PHARMACOLOGICAL EVALUATION AND CHARACTERIZATION OF THE ANTIULCER CONSTITUENTS OF STEM BARK EXTRACT OF *Bridelia ferruginea* Benth (Euphorbiaceae)

BY

NNAMANI, MARCELLUS EJIKE
(PG/Ph.D/10/52610)

DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY
FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF NIGERIA, NSUKKA

DECEMBER, 2015

PHARMACOLOGICAL EVALUATION AND CHARACTERIZATION OF THE ANTIULCER CONSTITUENTS OF STEM BARK EXTRACT OF *Bridelia ferruginea* Benth (Euphorbiaceae)

BY

NNAMANI, MARCELLUS EJIKE
(PG/Ph.D/10/52610)

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACOLOGY AND TOXICOLOGY OF THE UNIVERSITY OF NIGERIA, NSUKKA

SUPERVISORS: PROF. P. A. AKAH & PROF. C. O. OKOLI

DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY
FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF NIGERIA, NSUKKA

DECEMBER, 2015

TITLE

PHARMACOLOGICAL EVALUATION AND CHARACTERIZATION OF THE ANTIULCER CONSTITUENTS OF STEM BARK EXTRACT OF *Bridelia ferruginea* Benth (Euphorbiaceae)

CERTIFICATION

This is to certify that Nnamani Marcellus Ejike, a postgraduate student with registration number PG/PhD/10/52610, of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka has satisfactorily completed the requirements for the award of the degree of Doctor of Philosophy (PhD) in Pharmacology and Toxicology. The work embodied in this project is original and has not been submitted in part or full to this or any other University.

Prof. P. A. Akah
(Supervisor)

Prof. C. O. Okoli
(Supervisor)

Dr. A. C. Ezike
(Head of Department)

DEDICATION

To the members of my family and friends for their support and encouragement.

ACKNOWLEDGEMENT

I am very grateful to God Almighty for granting me the grace to successfully complete this work.

My profound gratitude also goes to my Supervisors; Prof. P.A. Akah and Prof. C.O. Okoli for their selfless assistance, unalloyed support, guidance and advice throughout the course of this work.

My gratitude goes to Dr. M. Tchimene for all the sacrifices he made throughout the course of this work. I remain grateful to the entire staff of International Centre for Ethnomedicine and Drug Development, Nsukka, for their technical assistance throughout the course of this work.

I wish to express my gratitude to all my friends and colleagues especially Dr. C.S. Nworu, Dr. F.B.C. Okoye and Dr. K.G. Ngwoke for their assistance. In the same vein, my thanks go to Dr. S.O. Eze and Mr. A.L. Ezugwu of Department of Biochemistry; University of Nigeria Nsukka, Dr. S.O. Udegbunam of Department of Veterinary Surgery; University of Nigeria Nsukka, Mr. E.O. Eze of Department of Crop Science; University of Nigeria Nsukka, Mr. D. Ugwu, of Department of Chemistry; University of Nigeria Nsukka for their assistance.

I am specially indebted to my wife and children for all their prayers, patience, support and above all their sacrifices to ensuring that the work is completed.

I also wish to thank my sister in-law, Dr (Mrs) P. Nnamani and her husband, Dr. C. Nnamani for being the source of inspiration to attaining this academic status.

Moreover, I am indebted to Pharm. D.D. Gimba for his fatherly advice, support and encouragement.

Finally, I thank all the members of staff, Department of Pharmacology & Toxicology especially the laboratory staff for their assistance.

Nnamani, M. Ejike
December, 2015

TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION

CHAPTER TWO: MATERIALS AND METHODS

LIST OF ABREVIATIONS

^{13}C-NMR: Carbon-13 nuclear magnetic resonance

AC: Absorbance of control

ACTH: Adrenocorticotropic hormone

AMF: Aqueous methanol fraction

ANOVA: Analysis of variance

As: Absorbance of sample

ATP: Adenosine triphosphate

BF1: Isolated compound 1

BF2: Isolated compound 2.

BFSB: *Bridelia ferruginea* stem bark

CagA: Cytotoxin-associated gene-A

CD_3Cl: Deuterated chloroform

CD_3OD: Deuterated methanol

CF: Chloroform fraction

CGRP: Calcitonin gene-related peptide

CGRP: Calcitonin gene-related peptide

cNOS: Constitutive nitric oxide synthase

COSY: Correlation spectroscopy

COX: Cyclooxygenase

CRF: Corticotrophin-releasing factor

DEPT: Distortionless enhancement by polarization transfer

DPPH: 1, 1-diphenyl picrylhydrazil

EGF: Epidermal growth factor

EGF-R: Epidermal growth factor receptor

HCl: Hydrochloric acid

HMBC: Heteronuclear multiple bond correlation

H-NMR: Proton nuclear magnetic resonance

HO-1: Hemeoxygenase-1

HP: *Helicobacter pylori*

HSP: heat shock protein

HSQC: Heteronuclear single-quantum correlation spectroscopy

IBD: Inflammatory bowel disease

IL: Interleukin

iNOS: Inducible nitric oxide synthase

InterCEDD: International Centre for Ethnomedicine and Drug Development

KCl: Potasium chloride

LD: lethality dose

L-NAME: N-nitro-L-arginine methylester

MAPK: Mitogen-activated protein kinase

ME: Methanol extract

$MgCl_2$: Magnesium chloride

MOS: Mitochondrial oxidative stress

MPO: Myeloperoxidase

NEM: N-ethylmaleimide

NO: Nitric oxide

NP-SH: Non-protein sulfhydryl

NSAIDs: Non-steroidal antiiflammatory drugs

PARs: Proteinase-activated receptors

PGE: Prostaglandin E

PGHS: Prostaglandin H synthase

PGI: Prostacyclin

PGs: Prostaglandins

PHG: Portal hypertensive gastropathy

PPIs: Proton pump inhibitors

PUD: Peptic ulcer disease

ROS: Reactive oxygen species

SEM: Standard error of mean

SRMD: Stress related mucosal disease

TFF: Trefoil factor family

TLC: Thin layer chromatography

TMS: Tetramethylsilane

TNF: Tumuor necrosis factor

ULI: Ulcerative lesion index

USA: United States of America

VacA: vacuolating cytotoxin-A

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Peptic ulcer disease (PUD) is a sore in the lining of the stomach or duodenal mucosa. The search for an ideal antiulcer drug continues and has also been extended to medicinal plants. *Bridelia ferruginea* Benth (Euphorbiaceae) is a plant used in traditional medical practice in South West Nigeria. This study was aimed at evaluating the antiulcer activity and mechanisms of the extract of the stem bark and to isolate the bioactive constituents responsible for the antiulcer activity. Methanol extract (ME) was obtained by cold maceration and concentration *in vacuo*. ME was partitioned in chloroform-methanol-water (2:2:1) mixture to obtain the chloroform (CF) and aqueous methanol (AMF) fractions. The extract and fractions were subjected to biological activity-guided screening using indomethacin-induced ulcer as activity-guide. Based on higher ulcer protection given by CF, it was fractionated in a silica gel and eluted with gradient mixtures of n-hexane-ethyl acetate to obtain six broad fractions (I ï VI). Fractions III and VI offered the highest protection on screening for biological activity. Purification of fractions III and VI in a sephadex LH-20 column with methanol as eluent gave compounds I (BF1) and II (BF2) respectively. The antiulcer activity of BF1 and BF2 was done using the activity-guide and the structural identities established using nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) and electron impact mass (EIM) spectroscopies. The extract was subjected to phytochemical analysis using conventional methods. The oral acute toxicity of ME was determined in mice. The antiulcer activity of ME, bioactive column fractions and isolated compounds was investigated using indomethacin, ethanol, cold-restraint stress and pyloric ligation-induced ulcers in rats. The mechanisms of antiulcer activity were studied using gastric acid secretion induced by pyloric ligation in rats, proton pump inhibition using inhibition of H^+ K^+ ATPase activity *in vitro,* determination of the roles of endogenous nitric oxide and sulfhydryl compounds using the effects

of L-NAME (L-nitroarginine methylester) and NEM (N-ethylmaleimide) respectively on ulcer indices in ethanol-induced ulcer and antioxidant activity using DPPH radical scavenging activity. The results showed that ME tested positive to saponins, reducing sugars, tannins, carbohydrates, flavonoids, glycosides, alkaloids, steroids, proteins and terpenoids. No lethality was observed in the mice on oral administration of doses up to 5000 mg/kg. There were no obvious signs of abnormal behavioural changes in the mice. The extract, fractions and isolated compounds produced significant ($p < 0.05$) dose-related inhibition of indomethacin, ethanol, cold restraint stress and pyloric ligation-induced ulcers. The isolated compounds, BF1 and BF2, significantly ($p < 0.05$) decreased the total acid and volume of gastric secretion and elevated the pH. The fractions and isolated compounds significantly ($p < 0.05$) inhibited the activity of $H^+ K^+$ ATPase in a dose-dependent manner. The isolated compounds, BF1 and BF2 did not increase gastric lesion indices in L-NAME pre-treated rats but they significantly ($p < 0.05$) increased ulcer indices in the NEM pre-treated rats. The extract, fractions and isolated compounds scavenged DPPH radical in a dose-dependent manner. Comparison of the spectral data of BF1 and BF2 with the published libraries of isolated compounds revealed their identities to be ɓ-sitosterol and ɓ-sitosterol-3-O-ɓD-glucopyranoside respectively.

CHAPTER ONE

1.0 Introduction

1.1 Peptic ulcer disease

Peptic ulcer is a sore that forms in the lining of the stomach or the duodenum. It encompasses gastric and duodenal ulcers and is the most prevalent gastrointestinal disorder (Prabha *et al.*, 2011). Clinically, it presents as abdominal stress most often in the upper quandrant of the abdomen and epigastric region (Mayty, 2003; Ezekwesili *et al.,* 2014). Peptic ulcer disease (PUD) is a chronic disease which impairs quality of life with increased morbidity and mortality (Shobha and Jamadar, 2013). It is associated with necrosis, infiltration of neutrophils, reduction in blood flow, and induction of oxidative stress as well as secretion of inflammatory mediators (De Souza *et al.*, 2011; Viana *et al.,* 2013). Ulceration refers to a process of inflammation in which an epithelial surface of the skin, gastric epithelium, colonic mucosa, and bladder epithelium has become necrotic and eroded, often associated with subepithelial acute and chronic inflammation (Tripathi, 2008; Yadav *et al.*, 2013).

PUD may manifest as superficial, deep or perforated erosions equal to or greater than 0.5 cm of the mucosal lining of the stomach, pyloric channels, duodenum and at or near the site of surgical anastomosis (post-operative ulcers) (Dhasan *et al.*, 2010; Kalra *et al.*, 2011). The major types of peptic ulcer disease are gastric and duodenal ulcers. About 98% of peptic ulcers are either in the first portion of the duodenum or in the stomach in a ratio of about 4:1 (James and Vinay, 2003).

PUD continues to be an important clinical issue because of common use of non-steroidal antiinflammatory drugs (NSAIDs) and low doses of acetylsalicylic acid. A rare but increasingly problematic issue is *Helicobacter pylori*-negative and NSAID-negative ulcers (Malfertheiner *et al.*, 2009).

Other unavoidable factors associated with higher incidence of peptic ulcer include gender, as there is higher prevalence of the disease among women than men (Malfertheiner *et al.*, 2009). People over the age of 60 are also more prone to peptic ulcer disease (Morsy and Azza, 2011). In addition, ethnic backgrounds as African-Americans or Hispanics have 2-fold higher risk of developing gastric ulcer. Furthermore, patients suffering from other diseases as congestive heart failure have higher incidence of having peptic ulcer (Morsy and Azza, 2011). Type O blood group has also been associated with increased incidence of the disease (Morsy and Azza, 2011). Genetics is another unavoidable risk factor for peptic ulcer (Sun *et al.*, 2009). Pepsinogen C gene polymorphism, for example, is significantly associated with development of peptic ulcer (Sun *et al.*, 2009). Other relatively rarer predisposing factors to development of peptic ulcer include Crohnôs disease of the stomach, eosinophilic gastritis, systemic mastocytosis, radiation damage and viral infections by cytomegalovirus or herpes simplex (Malfertheiner *et al.*, 2009).

However, under certain conditions, some risk factors may contribute to mucosal injury and initiation of gastric ulcer, such as Zollinger-Ellison syndrome (increased hydrochloric acid secretion), psychological stress, liver cirrhosis, chronic obstructive pulmonary disease, renal failure, organ transplantation and rheumatoid arthritis (Morsy and Azza, 2011).

1.2 Epidemiology

Ulcer disease, which can occur in the esophagus, stomach, and/or in the duodenum, is one of the main prevalent still unresolved medical problems facing numerous patients in a wide range of age of both sexes worldwide (Yadav *et al.,* 2013).

Peptic ulcer was considered to be a disease of the young and middle aged adults but it affects all age groups even children. The epidemiological reports showed that peptic ulcers are high ranking global health challenges affecting approximately 8-10% of the population (Willemijntje and Pankaj, 2006; Kelly *et al.,* 2009) and it is also estimated that 50% of healthy individuals experience symptoms of peptic ulcer on daily basis (Willemijntje and Pankaj, 2006). Gastric hyperacidity and ulcer are major recurrent diseases of the gastrointestinal tract affecting all geographical regions (Brown, 2000; Siddaraju and Dharmesh, 2007).

Report shows that peptic ulcer disease is common in South West Nigeria, accounting for about 82.7% of all patients with symptom of dyspepsia (Adeniyi *et al*., 2012). *Helicobacter pylori* positive patients account for 100% duodenal ulcer and 82% gastric ulcer in Nigeria (Ndububa *et al*., 2001). Study shows that there has been a noticeable change in disease manifestation in the upper gastrointestinal track in Nigeria resulting in a reduction in the incidence of duodenal ulcer and increase in the prevalence of gastric ulcer, and gastroeosophageal reflux disease (Nwokediuko *et al*., 2012). This trend may be due to widespread and indiscriminate use of potent acid suppressant, changes in diets, sedentary life style and increasing obesity (Nwokediuko *et al*., 2012).

The incidence of PUD associated with *Helicobacter pylori* infection is decreasing especially in Western countries (Arents *et al.,* 2004; Arroyo *et al.,* 2004; Jang *et al.*, 2007). The declining global prevalence of PUD might be because of the decreasing prevalence of *H. pylori* infection (Wong *et al.,* 2004). This could lead to a relative increase in the number of patients with this

NSAID-associated and idiopathic peptic ulcer disease (IPUD (Arents *et al.,* 2004). NSAID use contributes to an increased proportion of peptic ulcer disease (Lassen *et al.*, 2006).

1.3 Signs/symptoms

There are distinguishing clinical features of duodenal and gastric ulcers. These are shown in Table 1 (Harsh, 2005).

Table 1. Clinical features of duodenal and gastric ulcers

S/NO	Duodenal ulcer	Gastric ulcer
1	Pain-food relief pattern	Food-pain patern
2	Night pain common	No night pain
3	No vomiting	Vomiting common
4	Melaena more common than heamatemesis	Haematemesis more common
5	No loss of weight	Significant loss of weight
6	No particular choice of diet	Patients choose bland diet devoid of fried foods and curries
7	Deep tenderness in the right hypochondrium	Deep tenderness in the midline in epigastrium
8	Marked seasonal variation	No seanonal variation
9	Occurs more commonly in people at greater stress	More often in laboring groups

1.4 Diagnosis of peptic ulcer disease

Peptic ulcer disease can be diagnosed by the symptoms and signs experienced by individual patients. Other ways of diagnosis include physical examination, laboratory studies and methods of visualization of ulcers like endoscopy (UMHS, 2005).

1.5 Complications of peptic ulcer disease

It is reported that about 4,500 people in United Kingdom and 15,000 people in the USA die each year from the complications of peptic ulcer disease (Valle, 2005). The most serious complications of peptic ulcer disease include hemorrhage, perforation, penetration, and gastric outlet obstruction (Ahmad *et al.*, 2014).

The signs and symptoms of complicated ulcer are:

1. Gastrointestinal bleeding which presents with heme positive stool, melena, hematemesis and anemia.
2. Intestinal obstruction. There are nausea and vomiting.
3. Penetration or perforation with severe abdominal pain.
4. Cancer.

1.6 Laboratory tests

The laboratory tests that are required in the investigation of peptic ulcer disease are the invasive and non-invasive tests for *H. pylori*. The non-invasive *H. pylori* tests are tests that identify active infection and invasive tests that detect antibodies (UMHS, 2005). This distinction is important because antibodies (positive immune response) only indicate the presence of *H. pylori* at some time (UMHS, 2005). Antibody tests do not differentiate between previously eradicated *H. pylori* infection and currently active one. Tests for antibodies are simpler to administer, provide a faster result and less expensive than tests for active infection. Tests for active *H. pylori* include fecal *H. pylori* antigen tests and urea breath tests (UMHS, 2005). The stool antigen tests have sensitivity and specificity of more than 90% in untreated patients with suspected *H. pylori* infection (UMHS, 2005).

For the urea breath test, the patient drinks an oral preparation containing ^{13}C or ^{14}C-labelled urea. This test has a sensitivity and specificity of more than 90% for active infection (UMHS, 2005). This test requires more patient preparation and is more expensive. A number of drugs like antibiotics, bismuth and proton pump inhibitors can adversely affect the accuracy of urea breath tests. Patients undergoing this test are expected to fast for at least 6 hours (UMHS, 2005).

1.7 Visualization of ulcers

There are techniques for the visualization of ulcers. These include:

1. **Endosocopy**

PUD cannot be diagnosed on symptoms alone: endoscopic evidence is required to establish a diagnosis (Zhaoshen *et al.*, 2010). This involves the use of an endoscope which is guided into the throat and down into the esophagus and finally into the stomach and upper intestines. After successful introduction of the instrument, the inner lining of these organs can be observed from

the camera on a television screen. During endoscopic technique, biopsy can be taken and tested for *H. pylori*. This technique has high specificity and sensitivity for PUD. It also offers an opportunity for biopsy and histological examination of questionable lesions. The major limitation of this technique is the high cost, which very few can afford. It is indicated for patients older than 45 years especially with alarm symptoms (American Society for Gastrointestinal Endoscopy, 1999).

The prevalence of endoscopically confirmed PUD is higher in China (17.2%) than in Europe (4 ï 6%) (Zhaoshen *et al.*, 2010). Almost three quarters of individuals with PUD in China are asymptomatic, and no gastrointestinal symptom patterns are predictive of PUD (Zhaoshen *et al*., 2010).

II. Radiography

This method is less accurate than endoscopy for the diagnosis of small ulcers. This involves the radiological study of the stomach, duodenum and esophagus. It is carried out by administering a barium meal to the patient and obtaining the X-ray of the gastrointestinal tract. The presence of ulcer is outlined on the X-ray (American Society for Gastrointestinal Endoscopy, 1999).

1.8 Pathophysiology of peptic ulcer disease

There are several factors associated with the development of peptic ulcer disease. The gastrointestinal problems caused by different etiologies were observed to be associated with the alterations of various physiological parameters such as reactive oxygen species, nitric oxide synthase, lipid peroxidation, and secretion of excessive gastric acid (Carr *et al.*, 2002; Yadav *et al.*, 2013). In 80% of the cases, gastric ulcer is caused primarily by the use of non-steroidal

antiinflammatory drug, *H. pylori* infection (10%) and the intake of very spicy and fast food (about 8- 10%) (Abdulla *et al.*, 2010; Yadav *et al.*, 2013).

Under normal conditions, mucosal integrity is maintained by defense mechanisms, which include an epithelial ñbarrierò, mucus secretion, bicarbonate, prostaglandins, nitric oxide, growth factors, heat-shock proteins and continuous blood flow (de-Faria *et al.*, 2012). The defensive barriers in the gastric wall that resist gastric acid and pepsin involve mucus and the bicarbonates grade in the mucus, the tight junction structure among gastric epithelial cells, and the blood flow in mucosa that provides oxygen and nutrition to mucosa and support the turnover of gastric epithelium and mucus (Wang and Wang, 2011).

A peptic ulcer results from an imbalance between some endogenous aggressive factor(s) (hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species (ROS)) and cytoprotective factors, which include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), adequate mucosal blood flow, cell renewal and migration, non-enzymatic and enzymatic antioxidants and some growth factors (Bhattacharjee *et al.*, 2002; Laine *et al.*, 2008; Raju *et al.*, 2009; Wasman *et al.,* 2010; Beserra *et al.*, 2011; Prabha *et al.*, 2011). Other aggressive factors which are exogenous are consumption of NSAIDs, alcohols and stressful conditions (Viana, 2013).

The pathogenesis of ulcers is multifactorial and includes diverse factors such as a stressful lifestyle, alcohol consumption, use of steroidal and nonsteroidal antiinflammatory drugs (NSAIDs) and drugs which stimulate gastric acid and pepsin secretion, *H. pylori* infections, smoking, lower socioeconomic status and family history (Bandyopadhyay *et al.*, 2001; Prabha *et al.,* 2011).

1.9. Risk factors for peptic ulcer diease

1.9.1 Acid secretion

Gastric acid has been known for many decades to be a key factor in normal upper gastrointestinal functions such as protein digestion, calcium and iron absorption, as well as providing some protection against bacterial infections (Olbe *et al.,* 2003). However, inappropriate levels of gastric acid underlie several pathological conditions, including gastric ulcer (Olbe *et al*., 2003). Hyperchlorhydria is a condition characterized by uncontrolled hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through the proton pump (Yadav *et al*., 2012). Gastric hydrochloric acid secretion is modulated by neural and hormonal stimulation of receptors on the basolateral membrane as well as by activation of enzymes located on the surface of parietal cells (Alex and Walter, 2009).

1.9.2 *Helicobacter pylori* (HP)

Helicobacter pylori is the most important etiological agent of chronic active type B gastritis and peptic ulcer diseases, and is linked to gastric carcinoma (Atherton, 2006). The prevalence of *H. pylori* is about 40% in developed countries and 80ï 90% in the developing world (Perez-Perez *et al.,* 2004). Once acquired, *H. pylori* infection usually persists for life unless treated by antimicrobial therapy (Israel *et al.*, 2009). Current data shows that *H. pylori* infection plays a major role in peptic ulcer disease and non-ulcer dyspepsia (Itoh *et al.*, 2005). Apart from these diseases, *H. pylori* is thought to play a role in the etiology of atrophic gastritis, gastric adenocarcinoma and lymphoma (Martyn *et al.*, 2007; Ahmet *et al.*, 2011). There is significant association between *H. pylori* infection and portal hypertensive gastropathy (PHG) in cirrhotic patients which is also related to severity of PHG (Sathar *et al.*, 2014).

H. pylori is a bacterium that colonizes the human stomach and can establish a long-term infection of the gastric mucosa (Martyn *et al.*, 2007). The surfaces of the cells lining the stomach contain a protein, called decay-accelerating factor, which acts as a receptor for the bacterium and it can survive in the highly acidic medium of the stomach by producing urease, an enzyme that generates ammonia to neutralize the acid (Morsy and Azza, 2011). Other toxic products from the metabolism of urea by urease include ammonium chloride and monochloramine (James and Vinay, 2003).

This bacterium then produces a number of toxins causing inflammation and damage to the stomach, leading to ulcers especially in predisposed individuals. The bacterium also alters certain immune factors that allow them to evade detection by the immune system and cause persistent inflammation (Morsy and Azza, 2011).

There is evidence to support the existence of distinct genetic lineages of *H. pylori* and this genetic variation may play a role in its pathogenicity (Hatakeyama, 2006). Among genes related to pathogenicity, the cytotoxin-associated (cagA) gene and the vacuolating cytotoxin (vacA) genes of *H. pylori* have been studied most extensively (Hatakeyama, 2006). The cagA gene, which is not present in all strains, is considered to be a marker for the presence of a pathogenicity island of approximately 35 000 bp that encodes a type IV secretion system that transfers the CagA protein into the host cells (Hatakeyama, 2006). Infection with cagA – positive *H. pylori* strains increases the risk for the development of atrophic gastritis and gastric cancer (Martyn *et al.,* 2007). The vacA gene encodes a vacuolating cytotoxin that is excreted by *H. pylori* and damages epithelial cells (Martyn *et al.*, 2007).

It is well known that *H. pylori* is associated with alterations in the gastric epithelial cell cycle and apoptosis, higher levels of mononuclear and neutrophilic infiltrates, more severe atrophy and

intestinal metaplasia (Antonio and Gaetano, 2004). *H. pylori* causes increased production of proinflammatory cytokines such as interleukin (IL-1, IL-6 and IL-8) and tumor necrosis factor (TNF) (Dundon *et al.*, 2001). The infection of the antrum leads to depletion of antral somatostatin and increased gastrin release from G cells (Willemijnte and Pankaj, 2006). Then, the gastrin stimulates G-receptors of the parietal cells leading to increased acid secretion. The increased acid secretion further damages the duodenal mucosa. Persistent damage of the duodenal mucosa stimulates the development of patches of gastric metaplasia in the duodenum, which in turn are colonised by *H. pylori* (Dundon *et al.,* 2001).

Some strains of *H. pylori* that produce vacuolating toxin (VacA) and cytotoxin associated geneA (CagA) cause more intense tissue inflammation and cytokine production (Israel *et al.*, 2001). Cytotoxin associated geneA (CagA) is a powerful stimulus for the production of interleukin-8 (IL-8) by the epithelial cells (Israel *et al.*, 2001).

1.9.3 Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal antiinflammatory drugs (NSAIDs) are used to treat long term painful conditions like arthritis. They include such agents as ibuprofen, indomethacin, aspirin and diclofenac. They are known to cause mucosal damage by inhibiting cycloxygenase pathway of arachidonic acid metabolism thereby reducing the formation of cytoprotective prostaglandins (PGE_1, PGE_2 and PGI_2) and increasing the overproduction of leukotrienes and other products of the 5-lipoxygenase pathway (Kalra *et al.*, 2011). This inhibition interferes with the protective mechanisms such as mucus content, surface active phospholipids, bicarbonate secretion, and mucosal blood flow and proliferation (Kalra *et al.*, 2011). The continuous generation of prostaglandins by cyclooxygenase isoenzymes in the gastric mucosa helps to maintain an adequate mucosal blood

flow and also stimulates the generation of mucus (Roslida *et al.*, 2010). NSAIDs inhibit cyclooxygenase and thereby reduce the intrinsic ability of the mucosa to resist injury induced by endogenous and exogenous aggressors (Heeba *et al.*, 2009). It was equally reported that these have the ability to cause gastroduodenal ulceration which is related to their ability to suppress prostaglandin synthesis and increase acid secretion (Jince *et al.,* 2010).

While cylooxygenase-1 (COX-1) is the predominant isoform expressed in the healthy gastric mucosa, COX-2 expression can be up-regulated very rapidly (Wallace, 2008). Substantial increase of COX-2 expression can be seen following a mucosal exposure to a hapten, induction of ischemia or when COX-1 activity is suppressed by aspirin (Wallace, 2008). This upregulation of COX-2 appears to be a defensive and antiinflammatory response aimed at enhancing mucosal defense (Wallace, 2008).

