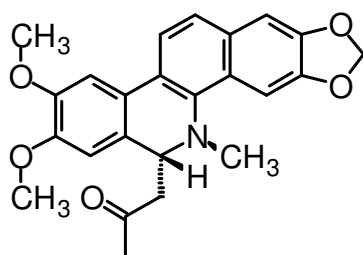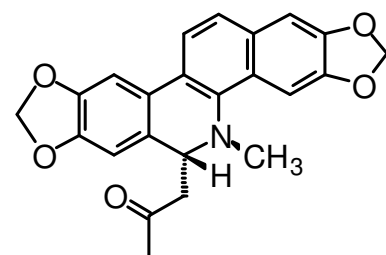Koenimbine
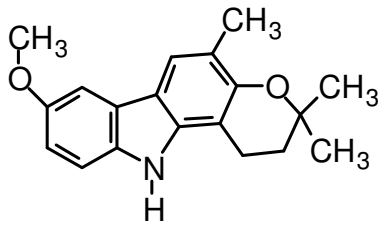
Koenine

(S)-boldine

Laurotetanine

Magnocurarine

(S)- reticuline

8-acetonyldihydronitidine

8-acetonyldihydroavicine

Fig. 2.10. Chemical structures of bioactive alkaloids against diarrhoeal mechanisms

2.15.3. Phenolic

Phytophenolic compounds are widely distributed as secondary metabolites of medicinal plants, as well as in some edible plants (Naczk and Shahidi, 2004). The consumption of diet rich in phenolic compounds has been hypothesized to be important in health promotion and disease prevention in humans and animals (Ramful *et al*, 2010). Phenolic compounds are characterized as aromatic metabolites that have one or more acidic hydroxyl groups attached to the phenyl ring. The sub-class of phenolics compounds are presented in Fig. 2.8. This group of compounds exhibits numerous biological activities directly or indirectly on intestinal epithelium which contribute to alleviation of diarrhoea symptoms.
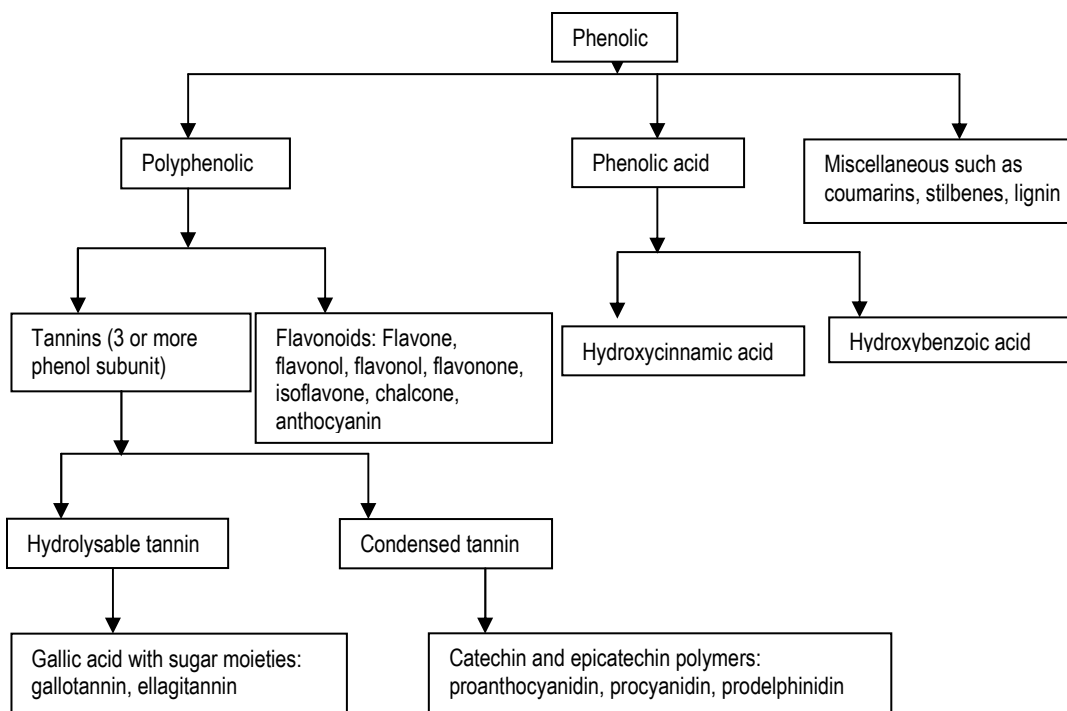

Fig. 2.11 Sub-classes of biologically important group of phenolic compounds.