The mechanism by which indomethacin induces ulcer is by inducing H^+ K^+ATPase in gastric parietal cells which increases gastric acid secretion and decreases pH (Prabha *et al.,* 2011). Indomethacin decreases protein content of the gastric mucosa due to damage in the gastric mucosa which results in the leakage of protein into the gastric juice and significant decrease in the levels of glycoproteins like sialic acid and hexosamine of the gastric mucosa (Prabha *et al.*, 2011). It has also been reported that NSAIDs, especially indomethacin, inactivates gastric peroxidase to induce reactive-oxygen-mediated gastric mucosa injury, and that indomethacin-induced oxidative damage by ROS showed increased lipid peroxidation and thiol depletion (Yadav *et al.*, 2013). The gastrointestinal irritant properties of the NSAIDs such as indomethacin or piroxicam are the major impediments to their use as antiinflammatory drugs (Chiba *et al.*, 2008). Gastric ulcers associated with the use of NSAIDs in painful diseases of rheumatic and non-rheumatic origin remain a major problem in the clinical field (Hawkey and Langman, 2003).

Synthetic NSAIDs like diclofenac cause mucosal damage by interfering with prostaglandin (PG) synthesis, increasing acid secretion and back diffusion of H^+ ions; thus leading to breaking up of mucosal barrier (Roslida *et al.*, 2010).

NSAID can directly damage the gastric epithelium by intracellular accumulation of drug in an unionized state and reduce the hydrophobicity of the mucus gel layer by changing the action of surface active phospholipids (Jain and Panigrahi, 2012). The enzymes such as catalase and glutathione peroxidase provide defense against damage of gastric mucosa after administration of NSAIDs and also decrease lipid peroxide level in rats (Roldao *et al.*, 2008).

Neutrophil adherence to the endothelium of gastric microcirculation is critical in NSAID injury and its adherence damages the mucosa by liberating oxygen free radicals, releasing proteases and obstructing capillary blood flow (Jain and Panigrahi, 2012). NSAIDs might induce the synthesis of tumour necrosis factor (TNFŬ) and leukotrienes and these inflammatory mediators stimulate neutrophil adherence by up-regulation of adhesion molecules (Jain and Panigrahi, 2012).

There are some risk factors for NSAIDs ï induced ulcers and they are as follows:

1. Past history of peptic ulcer disease
2. Past history of adverse event with NSAIDs
3. Concomitant use of corticosteroid
4. High or multiple NSAIDs.
5. Individual NSAIDs. Some are higher than others in GI toxicity. Azapropazone and piroxicam are highest compared with ibuprofen.
6. Age. The risk is high at above 60 years.

1.9.4 Free radicals

Oxidative stress is considered to be associated with a number of diseases and has been implicated in the normal aging process (Gems and Patridge, 2008; Omotade and Seun, 2012). Generation of reactive oxygen species (ROS) causes mitochondrial dysfunction which induces cell death in a myriad of pathological conditions (Ott *et al.*, 2007; Samik *et al*., 2011). Development of mitochondrial oxidative stress (MOS) due to increased production of ROS impairs respiratory chain function and depletes cellular ATP leading to apoptosis (Samik *et al*., 2011). MOS disrupts cellular integrity and promotes cell death by down-regulation of survivin, epidermal growth factors (EGF), basic fibroblast growth factor (B_{cl2} and B_{clxl}) leading to tissue injury, organ damage and pathology (Maity *et al.*, 2008; Samik *et al*., 2011). The mitochondrial apoptotic pathway contributes significantly to the pathogenesis of traumatic brain injury and sepsis (Bayir and Kagan, 2008), ischemia and hemorrhagic stroke, acute and degenerative cardiac myocyte death (Hoye *et al.*, 2008), alcoholic liver injury (Bailey and Cunningham, 2002), gastric mucosal injury (Maity *et al.*, 2008) and liver injury in malaria (Guha *et al*., 2006). Free radicals are involved in the progression of ulcers (Prabha *et al.*, 2011). Reactive oxygen species such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite radicals have been reported to play a significant role in oxidative stress related to the pathogenesis of various important diseases (Finkel and Holbrook, 2000; Onasanwo *et al.*, 2011; Rifat-uz-Zaman *et al*., 2013). Lipid peroxidation is an important factor in the deteriorating reaction in food during storage and processing and it is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing and arteriosclerosis (Onasanwo *et al.*, 2011). Reactive oxygen species and lipid radicals are generated during lipid peroxidation and metabolism (Rifat-uz-Zaman *et al*., 2013). The role of ROS and free radicals in tissue damage in such diseases is becoming increasingly recognized (Onasanwo *et al.*, 2011). ROS has been

implicated in the development of mucosal lesions (Bandyopadhyay *et al.,* 2006; Onasanwo *et al.*, 2011). Antioxidants are known to play a significant role in repairing gastric damage (Onasanwo *et al.*, 2011). Antioxidant parameters are reported to be reduced in the stomach tissue that is damaged by indomethacin (Onasanwo *et al.*, 2011). Oxidative free radicals have been implicated in mediating NSAIDs, *H. pylori*, ethanol and cold restraint stress-induced gastric injury (Dharmani *et al.,* 2005; Shobha and Jamadar, 2013). The roles of oxygen radicals have also been determined in the etiology and pathogenesis of indomethacin-induced gastric damage (Onasanwo *et al.*, 2011). The mechanisms of the antioxidant activity may be attributed to a metal-chelating ability, strong hydrogen-donating ability and their effectiveness as scavengers of hydrogen peroxide, superoxide anion and nitric oxide free radicals (Onasanwo *et al.*, 2011).

Exposure to these radicals such as superoxide species, through smoking, environmental pollutants (automobiles, asbestos and industries), excess alcohol, microbial infection and ionizing radiation is a risk factor in PUD development (Omotade and Seun, 2012). Studies have shown that oxidative stress is also involved in the pathogenesis of peptic ulcers (Bairy *et al.*, 2002). Reactive oxygen species generated in the cells of aerobic organisms due to many factors have been implicated in the pathogenesis of many conditions like Parkinsonôs, Alzheimerôs, Huntingtonôs diseases, liver cirrhosis, ulcer, artherosclerosis and cancer (Ajaikumar *et al.*, 2005). The imbalance between ROS accumulation and defense mechanisms in the body contributes to oxidative stress-induced diseases such as cancer, ulcer, inflammation and brain dysfunction (Siddaraju and Dharmesh, 2007). ROS especially hydroxyl radicals (OH^-) play a major role in causing oxidative damage to mucosa which in turn leads to ulcer formation (Siddaraju and Dharmesh, 2007). There is a 1000-fold higher production of ROS in the stomach compared to that in other tissues/plasma which makes the stomach most susceptible to damage by ROS

(Siddaraju and Dharmesh, 2007). ROS equally are involved in the multi-step process towards the development of gastric adenocarcinoma from ulcerous condition (Oliveira, 2003).

1.9.5 Life style risk factors

1.9.5.1 Smoking

Smoking may lead to initiation of ulceration, slow ulcer healing and an increased risk of gastric ulcer recurrence (Morsy and Azza, 2011). Smoking may have an inconsistent effect on gastric acid secretion; however it reduces prostaglandin and bicarbonate production, reduces mucosal blood flow, interferes with the action of histamine H_2 receptor antagonists and accelerates gastric emptying of liquids (Morsy and Azza, 2011).

1.9.5.2 Alcohol

Ethanol as an ulcerogen exerts its action by inducing intense damage in gastric mucosa through the promotion of disturbances of mucosal microcirculation, ischemia and appearance of free radicals, endothelin release, degranulation of mast cells, and inhibition of prostaglandins and decrease of gastric mucus production (Roslida *et al.*, 2010). With cessation of blood flow and loss of delivery of needed oxygen and nutrients, the tissue would be more susceptible to ethanol injury. Ethanol-induced gastric mucosal damage has also been associated with a significant depletion of non-protein sulphydryl (NP-SH) concentrations in the gastric mucosa (Roslida *et al.*, 2010). Leukorienes are one of the important causes of ulceration induced by ethanol (Goel and Sairam, 2002).

The mucosal injury due to ethanol administration consists mainly of separation of the surface epithelium from the underlying lamina propia with complete loss of epithelium resulting in rapid penetration into gastro-duodenal mucosa causing membrane damage (Solanki and Shah, 2013). The subsequent increase in mucosal permeability together with the release of vasoactive products from mast cells, macrophages and other blood cells may lead to vascular injury, necrosis and ulceration (Solanki and Shah, 2013). It is suggested that chronic ethanol administration induces oxidative stress, mainly increasing lipid peroxidation of the cell membrane and this leads to increased membrane fluidity, disturbances of calcium homeostasis and finally cell death (Nordmann, 2000; Solanki and Shah, 2013).

1.9.5.3 Stress

Stress related mucosal disease (SRMD) occurs in critically ill patients: not all patients in an intensive care setting but only those who are extremely ill, such as those with severe trauma, burns greater than one third of the body surface area, major intracranial disease, major surgery, and severe medical illness (Laine *et al*, 2008). Stress-related gastric mucosal injury appears to be related to local ischemia, although progression to significant mucosal injury requires acid (Laine *et al*, 2008). In the absence of acid, gross gastric lesions were minimal, involving 4% of the body and 3% of the antrum surface area. COX-2 expression is therefore assumed to be up-regulated as one of the protective mechanisms when the stomach is exposed to stress such as ischemia/reperfusion (Laine *et al*, 2008).

Reduced local mucosal nitric oxide (NO) generation and increased endothelin-1 (a potent vasoconstrictor) also appear to play an important role in the mechanisms of SRMD (Björne *et al.*, 2005). Endogenous NO plays a dual role in ischemia/reperfusion-induced gastric injury:

constitutive NO synthase/NO is protective, whereas inducible NO synthase/NO is proulcerogenic. The gene expression of inducible NO synthase is markedly up-regulated in the stomach following ischemia/reperfusion, accompanied by a significant increase in the mucosal NO content and supporting a pathogenic role (Kobata *et al.*, 2007). The incidence of SRMD-associated clinically significant bleeding appears to have markedly decreased in the past few decades, most likely because of better care of critically ill patients (Spirt *et al.*, 2004). Critically ill patients may have a decrease in gastric mucosal blood flow through systemic hypotension, but, in addition, patients may have splanchnic hypoperfusion even when systemic hemodynamics are relatively maintained (Spirt *et al.*, 2004). Of interest, mild stress prevents gastric lesions that occur in response to severe stress. This effect is mediated by prostaglandins derived from both COX-1 and COX-2, probably in both the brain and the stomach, and the effect is functionally associated with prevention of the decrease in body temperature that occurs during severe stress (Tanaka *et al.*, 2007). Phospholipase A_2 plays a role, triggering central PG production by COX-1 and COX-2, and thyrotropin-releasing hormone also may be involved in the protective action of mild stress (Laine *et al*, 2008).

The increase in gastric secretion has been considered as a pathogenic mechanism responsible for stress-induced gastric lesions (Onasanwo *et al.*, 2011)**.** Acute stress generates ROS causing gastric injury by various mechanisms (Shobha and Jamadar; 2013). Cold exposure, severe form of stress, mobilizes cathecolaminergic systems in brain area associated with behavioral responses to aversive stimuli and neuroendocrine responses of the hypothalamus-pituitary-adrenal axis including changes in corticotrophin-releasing factor (CRF), adrenocorticotropic hormone (ACTH) plasma levels and adrenergic receptors in the pituitary gland (Landeira-Fernadez, 2004). Stress causes the digestive tract to slow down and more gastric acid is allowed to accumulate in

the stomach (Morsy and Azza, 2011). Increased stomach acidity may predispose to or aggravate an already present ulcer. Stress can also cause changes in appetite, leading to over-eating or lack of appetite (Morsy and Azza, 2011). Overeating causes the stomach to produce more acid while lack of appetite will subject the stomach mucosa to the acid produced in an empty stomach (Morsy and Azza, 2011).

Although psychological stress is no longer considered a direct cause of ulcers, it surely can delay the healing and aggravate already existing gastric ulcers (Morsy and Azza, 2011).

1.10 Endogenous gastro-protective mechanisms

1.10.1 Physiological gastric mucosal barrier

The mechanisms of gastric mucosal defense include several local and neurohormonal protective factors which allow the mucosa to resist frequent exposures to damaging factors (Laine *et al.*, 2008). Gastric mucosal protection is aimed at three main functions either in isolation or in combination which involve antisecretory, cytoprotective and antioxidant activities (Al Mofleh, 2010).

1.10.2 Mucus-bicarbonate-phospholipid barrier

The surface of gastric mucosa is covered by a layer; formed by mucus gel, bicarbonate anions and surfactant phospholipids which are capable of retaining the bicarbonate ions secreted by surface epithelial cells to maintain a microenvironment with a pH near to 7 at the mucus-mucosa interface (Matteo *et al.*, 2011). The mucus layer is also able to prevent the penetration of pepsin, thereby protecting the proteolytic digestion of epithelium (Allen and Flemstrom, 2005). In addition, the luminal surface of mucus gel is covered by a film of surfactant phospholipids which confers hydrophobic properties to the mucus layer (Lichtenberger, 1999). The mucus gel is

secreted by surface epithelial cells and it is composed of a large amount of water (about 95%) and various kinds of mucin glycoproteins, the production of which may vary in different regions of the gastric mucosa (Ho *et al*., 2004; Allen and Flemstrom, 2005). Gel-forming mucin units polymerize into large mucin multimers, which are essential for gel formation. The mucus gel is secreted along with low-molecular weight trefoil factor family (TFF) peptides, which play a relevant role in the formation of the mucus layer (Newton *et al*., 2000). For example, TFF2 is known to increase the viscosity of gastric mucin and stabilize the gel network (Thim *et al.,* 2002). The secretion of gastric mucus is regulated also by various gastrointestinal hormones, including gastrin and secretin, as well as prostaglandins and acetylcholine (Allen and Flemstrom, 2005).

The secretion of bicarbonate into the mucus gel layer is essential to maintain a pH gradient at the epithelial surface, which represents a first line of defense against gastric acid (Allen and Flemstrom, 2005). Bicarbonate secretion from the apical membrane of surface epithelial cells is mediated by a Cl^-/HCO_3^- anion exchanger, and stimulated by various factors, including prostaglandins (via EP1 receptors), luminal acid, corticotropin-releasing factor, melatonin, uroguanylin and orexin A (Allen and Flemstrom, 2005; Montrose *et al.*, 2006).

The mucus-bicarbonate barrier is the only system which segregates the epithelium from the gastric lumen. Therefore, when this protective barrier breaks down during pathological events or upon exposure to ulcerogens/irritants, a second line of protective mechanisms that involve intracellular acid neutralization, rapid epithelial repair, and maintenance of mucosal blood flow will be activated (Matteo *et al.*, 2011). Glycoproteins like sialic acid and hexosamine increase the production of mucus and thus prevent gastric ulceration (Prabha *et al.,* 2011).

1.10.3 Epithelial cells

The continuous layer of surface epithelial cells represents the next line of mucosal defense. This epithelial tissue is responsible for the production of mucus, bicarbonate and other components of the gastric mucosal barrier. These cells are hydrophobic in nature, with the ability to repel acid- and water-soluble irritants/ulcerogenic agents, due to the presence of phospholipids on their surface (Lichtenberger, 1999). Surface epithelial cells are also closely interconnected by tight junctions, forming a continuous barrier, which prevents back diffusion of acid and pepsin (Allen and Flemstrom, 2005). Another relevant protective factor, available in the epithelial cells, is represented by heat shock proteins, which are activated in response to stress, including temperature increments, oxidative stress and cytotoxic agents (Tanaka *et al.*, 2007). These proteins can prevent protein denaturation and protect cells against injury.

Cathelicidin and beta-defensin are cationic peptides which play a relevant role in the innate defensive system at the mucosal surface, preventing bacterial colonization (Yang *et al.*, 2006). In addition, trefoil factor (TFFs) secreted by epithelial cells regulate the re-epitheliazation process and promotes mucosal protective actions (Taupin and Podolsky, 2003).

1.10.4 Mucosal cell renewal

The integrity of gastric epithelium is maintained by a continuous process of cell renewal ensured by mucosal progenitor cells. These cells are subjected to a continuous, well-coordinated and controlled proliferation, which ensures the replacement of damaged or aged cells on the epithelial surface (Matteo *et al.*, 2011). The process of complete epithelial cells renewal takes about 3-7 days, while the overall glandular cell replacement requires months (Matteo *et al.*, 2011). However, the restitution of surface epithelium after damage occurs very rapidly and

results in the migration of preserved cells located in the neck area of gastric glands (Laine *et al.*, 2008).

The process of cell turnover is regulated by growth factors (Matteo *et al.*, 2011). In particular, a marked expression of epidermal growth factor receptor (EGF-R) has been detected in gastric progenitor cells (Matteo *et al*., 2011). Such a receptor can be activated by mitogenic growth factors, such as transforming growth factor-Ŭ (TGF-Ŭ) and insulin-like growth factor-1 (IGF-1) (Nguyen *et al.*, 2007). In addition, PGE_2 and gastrin are able to transactivate the EGF-R and promote the activation of mitogen-activated protein kinase (MAPK) pathway, with consequent stimulation of cell proliferation (Pai *et al.*, 2002). Notably, the presence of EGF has not been detected in the normal mucosa, although it is contained in the gastric juice, as a product of salivary and esophageal glands, and can stimulate mucosal cell proliferation in case of injury (Milani and Calabrò, 2001). In addition, mucosal progenitor cells do express survivin, an antiapoptotic factor, which inhibits apoptotic cell death (Chiou *et al.,* 2005).

1.10.5 Mucosal blood flow

Mucosal ischemia triggers gastric ulcer by inducing tissue necrosis, free radical formation and cessation of nutrient transport, all resulting from vascular and microvascular injury such as thrombi, constriction or other occlusions (Morsy and Azza, 2011).

Mucosal blood flow is essential to deliver oxygen and nutrients, and to remove toxic metabolites from gastric mucosa. Arteries embedded into the muscularis mucosae branch into capillaries, which then enter the lamina propria and travel toward the proximity of glandular epithelial cells (Matteo *et al.*, 2011). Endothelial cells, lining these microvessels, produce NO and prostacyclin (PGI_2), which are potent vasodilators, thus protecting the gastric mucosa against damage and

counteracting the detrimental effects of various vasoconstrictors such as leukotriene C_4, thromboxane A_2, and endothelin (Matteo *et al.*, 2011). In addition, NO and PGI_2 maintain the viability of endothelial cells and inhibit platelet and leukocyte adhesion to the microvasculature, thereby preventing the occurrence of micro ischaemic phenomena (Laine *et al.*, 2008). When the gastric mucosa is exposed to irritants or acid back-diffusion, a massive and rapid increase in mucosal blood flow occurs (Laine *et al.*, 2008). This process allows removal and dilution of back diffusing acid or noxious agents. The increase in blood flow is a mechanism for preventing gastric mucosal cell injury and its decrease results in the development of tissue necrosis. The increase in mucosal blood flow is mediated by NO release and there is experimental evidence demonstrating that NO protects the gastric mucosa against injury induced by ethanol or endothelin 1 while the inhibition of NO synthase enhances mucosal injury (Holzer, 2007).

1.10.6 Sensory innervation

The vasculature of gastric mucosa and submucosa is innervated by extrinsic primary afferent sensory neurons, which are arranged in a plexus at the base of the mucosal layer (Holzer, 2007). The nerve fibers stemming from this plexus run along with capillary vessels and reach the basal membrane of surface epithelial cells and can detect luminal acidity or back-diffusing acid through acid-sensing channels (Matteo *et al.*, 2011). The activation of such sensory nerves modulates the contractile tone of submucosal arterioles leading to the release of calcitonin gene-related peptide (CGRP) and substance P from nerve terminals surrounding large submucosal vessels (Holzer, 2007). CGRP then contributes to the maintenance of mucosal integrity through the vasodilation of submucosal vessels mediated by NO release. Sensory innervation plays a prominent role in the protection of gastric mucosa from injury as demonstrated by studies where

the ablation of sensory transmission with capsaicin impaired the vaso dilatatory response and increased the sensitivity of gastric mucosa to noxious agents (Holzer, 2007).

1.10.7 Prostaglandins

The gastric mucosa represents a source of continuous prostaglandin (PGE_2 and PGI_2) production which are crucial factors for the maintenance of mucosal integrity and protection against noxious agents (Halter *et al.*, 2001; Brzozowski *et al.*, 2005a). It has been demonstrated that prostaglandins have the potential to stimulate almost all the mucosal defense mechanisms (Brzozowski *et al.*, 2005a). They reduce acid output, stimulate mucus, bicarbonate and phospholipid production, increase mucosal blood flow and accelerate epithelial cell restitution and mucosal healing (Brzozowski *et al.,* 2005a). Prostaglandins are also known to inhibit mast cell activation as well as leukocyte and platelet adhesion to the vascular endothelium (Halter *et al.,* 2001; Brzozowski *et al.,* 2005a). The beneficial effects of prostaglandins (PGE_2) have been shown to be mediated by activation of specific EP receptor subtypes and the activation of EP_1 receptors mediates the most important protective effects of prostaglandins, via increase in bicarbonate secretion, mucosal blood flow in the damaged mucosa and a decrease in gastric motility (Takeuchi *et al.*, 2002). Other EP receptor subtypes are also involved in the protective actions of PGE_2 such as EP_3 receptors that inhibit the gastric acid secretion and EP_4 receptors that stimulate the secretion of mucus (Kato *et al.,* 2005).

1.10.8 Neurohormonal mechanisms

Gastric mucosal defense is supported by mechanisms activated, at least in part, by the central nervous system and hormonal factors (Laine *et al.,* 2008). Studies have demonstrated that central vagal activation stimulates mucus secretion and increased intracellular pH in the surface epithelial cells of the stomach (Chatzaki *et al.,* 2006). In addition, the corticotrophin releasing factor (CRF) pathway is involved in endocrine responses to stress (Chatzaki *et al.*, 2006). Moreover, peripheral CRF contributes significantly to the regulation of gastric defense mechanisms especially the CRF_2 receptor known to mediate antiapoptotic effects in gastric epithelial cells and to inhibit gastric emptying and motility (Chatzaki *et al.*, 2006). Other hormone mediators include gastrin-17, cholecystokinin, thyrotropin-releasing hormone, bombesin, epidermal growth factor (EGF), peptide ɔ and neurokinin A, play significant roles in the regulation of gastric protective mechanisms, which can be blunted by afferent nerve ablation, CGRP receptor blockade, and inhibition of NO synthase (Peskar, 2001; Moszik *et al.*, 2001). Ghrelin, a hormone peptide produced in rodents and humans, is involved in the regulation of growth hormone secretion and appetite stimulation (Brzozowski *et al.*, 2005b). Moreover, it is also able to exert significant protective effects at gastric level, through the enhancement of mucosal blood flow via stimulation of NO and calcitonin gene related peptide (CGRP) release from sensory afferent nerves (Brzozowski *et al.*, 2005b). Glucocorticoids have been shown to support the mechanisms of protection at gastric level (Matteo *et al.*, 2011). These hormones are involved in the response to stress and represent potent gastroprotective factors against injury (Filaretova *et al.,* 1998). This gastroprotective activity of glucocorticoids was supported by the studies where glucocorticoid antagonists enhanced the severity of stress-induced erosions (Filaretova *et al.*, 2001). The mechanisms through which glucocorticoids exert their protective effects include the maintenance of glucose homeostasis, increase in mucosal blood flow, mucus

secretion and attenuation of both enhanced gastric motility and microvascular permeability (Filaretova *et al.*, 2007).

1.10.9 Mucosal immune system

The mucosal immune system contributes to mucosal defense against exogenous and endogenous irritants and its impairment can lead to mucosal injury and impairment of endogenous cytoprotective repair mechanisms (Morsy and Azza, 2011). The mucosal immune system is coordinated by innate and adaptive immune response regulated by several mediators released from immuno-regulatory cells. Neutrophils and macrophages infiltrate into the gastric mucosa as a response to HP infection where they release lysosomal enzymes, leukotrienes and reactive oxygen species which impair mucosal defense and drive the immunopathogenetic process of ulcerogenesis (Morsy and Azza, 2011). Bacterial antigens activate T and B lymphocytes and pro-inflammatory cytokines regulate the local and systemic immune response with release of further cytokines and antibodies (Malfertheiner *et al.,* 2009). The type of T-cell response can change the outcome of this infection because T-helper predominant response cause mucosal damage while a high regulatory T-cell response with interleukin-10 release confers gastric ulcer protection (Malfertheiner *et al.*, 2009).

1.11 Endogenous gastro-protective mediators

1.11.1 Nitric Oxide

Nishida *et al* (1998), in their study reported that NO produced by constitutive Nitric Oxide Synthase (cNOS) is cytoprotective and NO produced by inducible nitric oxide synthase (iNOS)

is cytotoxic. Indomethacin affects the tissue levels of NO. It was reported that indomethacin caused up-regulation of endothelin that leads to decreased production of gastric mucosal cNOS. In addition, indomethacin enhances the activity of myeloperoxidase which in turn facilitates the increase in serum NO as the MPO/H2O2 system serves as a major catalytic sink for NO thereby preventing NO feedback inhibition (Galijasevic *et al.,* 2003). Indomethacin-induced gastric injury might be mediated, at least in part, by the reduction in tissue cNOS-derived NO content and enhanced production of iNOS-derived NO in serum (Tarek *et al.*, 2008).

The level of NO was significantly elevated in the serum in ulcerated rats when compared to normal rats. Tarek *et* al, (2008) and Motawi *et al*, (2008) in their studies reported that the ulcerated rats exhibited marked reduction in tissue NO, which further adds to the enhanced neutrophil infiltration.

Studies have shown that in the digestive system, NO produced by cNOS is cytoprotective and NO produced by iNOS is cytotoxic (Motawi *et al.*, 2008). It is well known that NO is involved in the modulation of gastric mucosal integrity and is important in the regulation of acid and alkaline secretion, mucus secretion and gastric mucosal blood flow (Chandranath *et al*, 2002). It has been suggested that NO augments the release of mucus in stomach (Roslida *et al.*, 2010). On the other hand, it was reported that inhibition of NO by nitric oxide synthase (NOS) inhibitors decreased acid secretion in mice and dogs (Roslida *et al.*, 2010). The NO pathway is thought to be involved in promotion of gastric acid secretion through ECL (enterochromaffin-like) cells (Hasebe *et al.*, 2001).

1.11.2 Heat shock protein (HSP)

There have been reports showing the protective role of inducible heat shock protein (HSP) 70 in gastric epithelial cells and that polymorphism of HSP70 gene is not directly associated with the

susceptibility to peptic ulcer (Tahara *et al.*, 2012). There are genetic evidences that HSP70 is protective against gastric ulcer, inflammatory bowel disease-related colitis and lesions of small intestine (Mizushima, 2010). HSPs function mostly as molecular chaperones and are induced by various stresses. HSP70 stimulate ulcer healing (Mizushima, 2010). The A to G transition at position 1267 of the HSPA1B gene was shown to correlate with changes in the level of HSPA mRNA expression (Ghorbani *et al.,* 2014). Thus the HSPA1BA1267G polymorphism may be a marker of susceptibility to peptic ulcer (Ghorbani *et al.,* 2014). Therefore, the abnormality in acid secretion may not be the primary disorder but impairment of the defensive mechanisms (Bigheti *et al*., 2005). When cells are exposed to gastric irritants, expression of heat shock proteins (HSP-70) is induced making the cells resistant to the irritants (de-Faria *et al.*, 2012). HSP-70 accelerates the process of healing by increasing the level of PGE_2 and expression of growth factor (GF) thereby stimulating cell proliferation at the gastric margin and angiogenesis in granulation tissue (Ishihara *et al.*, 2011).

1.11.3 Prostaglandins

In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair (Jince *et al.*, 2010). Prostaglandins can also inhibit the release of a number of inflammatory mediators, such as tumor necrosis factor-alpha from macrophages and interleukin-8 from neutrophils (Martin and Wallace, 2006; Morsy *et al.*, 2010). Tumor necrosis factor-alpha promotes gastric epithelial cell apoptosis and triggers activation of adhesion molecules and leucocyte recruitment, leading to microvascular perturbations (Morsy *et al.*, 2010). Prostaglandin E receptors have a prominent role in mucosal protection and gastric ulcer healing (Takeuchi,

2010). Prostaglandins accelerate ulcer healing in experimental models and the mechanisms responsible for this effect are not fully understood, but it is related to the ability of prostaglandins to reduce gastric acid secretion which contributes to acceleration of ulcer healing (Wallace, 2008). Phenolic compounds, such as tannins and flavonols, have dual effect on prostaglandin biosynthesis; low concentrations stimulate and high concentrations inhibit (de-Faria *et al.*, 2012). These substances stimulate prostaglandin synthesis by reducing substrates for the oxidized intermediates of prostaglandin H synthase (PGHS), thereby accelerating the peroxidase cycle and by protecting PGHC from self-catalyzed inactivation by removing the free radical of PG (de-Faria *et al.*, 2012). It has been reported that PGE_2 not only prevents the formation of irritant-induced gastric ulcer but also enhances gastric ulcer healing. For the effective treatment of gastric ulcers, not only the prevention of further ulcer formation, but also the enhancement of ulcer healing is important. The ability of prostaglandins to stimulate mucus and bicarbonate secretion may also significantly contribute to the promotion of ulcer healing. The endogenous prostaglandins that contribute to ulcer healing are mainly derived from COX-2 the beneficial effects of PGE_2 on gastric ulcer healing in rodents appear to be mediated via EP_4 receptor (Wallace, 2008).

1.11.4 Lipoxins

Lipoxins are biochemical product of conversion of arachidonic acid by cyclooxygenase-2 and 5-lipoxygenase enzymes. Lipoxin-A_4 is an endogenous mediator contributing to resolution of the inflammatory state and thus has an important role in mucosal defense (Morsy and Azza, 2011).

Lipoxin A_4 protects the stomach from aspirin-induced damage via suppressing leukocyte adherence within gastric micro-circulation (Lim *et al.*, 2009). In addition, Lipoxin A_4 can inhibit

inflammatory pain processing and regulate trans-epithelial electrical resistance. Antagonism of Lipoxin A_4 receptor can significantly exacerbate gastric ulcer (Lim *et al*., 2009).

1.11.5 Mucus

Mucus is secreted during superficial damage and provides favorable micro environment during repair process (Kalra *et al.*, 2011). Mucin, a major part of mucus, is a viscous glycoprotein sheet that covers the gastric mucosa and acts as a barrier against ulcerogens (Kalra *et al.*, 2011). The gastric mucus coat is important in preventing and facilitating the repair of the gastric epithelium (Onasanwo *et al.,* 2011). The synthesis of mucus that strengthens the mucosal barrier against harmful agents also has an important function in gastric protection (Andreo *et al.*, 2006).

1.11.6 Sulfhydryl group

Accordingly, the literature reports that endogenous non-protein sulfhydryl (NP-SH) compounds are key compounds in the mucosal protection against ethanol-induced gastric injury (Roslida *et al.*, 2010). Usually the growth in damage is accompanied by decrease of the concentration of mucosal NP-SH compounds, because the SH-groups bind the free radicals formed due to the action of noxious agents (Andreo *et al.*, 2006).

NP-SHs compounds may be involved in scavenging oxygen-derived free radicals and controlling the production and nature of mucus (Roslida *et al.*, 2010). The sulphydryl compounds bind to free radicals that are formed following tissue injury by noxious agents. These agents may also

protect mucus, since mucus subunits are joined by disulfide bridges that, if reduced, render mucus water-soluble (Roslida *et al.*, 2010).

1.11.7 Hydrogen sulfide

Hydrogen sulfide, another gaseous mediator generated endogenously by cystathionine ɓ-synthase and cystathionine ɔ-lyase, causes vasodilatation, decreases adhesion of leukocyte to vascular endothelium, inhibits nonsteroidal anti-inflammatory drugs-induced gastric mucosal injury and inhibits expression of tumor necrosis factor Ŭ (Fiorucci *et al.*, 2006; Tulassay and Herszenyi, 2010). Despite the protective role of this gas against mucosal injury, it is suspected that hydrogen sulphide may contribute to the pro-inflammatory actions in *H. pylori* infection (Morsy and Azza, 2011). Nevertheless, with non-steroidal antiinflammatory drugs, hydrogen sulfide provide gastric protection by inducing up-regulation of antiinflammatory and cytoprotective genes, including hemeoxygenase-1, vascular endothelial growth factor, insulin-like growth factor receptor and several genes associated with the transforming growth factor- ɓ-receptor signaling pathway (Lim *et al.*, 2009).

1.11.8 Hemeoxygenase-1 enzyme

Hemeoxygenase-1 (HO-1), encoded by Hmox1 gene and ubiquitously expressed, is one of the inherent cytoprotective factors involved in repair mechanisms following injury (Gozzelino *et al.*, 2010). Hemeoxygenase-1 is the rate-limiting and inducible enzyme that catalyzes the breakdown of heme into carbon monoxide, iron and biliverdin (Samik *et al.*, 2011; Wu *et al.*, 2011; Morsy and Azza, 2011; Bekyarova, *et al.*, 2013). Biliverdin is converted to bilirubin by biliverdin reductase (Samik *et al.*, 2011). These reaction products of hemeoxygenase-1 (HO-1) have potent

antiinflammatory, antioxidative and vasodilative properties and play an important role in the protection of tissues from several stresses as evidenced from the report that the cytotoxic effects of oxidative stress are worsened in cells that lack HO-1 (Akamatsu *et al.*, 2004; Ueda *et al.*, 2008; Ferreira *et al.*, 2008; Gozzelino *et al.*, 2010 and Wu *et al.,* 2011). Although HO-1 is expressed at low levels in most tissues under normal basal conditions, it is highly inducible in response to various pathophysiological stresses (Wu *et al*., 2011). Hemeoxygenase isoform 1 is a phase II drug detoxifying enzyme induced in response to stress, oxidative stressors, ultraviolet irradiation, inflammatory cytokines, heavy metals, free heme and nonsteroidal antiinflammatory drugs (Inoue *et al.*, 2008; Li *et al.*, 2008 Samik *et al.*, 2011; Morsy and Azza, 2011; Beckam *et al.*, 2011). Up-regulation of hemeoxygenase-1 offers antiapoptotic resistance to the cells due to potent antioxidant effect of bilirubin, biliverdin and carbon monoxide formed (Morsy and Azza, 2011). Hemeoxygenase was shown to protect gastric mucosal cells against nonsteroidal antiinflammatory drugs (Ueda *et al.*, 2008). The mitochondrial translocation of HO-1 resulted in the prevention of NSAID-induced mitochondrial dysfunction, oxidative stress and gastric mucosal injury (Samik *et al.*, 2011). Heat shock proteins such as HSP32 known as heme oxygenase-1 (HO-1) induced following burn, is an adaptive response which can protect gastric mucosa and liver against further oxidative damage (Bekyarova *et al.*, 2013).

1.11.9 Neuropeptides

Neuropeptides involved in gastro-protection are cholecystokinin, gastrin 17, bombesin, corticotrophin-releasing factor, peptide ɔ, ghrelin, orexins and intragastric peptone (Morsy and Azza, 2011). Recent advances in understanding the nature of factors involved in the maintenance of gastric mucosal integrity revealed that the extent of mucosal protection against ethanol

cytotoxicity is influenced by ghrelin, a 28-amino acid peptide hormone produced mainly in the stomach (Konturek *et al.*, 2004; Sibilia *et al.*, 2008). This endogenous ligand for the growth hormone secretagogue receptor has been implicated in the control of local inflammations, healing experimentally induced gastric ulcers, and the protection of gastric mucosa against acute injury by ethanol (Konturek *et al.*, 2004; Sibilia *et al.*, 2008; Ceranowicz *et al.*, 2009). Moreover, ghrelin emerged as an important regulator of the cross-talk between NOS and COX enzyme systems (Sibilia *et al.*, 2008), the products of which, NO and PGE_2, play direct cytoprotective role in maintaining the gastric mucosal integrity under normal physiological conditions (Peskar, 2001; Bronislaw and Amalia, 2010). Ghrelin mediates its gastro-protective effects via prostaglandin E_2, stimulation of nitric oxide production and calcitonin gene related peptide release from sensory afferent nerves as well as enhancement of gastric mucosal blood flow (Morsy and Azza, 2011). Orexins especially orexin-A prevents mucosal injury and gastric ulceration through several mechanisms such as increase in gastric blood flow, elevation of luminal nitric oxide, reduction of lipid peroxidation, generation of prostaglandin E_2 and enhancement of vagal and sensory nerve activities (Nayeb-Hashemi and Kaunitz, 2009).

1.11.10 Peroxisome proliferation-activated receptor

Peroxisome proliferation-activated receptors (Ŭ, ɓ ad ɔ) are nuclear transcription factors that are expressed in the gastrointestinal tract, liver, skeletal muscle, heart, adipose tissue, breast and skin (Morsy and Azza, 2011). These receptors inhibit nuclear factor-əB, the activation of certain inflammatory response genes and regulate transcription of target genes involved in lipid and lipoprotein metabolism, glucose homeostasis and cell differentiation (Morsy and Azza, 2011). Activation of peroxisome proliferation-activated receptors during non-steroidal antiinflammatory

drugs administration blocks the production of inflammatory response markers, such as endothelin-1, vascular cell adhesion molecule-1 in endothelial cells and tissue factors such as matrix metalloproteinase-3 and tumor necrosis factor- Ŭ in macrophages (Lim *et al.,* 2009). These antiinflammatory actions are mediated by inhibition of pro-inflammatory transcription pathways as nuclear factor-əB, activator protein-1 and nuclear factor of activated T cells (Lim *et al.*, 2009).

1.11.11 Growth factors

Growth factors activated as a response to tissue injury are stimuli for cell proliferation, division, migration and re-epitheliazation (Morsy and Azza, 2011). Healing requires angiogenesis in the granulation tissue at the base of the ulcer, together with replication of epithelial cells at the ulcer margins and subsequent re-establishment of glandular architecture (Wallace, 2005). Epithelial and endothelial cell proliferation is largely driven by growth factors (Wallace, 2005). The major growth factor receptor expressed in gastric progenitor cells is the epidermal growth factor receptor (EGF-R) (Laine *et al*., 2008). Epidermal growth factor (EGF) itself is absent in normal gastric mucosa, however it is present in the gastric lumen, derived from salivary and esophageal glands, and can stimulate progenitor cell proliferation in case of injury (Laine *et al*., 2008). The healing of chronic gastric ulcers involves the participation of EGF (Takayama *et al.,* 2011).

Growth factors such as epidermal growth factor, hepatocyte growth factor, platelet derived growth factor and basic fibroblast growth factor activate epithelial cell migration and proliferation and accelerate ulcer healing by binding to their specific receptors on the cell surface, triggering a number of intracellular signaling events that result in cell migration and proliferation. In the stomach, epidermal growth factor triggers mitogenic response and is important for epithelial cell proliferation, migration, re-epitheliazation and reconstruction of

gastric glands (Tulassay and Herszenyi, 2010). Vascular endothelial growth factor is important for angiogenesis, vascular remodeling and mucosal regeneration (Morsy and Azza, 2011). Transforming growth factor- Ŭ protects against gastric mucosal injury and promotes wound healing. Receptors for epidermal and transforming growth factors are expressed in gastric progenitor cells and are trans-activated by gastrin and prostaglandin E_2 that trigger cell proliferation and repair of gastric mucosa (Tulassay and Herszenyi, 2010).

1.11.12 Proteinase-activated receptors (PARs)

PARs have a role in regulating secretion by epithelia of the salivary glands, stomach, pancreas and intestine (Wallace, 2005). Proteinase-activated-2 receptors are expressed throughout the gastrointestinal tract, especially in the epithelial cells and sensory afferent neurons and in the stomach, the activation of these receptors triggers mucus secretion and reduces the extent of stomach endothelial damage induced by non-steroidal antiinflammatory drugs (Morsy and Azza, 2011). This may be through modulating sensory afferent nerves and the release of vascular endothelial growth factor from platelets, which affect new blood vessel angiogenesis that promote ulcer healing (Yoshida and Yoshikawa, 2008).

The proteases that activate PARs are often generated and released during injury and inflammation, and activated PARs orchestrate tissue responses to injury, including hemostasis, inflammation, pain, and repair (Atsufumi *et al.*, 2004). On activation, protease-activated receptor (PAR)-2 modulates multiple gastric functions and exerts mucosal protection via activation of sensory neurons (Toyoda *et al.*, 2003; Atsufumi *et al.*, 2004; Kawabata *et al.*, 2004). The PAR-1 agonist, given systemically, protects against gastric mucosal injury via COX-1-dependent formation of prostanoids, modulating multiple gastric functions and the underlying mechanism is entirely different from that for PAR-2 (Atsufumi *et al.*, 2004).

1.12 Therapeutic interventions

Despite progress in diagnosis and treatment, peptic ulcer disease (PUD) remains a common reason for hospitalization and operation (Wang *et al.*, 2010). Treatment of PUD currently targets reduction of acid secretion, neutralization of secreted acids and enhancement of mucosal integrity (Jain *et al.*, 2007). Many of these drugs can promote both wide range side effects and low efficacy in PUD treatment (Jain *et al.*, 2007).

1.12.1 Goals of therapy

The goals of treatment of peptic ulcer disease are the following:

A. Relief of pain
B. Healing
C. Prevention of relapses
D. Prevention of complications

1.12.2 Conventional antiulcer drugs

The main therapeutic target is the control of gastric secretion using antacids, H_2 receptor blockers like ranitidine, famotidine, anticholinergics like pirenzepin, telezipine or proton pump blockers like omeprazole, lansoprazole (Rao *et al.,* 2003). The prevention or cure of peptic ulcers is one of the most challenging problems in medicine because gastric ulcer therapy faces drawbacks and most of the drugs currently available in the market showed limited efficacy against gastric diseases and are often associated with severe side-effects (Bandyopadhyay *et al.,* 2002; Prabha *et al.*, 2011).

1. Antacids

The only relief from the pain of ulcers has been provided for decades by the neutralization of gastric acid with antacids. Antacids by their alkaline nature act by neutralizing the gastric acid. This neutralization reaction weakens the corrosive effects of the acids and reduces ulcer pains. They also strengthen the mucosal defensive mechanism through their stimulation of prostaglandin production in the mucosa (Mc Quaid, 2004). Moreover, neutralization reduces the peptic activity in the gastric juice and inactivates pepsin at pH above 5 (Arthur and John, 2000).

2. Cytoprotective agents

a. Prostaglandin analogs- Misoprostol

PGE_2 and PGI_2 are the major prostaglandins synthesized by the gastric mucosa. They protect the gastric mucosa against noxious agents by cytoprotective effects that involve reduced acid secretion, increased mucus and bicarbonate secretion as well as increased mucosal blood flow (Willemijntje and Pankaj, 2006). They bind to prostaglandin receptor (EP_3) on parietal cells and stimulate the G1 pathway thereby decreasing intracellular cyclic AMP and gastric acid secretion. Clinically, acid suppression is the main effects of prostaglandin. Misoprostol is a synthetic analog of PGE_2 with higher potency, duration of activity, oral bioavailability and safety (Willemijntje and Pankaj, 2006). Misoprostol is a pro-drug that is rapidly and extensively de-esterified in the parietal cells to form misoprostol acid; active metabolite. Its bioavailability is reduced by antacids and foods; hence should not be taken with food. It is contraindicated in patients with inflammatory bowel disease and pregnancy because it exacerbates inflammatory bowel disease (IBD) and increases uterine contractility. It is used in patients that need NSAIDs to prevent NSAID-induced ulcers (Willemijntje and Pankaj, 2006)

b. Sucralfate

Pepsin mediates the hydrolysis of mucosal proteins thereby contributing to mucosal erosion and ulcerations. This process can be inhibited by sulfated polysaccharides. In an acidic medium, sucralfate inhibits pepsin mediated hydrolysis of mucosal proteins by undergoing extensive cross-linkage to form viscous, sticky polymer that adheres to epithelial cells and ulcer craters (Willemijntje and Pankaj, 2006). Sucralfate equally stimulates local production of prostaglandins, epidermal growth factors and binds bile salts. Sucralfate is a better prophylactic agent against stress-induced ulcers in critically ill patients than proton pump inhibitors (PPIs) and H_2ï receptor antagonist because increased gastric pH is a factor in the development of nosocomial pneumonia in such patients (Willemijntje and Pankaj, 2006). Other conditions where acid suppression is not effective but utilizes sucralfate are oral mucositis, bile reflux gastropathy, radiation proctitis and solitary rectal ulcers.

c. Rebamipide

This is used for the treatment of ulcers especially in parts of Asia. It exerts cytoprotective effects by increasing the production of PGs in gastric mucosa and by scavenging reactive oxygen species (ROS).

d. Ecabet

This also is used for treatment of peptic ulcer disease mostly in Japan. It increases the formation of PGE_2 and PGI_2

e. Carbenoxolone

This is a derivative of glycyrrhizic acid found in liquorice root. It is used for ulcer treatment especially in Europe. It enhances the composition and quantity of mucin.

f. Bismuth compounds

These bind to the base of ulcers, promote mucin and bicarbonate secretion as well as exert antibacterial action. They are used with antibiotics to eradicate *H. pylori.*

3 Histamine H_2 – receptor antagonists

These include cimetidine, ranitidine, famotidine and nizatidine which differ in their pharmacokinetics and propensity to cause drug interaction. They reversibly and predominantly inhibit basal gastric acid secretion by competing with histamine for binding to H_2-receptor on the basolateral membranes of the parietal cells (Willemijntje and Pankaj, 2006). They are less potent than proton pump inhibitors but yet suppress 24 hour gastric acid secretion by about 70% (Willemijntje and Pankaj, 2006).

4 Eradication of *Helicobacter pylori*

Therapeutic interventions to eradicate *H. pylori* are needed to prevent ulcer formation and its transformation to gastric cancer, one of the major complications of chronic gastric ulcer. *H. pylori* eradication therapy comprises a combination of two or more drugs including antimicrobials, proton pump inhibitors and gastro-protective agents (Morsy and Azza, 2011).

Several eradication methods have been suggested. Dual eradication therapy using proton pump inhibitor with amoxicillin has been tried (Graham *et al.*, 2010). Triple eradication therapy employing two antimicrobials together with proton pump inhibitor also showed some success, but not enough to be considered first-line treatment (Castillo-Juarez *et al.*, 2009; Morsy and Azza, 2011). Quadruple *H. pylori* eradication was also successfully tried and consisted of two

antimicrobials, proton pump inhibitor and the gastro-protective agent; colloidal bismuth subcitrate (Zheng *et al.*, 2010). Nowadays, the first line of *H. pylori* eradication therapy is a regimen of 7 or 14 days consisting of a proton pump inhibitor such as omeprazole in combination with clarithromycin and metronidazole. A second regimen that is equally effective uses omeprazole as previously mentioned, together with lower dose of clarithromycin and substituting metronidazole with amoxicillin (Morsy and Azza, 2011).

The conventional eradication triple therapy combines two antibiotics and a proton pump inhibitor. The success rate following this therapy is approximately 80% and is constantly decreasing worldwide, mainly due to antibiotic resistance (Wolle and Malfertheiner, 2007). However, these therapies involve taking too many drugs, which may cause side effects that, in addition to significant cost of the treatment, promote insufficient patient compliance. It has been reported that a 10-day course of levofloxacin triple therapy appeared to be more effective and better tolerated than a 10-day bismuth-based quadruple therapy in the treatment of persistent *H. pylori* infection (Karathapanis *et al.,* 2009). These factors, together with antibiotic resistance, indicate the need to find new anti-*H. pylori* treatments.

Some genetic predispositions for *H. pylori* infection cure rate may exist as exemplified in cytochrome P450-2C19 polymorphism that seems to predict the cure of the infection and predisposition to gastric ulcer (Lay and Lin, 2010). Another example is cytokine genes polymorphism that was significantly associated with persistent infection (Abdiev *et al.*, 2010). Polymorphism of multidrug resistance protein 1 also was reported to influence *H. pylori*-induced gastric inflammation (Tahara *et al*., 2011). Such genetic predisposition gives us hope that the infection predisposing to peptic ulcer and gastric cancer may some day be a target for preventive gene therapy in the near future. Attempts to develop effective vaccination against this bacterium

reached phase I and II clinical trials, and may present effective preventive strategy in preventing gastric ulcer formation and, more importantly, preventing gastric cancer in the future (Majumdar *et al.,* 2011).

Studies have also shown that ingesting lactic acid bacteria exerts a suppressive effect on *H. pylori* infection in both humans and animals while supplementing with *Lactobacillus* and *Bifidobacterium*- containing yoghurt (AB-yoghurt) was shown to improve the rates of eradication of *H. pylori* in humans (Kuan-Yuan *et al.*, 2004). Elimination of *H. pylori* infection leads to a significant increase in the levels of the powerful appetite-stimulating hormone, ghrelin in the tissues of the stomach where it is produced (Tatsuguchi *et al.*, 2004). This may be responsible for the increase in weight of patients undergoing treatment for the eradication of *H. pylori* (Baena *et al*., 2002; Murray *et al.*, 2003). Study has shown that modest consumption of wine and beer (approximately 7 units/week) protects against *H. pylori* infection, presumably by facilitating eradication of the organism (Liam *et al.*, 2002).The major draws back for this multiple therapy are compliance, cost and unpleasant side effects.

5. Proton pump inhibitors (PPIs)

Proton pump inhibitors (PPI); omeprazole and lansoprazole, have an excellent safety profile and have become one of the most commonly prescribed classes of drugs in primary and specialty care. Their mechanism of action involves inhibition of the H^+ K^+ATPase enzyme that is present in gastric mucosal parietal cells (de-Faria *et al.*, 2012). PPIs are the most potent inhibitors of gastric acid secretion available, with efficacy superior to histamine-2 receptor antagonists (Sheen and Tridafilopoulus, 2011).

These drugs have been proven not only to prevent NSAID-induced upper gastrointestinal injury, but also to promote the healing process once the damage has occurred, even in the presence of a continued NSAID administration (Matteo *et al.*, 2011). The beneficial effects of PPIs can be largely ascribed to their ability to maintain a sustained inhibition of gastric acid secretion. There is also evidence to suggest that pharmacodynamic properties unrelated to acid inhibition may contribute to the therapeutic actions of these drugs (Blandizzi *et al.*, 2008).

1.12.3 Non-specific therapeutic measures

This approach plays adjunctive roles in the treatment and management of PUD. The following measures are involved in this approach.

I. Avoidance of smoking. Reduction of smoking or cessation is associated with the relief of existing gastric ulcer symptoms.
II. Stress management. This will enhance ulcer healing and can be achieved by engaging in activities that will reduce both physical and psychological stresses.
III. Restriction of Alcohol Consumption. People predisposed to gastric ulcer may dilute alcoholic beverages to reduce their concentration, restrict the number of drinks, replace wine with less toxic content or avoid non-alcoholic drinks (UMHS, 2005).

1.12.4 Herbal and natural products for the treatment of peptic ulcer disease

Reports on clinical evaluation of conventional antiulcerogenic drugs showed that there are incidences of relapses, adverse effects and danger of drug interactions during ulcer therapy (Goel and Sairam, 2002). The high cost of newly available drugs for peptic ulcer disease is responsible for the persistence, morbidity and mortality of the disease in third world countries due to low per

capita income (Shayne, 2002). As a result, the search for an ideal antiulcer drug continues and has also been extended to herbs for new and novel molecules which afford better protection and reduction in incidence of relapse (Goel and Sairam, 2002).

Herbal drugs are used widely even when their biologically active compounds are unknown because of their assumed effectiveness, availability, lesser side-effects and relative low cost (Prabha *et al.,* 2011; Ezekwesili *et al*., 2014). About 60% of the worldôs population relies almost entirely on plants for medications and natural products, which have long been recognized as an important source of therapeutically effective medicine (Ajaikumar *et al.*, 2005).

Efforts are focused on more effective and safer new antiulcer agents from natural products where some plant extracts can be valuable source of new therapeutics in the treatment of PUD (Schmeda and Yesilada, 2005). The beneficial effects of herbal and plant extracts isolated or in combination, in the prevention of gastric injury have been evaluated in several experimental studies involving antisecretory, cytoprotective and antioxidant mechanisms (Al Mofleh, 2010). Herbs, medicinal plants, spices, vegetables and crude drug substances are considered to be a potential source to control various diseases including gastric ulcer (Awaad *et al*., 2013). Plants with antiulcerogenic activity were used either as raw materials which are obtained by extraction with solvents or as individual isolated compounds (Awaad *et al*., 2013).

The roots of *Saussurea lappa* C.B. Clarke (Asteraceae) were found to have protective activity against peptic ulcer through its cytoprotective effect (Sutar *et al*., 2011).

Cissampelos mucronata leaf extract possesses antiulcer activity against indomethacin, histamine and stress-induced ulcers in rats (Akah and Nwafor, 1999).

The ethanol extract of *Zizyphus lotus* (L.) Lam (Rhamnaceae) root possesses antiulcerogenic activity through increase in prostaglandin synthesis (Jadhav and Prasanna, 2011). Its antiulcer activity was attributed to flavonoid constituents (Jadhav and Prasanna, 2011).

It was reported that root bark, leaves and fruits extracts of *Zizyphus lotus* (L.) Lam possess significant and dose-dependent inhibition of acute ulcer induced by HCl/ethanol (Wahida *et al*., 2007).

Extracts of *Quassia amara* L. (Simaroubaceae) bark was reported to possess antiulcerogenic effect in acute ulcer induction models related to an increase in gastric barrier mucus and non-protein sulfhydryl groups (Garcia-Barrantes and Badilla, 2011).