Phenolic compounds with antidiarrhoeal activities against some of the mechanisms described above include:

- 2(*S*) -5'- (-1''',1'''-dimethylallyl) -8- (3'',3''-dimethylallyl) -2',4',5,7-tetrahydroxyflavanone; 2(*S*) -5'- (1''',1'''-dimethylallyl) -8- (3'',3''-dimethylallyl) -2'-methoxy-4',5,7-trihydroxyflavanone and 5'- (1''',1'''-dimethylallyl) -8- (3'',3''-dimethylallyl) -2',4',5,7-tetrahydroxyflavone] from *Dalea scandens* var. *paucifolia* with MIC of 1.56 µg/ml against standard and Methicillin-resistant *S. aureus* (MRSA) (Nanayakkara *et al*., 2002).
- Moracin T isolated from *Morus mesozygia* was reported to exhibit antimicrobial activities with MIC of 5.0 µg/ml against *E. coli*, S. *dysenterica*, P. *aerigunosa*, S. *typhi*, and 10 µg/ml against *S. aureus*, *C. albicans* (Kuete *et al*., 2009).
- Isoquercitrin, catechin and epicatechin isolated from *Chiranthodendron pentadactylon* flowers have antisecretory effect on *Vibrio cholerae* toxin induced intestinal fluid accumulation with ID_{50} of 19.2, 51.7 and 8.3 µg/ml against loperamide (positive control) with ID_{50} of 6.1 µg/ml.

- Davidigenin isolated from *Mascarenhasia arborescens* inhibits histamine induced contractile response of rat ileum and Ach induced contractile response on rat duodenum. The compound was reported to have non-competitive concentration dependent inhibitory activity (Desire *et al*., 2010).

- Vitexin isolated from *Aloysia citriodora* has also been reported to have antispasmolytic activities (Ragone *et al*., 2007).

- Quercetin has 90.7±0.3% and 79.6±2.3% inhibition of Ach and the depolarized KCl induced contractions on guinea pigs isolated ileum at IC_{50} <0.1 μg/ml respectively (Cimanga *et al*., 2010).

- Quercitrin was reported to have spasmolytic activities of 82.3±2.3% and 72.1±0.6% against Ach and the depolarized KCl induced contractions on guinea pigs isolated ileum at IC_{50} <0.01 μg/ml respectively (Cimanga *et al*., 2010).

- Spasmolytic activities of rutin were also reported as 93.4±1.6% and 86.3±2.1% against Ach and the depolarized KCl induced contractions on guinea pigs isolated ileum at IC_{50} <0.01 μg/ml respectively (Cimanga *et al*., 2010).

- Luteolin isolated from *Pogonatherum crinitum* has iNOS inhibitory activity with E_{max} equals 100.00±0.00% and IC_{50} of 10.41 μM while Kaempferol isolated from the same plant has iNOS inhibitory activity with E_{max} of 95.12±1.15% and the IC_{50} equals 10.61±0.44 μM (Wang *et al*., 2008)

- Stilbenoids such as r-2-viniferin, trans-amurensin and cis-amurensin isolated from *Vitis amurensis* have LOX inhibitory activity with IC_{50} of 6.39±0.08 μM, 12.1±0.32 μM and 16.3±0.52 μM respectively (Ha *et al*., 2009).

- Laurentixanthone A isolated from *Vismia laurentii* has good activities with MIC of 4.88 μg/ml against *S. dysenterica*, *S. flexneri*, *B. substilis* (Nguemeving *et al*., 2006).

- 5,7-dihydroxy-2-[14-methoxy-15-propyl phenyl]-4H-chromen-4-one isolated from *Leuca aspera* has superoxide radical scavenging activity of 75.4±0.31% and lipid peroxidation inhibition of 68.7±0.41% at a concentration of 40 ppm (Meghashri *et al*., 2010).

- Lipid peroxidation inhibitory activity of foeniculoside X isolated from *Foeniculum vulgare* at concentration of 10^{-5}M was reported to be 6.30 nm TBARS/mg LDL (Marino *et al*., 2007).