Ethanol extract of coconut seed (*Cocus nucifera* L., Arecaceae) possesses antiulcer activity (Anosike and Obidoa, 2010).

The ethanol extract of *Encholirium spectabile* Mart. (Bromeliaceae) aerial parts produces antiulcerogenic effect against ulceration induced by absolute ethanol, ethanol/HCl, ibuprofen and ischemia/reperfusion due to the activation of antioxidant systems and the involvement of prostaglandins and the NO synthase pathway (Carvalho *et al*., 2010).

The methanol extract of *Cissus quadrangularis* L. (Vitaceae) demostrated protective activity against gastric ulceration using aspirin-induced gastric ulcer model (Shanthi *et al*., 2010). Alcohol extract of *Gynura procumbens* Merr. (Asteraceae) leaves exhibits significant protection against ethanol-induced injury (Mahmood *et al*., 2010).

Zingiber officinale (Roscoe) (Zingiberaceae) rhizome extracts show antiulcerogenic activity against ethanol and acetic acid-induced ulcer and also prevented the oxidative damage of gastric mucosa by blocking lipid peroxidation, decreasing superoxide dismutase and increasing catalase activity (Arun *et al*., 2010). Other workers showed that ethanol extract possesses good protective

effect against indomethacin-induced gastric ulcer in rats (Anosike *et al*., 2009). Studies further show that ginger extract possesses antiulcer properties through augmentation of mucin secretion and decreased cell shedding (Agrawal *et al*., 2000; Moshen *et al*., 2006).

The extract of leaves of *Butea frondosa* Roxb (Fabaceae) show protective effects against ulcers induced by HCl (Londonkar and Ranirukmini, 2010).

Ethanol extracts of leaves of *Parkia platycephala* Benth. (Leguminosae) show gastroprotective activity against ethanol- and ethanol/HCl-induced gastric damage mediated in part, by the nitric oxide release (Fernandes *et al*., 2010). The extract protects against lesions induced by ischemiaï reperfusion with antioxidant effect through increase in catalase activity (Fernandes *et al*., 2010).

Extracts of leaves of *Anacardium humile* St. Hil (Anacardiaceae) protect gastric mucosa against absolute alcohol-induced ulcer and this protection might be due to increased PGE_2 and mucous production (Ferreira *et al*., 2010).

Bark extract of Red mangrove, *Rhizophora mangle* L. (Rhizophoraceae) produces gastric protection through increase in mucus content and a proportional increase in proteins (Sa´ nchez *et al*., 2001). It shows gastroprotective and antisecretory effect in addition to increase in PGE_2 levels in a dose dependent manner (Sa´ nchez *et al*., 2010).

The bark of *Excoecaria agallocha* L. (Euphorbiaceae) extract decreases the acidity and increases the mucosal defense in the gastric areas (Thirunavukkarasu *et al*., 2009).

The extracts of *Erythrina indica* L. (Febaceae) leaves possess antiulcer properties against pylorus-ligated and indomethacin-induced ulceration and the effect may be attributed to the presence of polyphenolic compounds (Sachin and Archana, 2009).

Liquorice or *Glycyrrhiza glabra* L. (Fabaceae) leaves, roots and seeds extracts have significant mucosal protective and antioxidant effects on the gastric mucosa in rats (Ligha and Fawehinmi, 2009).

The extracts of bark of *Virola surinamensis* Kuntze (Myristicaceae) inhibit mucosal injury induced by indomethacin, stress and pylorus ligature (Hiruma-Lima *et al*., 2009).

The stem bark of *Combretum leprosum* Mart. & Eiche (Combretaceae) possesses gastroprotective and anti-ulcerogenic effects which are related to the inhibition of the gastric acid secretion and an increase of mucosal defensive factors (Nunes *et al*., 2009).

Gymnosporia rothiana (Walp.) (Celastraceae) leaves extracts show significant reduction in ulcer lesion index against ethanol and indomethacin-induced gastric injury by decreasing the volume and elevating the pH of gastric content, increasing prostaglandin and free radical scavenging activity which are attributed to its high levels of terpenoids such as ɓ-amyrin, lupeol and friedelin (Jain and Surana, 2009a).

Spathodea falcate (Bignoniaceae) bark possesses cytoprotective effect against gastric lesions induced by ethanol and indomethacin through decrease in volume and increase in pH of gastric content (Jain and Surana, 2009b).

A polyherbal formulation of *Glycyrrhiza glabra* L. (Fabaceae; Root), *Saussurea lappa* Clarke (Asteraceae; Root), *Aegle marmelos* (Rutaceae; Fruit) and *Santalum album* L. (Santalaceae) stem has been reported to possess antiulcerogenic activity against ulcers induced by cold restraint stress and aspirin (Muralidhar *et al*., 2009). The cytoprotective effect of this product may be by the enhancement of defensive mechanism through an improvement of gastric cytoprotection as well as acid inhibition (Muralidhar *et al*., 2009).

Other plants reported to possess antiulcerogenic activities include *Trixis divaricata* Spreng. (Asteraceae) (Pereira *et al*., 2005), *Byrsonima crassa* Niedenzu (IK) (Malpighiaceae) leaves (Sannomiya *et al*., 2005), *Zataria multiflora* Boiss. (Lamiaceae) (Minaiyan *et al*., 2005), *Pausinystalia macroceras* (K. Schum.) stem bark (Nwafor *et al*., 2005), *Ageratum conyzoides* L. (Asteraceae) leaves (Mahmood *et al*., 2005), *Ocimum sanctum* Linn. (Labiatae) (Singh and Majumdar, 1999) and *Curcuma longa* Linnaeus, (Zingiberaceae) (Rafatullah *et al*., 1990), *Momordica species* (Alam *et al.,* 2009; Dhasan *et al.*, 2010; Vijayakumar *et al.*, 2011), extracts of *Moringa oleifera* (Devaraj *et al.*, 2007, Kansara and Singhal, 2013) and extracts of the leaves and barks of *Sesbania species* (Sertie *et al.*, 2001; Bhalke *et al.*, 2010).

Other plants that possess antiulcerogenic activities are shown in Table 2.

Plant extracts have shown some activities against *H*. p*ylori* activity thereby offering a veritable source of antibacterial agents for the eradication of *H*. *pylori*.

Extracts of *Impatiens balsamina* L. (Balsaminaceae) possess anti-*H*. *pylori* (Yuan-Chuen *et al*., 2011).

The ethanol extracts of *Bixa orellana* L. (Bixaceae) seed, *Chamomilla recutita* L. (Asteraceae) inflorescence, *Ilex paraguariensis* A. (Aquifoliaceae) leaves, *Malva sylvestris* L. (Malvaceae) inflorescence and leaves, *Plantago major* L. (Plantaginaceae) aerial parts and *Rheum rhaponticum* L. (Polygonaceae; Root) possess inhibitory activity against *in vitro* growth of *H*. *pylori* (Cogo *et al*., 2010).

Carotenoids isolated from *Malus domestica* Borkh. (Rosaceae) peel extracts exhibit potent anti-*H*. *pylori* activity (Molna´ *et al*., 2010).

Aqueous extract of *Enantia chlorantha* Oliv. (Annonaceae) stem bark possesses both *in vitro* and *in vivo* activities against *H*. *pylori* (Tan *et al*., 2010).

Nigella sativa L. (Ranunculaceae) seeds extract inhibit the growth of *H. pylori* (OôMahony *et al.,* 2005).

Solanum lyratum Thunb, *Solanum erianthum* and *Solanum torvum* (Solanaceae) extracts show inhibitory activity against *H. pylori* (Hsu *et al*., 2010a, b).

Table 2: Plants with antiulcerogenic effects

S/No	Name of plant	Family	Parts used	References
1	*Adhatoda vasica* Nees	Acanthecaceae	Leaves	Shrivastava *et al*., 2006
2	*Ageratum conyzoides* L.	Asteraceae	Leaves	Mahmood *et al*., 2005.
3	*Alchornea castaneaefolia*	Euphobiaceae	Leaves	Hiruma-Lima *et al.,* 2006.
4	*Aloe vera*	Xanthorrhoeaceae	Leaves	Subramanian *et al*., 2007.
5	*Anacardium humile* St. Hil.	Anacardiaceae	Leaves	Ferreira *et al*., 2010.
6	*Butea frondosa* Roxb.	Fabaceae	Leaves	Londonkar and Ranirukmini., 2010
7	*Carica papaya* L.	Caricaceae	Fruit*s*	Ologundudu *et al*., 2008
8	*Caryocar coriaceum*	Caryocaraceae	Fruits	Oliveira *et al*., 2009.
9	*Ceiba pentandra*	Bombacaceae	Stem bark	Ibara *et al*., 2007.
10	*Cissus quadrangularis* L	Vitaceae	Stem bark	Shanthi *et al*., 2010
11	*Coccinia gramdis* Inn.	Cucurbitaceae	Leaves	Mazumder *et al.,* 2008.
12	*Cocus nucifera* L	Arecaceae	Seed	Anosike and Obidoa, 2010
13	*Combretum leprosum* Mart. & Eiche	Combretaceae	Stem bark	Nunes *et al*., 2009.

14	*Croton zehntneri*	Euphorbiaceae	Leaves	Oliveira *et al*., 2009.
15	*Encholirium spectabile* Mart	Bromeliaceae	Aerial parts	Carvalho *et al*., 2010
16	*Erythrina indica L.*	Fabaceae	Leaves	Sachin and Archana, 2009.
17	*Excoecaria agallocha* L.	Euphorbiaceae	Stem bark	Thirunavukkarasu *et al.,* 2009
18	*Glycyrrhiza glabra* L.	Fabaceae	Leaves, roots and seeds	Ligha and Fawehinmi, 2009.
19	*Gymnosporia rothiana* Walp.	Celastraceae	Leaves	Jain and Surana, 2009a
20	*Gynura procumbens* (Merr.)	Asteraceae	Leaves	Mahmood *et al*., 2010
21	*Helicrysum mechowianum*	Asteraceae	Leaves	Ibara *et al*., 2007.
22	*Himatanthus drasticus*	Apocynaceae	Latex	Oliveira *et al*., 2009.
23	*Indigofera truxillensis* Kunth	Fabaceae	Arial part*s*	Cola-Miranda *et al*., 2006.
24	*Lasiathera Africana* P. Beauv.	Icacinaceae	Leaves	Okokon *et al*., 2009.
25	*Linum usitatissimum* Linnaeus	Linaceae	Arial parts	Dugani *et al*., 2008.
26	*Matricaria chamomilla* L.	Asteraceae	Flowers	Karbalay-Doust and Noorafshan, 2009.
27	*Menthe arvensis* L.	Lamiaceae	Leaves	Londonkar and Poddar, 2009.
28	*Morus alba* L.	Moraaceae	Leaves	Abdulla *et al*., 2009.

29	*Orostachys japonicas*	Crassulaceae	Arial parts	Jung *et al*., 2007.
30	*Parkia platycephala* Benth	Leguminosae	Leaves	Fernandes *et al*., 2010
31	*Polyalthia longifolia*	Annonaceae	Leaves	Malairajan *et al*., 2008.
32	*Quassia amara* L.	Simaroubaceae	Stem bark	Garcia-Barrantes and Badilla, 2011
33	*Rhizophora mangle* L.	Rhizophoraceae	Stem bark	Sanchez *et al*., 2001.
34	*Solanum nigrum*	Solanaceae	Fruits	Jainu and Shyamala, 2006.
35	*Spathodea falcate*	Bignoniaceae	Stem bark	Jain and Surana, 2009b
36	*Strychnos potatorum* Linn.	Loganiaceae	Seeds	Sanmugapriya and Venkataraman, 2007
37	*Stryphnodendron rotundifolium*	Fabaceae	Leaves	Oliveira *et al*., 2009.
38	*Syzygium aromaticum* L.	Myrtaceae	Flower buds	Magaji *et al*., 2007.
39	*Terminalia chebula* Retz.	Combretceae	Fruits	Raju *et al*., 2009.
40	*Tripleurospermum disciforme*	Asteraceae	Flowers	Minaiyan *et al*., 2006.
41	*Vanillosmopsis arborea*	Asteraceae	Stem bark	Oliveira *et al*., 2009.
42	*Virola surinamensis* Kuntze	Myristicaceae	Stem bark	Hiruma-Lima *et al*., 2009.
43	*Zinger officinale* (Roscoe)	Zingiberaceae	Rhizome	Agrawal *et al*., 2000 Moshen *et al*., 2006 Anosike *et al*., 2009 Arun *et al*., 2010

Prunus mume Siebold and Zucc. (Rosaceae) extracts were reported to possess inhibitory effect on *H. pylori* infection and antiinflammatory effect on gastric mucosa (Enomoto *et al*., 2010).

Fresh broccoli sprout methanol extract of *Brassica oleracea* L., (Brassicaceae) possesses inhibitory effect on *H. pylori* (Moon *et al*., 2010).

Byrsonima crassa Nied. (Malpighiaceae) methanol and chloroform extracts inhibit the growth of *H. pylori in vitro* (Bonacorsi *et al*., 2009).

Curcumin from *Curcuma longa* Linnaeus (Zingiberaceae) has been reported to inhibit *H. pylori* growth (Chowdhury *et al*., 2009).

Methanol extracts of *Lycopodium cernuum* Linn (Lycopodiaceae) were reported to possess anti-*H. pylori* activity (Ndip *et al*., 2007; Ndip *et al*., 2008).

Essential oil obtained from the dried aerial parts of *Thymus caramanicus* Jalas (Lamiaceae) show inhibitory activity against *H. pylori* (Fereshteh *et al*., 2009).

Crude essential oil of *Dittrichia viscosa* L. (Asteraceae) and its oxygenated fractions exhibit anti-*H. pylori* activity (Miguel *et al*., 2008).

Methanol extracts of some plants such as *Ageratum conyzoides* (Asteraceae), *Scleria striatinux* (Cyperaceae), *Lycopodium cernua* (Linn) (Lycopodiaceae), *Acanthus montanus* (Acanthaceae),

Eryngium foetidium (Apiaceae), *Tapeinachilus ananassae* (Costaceae), *Euphorbiahirta* (Euphorbiaceae), *Emilia coccinea* (Asteraceae) and *Scleria verrucosa* (Cyperaceae) possess anti-*H. pylori* activities (Ndip *et al.,* 2007).

Bark of *Tabebuia impetiginosa* (Bignoniaceae) exhibits strong activity against *H. pylori* (Park *et al*., 2006).

Crude extracts as well as the isolated compounds from *Santalum album* (Santalaceae) possess antibacterial activity against *H. pylori* (Ochi *et al*., 2005).

Methanol extracts of both *Sanguinaria canadensis* L. (Papaveraceae; rhizomes) and *Hydrastis canadensis* L. (Ranunculaceae; roots and rhizomes) inhibit the growth of *H. pylori in vitro* (Mahady *et al.,* 2003).

The plant extracts with antiulcerogenic properties are due to the presence in part or combination of alkaloids, terpenoids, saponins, phenolic compounds and polysaccharides (Awaad *et al*., 2013). Natural products exhibit their antiulcerogenic activities through different mechanisms; either prophylactically or therapeutically or both. The prophylactic products possess their effect by their antioxidant potentials or their antiinflammatory activity while the therapeutic agents either have antisecretory or healing effects (Awaad *et al*., 2013). In addition to these, the anti-*H. pylori* activity of some plant extracts may explain their antiulcerogenic activity (Awaad *et al*., 2013). The prophylactic (gastroprotective or cytoprotective) mechanism is based on the ability to strengthen defensive factors like prostaglandin synthesis, somatostatin synthesis and an inhibitory effect on gastrin secretion (Muralidhar *et al*., 2009; Thirunavukkarasu *et al.,* 2009; Ferreira *et al*., 2010). The participation of the antioxidant mechanisms on the gastroprotective effect prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and by

significant decrease in superoxide dismutase and increase in catalase activity (Carvalho *et al*., 2010; Almeida *et al*., 2011).

1.12.5 Non-conventional gastro-protective drugs

A number of drugs, other than traditional anti-ulcer medications have been evaluated for anti-ulcer potentials and proved to possess antiulcerogenic activity. Stress causing hypertension may concomitantly predispose to gastric ulcer (Morsy and Azza, 2011). The antihypertensive drugs such as angiotensin (AT_1) receptor blockers, telmisartan and candesartan can prevent gastric ulcer formation, with telmisartan having higher potency than candesartan (Morsy *et al*., 2009). The protective effect of telmisartan on gastric mucosal ulceration induced by non-steroidal antiinflammatory drugs is possibly through its antioxidant action and activation of peroxisome proliferation-activated receptor ɔ (Morsy *et al.,* 2009).

Gastric ulcer is also commonly seen concurrently in type 2 diabetic patients (Morsy *et al*., 2010). The possible gastro-protective effects of insulin sensitizers, thiazolidinediones (eg. rosiglitazone) and metformin are attributed to their ability to ameliorate oxidative stress and inflammation, antisecretory actions, enhanced mucosal protection, antioxidant activity, increased mucin concentrations and gastric mucosal nitric oxide levels rendering them attractive candidates for the prevention of gastric ulcer in patients with type 2 diabetes (Morsy *et al*., 2010). In addition, rosiglitazone increased gastric juice pH, providing superior gastro-protection to metformin (Morsy *et al*., 2010).

Another antidiabetic drug, pioglitazone has a gastroprotective activity being an agonist of peroxisome proliferation-activated receptor ɔ and exerts strong effect in preventing the formation of gastric ulcers and healing of already existing ones (Konturek *et al.,* 2010). This gastric ulcer

preventing/healing effect of pioglitazone is, at least in part, mediated by endogenous nitric oxide (Konturek *et al.,* 2010). Astonishingly, under diabetic conditions, pioglitazone gastro-protective effect is decreased. The attenuation of pioglitazone action is possibly due to reduction in nitric oxide production, angiogenesis and increased expression and release of pro-inflammatory cytokines under diabetic conditions (Konturek *et al*., 2010).

Organoselenium compounds have shown not only gastro-protective and ulcer healing effects, but also possessed antibacterial effect against *H. pylori* (Santhosh *et al*., 2010). When tested on indomethacin-induced gastric ulcer in mice, melatonin demonstrated gastro-protective effects via angiogenic properties through up-regulation of matrix metalloproteinase-2; an important regulator of angiogenesis (Ganguly *et al.,* 2010).

1.13 Botanical profile of *Bridelia ferruginea*

1.13.1 Plant taxonomy

Kingdom ..... Plantae

Division..... Angiospermae

Class..... Archichlamydeae

Order..... Geraniales

Family..... Euphorbiaceae

Genus ..... *Bridelia*

Specie..... *ferruginea*

Vernacular namesé é é é é é é é é é é é .ñKirniò, ñKizniò (Hausa)

ñMarehiò (Fulani)

ñIralodanò (Yoruba),

ñOlaò (Ibo)

ñKensangeò (Boki) (Ekanem *et al*., 2008).

1.13.2 Plant description

This is a plant (Figure 1) which grows up to 3-4 m high and may be 27.5 cm in width (Olatunji *et al.,* 2010; Ezike *et al.*, 2011). The stem is often crooked with branches occurring at the lower regions (Figure 2). The bark is gray, rough and often scaly (Rashid *et al*, 2000). The plant often bears spines and may be crimson coloured. The leaves (Figure 3) may be small to medium sized,

Figure 1: *B. ferruginea* plant

Figure 2: *B. ferruginea* stem

Figure 3: *B. ferruginea* fruits and leaves

simple, alternate, spiral or distichous, broadly elliptic and pubescent (GHP, 1992). They are pinnately veined with entire margin and an acuminate or acute apex (GHP, 1992). The flowers are creamy-yellow and sweet scented and appear between February and August, while the fruits occur in July ï September (Iwu, 1993).

1.13.3 Geographical distribution

Bridelia species belong to the family Euphorbiaceae and comprise approximately 60-70 species found in Asia, Africa and Australia (Rashid *et al*., 2000; Kathriarachchi *et al*., 2005; Ngueyem *et al.*, 2009). It occurs commonly in the guinea savannah and coastal plains of Africa particularly Burkina Faso, Cote dôIvoire, Ghana, Nigeria and Togo as well as Asia and Australia (GHP, 1992; Addae-Mensah, 1992).

1.13.4 Ethnomedicinal uses

Bridelia ferruginea is utilized in traditional African Medicine in treating disease conditions such as arthritis, bruises, boils, dislocation, burns, fever, headaches, stiffness, rheumatic pains and oedema (Olumayokun *et al.*, 2012). Other uses include intestinal disorders, diabetes, thrush, epilepsy, infectious diseases including sexually transmitted diseases, skin diseases and eruption, skin cancers, roundworm (Cimanga *et al*., 2001). It is also an antidote for arrow poison, (Ngueyem *et al.,* 2009) and used as antiinflammatory (Olajide *et al*., 2003) and antitumor agent (Rhashid *et al.*, 2000).

In Ezimo, Udenu LGA, Enugu State Nigeria, the fresh stem bark paste is used to arrest bleeding from fresh wound. This is accomplished by placing a freshly prepared paste of the stem bark on the wound. It is used also to treat gastrointestinal disorders.

1.13.5 Literature review

Reports on the plant have shown that aqueous leaf and root extracts of the plant possess hypoglycemic activities (Addae-Mensah and Achenbach, 1985; Onunkwo *et al.*, 1996; Bakoma *et al.*, 2011).

Ethnopharmacological reports have shown that the stem bark extract possess antiulcer properties (Ezike *et al.*, 2011) and antiinflammatory and antibacterial (Olajide *et al.*, 2003) properties. Research further indicates that extract of the stem bark possesses antioxidant properties (Adetutu *et al.*, 2011), antipyretic and analgesic activities (Akuodor *et al.,* 2011). The aqueous stem bark extract possesses antihypertensive, diuretic and sedative actions (Nene-Bi *et al.*, 2010; Nene-Bi *et al.*, 2012). The stem bark extracts possess antioxidative and neuroprotective activities (Omotade, 2012). Rutin, one of the active constituents of *B. ferruginea* lowered blood sugar level of fasted rabbits (Addae-Mensah and Achenbach, 1985).

Furthermore, studies have shown that the aqueous extract of *B. ferruginea* stem bark reduces vascular permeability in both cyclophosphamide-induced hemorrhagic cystitis and acetic acid-induced vascular permeability in rats and mice (Olajide *et al.*, 2000). *B. ferruginea* also produced stabilization of erythrocytes exposed to heat and stress-induced lysis (Olajide *et al.*, 2000). Studies have shown that the stem bark extracts exhibit antiinflammatory properties, which were attributed to the suppression of up-regulation of tumour necrosis factor alpha (TNFŬ) (Olajide *et al.*, 2003). *B. ferruginea* stem bark extract inhibits xanthine oxidase and possesses superoxide scavenging activity due the presence of 3-O-methylquercetin, myricetin, ferrugin and quercetin 3-O-glucoside (Cimanga *et al.*, 2001).

The stem bark and leaf extracts have contractile effects on the smooth muscle of the bladder (Onoruvwe *et al.*, 2001). The extracts of *B. ferruginea* possess anti-thrombotic effects

(Olumayokum, 1999). Leaf extracts of *B. ferruginea* show the presence of two coumestanflavonoids, bridelilactone and bridelilactoside (Okunji, 1982). There are aesculetin and scopoletin as well as flavonoids from the petroleum spirit extract of the leaf (Okunji, 1982). Petrol extract and aqueous methanol extracts contain respectively terpenoids and flavonoid glycosides (quercetin-3-neohesperiaoside (rutin)) (Addae-Mensah and Achenbach, 1985). Chemical and pharmacological studies of *Bridelia species* have shown the presence of flavonoids, sesquiterpenes, triterpenoids, and phenolic compounds (Ngueyem *et al.,* 2009). *Bridelia species* possess variety of biological activities including antiamebic, antianemic, antibacterial, anticonvulsant, anti-diabetic, antidiarrhoeal, antihelmintic, antiinflammatory, antimalarial, antinociceptive, antiviral, and hypoglycemic (Ngueyem *et al.,* 2009).

1.14 Aims of the study

This study was aimed at investigating the antiulcer activity of extract of the stem bark of *Bridelia ferruginea* Benth and isolating the phytoactive principle responsible for the antiulcer activity.

The specific objectives were to:

a) elucidate the antiulcer activity profile of the extract using rodent models of experimentally-induced ulcer;
b) investigate the likely mechanisms of antiulcer effects using *in vitro* models;
c) isolate and characterize the antiulcer phytoconstituents.

CHAPTER TWO

2. Materials and methods

2.1 Materials

2.1.1 Animals

Adult male albino rats (120ï 150 g) and mice (30 ï 32 g) of both sexes were used. The rats and mice were obtained from the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The animals were handled according to standard protocols.

2.1.2 Chemicals, solvents and reagents

All the chemicals, reagents and solvents used (chloroform, methylene chloride, ethylacetate, formic acid, n-hexane, methanol, sephadex LH-20, silica gel and acetone) were of analytical grade.

2.1.3 Drugs

Indomethacins, carbenoxolone, N-ethylemaleimide (NEM), L-Nitroarginine methylester (L-NAME) were purchased from Sigmaï Aldrich (St. Louis, MD, USA) while cimetidine and omeprazole were manufactured by Jiangx Xier Kangtal Pharmaceutical Co Ltd Ind. North Zone New Technology Zone Jiangxi, China.

2.2 Methods

2.2.1 Collection of plant materials

Fresh stems of *B. ferruginea* were cut from plants growing in bushes in Ezimo, Udenu L.G.A., Enugu State, Nigeria, in October 2010. The plant was identified and authenticated at the International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria.

A voucher specimen (BFSB2011) of the plant was kept in the herbarium of the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The bark was separated from the stem, cleared of the dead outer parts and thorns, cut into smaller pieces and dried away from direct sunlight for 1 week. The dried plant material was milled into powdered form.

2.2.2 Extraction

The powdered plant material (8 kg) was extracted by cold maceration in methanol (100%) for 96 h. It was filtered and washed with methanol. Removal of the solvent in *vacuo* in a rotary evaporator gave the methanol extract (2.01 kg).

2.2.3 Phytochemical analysis of extract

The methanol extract (ME) was analysed for the presence of carbohydrate, tannins, flavonoids, terpenes, steroids, saponins, anthraquinones and alkaloids (Evans, 1996).

Test for flavonoids

5 ml of the methanol extract was added to 1 ml of concentrated sulphuric acid and 0.5 g of magnesium. A pink or red coloration that disappears on standing for 3 minutes indicates the presence of flavonoids.

Test for tannins

About 1 ml of the methanol extract was added to 2 ml distilledwater in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or blue-black coloration (Gallic tannins).

2 ml of the extract was added to 2 ml of distilled water and 1 to 2 drops of diluted ferric chloride solution added. A dark green coloration indicates the presence of tannins.

Test for saponins

To 1 ml of methanol extract was added few ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 minutes.

Test for alkaloids

a. 10 ml of concentrated etheric solution was evaporated to dryness. The dry residue was added to 1.5 ml HCl (2%) acid solution. Thereafter, 1 to 2 drops of Mayerôs reagent and Wagnerôs reagent were added. Yellow-white precipitate indicates the presence of the alkaloidal base.
b. 20 ml of methanol extract was evaporated to dryness. The residue was dissolved in 5 ml of HCl (2N) and filtered. A few drops of Mayerôs reagent and Wagnerôs reagent were added. The presence of precipitate indicates the alkaloids.
c. To 15 ml of the methanol extract was added 2 ml of 10% ammonium hydroxide solution (pH 7). The alkaloid was extracted 3 times with 10 ml chloroform. The chloroform layer was washed 3 times with 2 ml HCl (10%). This was divided into two portions. To one part was added Mayerôs reagent and to the second portion was added Wagnerôs reagent. The formation of a brown or white precipitate was regarded as positive for the presence of alkaloids.

Test for steroids

10 ml of the methanol extract was evaporated to dryness. The residue was dissolved in 0.5 ml of hot acetic anhydride. The solution was filtered and 0.5 ml of the filterate was added

chloroform. Thereafter, Libermann Burchardt reagent was added. The appearance at the interphase of a blue-green ring indicates the presence of steroids.

Test for anthraquinones

2 ml of methanol extract was added 2 ml of ammonium hydroxide solution (10%). A bright pink colour indicates the presence of anthraquinones (Borntragerôs test).

Test for terpenoids

To 5 ml of methanol extract were added 2 ml of chloroform and 3 ml of sulphuric acid. Formation of of reddish brown color indicates the presence of terpenoids.

Test for reducing sugars

a. About 1 ml of methanol extract was added 1 ml of distilled water. 20 drops of boiling Fehlingôs solution (A and B) in a test tube were added. The formation of a red-brick precipitate in the bottom of the tube indicates the presence of reducing sugars.
b. To 2 ml of methanol extract was added 5-8 drops of boiling Fehlingôs solution. A red-brick precipitate shows the presence of reducing sugars.

2.2.4 Fractionation of methanol extract

About 2 kg of the extract was partitioned in a mixture of chloroform-methanol-water (2:2:1). The resulting chloroform layer was concentrated in *vacuo* in a rotary evaporator to obtain (38.1 g) chloroform fraction (CF). The aqueous-methanol portion was freeze-dried to obtain 126.5 g of the aqueous methanol fraction (AMF).

2.2.5 Biological activity guided studies

The extract and fractions were screened for antiulcer activity in bioactivity-guided experiments using indomethacin-induced ulcers model. The chloroform fraction offered higher protection than the aqueous methanol fraction. Based on this, about 38 g of the chloroform fraction was further fractionated in a silica gel (60-200 mesh) column eluted with gradient (100: 0; 95: 5; 90:10; 80: 20) mixtures of n-hexane-ethylacetate.

About 37 fractions of 100 ml each were collected. The fractions were pooled together into 6 broad fractions (I ï VI) based on the similarity of constituents visualized on silica gel coated TLC plates developed with n-hexane-ethylacetate (7:3). The fractions were screened for antiulcer activity using the activity guide. Fractions III and VI gave comparably higher protection against ulcer. Further purification of fractions III and VI by column chromatography using sephadex LH-20 with methanol as eluent yielded white waxy amorphous powders (compound I; 2.60 g) and (compound II; 2.85 g) respectively. The antiulcer activity of compounds I and II was evaluated using the model. The fractionation and bioactivity-guided experiments are schematically shown in Figure 4.

2.2.7 Characterization of isolated compounds

The molecular weights of the compounds were determined by Electron Impact Mass Spectroscopy (EI-MS). Electron impact mass spectra were measured on a Finnigan MAT 8430 mass spectrometer. In this technique, ionization occurs in the ion source by collision of the sample molecules with electrons emitted from a filament by a thermoionic process. In EI-MS, the energy for ionization (70 eV) was achieved by accelerating the electrons produced by

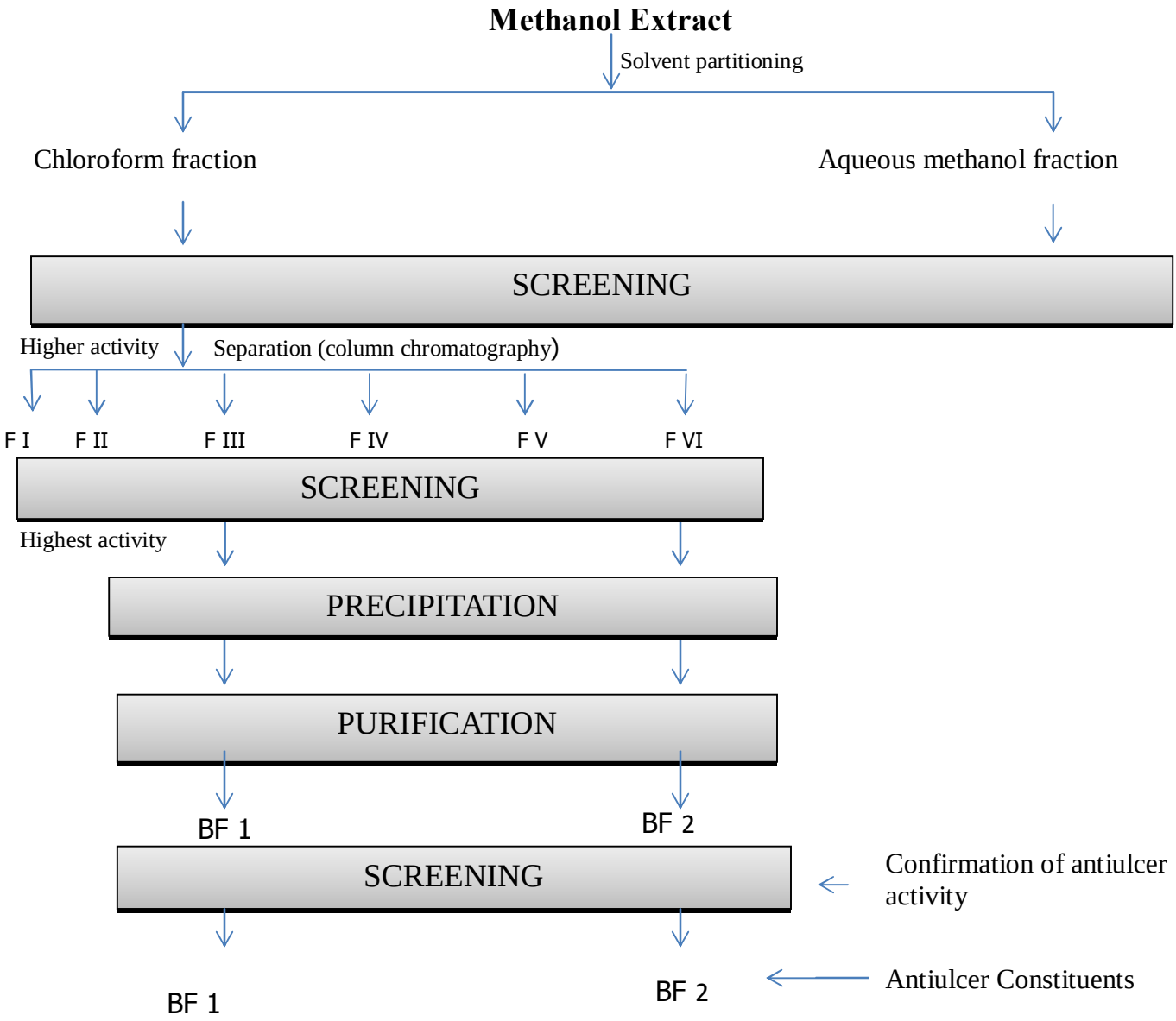


Figure 4: Fractionation and bioactivity-guided scheme

the filament through a potential drop of 70 V, applied between the filament and the chamber. Ionisation efficiency in EI-MS was in the order of one ion produced for every 10,000 molecules.

The molecular structures of the compounds were elucidated using ^{1}H-NMR and ^{13}C-NMR. The spectra were recorded at 300K on ARX 600 MHz NMR spectrometers. The one dimensional (1D) and two dimensional (2D) spectra were obtained using the standard Bruker software. Sample BF1 was dissolved in deuterated chloroform (CD_3Cl) while sample BF2 was dissolved in deuterated methanol (CD_3OD), the choice of which was dependent on the solubility of the samples. Tetramethylsilane (TMS) was used as internal standard reference signal. The observed chemical shifts (ŭ) were recorded in ppm and the coupling constants (*J*) were recorded in Hz.

2.3 Pharmacological tests

2.3.1 Acute toxicity test

The acute toxicity and lethality (LD_{50}) of ME was determined in albino mice of either sex (30 - 32 g) using the oral route by the method of Lorke (1983). In phase I of the test adult albino mice of either sex (30 ï 32 g) were divided into three groups (n = 3). The animals received one of 10, 100 or 1000 mg/kg of ME. 24 h later, based on zero death/mortality, further doses of 1600, 2900 and 5000 mg/kg were administered orally to different mice in phase II of the test and the animals were observed for 24 h for deaths.

2.3.2 Antiulcer activity tests

2.3.2.1 Indomethacin-induced ulcer test

This was carried out according to the method described by Jose *et al* (2010) with some modifications. The adult albino rats used for this test were starved of food for 24 h prior to the

experiment but allowed free access to water. The rats were randomly allotted to 13 groups of five animals each. Treatment groups (1-11) received oral doses of one of ME, CF, AMF, fractions I-VI, isolated compounds; BF1 and BF2 at 100 or 300 mg/kg. Groups 12 and 13 were the control groups and received either 1% Tween-80 (1 ml) or cimetidine (100 mg/kg). One hour after treatmrnt, indomethacin (100 mg/kg) was given to the animals orally. Four hours later, the animals were sacrificed by cervical dislocation and their stomachs removed and opened along the greater curvature. The open stomachs were rinsed carefully under a running tap and pinned on a cork board and examined with a hand lens (x10).

The ulcers were classified modification Main and Whitle, (1975) as:

Level I = ulcer area <1 mm

Level II = ulcer area 1ï 3 mm

Level III = ulcer area >3 mm

Ulcer index was calculated as: 1 x N1 + 2 x N2 + 3 x N3

Where N1 = no of ulcers in level I

N2 = no of ulcers in level 2

N3 = no of ulcers in level 3

Percentage curative ratio (% C) = 100 $[1-UI_t/UI_c]$ (Suziki *et al,* 1976)

Where UIt = ulcer index of animals treated with test sample

UIc = ulcer index of animals treated with vehicle.

2.3.2.2 Absolute ethanol-induced ulcer test

This was carried out according to the method described by Jose *et al* (2010) with some modifications. Adult albino rats were used for this study. The rats were starved of food for 24 h prior to the experiment but allowed free access to water. The rats were randomly alloted o 13 groups of five animals each. Treatment groups (1-11) received oral doses of one of ME, CF, AMF, fractions I-VI, isolated compounds; BF_1 and BF_2 at 100 or 300 mg/kg. Control groups (12 and 13) received either 1% Tween-80 (1 ml) or cimetidine (100 mg/kg). One hour after treatment, each animal received 1 ml of absolute ethanol orally (Robert, 1979). One hour after ethanol administration, the animals were sacrificed by cervical dislocation and their stomachs removed and opened along the greater curvature. The open stomachs were rinsed carefully under a running tap, pinned on a cork board and examined with a hand lens (x10). Erosions formed on the glandular portion of the stomach were observed and each stomach given severity rating on a 0-7 scale based on the ulcers (Ezike *et al*., 2014).

Where 0 = no ulcer,

1= One slight ulcer,

2 = More than one grade 1 ulcer,

3 = One ulcer of length Ò0.5 cm,

4 = One ulcer of length >0.5 cm,

5 = More than one grade 3 ulcer,

6 = More than one grade 4 ulcer,

7 = Complete hemorrhagic lesion of the mucosa.

Mean ulcer score for each group was calculated and expressed as the Ulcer Index (UI) (Ezike *et al*., 2014).

Ulcer protection % = 100 (1-y / z)

Where y = Ulcer index of treated group

z = Ulcer index of control group (Ezike *et al*., 2009).

2.3.2.3 Cold restraint stress-induced gastric ulcers test

This was carried out according to the method described by Viana *et al.* (2013) with some modifications. Adult albino rats were starved of food for 24 h prior to the experiment but allowed free access to water. The rats were randomly allotted to seven groups of five animals each. Treatment groups (1-5) received oral doses of one of ME, fraction III, fraction VI, isolated compounds; BF_1 and BF_2 at 100 or 300 mg/kg. Control groups (6 and 7) received either 1% Tween-80 (1 ml) or cimetidine (100 mg/kg). The test was restricted to these fractions because other fractions did not show activity in earlier models. One hour after treatment the rats were individually restrained in plastic bottles and placed inside the cages and maintained at 4 ± 1°C. After 4 h, the animals were sacrificed by cervical dislocation and their stomachs removed and opened along the greater curvature. The opened stomachs were rinsed carefully under a running tap and pinned on a cork board and examined with a hand lens (x10).

The ulcers were classified, and ulcer index and percentage curative ratio calculated as in section 2.3.2.1

2.2.2.4 Pyloric ligation-induced ulcer test

The pyloric ligation-induced ulcer model was performed using the method described by Shay *et al* (1945) with some modifications. Adult albino rats were starved of food for 24 h prior to the experiment but allowed free access to water. The rats were randomly allotted to four groups of

five animals each. The animals were anesthetized i.p with phenobarbital sodium (35 mg/kg), the abdomen incised and the pylorus ligated. Immediately after the pylorus ligature, the treatment groups (1 and 2) received one of BF1 and BF2 at 100 or 300 mg/kg administered intraduodenally. Control animals (3 and 4) received either 1% Tween-80 (1 ml) or cimetidine (100 mg/kg). This model was restricted to the isolated compounds to further evaluate their effect on this model. The stomachs were replaced carefully and the animals were allowed to recover. Four hours later, the animals were sacrificed by cervical dislocation; the abdomens opened and another ligature placed around the esophagus close to the diaphragm. The stomachs were removed and opened along the greater curvature, rinsed carefully under a running tap and pinned on a cork board for examination with a hand lens (x10).

The ulcers were classified, and ulcer index and percentage curative ratio calculated as in section 2.3.2.1

2.3.3 Tests for antiulcer mechanisms

2.3.3.1 Gastric acid secretion test

The gastric contents of each rat in experiment 2.2.2.4 were collected and drained into a graduated centrifuge tube and centrifuged at 2000 × g for 15 min. The volume and pH of the supernatant were measured with a measuring cylinder and digital pH meter respectively and recorded. Total acidity of the gastric juice was determined.

Determination of total acidity: An aliquot of 1 ml gastric juice was diluted with 1 ml of distilled water in a 50 ml conical flask. Two drops of phenolphthalein indicator was added to the flask and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH used was noted. The total acidity was expressed as Meq/L by the formula:

TA = n x 0.01 x 36.5 x 1000

Where;

n = volume of NaOH consumed

36.5 = molecular weight of NaOH

0.01 = normality of NaOH

1000 = the factor (to be represented in litre) (Trease and Evans, 1992).

2.3.3.2 H^+K^+ATPase inhibition activity test

I. Preparation of enzyme

The H^+K^+ATPase used in this study were prepared from fresh goat stomach using the method described by Rajesh *et al* (2013) with some modifications. Fresh goat stomach was obtained from a local slaughterhouse at Ikpa Market, Nsukka, Enugu State, Nigeria. The stomach was cut opened and the mucosa at the gastric fundus was cut off and the inner layer scraped out for parietal cells (Sachs *et al.,* 1978). The cells obtained were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 and centrifuged at 4000 g for 15 min. The supernatant (enzyme) was used to determine the H^+K^+ATPase inhibition. Protein content of the supernatant was calculated according to Lowry *et al* (1951).

II. Calculation of protein content of cell extract

The absorbance of the supernatant was measured spectrophotometrically at 750 nm in triplicates. Using the standard protein curve, the protein content was calculated from the formula:

$A = CLC$

where A = absorbance (0.911), **€** = extinction coefficient (0.796), L = path length (1) and C = concentration. The standard curve is shown in appendix 1.

III. Assessment of H^+K^+ATPase inhibition

The reaction mixture containing 0.1 ml (about 114 µg) of the supernatant and fraction III, fraction VI, isolated compounds (BF1 and BF2) and omeprazole at 100, 200 or 300 µg were pre-incubated for 60 min at 37°C. The reaction was initiated by adding substrate 2 mM ATP (200 µL), 2 mM $MgCl_2$ (200 µL) and 10 mM KCl (200 µL). After 30 min of incubation at 37°C, the reaction was stopped by the addition of assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid followed by centrifugation at 2000 g for 10 min. The inorganic phosphate released was measured spectrophotometrically at 660 nm by the method of Fiske-Subbarow (Fiske and Subbarow, 1925). Enzyme activity was calculated as micro mole of Pi released per hour at various doses of the fraction.

Enzyme blockade (%) = 100 [(Activity of control ï Activity of test) / **(**Activity of control)]

2.3.3.3 Role of endogenous nitric oxide [NO] and sulfhydryl compounds [SH]

I. L-NAME-pre-treated rats

The test was performed according to the method described by Arrieta *et al* (2003) with slight modifications. The animals were randomly allotted to eight groups of five animals each. All the animals were fasted for 24 h prior to commencement of treatment. Groups 1 ï IV were pretreated with L-Nitroarginine Methylester (L-NAME; 70 mg/kg) while groups V ï VIII were pretreated with normal saline (1 ml) intra-peritoneally. Thirty minutes after L-NAME or normal saline

pretreatment, groups 1, II, V and VI received an oral doses of BF1 or BF2 (300 mg/kg) and control groups (III, IV, VII and VIII) received oral vehicle (1% Tween 80) or carbenoxolone (100 mg/kg). Thirty minutes later, each animal in groups (I ï VIII) received 1 ml of absolute ethanol orally. One hour after ethanol administration, the animals were sacrificed by cervical dislocation and their stomachs removed and opened along the greater curvature. The open stomachs were rinsed carefully under a running tap, pinned on a cork board and examined with a hand lens (x10).

The ulcers were classified, and ulcer index and percentage curative ratio calculated as in section 2.3.2.1

II. NEM-pre-treated rats

The test was performed according to the method described by Arrieta *et al* (2003) with slight modifications. The animals were randomly allotted to eight groups of five animals. All the animals were fasted for 24 h prior to the commencement of treatment. Groups 1 ï IV were pretreated with N-Ethylmaleimide (NEM; 10 mg/kg) while groups V ï VIII were pretreated with normal saline (1 ml) intra-peritoneally. Thirty minutes after NEM or normal saline pretreatment, the treatment groups (1, II, V and VI) received an oral administration of BFI or BF2 (300 mg/kg) and control groups (III, IV, VII and VIII) received oral vehicle (1% Tween 80) or carbenoxolone (100 mg/kg). Thirty minutes later, each animal in groups (I ï VIII) received 1 ml of absolute ethanol orally. One hour after ethanol administration, the animals were sacrificed by cervical dislocation and their stomachs removed and opened along the greater curvature. The open stomachs were rinsed carefully under a running tap, pinned on a cork board and examined with a hand lens (x10).

The ulcers were classified, and ulcer index and percentage curative ratio calculated as in section 2.3.2.1

2.3.3.4 Antioxidant (DPPH radical scavenging) activity test

The antioxidant activity of the extract/isolates was evaluated using 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging as described by Sajjad *et al* (2006) and Patel *et al* (2010) with slight modification. DPPH solution (0.6 mM) was freshly prepared using methanol as solvent. DPPH solution (0.5 ml) was mixed with different concentrations (10, 20, 40, 60, 80, 100, 200 and 300 µg/ml) of ME, Fraction III, Fraction VI, isolated compounds (BF1 and BF2) or ascorbic acid. The volume of the solution was adjusted with methanol to a final volume of 5 ml. After incubation in the dark for 30 min at room temperature, the absorbances of the mixtures were measured at 520 nm against methanol as blank using UV-spectrophotometer. Absorbance measurements were done in triplicates. The antioxidant activities of the extract and fractions were evaluated by comparing their absorbances with that of the control (containing 0.5 ml of DPPH solution and 4.5 ml of methanol). The antioxidant activity was obtained using the relationship shown below. The concentrations that produce 50% inhibition (IC_{50}) of free radical were also obtained from dose response curve.

Antioxidant activity = 100 [(AC - AS) / AC]

AC = Absorbance of control.

AS= Absorbance of sample.

2.4 Statistical analysis

Data were analyzed using One-Way Analysis of variance using GraphPad prism version 5.0 manufactured by GraphPad Software Inc., USA. Differences between means were determined using Bonferroni's post hoc test for multiple comparison and regarded significant at $p < 0.05$. Results are expressed as Mean ± SEM.

CHAPTER THREE

Results

3.1 Extraction and fractionation of extract

The result of the extraction and fractionation processes is shown in Table 3.

3.2 Phytochemical constituents of extract

The result of the tests is shown in Table 4.

3.3 Acute toxicity and lethality of extract

No lethality was observed in the mice upon oral administration of doses up to 5000 mg/kg. The extracts did not produce any obvious behavioral changes or signs of toxicity (e.g. convulsion, respiratory distress) during the 48 h observation period.

3.4 Physical characteristics of isolated compounds

The isolated compounds; BF1 and BF2 were white waxy amorphous powders. They were insoluble in water but soluble in chloroform.

3.5 Spectral data and structures of BF_1 and BF_2

The molar mass of BF_1 was deduced as 414 from the EI-MS spectroscopy based on the m/z peaks at 414, 415 and 416. The major fragment at m/z 396 occurred by a loss of 18 atomic mass unit (loss of H_2O molecule). This suggests the presence of OH group in the compound. BF1 was thus a steroid or triterpene with hydroxyl group at position 3. These observations suggested that BF1 was most likely a β-sitosterol. The ^{1}H-NMR spectrum of BF1 (Figure 5) showed a de-

Table 3 Extraction and fractionation yield

Material	ME	CF	AMF	I	II	III	IV	V	VI	BF1	BF2
Yield (g)	2010	38.1	126.5	1.08	1.2	9.5	1.5	1.65	11.8	2.6	2.85
% yield w/w	25.13	1.91	6.33	2.84	3.16	25.0	3.95	4.34	31.05	32.5	27.14

ME = Methanol extract, CF = Chloroform fraction, AMF = Aqueous methanol fraction, I ï VI = Fractions I ï VI, BF1 = isolated compound I and BF2 = isolated compound 2; yield (%) was calculated relative to weight of extracted plant material or weight of fractionated extract/fraction.

Table 4: Phytochemical constituents of methanol extract

Constituents	Inference
Alkaloids	++
Flavonoids	++
Reducing sugars	++
Saponins	++
Steroids	++
Tannins	++
Terpenoids	++

++: test strongly positive

MR (600 MHz, CDCl3) δ = 5.33 (s, 1H), 3.49 (d, *J*=11.0, 1H), 2.24 (m, 2H), 1.98 (m, 3H), 1.82 (dd, *J*=4.9, 15.0, 3H), 1.47 (m, 9H), 0.99 (d, *J*=3.7, 4H), 0.90 (d, *J*=6.5, 3H), 0.82 (m, 3H), 0.80 (m, 5H), 0.66 (s, 2H

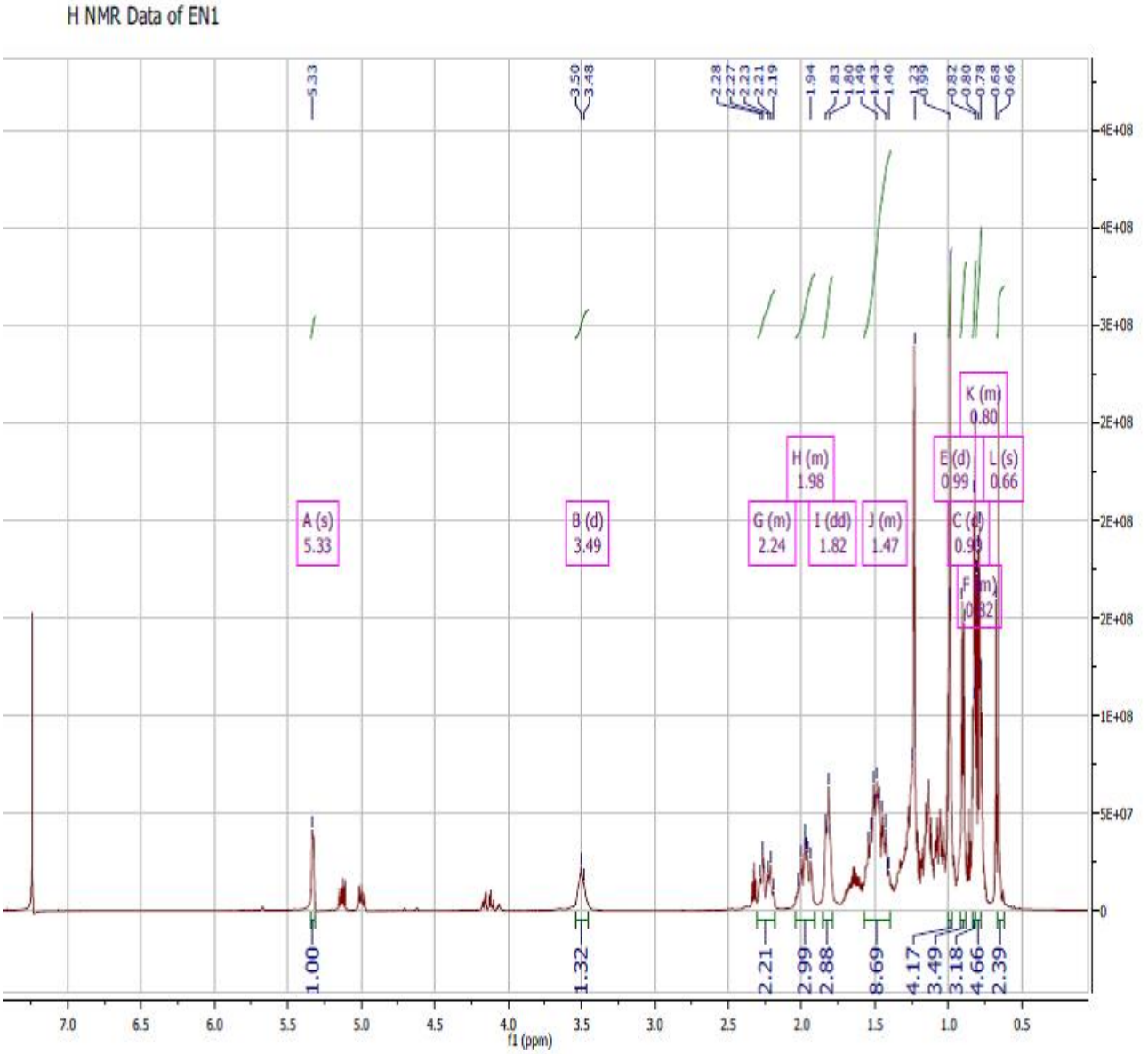


Figure 5: The 1D NMR spectra of BF_1 showing peak at δ_H 5.33 (brs, 1H), δ_H 3.49 suggestive of β-sitosterol

shielded proton peak at δ_H 5.33 (brs, 1H) assignable to the olefinic proton H-5 of the β-sitosterol. Another de-shielded proton at δ_H 3.49 was assignable to proton H-3. These two proton peaks showed correlation spectroscopy (COSY) (Figure 6) correlation with the other protons of the steroid nucleus. The NMR spectrum also showed methyl signals at 0.66 (s, 3H), 0.99 (s, 3H), 0.96 (d, *J*=6.5, 3H), 0.82 (d, *J*=6.5, 3H), 0.81 (d, *J*=6.5, 3H) and 0.80 (t, 3H) assignable to CH_3-18, CH_3-19, CH_3-21, CH_3-26, CH_3-27 and CH_3-29 respectively of the steroid nucleus. The compound was thus identified as β-sitosterol based on the comparison of the NMR data with that reported in the literature. The molecular structure of BF1 is shown in Figure 7.