- An *in vivo* lipid peroxidation inhibitory activity of arzanol isolated from *Helichrysum italicum* has also been reported (Rosa *et al*., 2011).
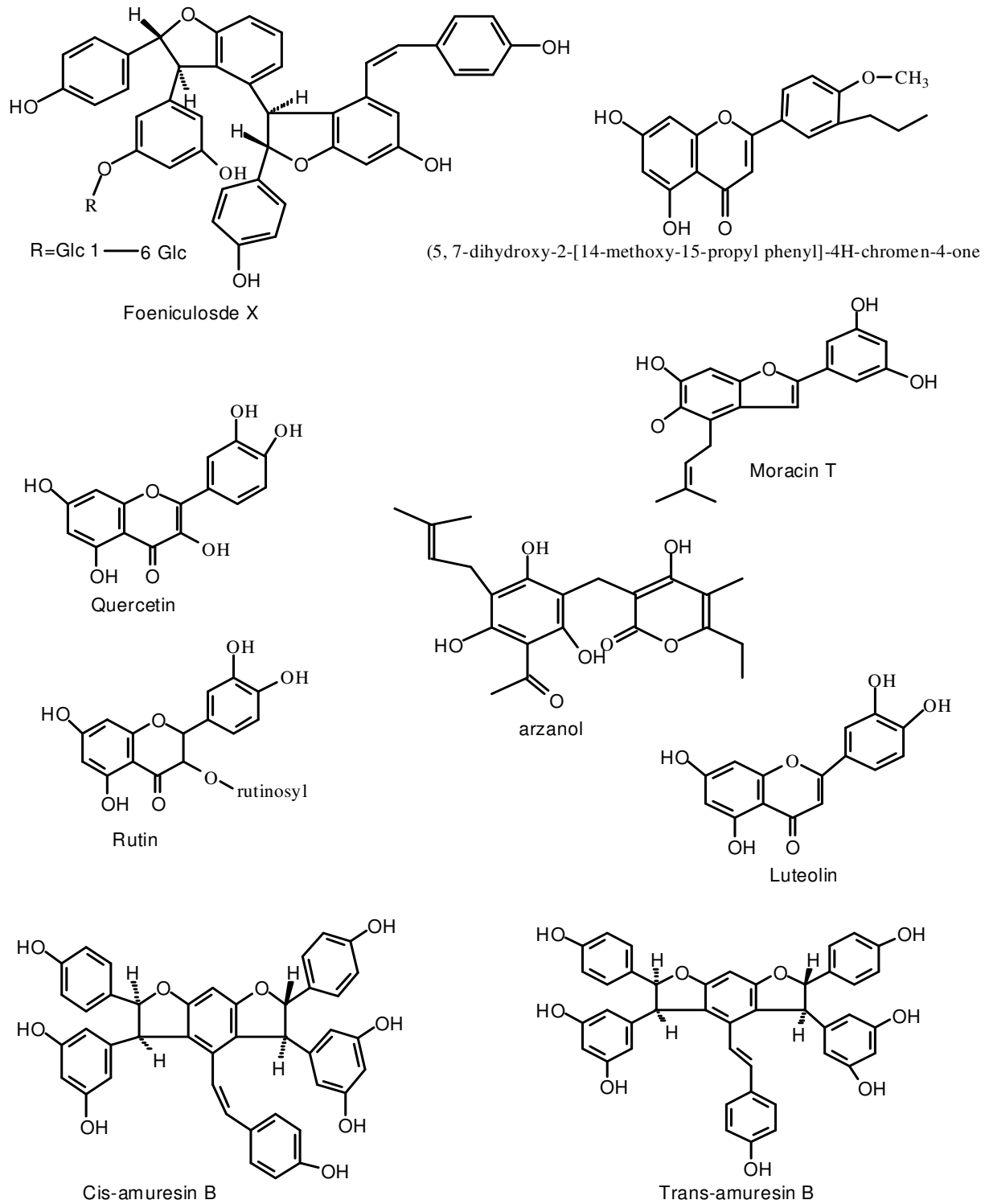
Fig. 2.12. Chemical structures of bioactive phenolics against diarrhoeal mechanisms

2.16. Ethnobotany and scientific investigation of plant species used traditionally in treating diarrhoea in South Africa

A survey on traditional practice in South Africa indicates that diarrhoea is one of the most prominent ailments being treated with medicinal plants (Dambisya and Tindimwebwa, 2003). At this stage, the scientific validation of their therapeutic potential, standardization, safety and mechanisms of actions of most of the plants is still lacking (Havagiray *et al.*, 2004). Ethnopharmacological properties and phytochemistry of the medicinal plants used for the treatment of diarrhoea in South Africa is reviewed and presented in Appendix 1.

2.17. Conclusion

Many medicinal plants are used in various traditional cultures of South Africa to treat diarrhoea and the associated complications. The traditional recipes include infusions, decoctions, and tinctures of the leaves, stems, roots, seeds and bark of medicinal plants. Several scientific methods have been used to evaluate and validate the traditional use of some of the plants as antidiarrhoeal agents. Such properties investigated as the potential antidiarrhoeal mechanism are the antimotility, antipropulsive, antioxidant, anti-inflammation, antispasmolytic, antimicrobial, antiprotozoal, and immunomodulatory activity of the medicinal plant preparations. Several of these aspects will be examined for some selected medicinal plants in this study.