The proton NMR spectrum of BF2 (Figure 8) was similar to that of BF1. The spectrum of BF2 also showed the presence of olefinic proton peak at δ_H 5.33 (brs, 1H) assignable to H-5 and the de-shielded proton signal at δ_H 3.45 (m, 1H) assignable to H-3. The spectrum also showed the presence of the methyl signals as previously described for BF1. The major difference, however, was in the presence of several other oxygenated proton peaks in the range of 2.80 to 4.50 ppm and carbon peaks in the range of 60 to 100 ppm. These additional signals suggested the presence of a sugar moiety. The signal at δ_H 4.22 (d, *J=7.8* 1H) was assignable to the anomeric proton H-1'. This proton was found to show COSY correlations with the other oxygenated protons. Analysis of the COSY spectrum of BF2 (Figure 9) showed that the signals at δ_H 2.89 (dd, *J*=8.5, 13, 1H), 3.11 (dd, *J*=4.8, 8.8, 1H), 3.02 (dd, *J*=5.2, 8.9, 1H) and 3.06 (m, 1H) were assignable to H-2', H-3', H-4' and H-5' respectively, while the signals at δ_H 3.40 (dd, *J*=5.9, 11.7, 1H) and 3.64 (dd, *J*=54.2, 11.7, 1H) were assignable to the two diastropic methylene protons HA-6' and HB-6'. The sugar moiety was confirmed as βD-glucose based on the coupling constant of the anomeric proton and the chemical shifts of the proton and carbon signals.

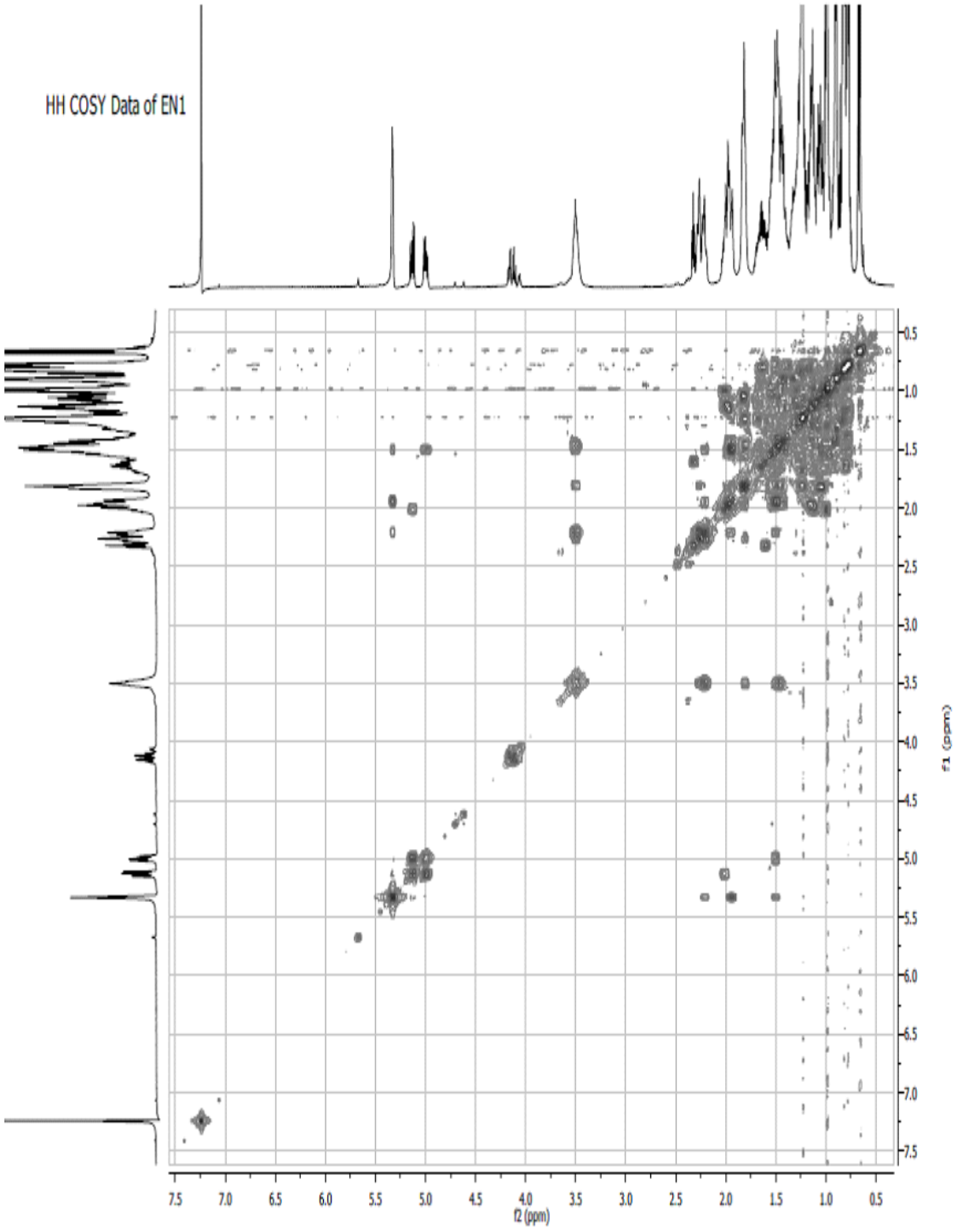


Figure 6: The COSY data of BF1 indicating the correlation with the other protons of the steroid nucleus

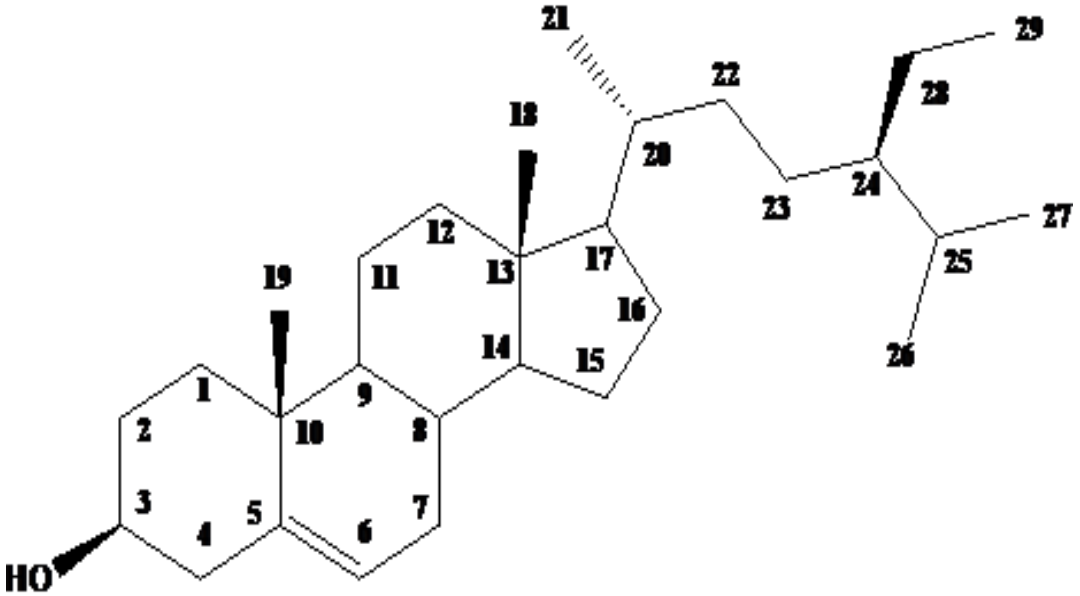


Figure 7: The molecular structure of compound BF1 (β-sitosterol)

^{1}H NMR (600 MHz, DMSO) δ = 5.33 (s, 1H), 4.88 (d, *J*=4.8, 1H), 4.86 (t, *J*=5.4, 2H), 4.42 (t, *J*=5.8, 1H), 4.22 (d, *J*=7.8, 1H), 3.64 (d, *J*=4.2, 1H), 3.46 (s, 1H), 3.40 (dd, *J*=5.9, 11.7, 2H), 3.11 (dd, *J*=4.8, 8.8, 1H), 3.06 (d, *J*=5.9, 1H), 3.02 (dd, *J*=5.2, 8.9, 1H), 2.89 (dd, *J*=8.5, 13.0, 1H), 2.36 (d, *J*=13.4, 1H), 2.12 (s, 1H), 1.94 (s, 2H), 1.80 (s, 3H), 1.63 (d, *J*=5.0, 1H), 1.49 (s, 5H), 1.40 (d, *J*=10.2, 2H), 1.23 (m, 3H), 1.14 (d, *J*=8.6, 3H), 0.99 (d, *J*=6.5, 3H), 0.95 (s, 4H), 0.90 (d, *J*=6.5, 3H), 0.82 (s, 2H), 0.65 (s, 2H).

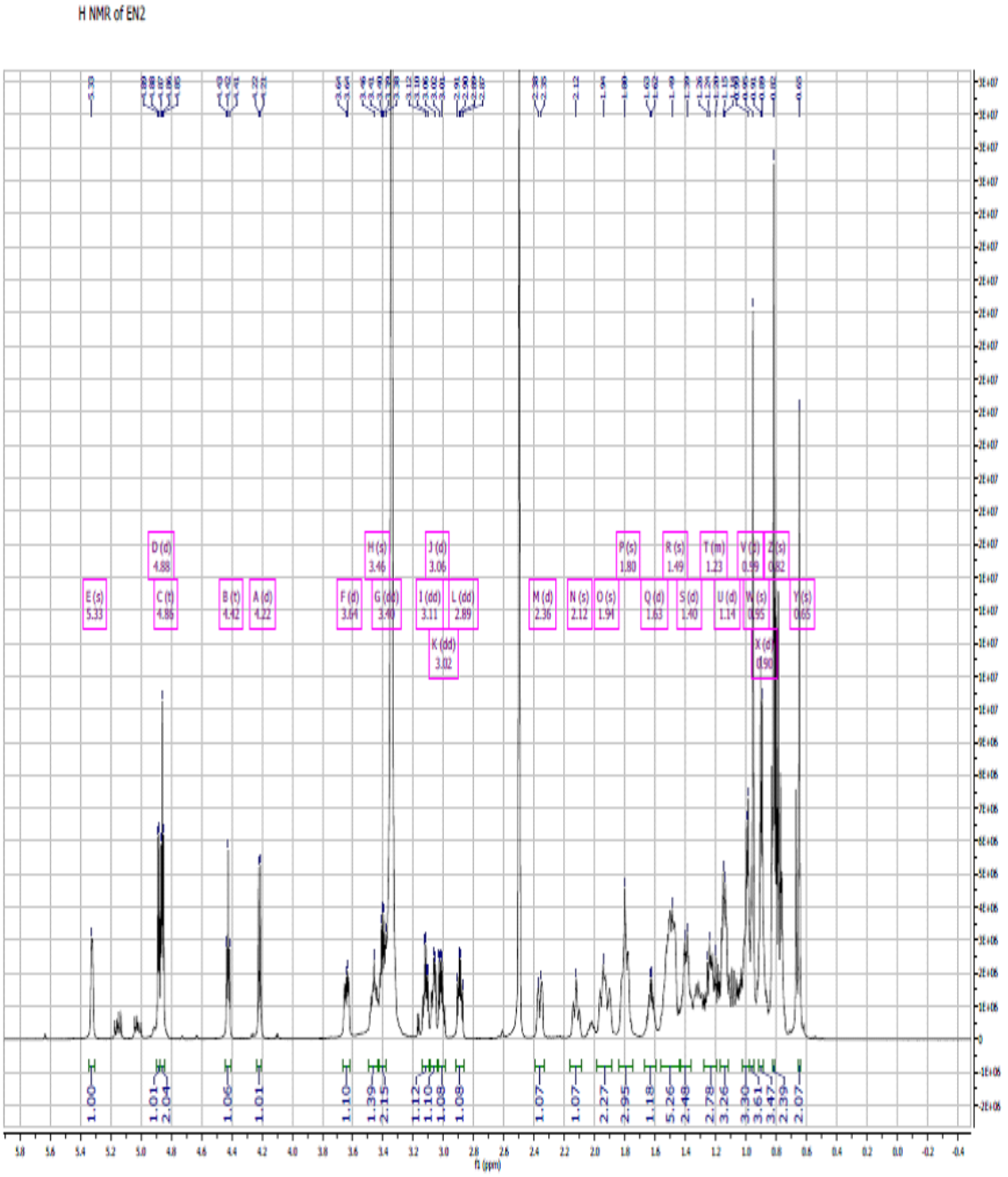


Figure 8: The 1D ^{1}H NMR spectra of BF2 showing peak at δ_H 5.33 (brs, 1H) and δ_H 3.45 (m, 1H) suggestive of β-Sitosterol-3-O-βD-glucopyranoside

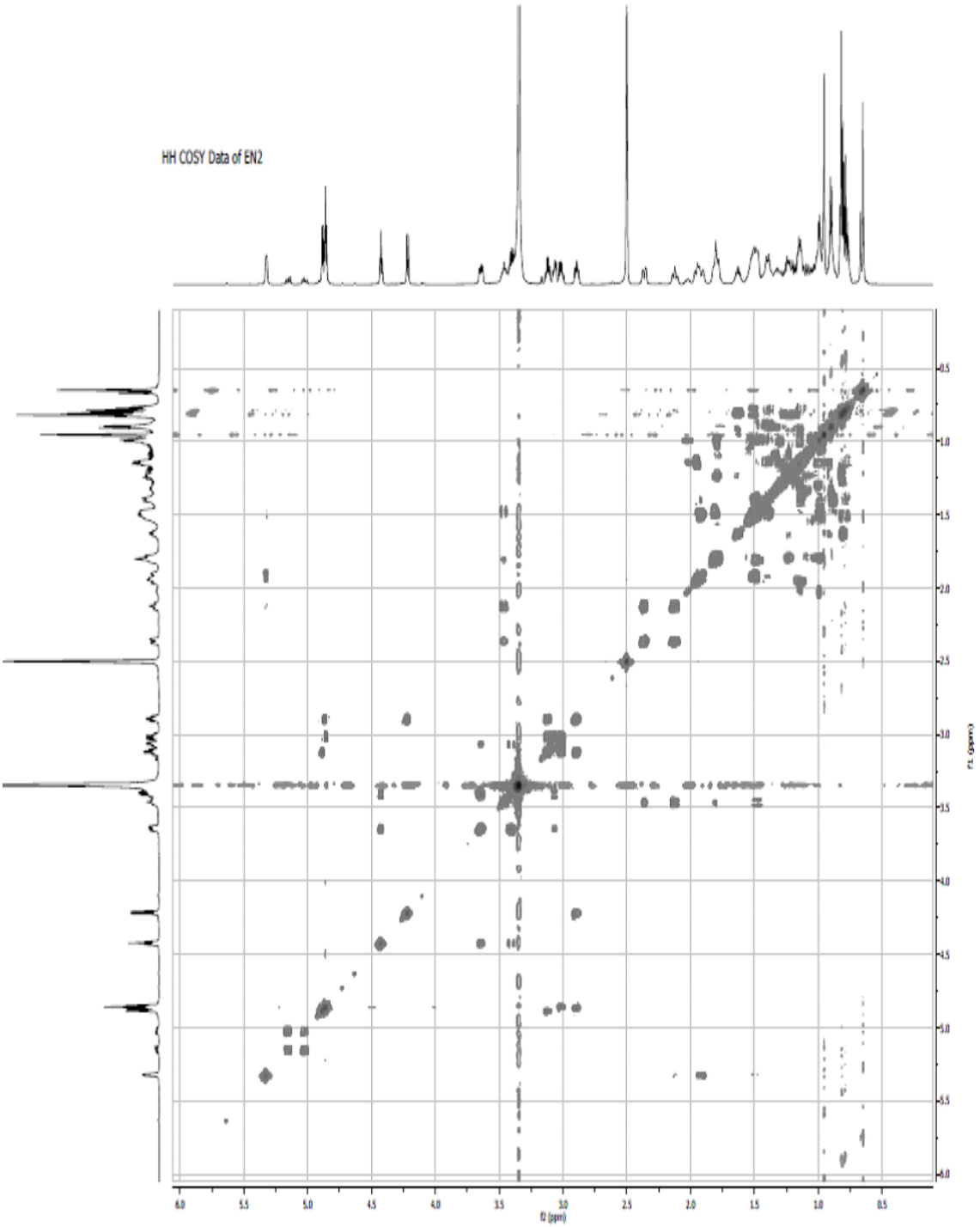


Figure 9: COSY data of BF2 indicating COSY correlations with the other oxygenated protons.

The ^{13}C-NMR spectrum of BF2 is shown in Figure 10. All the proton and carbon signals of BF2 as shown in Table 5 were assigned based on the analysis of the COSY, Distortionless Enhancement by Polarisation Transfer (DEPT), Heteronuclear Single-Quantum Correlation Spectroscopy (HSQC) (Figure 11) and Heteronuclear Multiple Bond Correlation (HMBC) (Figure 12). The attachment of the sugar moiety at position 3 of the steroid was confirmed by the correlation of the anomeric proton signals with C-3 (76.9) of the steroid nucleus in HMBC and that of the H-3 proton with C-1' (100.9) of the sugar moiety. This was also confirmed by the de-shielded position of the C-3 at δ_C 76.9 ppm. Based on the analysis of the NMR data and comparison with literature, BF2 was therefore confirmed as β-Sitosterol-3-O-βD-glucopyranoside. The molecular structure of BF2 is shown in Figure 13.

3.6 Effect of extract, fractions and isolated compounds on indomethacin-induced ulcers

The methanol extract (ME), chloroform fraction (CF), aqueous methanol fraction (AMF), fractions III, IV, V, VI and isolated compounds (BF_1 and BF_2) prevented the development of indomethacin-induced ulcers in a dose dependent manner. Fractions I and II showed no preventive activity. Fractions III and VI, as well as BF1and BF2 caused a significant ($p < 0.0001$) ulcer prevention (Table 6).

3.7 Effect of extract, fractions and isolated compounds on absolute ethanol-induced ulcers

The methanol extract (ME), chloroform fraction (CF), aqueous methanol fraction (AMF), fractions (I, II, III, IV, V, VI) and the isolated compounds (BF1 and BF2) caused significant prevention of the development of ulcers induced by absolute ethanol (except the chloroform fraction that showed less activity at 300 mg/kg). The aqueous methanol fraction (AMF) caused higher protective activity than the chloroform fraction (CF). Both of which offered significant protection than fractions I, II, III, IV, V and VI. Though the isolated compounds, BF1 and BF2

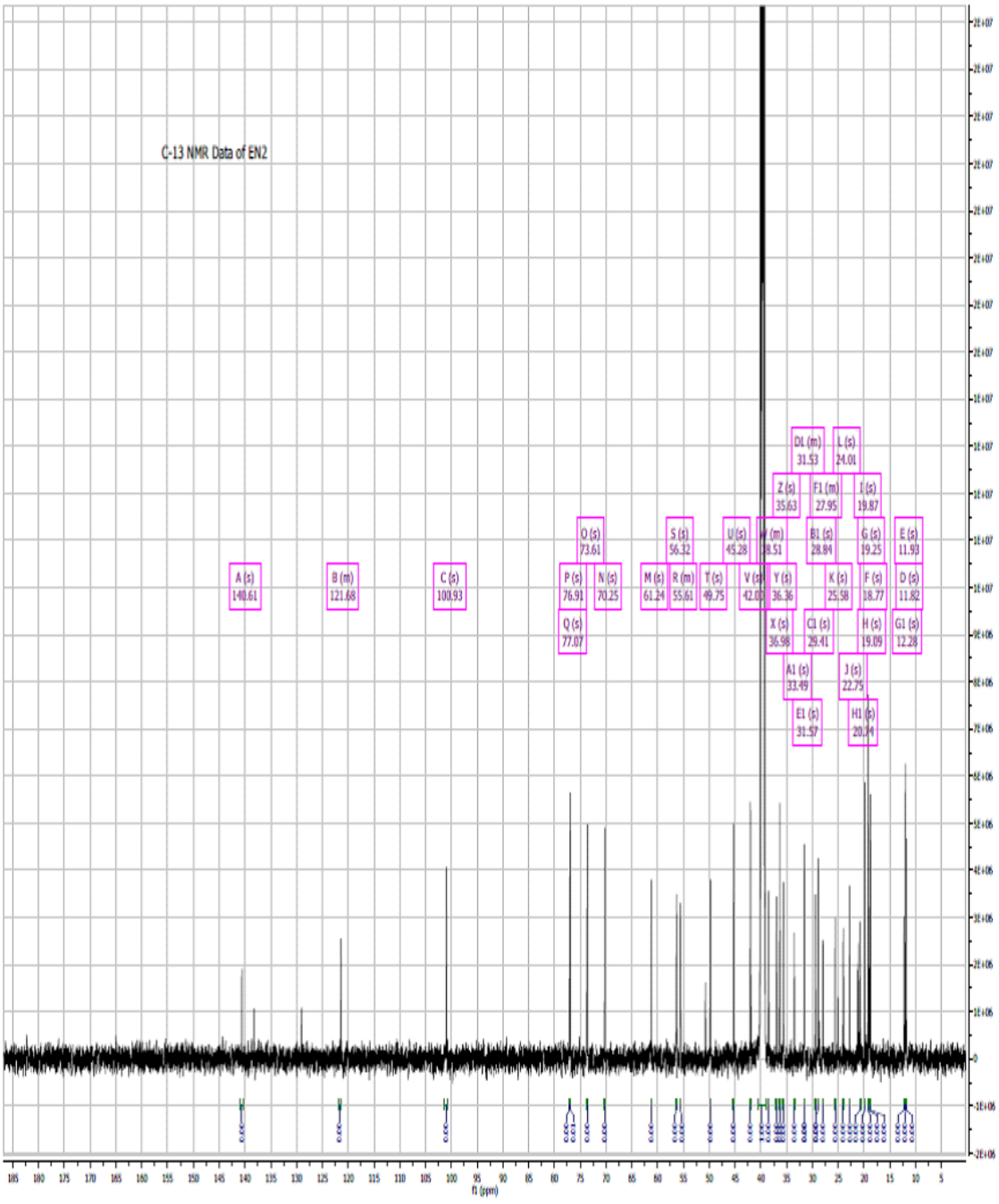


Figure 10: The ^{13}C- NMR of BF2 showing all the carbon signals suggestive of β-sitosterol-3-O-βD-glucopyranoside.

Table 5: NMR Data of β-sitosterol (BF1) and β-Sitosterol-3-O-β D-glucopyranoside (BF2)

Position	β-Sitosterol (BF1)*	β-Sitosterol-3-O-βD-glucopyranoside (BF2)#	
	δ_H	δ_H	δ_C
1		1.22 m (Ha) 0.93 m (Hb)	33.5
2	1.87 m (Ha) 1.47 m (Hb)	1.80 dd (Ha) 1.48 dd (Hb)	29.4
3	3.49 m	3.45 m	76.9
4	2.24 m	2.37 m (Ha) 2.11 m (Hb)	38.5
5		-	140.6
6	5.33 brs	5.33 brs	121.7
7	1.96 m (Ha) 1.47 m (Hb)	1.90 m (Ha) 1.51 m (Hb)	31.6
8			31.5
9			49.8
10			35.6
11			20.7
12			37.0
13			42.0
14			56.3
15			24.0
16			28.8
17			55.6
18	0.66 s	o.65 s	11.9
19	0.99 s	0.95 s	19.3
20			36.9
21	0.90 d (6.5)	0.90 d (6.5)	19.1
22			36.4
23			25.6
24			45.3
25			28.0
26	0.82 d (6.5)	0.82 d (6.5)	18.6
27	o.81 d (6.5)	0,82 d (6.5)	19.1
28			22.8
29	0.80 t	0,80 t	11.8
1'	-	4.22 d (7.8)	100.9
2'	-	2.89 dd (8.5, 13)	73.6
3'	-	3.11 dd (4.8, 8.8)	77.1
4'	-	3.02 dd (5.2, 8.9)	70.3
5'	-	3.06 m(5.9)	76.9
6'	-	3.64 dd (4.2, 11.7) 3.40 dd (5.9, 11.7)	61.3

*Spectra measured in $CDCl_3$; #Spectra measured in DMSO-d6.

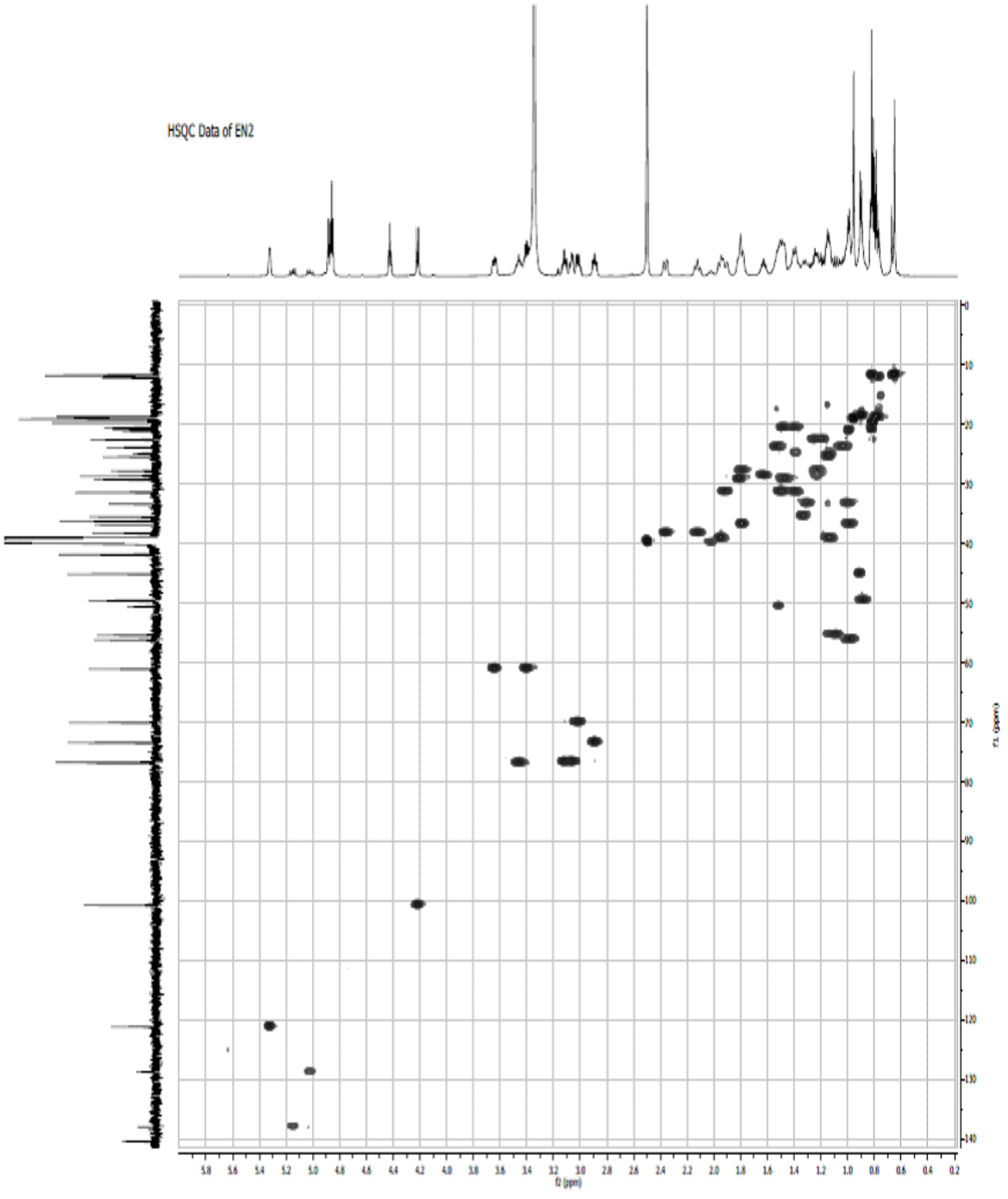


Figure 11: The HSQC data of BF2 indicating all the proton and carbon signals.

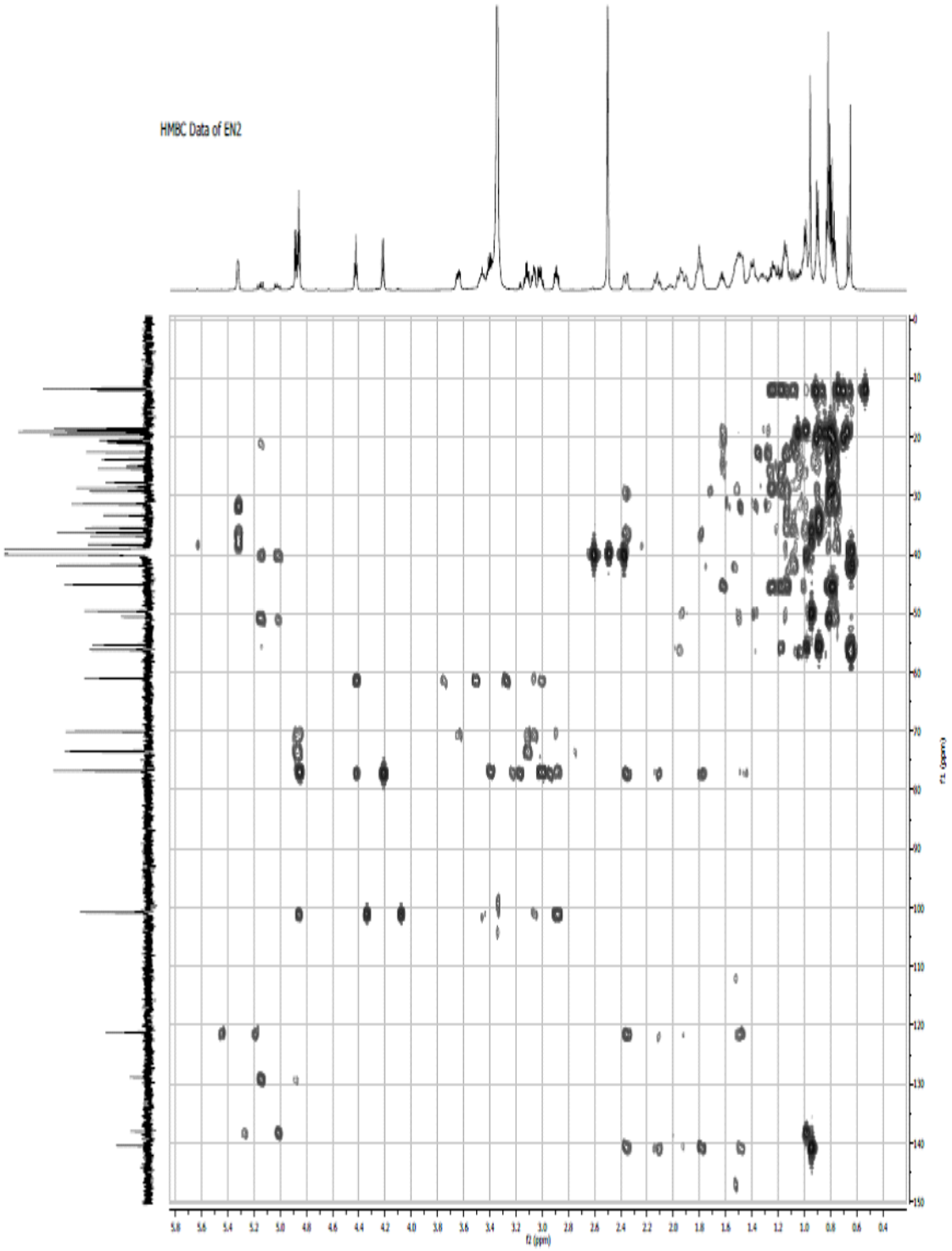


Figure 12: The HMBC data of BF2 indicating the correlation of the anomeric proton signals with C-3 (76.9) of the steroid nucleus.

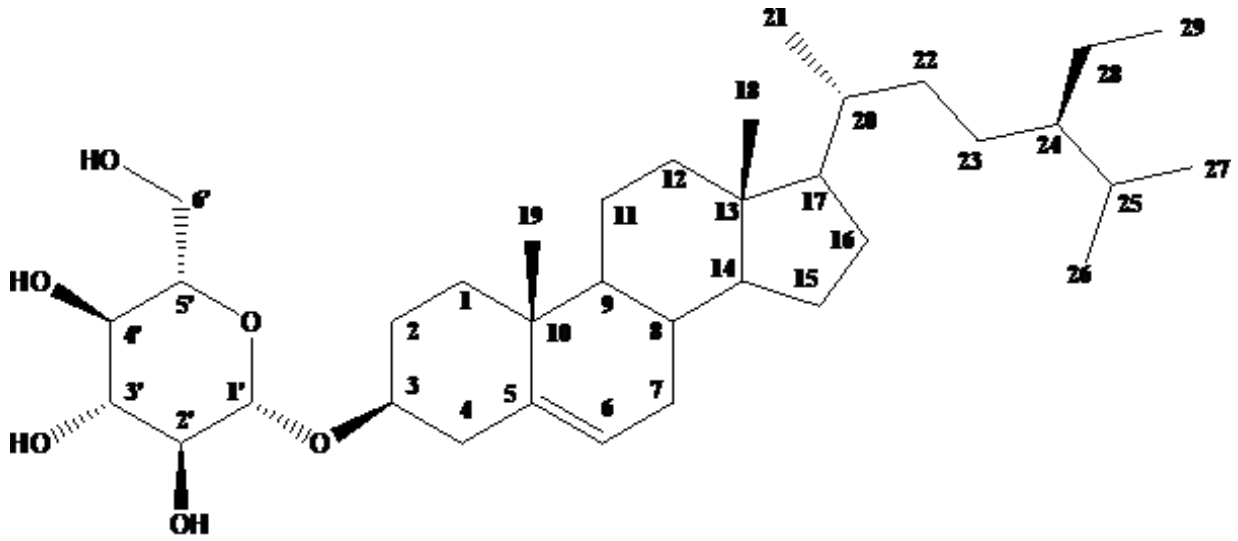


Figure 13: The molecular structure of BF2 (β-Sitosterol-3-O-β D-glucopyranoside)

Table 6: Effect of extract, fractions and isolated compounds on indomethacin-induced ulcers

Treatment	Dose (mg/kg)	Ulcer index	Curative ratio (%)
ME	300	0.23 ± 0.29[a]	88.61
CF	100	0.86 ± 0.10[a, b]	57.43
	300	0.24 ± 0.13	88.12
AMF	100	1.54 ± 0.29[a]	23.76
	300	0.88 ± 0.77	56.44
I	300	1.84 ± 0.04[a, b]	8.91
II	300	2.45± 0.02[a, b]	-21.29
III	300	0.35 ± 0.04[a, b]	82.67
IV	300	0.84 ± 0.02[a, b]	58.42
V	300	0.93 ± 0.03[a, b]	53.96
VI	300	0.78 ± 0.02[a, b]	61.39
BF1	100	0.41 ± 0.03[a]	79.70
	300	0.36 ± 0.02	82.18
BF2	100	0.61 ± 0.04[a]	69.80
	300	0.52 ± 0.07	74.26
Cimetidine	100	0.20 ± 0.11[a]	90.10
Control	-	2.02 ± 0.15	

[a] $p<0.0001$ compared to the control; [b]$p<0.0001$ compared to the cimetidine (One Way ANOVA; Bonferroni's post hoc); ME = Methanol extract; CF = chloroform fraction; AMF = Aqueous methanol fraction; I-VI = Fractions I ï VI; BF1 = Isolated compound 1; BF2 = Isolated compound 2.

produced dose dependent protection, there was no significant difference between the doses (100 and 300 mg/kg). The ulcer protective effects of BF1 and BF2 were not significantly ($p > 0.05$) higher than those of fractions III and VI respectively (Table 7).

3.8 Effect of extract, fractions and isolated compounds on cold restraint stress-induced ulcers

The methanol extract, fractions III and VI, and the isolated compounds (BF1 and BF2) caused a significant ($p< 0.0001$) dose-related reduction in ulcer lesion index in cold restraint stress-induced ulcers. The isolated compound, BF2 produced the greatest reduction with curative ratio of 95.8% (Table 8).

3.9 Effect of isolated compounds on pyloric ligation-induced ulcers

The isolated compounds; BF1 and BF2, produced a significant ($p < 0.0001$) but comparable reduction of ulcer index in pyloric ligation-induced ulcers. There was no significant difference in inhibitory activity of these compounds at 100 or 300 mg/kg (Table 9).

3.10 Effect of isolated compounds on gastric acid secretion

The isolated compounds, BF1 and BF2 caused a significant ($p < 0.0001$) decrease in the volume of gastric secretion. This decrease was dose-dependent for BF_1 but not for BF_2. Both compounds also produced significant ($p< 0.0001$) and dose-related increase in the pH of gastric contents. The increase in pH was comparable to that of cimetidine. On total acid, only BF_1 produced dose-related reduction (Table 10).

Table 7: Effect of extract, fractions and isolated compounds on absolute ethanol-induced ulcers

Treatment	Dose (mg/kg)	Ulcer index	Curative ratio (%)
ME	300	0.46 ± 0.32[a]	85.26
CF	100	1.51 ± 0.04[b]	51.60
	300	1.66 ± 0.50	46.79
AMF	100	0.91 ± 0.27[a]	70.83
	300	0.42 ± 0.20	86.54
I	300	2.00 ± 0.03[a, b]	35.90
II	300	2.14 ± 0.09[a, b]	31.41
III	300	1.78 ± 0.04[a, b]	42.95
IV	300	2.04 ± 0.14[a, b]	34.62
V	300	2.60 ± 0.06[a, b]	16.67
VI	300	2.02 ± 0.04[a, b]	35.26
BF1	100	1.80 ± 0.13[a, b]	42.31
	300	1.72 ± 0.10	44.87
BF2	100	2.08 ± 0.15[a, b]	33.33
	300	2.02 ± 0.05	35.26
Cimetidine	100	1.04 ± 0.05[a]	66.67
Control	-	3.12 ± 0.32	-

[a] $p<0.0001$ compared to the control; [b]$p<0.0001$ compared to the cimetidine (One Way ANOVA; Bonferroni's post hoc); ME = Methanol extract; CF = chloroform fraction; AMF = Aqueous methanol fraction; I-VI = Fractions I ï VI; BF1 = Isolated compound 1; BF2 = Isolated compound 2.

Table 8: Effect of extract, fractions and isolated compounds on cold restraint stress-induced ulcers

Treatment	Dose (mg/kg)	Ulcer index	Curative ratio (%)
ME	100	1.18 ± 0.64[a]	59.03
	300	0.54 ± 0.23[a]	81.25
Fraction III	100	1.50 ± 0.11[a, b]	47.92
	300	1.10 ± 0.08[a]	61.81
Fraction VI	100	0.54 ± 0.10[a]	81.25
	300	0.22 ± 0.09[a]	92.36
BF1	100	0.98 ± 0.13[a]	65.97
	300	0.84 ± 0.11[a]	70.83
BF2	100	0.46 ± 0.14[a]	84.03
	300	0.12 ± -0.06[a]	95.83
Cimetidine	100	0.20 ± 0.07[a]	93.06
Control	-	2.88 ± 0.62	-

[a]$p<0.0001$ compared to the control; [b]$p<0.0001$ compared to cimetidine (One Way ANOVA; Bonferroni's post hoc); ME = Methanol extract; BF1 = Isolated compound 1; BF2 = Isolated compound 2.

Table 9: Effect of isolated compounds on pyloric ligation-induced ulcer

Treatment	Dose (mg/kg)	Ulcer index	Curative ratio (%)
BF1	100	0.36 ± 0.07[a, b]	80.22
	300	0.22 ± 0.09[a, b]	87.91
BF2	100	0.30 ± 0.08[a, b]	83.52
	300	0.26 ± -0.05[a, b]	85.71
Cimetidine	100	0.52 ± 0.06[a, b]	71.43
Control	-	1.82 ± 0.10	-

[a]$p<0.0001$ compared to the control; [b]$p<0.0001$ compared to cimetidine (One way ANOVA; Bonferroni's post hoc); BF1 = isolated compound 1; BF2 = isolated compound 2.

Table 10: Effect of isolated compounds on gastric acid secretion

Treatment	Dose (mg/kg)	Vol. of secretion (ml)	pH	Total acid (Meq/L)
BF1	100	0.52 ± 0.11[a]	5.76 ± 0.01[a]	2160 ± 54.77[a]
	300	0.18 ± 0.08[a]	6.02 ± 0.08[a]	960 ± 41.83[a]
BF2	100	0.97 ± 0.10[a]	4.66 ± 0.09[a]	2370 ± 44.72[a]
	300	1.0 ± 0.07[a]	6.30 ± 0.07[a]	4000 ± 70.71[a]
Cimetidine	100	0.21 ± 0.02[a]	6.40 ± 0.07[a]	1010 ± 54.77[a]
Control	-	3.02 ± 0.15	4.50 ± 0.07	19940 ± 622.90

[a]$p<0.0001$ compared to the control (One way ANOVA; Bonferroni's post hoc); BF1 = Isolated compound 1; BF2 = Isolated compound 2

3.11 Effect of fractions and isolated compounds on $H^+ K^+$ ATPase

The fractions and isolated compounds inhibited the activity of $H^+ K^+$ ATPase in a dose dependent manner. Fractions III and VI produced a significantly ($p<0.05$) less inhibitory activity (IC_{50} 98.52 µg/ml and 77.02 µg/ml respectively) than omeprazole (IC_{50} 67.48 µg/ml) (Figure 14). The inhibitory activities of the fractions were less than that of BF1 and BF2. On their own, BF1 and BF2 evoked enzyme inhibition comparable to omeprazole (Figure 15).

3.12 Effect of BF1 and BF2 on gastric lesion in L-NAME pre-treated rats

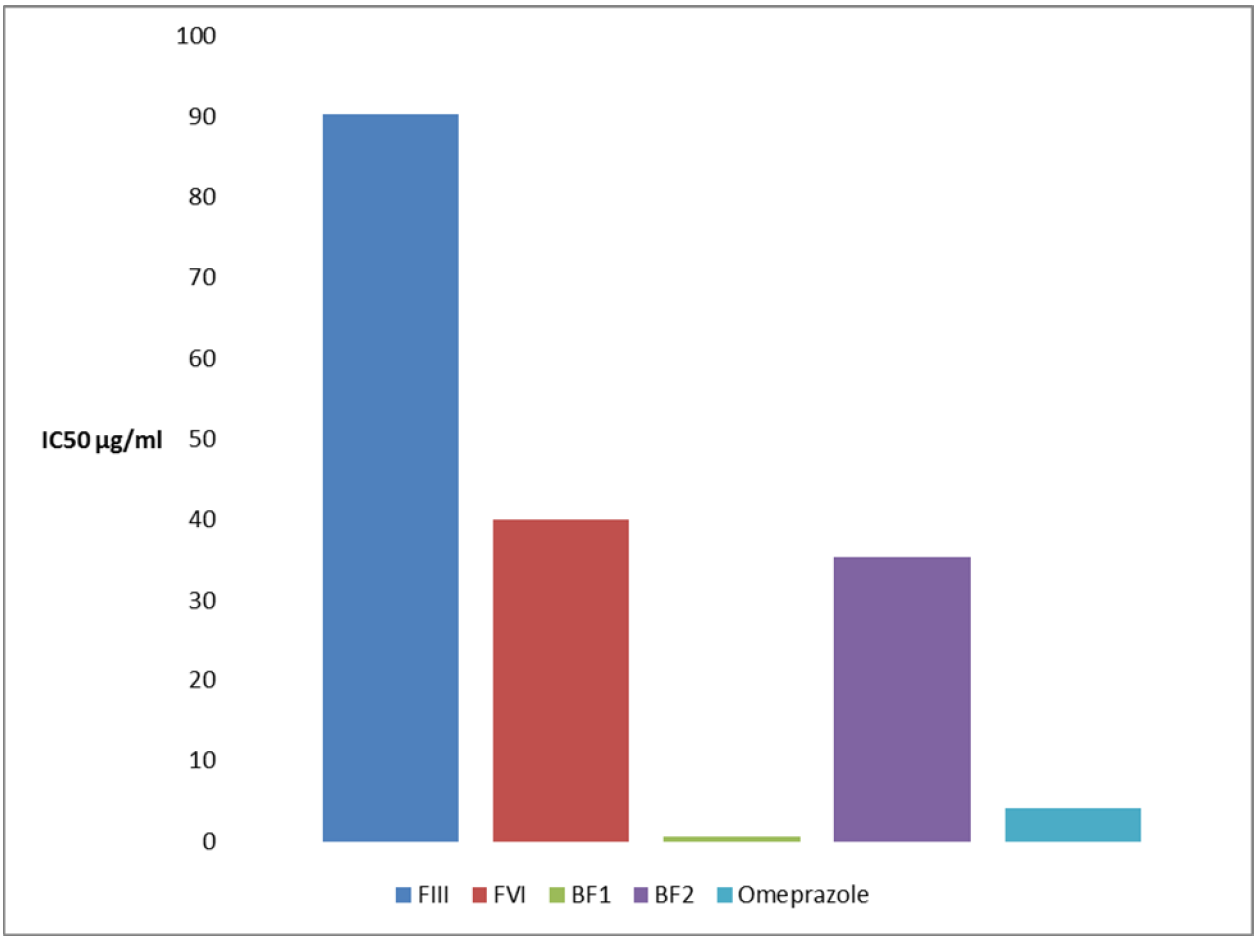
The isolated compounds, BF1 and BF2 did not produce increase in gastric lesion indices in the L-NAME pre-treated rats. The effect of BF1 and BF2 on gastric lesion in L-NAME pre-treated rats is shown in Table 12.

3.13 Effect of BF1 and BF2 on gastric lesion in NEM pre-treated rats

The isolated compounds BF1 and BF2 produced a significant ($p < 0.05$) increase in the ulcer indices in the NEM pre-treatment rats. The effect of BF1 and BF2 on gastric lesion in NEM pre-treated rats is shown in Table 13.

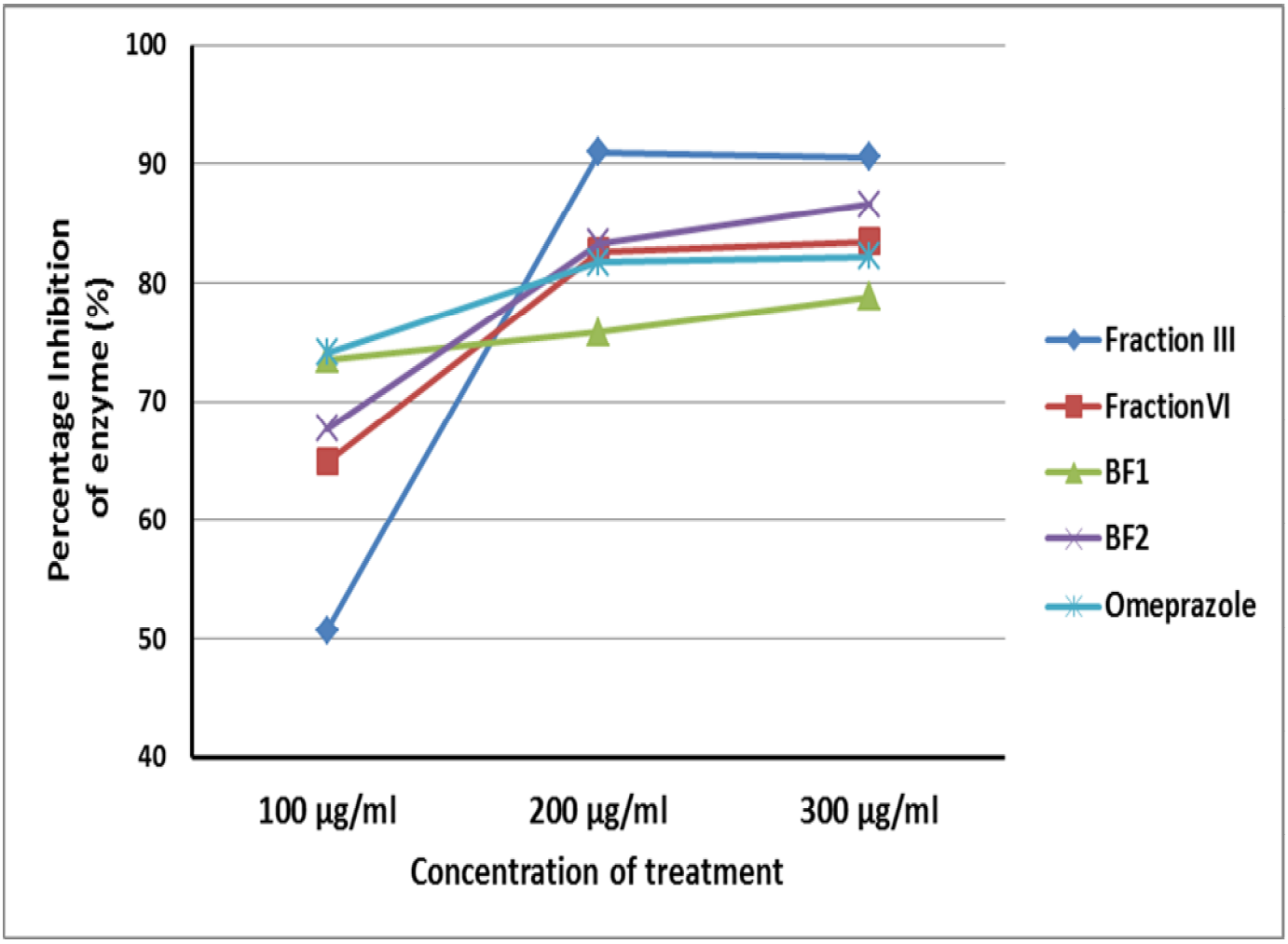
3.14 Effect of extract and fractions on DPPH free radical scavenging activity

The methanol extract (ME), fractions III and VI and isolated compounds BF1 and BF2 scavenged DPPH free radical in a dose-dependent manner. Fraction III produced a significant radical scavenging activity than BF1 whereas fraction VI and BF2 produced comparable effects. Fraction VI was the most active followed by BF2 (Figure 16). The IC_{50} of the extract, fractions and isolated compounds is shown in Figure 17.



FIII = Fraction III, FVI = Fraction VI, BF1 = isolated compound I and BF2 =isolated compound 2

Figure 14: The IC_{50} of fractions and isolated compound on $H^+ K^+$ ATPase activity.



BF1 = Isolated compound 1; BF2 = Isolated compound 2, FII = Fraction III; FVI = Fraction VI

Figure 15: Effect of fractions and isolated compounds on H^+K^+ATPase activity in vitro

Table 12: Effect of isolated compounds on endogenous nitric oxide

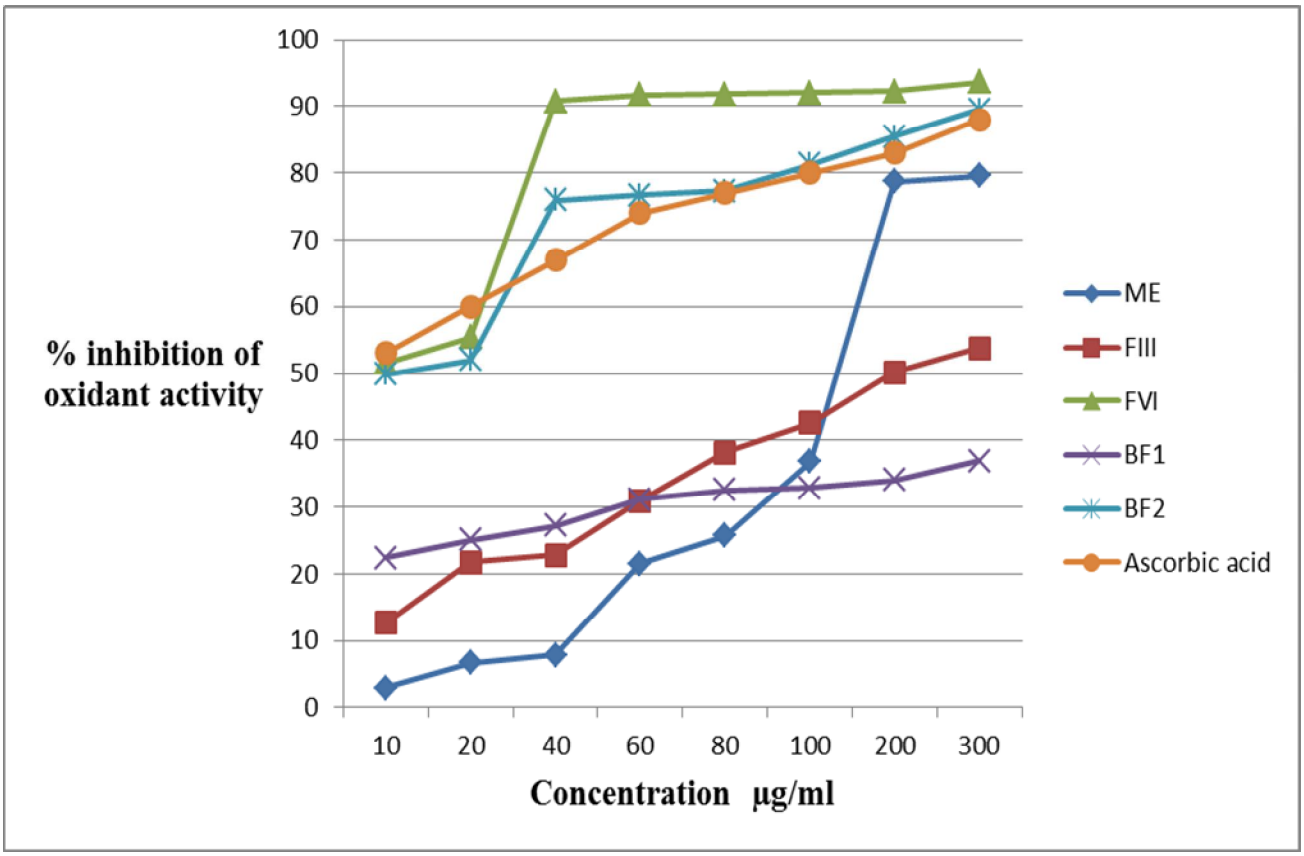
Treatment	Dose (mg/kg)	Ulcer index	
		Saline	L-NAME
BF1	300	0.48 ± 0.23 (76.70%)	0.40 ± 0.26[a] (89.01%)
BF2	300	0.94 ± 0.19 (54.37%)	0.36 ± 0.17[a] (90.11%)
Carbenoxolone	100	0.62 ± 0.27 (69.90%)	2.70 ± 0.12[a] (25.82%)
Control	-	2.06 ± 1.05	3.64 ± 1.66

[a]$p<0.0001$ compared to the control (One way ANOVA; Bonferroni's post hoc); Values in parenthesis are percentage inhibition of ulcer index calculated relative to the control; BF1 = Isolated compound 1; BF2 = Isolated compound 2.

Table 13: Effect of isolated compounds on endogenous sulfhydryl compounds

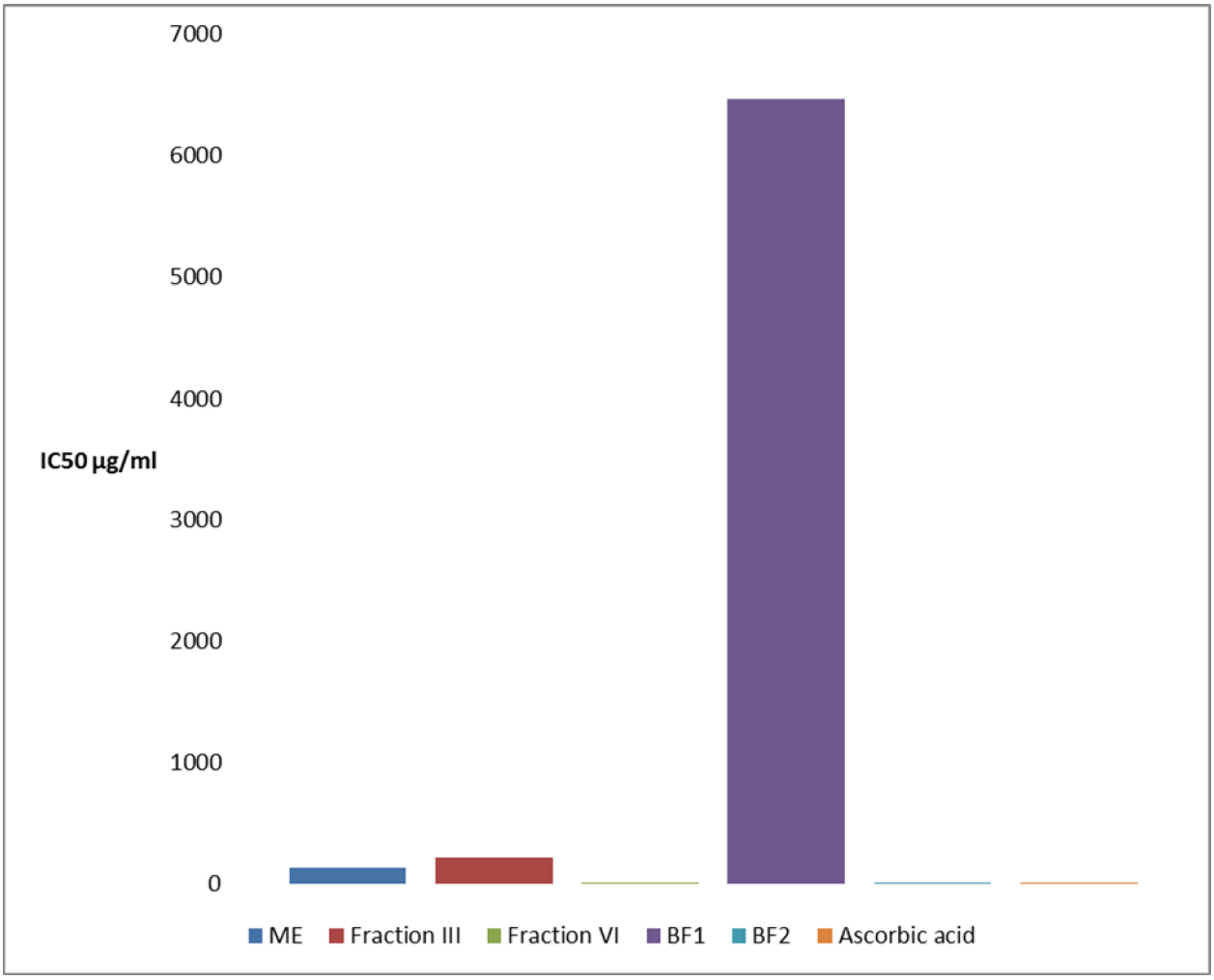
Treatment	Dose (mg/kg)	Ulcer index	
		Saline	NEM
BF1	300	0.48 ± 0.17 (84.81%)	1.20 ± 0.40[a] (68.25%)
BF2	300	0.23 ± 0.13 (92.72%)	0.78 ± 0.07[a] (79.37%)
Carbenoxolone	100	1.28 ± 0.67 (59.49%)	3.38 ± 0.29[a] (10.58%)
Control	-	3.16 ± 1.56	3.78 ± 0.23

[a]$p<0.0001$ compared to the control (One way ANOVA; Bonferroni's post hoc); Values in parenthesis are percentage inhibition of ulcer index calculated relative to the control; BF1 = Isolated compound 1; BF2 = Isolated compound 2.



ME = Methanol extract; BF1 = Isolated compound 1; BF2 = Isolated compound 2.
FII = Fraction III; FVI = Fraction VI.

Figure 16: Effect of extract, fractions and isolated compound on DPPH free radical scavenging activity.



ME = Methanol extract, BF1 = isolated compound I and BF2 =isolated compound 2

Figure 17: The IC_{50} of extract, fractions and isolated compound on DPPH free radical scavenging activity.

CHAPTER FOUR

4 Discussions and Conclusion

4.1 Discussion

The antiulcer properties of the stem bark extract of *B. ferruginea* have been evaluated. Findings from this study show that the stem bark extract of *B. ferruginea* contains biologically active constituents that may possess potent antiulcer properties. The extract, its solvent fractions and isolated compounds protected the rat from ulcers induced by indomethacin, absolute ethanol, cold restraint stress and pyloric ligation. They decreased the volume and total acid of gastric secretion, elevated the gastric pH, inhibited the activity of H^+ K^+ ATPase, inhibited increase in gastric lesion indices in L-NAME pre-treated rats, increased the ulcer lesion indices in NEM pre-treated rats and scavenged DPPH free radicals *in vitro*.

The gastroprotective activity may be attributed to cytoprotection and antisecretion that counteract the ulcerogenic effect of indomethacin. Indomethacin, a member of the non-steroidal antiinflammatory drugs (NSAIDs), causes gastroduodenal ulceration by suppressing the production of prostaglandins which increases the secretion of gastric acid (Wallace, 2001; Jince *et al*, 2010; Gege-Adebayo *et al*., 2013). Prostaglandins are endogenously produced via the cyclooxygenase pathway of arachidonic acid metabolism (Kushima *et al*., 2009) and play an important protective role in the stomach by stimulating the secretion of bicarbonate and mucus, maintaining the mucosal blood flow and regulating mucosal cell renewal (Jose *et al*., 2010; Roslida *et al*., 2010). Inhibition of prostaglandin production exposes the gastric mucosa to hypermotility and microvascular disturbances (Takeeda *et al*., 2004; Kushima *et al*., 2009), thus

promoting the activation and inýltration of neutrophils, reactive oxygen species (ROS*)* production and lipid peroxidation (Laine *et al*., 2008). The inhibition of prostaglandins interferes with the protective mechanisms involving mucus, surface active phospholipids, bicarbonate secretion, mucosal blood flow and mucosal proliferation (Kalra *et al*., 2011). Thus, suppression of prostaglandin synthesis by NSAIDs causes increased susceptibility to gastric mucosal lesions (Laine *et al*., 2008; Malfertheiner *et al*., 2009). Studies have also shown that generation of free radicals is a critical biochemical event in the pathogenesis of gastric ulceration by indomethacin (Lichtenberger, 2005; Inas *et al*., 2011; Ajani *et al*., 2014; Sabiu *et al*., 2015). Other mechanism through which indomethacin induces ulcers involves the reduction of antioxidant enzyme activity (Odabasoglu *et al.,* 2006). The particular mechanism responsible for the antiulcer activity of the extract and bioactive fractions and constituents is not known. Although depletion of gastric prostaglandin has not been shown to play any role in the pathogenesis of human ulcers, it is clearly implicated in drug-induced ulcers associated with NSAIDs. Thus, in addition to the likelihood of protecting against drug-induced ulcers, constituents of the extracts of this plant may particularly offer protection against ulcers of diverse aetiology. Few studies have also documented the antiulcer activity of the crude extract of the plant in indomethacin-induced ulcer (Ezike *et al*., 2011; Akuodor *et al*., 2012).

The antiulcerogenic activity against ethanol-induced ulcers in the rat may be attributed to cytoprotective activity. Ethanol-induced gastric ulceration has been used as a suitable model to study gastric ulcer (Marhuenda *et al*., 1993; Al-Qarawi *et al*., 2005; Bae *et al*., 2011). Ethanol induces gastric lesions because of its corrosive effect (Okpo *et al.,* 2011). Ethanol exerts direct damage on gastric mucosal cells (Pillai *et al*., 2010) which is predominant in the glandular part

of the stomach leading to the generation of reactive oxygen species (Gazzieri, 2007; Okpo *et al*., 2011; Shankara *et al*., 2014) and formation of characteristic necrotic lesions due to a reduction in the mucus production and bicarbonate secretion (Massignani *et al*., 2009). Reactive oxygen species generation by ethanol leads to imbalance between oxidant and antioxidant cellular processes causing severe damage to the vascular plexus and rupture of blood vessels. The resultant effects of these changes are haemorrhage, tissue necrosis and disruption of the protective mucosal barrier (Manjusha *et al*., 2014). Ethanol induces gastric mucosal damage by promoting disturbances of mucosal microcirculation, ischemia, endothelin release, degranulation of mast cells, inhibition of prostaglandins and decrease of gastric mucus production (Park *et al*., 2004; Arawwawala *et al*., 2010). Ethanol also increases the release of histamine and influx of calcium ions as well as stimulates the synthesis of leukotriene C4 (Okoli *et al*, 2009). It causes the solubilization of components of the mucus of the stomach, increases activity of xanthine oxidase and levels of the malondialdehyde, and decreases glutathione levels (Marrotta *et al*., 1999). Ethanol rapidly penetrates the gastric mucosa causing injury characterized by membrane damage, erosive hemorrhagic lesions with diffuse coagulative cell necrosis, cell exfoliation, multiple superficial erosions, marked vascular congestion and ulcer formation (Gazzieri *et al*., 2007; Li *et al*., 2008; Nassini *et al.,* 2010). In gastric ethanol injury, the endothelium is the first preferential target of ethanol damage (de-Faria *et al*., 2012). Hemorrhagic ulceration of the stomach in humans and experimental animals is reported to have been caused by ingestion of elevated amounts of ethanol (de-Faria *et al*., 2012). Alcohol causes peptic ulcers in human beings by damaging the mucous glands and the tight epithelial junctions between the gastric lining cells (Arthur and John, 2000). Ethanol also reduces the levels of endogenous sulfhydryl compounds (Nguemfo *et al.,* 2009) which are neutralized when they bind to free radicals

produced when tissues are injured by necrotizing agents like ethanol (Maity *et al*., 1998; Maria *et al*., 2011). The result of this study suggests that the extract, fractions and isolated compounds may mitigate ethanol-induced gastric lesions through cytoprotective activity.

The extract, fractions and isolated compounds produced antiulcerogenic activity against cold restraint stress-induced ulcers. Stress and distress have been shown to decrease upper gastrointestinal blood flow (Kauffman, 1997) and may render the human stomach and duodenum more susceptible to damage (Levenstein, 2002). Cold restraint stress-induced ulcer is an acceptable model for the induction of gastric ulcers where peripheral sympathetic activation and increased acid secretion are involved in the development of gastric lesions (Yadav *et al*., 2013). Stress caused by physiological and psychological factors (Viana *et al*., 2013) increases the generation of reactive oxygen species and gastric juice secretion leading to the development of gastric mucosal lesions (Bjarnason *et al*., 2007; Tamashiro *et al*., 2012). Stress-induced gastric lesions are mainly caused by oxidative damage due to hydroxyl radicals (Sener *et al*., 2005). Stress-related mucosal diseases are associated with critically ill patients such as those with severe trauma, shock, septicaemia, extensive burns, major surgery and intracranial lesions (Harsh, 2005; Laine *et al*., 2008). Intracranial lesion from brain trauma, intracranial surgery and brain tumours causes Cushingôs ulcers due to hypersecretion of gastric acid following excessive vagal stimulation of gastric glands (Harsh, 2005). The mechanisms of mucosal erosions in other etiological factors of stress ulcers where gastric acid secretion is normal or below normal may involve ischaemic hypoxic injury and depletion of the gastric mucus ñbarrierò which renders the mucosa susceptible to attack by acid-peptic secretion (Harsh, 2005). Few human studies have found that stress, anxiety and depression increase acid secretion (Feldman *et al*., 1992), impair

ulcer healing and promote ulcer relapse (Levenstein, 2002). There are several reports on increased perforated ulcer in the aftermath of societal catastrophies such as wars, economic collapse and earthquake due to the emotional stimuli associated with these conditions which increase highly peptic and acidic interdigestive gastric secretions (Arthur and John, 2000; Levenstein, 2002). The effectiveness of the extract, fractions and isolated compounds in this model suggests that the constituents of the plant may possess antisecretory properties associated with stress ulcers.

The isolated compounds protected the rat against pyloric ligation-induced ulcer and reduced the volume of gastric secretion and total acidity and elevated gastric pH. Pyloric ligation-induced ulcers is one of the widely used method for studying the effect of drugs on gastric secretion (Okpo *et al*., 2011) and this model shows the possible changes in the gastric parameters such as gastric secretion volume, total acidity, pH (Lakshimi *et al*., 2009), total hexose, hexosamine, fucose, sialic acid, total carbohydrate and proteins (Jince *et al*., 2010). Increase in total carbohydrate : protein ratio (TC:P) is a reliable index for mucin secretion which contributes to antiulcerogenic and cytoprotective effects (Venkataranganna *et al*., 1998; Rao *et al*., 2000; Jince *et al*., 2010). In this model, the ulceration is caused by high concentration of acid and pepsin (Tovey, 2015) through auto-digestion of the mucosa (Yadav *et al.,* 2011). Gastric acid accumulates in the stomach when the pyloric end is ligated causing the formation of gastric ulcers (Khare *et al*., 2008). In human beings, local irritants such as alcohol, cigarette smoking, heavily spiced foods and NSAIDs have longer duration of exposure at the pyloric antrum and the lesser curvature of the stomach, thus predisposing these sites to mucosal lesions more than other sites such as cardia, marginal ulcer and Meckelôs diverticulum (Harsh, 2005). Agents that reduce

gastric secretion volume and total acidity with elevation of pH offer gastroprotective effects against ulcers induced by this method. The result from this study indicates that the gastroprotective effect of the isolated compounds may be attributed to antisecretory mechanisms.

Antisecretory mechanisms involve the blockade of H_2-receptors and inhibition of H^+K^+ATPase on the basolateral membrane of parietal cells in the body and fundus of the stomach. These antisecretory mechanisms protect the development of ulcers due to increased acid secretion. Further evidence for the antisecretory activity of the fractions and isolated compounds is demonstrated by their potent inhibition of H^+ K^+ ATPase activity. H^+ K^+ ATPase (proton pump) is the common and final pathway of all stimulation of gastric acid secretion from the parietal cells of the gastric mucosa (Yadav *et al*., 2011). H^+ K^+ ATPase enzyme is located on the apical secretory membrane of parietal cell and responsible for acid secretion in the stomach. It catalyzes the electroneutral exchange of intracellular H^+ and extracellular K^+ coupled with the hydrolysis of the cytoplasmic ATP (Sidaraddu and Dharmesh, 2007; Yadav *et al*., 2011). Studies have shown that proton pump is up-regulated in ulcer conditions (Sidaraddu and Dharmesh, 2007). The present result showed comparable inhibition to omeprazole, a known anti- H^+ K^+ ATPase, that is currently used for the treatment and management of peptic ulcer disease and other acid related conditions. Therefore, the antiulcer activity of constituents of this plant may be due to antisecretory activity mediated via inhibition of the proton pump. This result corroborates similar reports on phytoconstituents that showed anti- H^+ K^+ ATPase activity with antiulcer effects (Yadav *et al*., 2011; Vaibhav *et al*., 2013; Rajesh *et al*., 2013).

The NO pathway has been reported to be involved in the promotion of gastric secretion through enterochromafin-like cells (Hasebe *et al*., 2001) and its inhibition decreases acid secretion (Roslida *et al*., 2010). Evaluation of the effects of BF1 and BF2 on the NO pathway showed that they did not cause any elevation in ulcer lesion index of L-NAME pre-treated rats. This suggests that nitric oxide (NO) pathway may not be involved in the gastroprotective activity of the compounds. NO is synthesized from L-arginine by the activity of NO synthase and is one of the major factors involved in acid, alkaline and mucus secretion (Chandranath *et al*., 2002) as well as regulation of the gastric blood flow and gastric microcirculation (Wallace, 2006). It is also a fundamental mediator underlying the gastric defense mechanism (Viana *et al*., 2013). Non-involvement of NO in the gastroprotective effects of the extract and fraction suggest the involvement of mechanism other than those mediated by NO in the antiulcer activity. Conversely, the isolated compounds completely lost their gastroprotective activity when N-ethylmaleimide (NEM), a non-selective sulfhydryl synthase inhibitor was co-administered leading to a profound elevation of ulcer lesion indices of rat subjected to ethanol-induced lesions. Studies have shown that the administration of glutathione depletors such as N-ethylmaleimide significantly potentiates the effects of ethanol on gastric mucosa injury (Luiz-Ferreira *et al*., 2010). Sulfhydryl compounds bind to free radicals generated during tissue injury caused by noxious agents and protect mucus through mucus disulfide bridges which if reduced renders mucus water soluble (Avila *et al*., 1996; Roslida *et al*., 2010). This result show that the gastroprotective activities of the compounds may be mediated through the participation of mucosal sulfhydryl compounds suggestive of cytoprotective.

Due to the role of free radicals in the pathogenesis of ulcers, the antioxidant activity of the extract and fractions was assayed. The extract, fractions, BF1 and BF2 scavenged DPPH free radicals which suggest that they possess antioxidant property. Free radicals have been implicated in mediating indomethacin, ethanol and cold restraint stress-induced ulcers (Dharmani *et al*., 2005; Prabha *et al*., 2011; Shobha and Jamadar, 2013). Oxygen free radicals play a vital role as second messengers in pro-inflammatory pathways (Moon *et al*., 2000). Lipid peroxidation and metabolism generate oxygen and lipid radicals (Kwiecien *et al*., 2001). Antioxidants play a significant role in repairing gastric damage and are reduced in the stomach tissue that is damaged by indomethacin (Onasanwo *et al*., 2011). They exert their activity by suppressing the production of free radicals, termination of chain reaction, scavenging free radicals and by repairing damaged cells (Sen *et al*., 2010). Reactive oxygen species such as superoxide, hydroxyl, hydroperoxyl, alkoxyl and peroxyl have been reported to be causative factors of gastric mucosal damage of different origins (Maity *et al*., 2003; Chattopadhyay *et al*., 2006; Bandyopadhay *et al*., 2006; Onasanwo *et al*., 2011) and are also involved in the progression of gastric ulcers (Prabha *et al*., 2011). It has been proposed that an imbalance between reactive oxygen species accumulation and defense mechanisms in the body causes oxidative stress-induced diseases such as cancer (Aruoma, 1998), ulcer, inflammation (Middleton *et al*., 2000) and brain dysfunction (Siddarju and Dharmesh, 2007). Therefore, the antioxidant property of the extract, fractions and isolated compounds may partly account for the antiulcer effects in indomethacin, ethanol and stress-induced ulcers and may limit gastric mucosal damage in humans when used in the treatment of ulcers. Several workers have also reported the antioxidant potential of the stem bark extract of *B. ferruginea* (Cimanga *et al*., 2001; Adetutu *et al*., 2011; Bothon *et al*., 2012; Olumayokun *et al*., 2012; Omotade *et al*., 2012; Ajiboye *et al*., 2013).

Bioassay-guided fractionation technique was employed to aid the systemic identification and isolation of the antiulcer constituents of this plant. Successive separation and screening tests narrowed the activity down to two isolates BF1 and BF2 which on characterization were identified as ɓ-sitosterol and ɓ-sitosterol-3-O-ɓD glucopyranoside respectively. Results from this study showed that both compounds protected treated rats against indomethacin, absolute ethanol, cold restraint stress and pyloric ligation-induced ulcers. ɓ-sitosterol-3-O-ɓD glucopyranoside was, however, more active than ɓ-sitosterol. These compounds have not been reported in the stem bark extract of *B. ferruginea*. ɓ-sitosterol and ɓ-sitosterol-3-O- ɓD glucopyranoside are steroids. ɓ-sitosterol-3-O- ɓD glucopyranoside is a ɓ-sitosterol with a sugar moiety at position 3 of the steroid. They are phytosterols which are steroid compounds composed of plant sterols and stanols related to cholesterol and differ in carbon chains and or presence or absence of double bond (Xiao *et al*., 1992). They may be useful biological and phytochemical markers of antiulcer activity for standardization of extract and fractions. Steroids such as ɓ-sitosterol and ɓ-carotene have been reported to protect against the development of gastric ulcers (Xiao *et al*., 1992). Reports have shown the gastroprotective activity of ɓ-sitosterol and sitosterol-3-O-ɓ-glucoside from root bark of *Hippocratea excelsa* in rat ethanol, aspirin, histamine and pyloric ligation-induced ulcers with sitosterol-glycoside being more effective than sitosterol (Navarrete *et al*., 2002). Also ɓ-sitosterol and cholesterol enhanced the gastroprotective effect of unsaturated phospholipids due to enhancement of the packing of the unsaturated phospholipids in the cell membranes (Romero and Lichtenberger, 1990). There are reports on the protective activity of sterolins (sterol glycosides) in unripe green banana (*Musa paradisiaca*) against aspirin-induced ulcer in rats, attributed to its four sitosterolins (sitoindoside I and II, sitosterol gentobioside and sitosterol myoinosityl-ɓ D-glucoside) (Ghosal and Saini, 1984; Ghosal, 1985). A related study

using mice restraint models, showed the ulceroprotective activity of a mixture of steryl- ɓ -D-glucosides (Okuyama and Yamazaki, 1983). Studies have also shown that certain dietary phospholipids (phosphatidyl choline (lethicin) and phosphatidyl ethanolamine (cephalin)) and phytosterols (ɓ-sitosterol, stigmasterol and an unidentified isomer of ɓ-sitosterol) have protective action against both gastric and duodenal ulceration, including ulceration due to non-steroidal anti-inflammatory drugs (NSAIDs). They also promoted ulcer healing both singly and in combination (Tovey, 2015). The results from this study showed that ɓ-sitosterol and ɓ-sitosterol-3-O-ɓD glucopyranoside may be the antiulcer constituents of *B. ferruginea* stem bark.

4.2 Conclusion

In conclusion, extract of stem bark of *B. ferruginea* may possess antiulcer properties mediated through cytoprotective, antisecretory, proton pump inhibitory and antioxidant effects. The antiulcer activities appear not to involve the nitric oxide pathway but may involve the participation of non-protein sulfhydryl compounds. Two compounds, ɓ-sitosterol and ɓ-sitosterol-3-O- ɓD glucopyranoside isolated from the extract demonstrated potent and significant antiulcer effect.

REFERENCES

Abdiev, S., Ahn, K. S., Khadjibaev, A., Malikov, Y., Bahramov, S., Rakhimov, B., Sakamoto, J., Kodera, Y., Nakao, A. and Hamajima, N. (2010): *Helicobacter pylori* infection and cytokine gene polymorphisms in Uzbeks. Nagoya *J. Med. Sci.* 72: 167-172.

Abdulla, M.A., Ali, H.M., Ahmed, K.A., Noor, S.M. and Ismail, S. (2009): Evaluation of the anti-ulcer activities of *Morus alba* extracts in experimentally-induced gastric ulcer in rats. *Biomed. Res*. 20 (1): 35ï 39.

Abdulla, M.A., Ahmed, K.A., Bayaty, F.H. and Masood, Y. (2010): Gastroprotective effect of *Phyllanthus niruri* leaf extract against ethanol-induced gastric mucosal injury in rats. *Afri. J. Pharm. Pharmacol*. 4 (5):226-30.

Addae-Mensah, I. and Achenbach, H. (1985): Terpenoids and flavonoids of *Bridelia ferruginea. Phytochem*. 24 (8): 1817-1819.

Addae-Mensah, I. (1992): Towards a rational scientific basis for herbal medicine: A phytochemistôs two decade contribution. Accra: Ghana University Press.

Adeniyi, B. A., Otegbayo, J. A., Lawal, T. O., Oluwasola, A. O., Odaibo, G. N., Okolo, C., Ola, S. O., Idowu, P. A., Akere, A. and Kehinde, A. O. (2012): *Afri. J. Microbiol. Res*. 6 (14): 3399-3402.

Adetutu, A., Morgan, W.A. and Corcoran, O. [2011]: Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extract of *Bridelia ferruginea* leaf, a wound-healing plant of Nigeria. *J. Ethnopharmacol*. 133(1): 116-119.

Agrawal, A.K., Rao, C.V., Sairam, K., Joshi, V.K. and Goel, R.K. (2000): Effect of *Piper longum* Linn, *Zingiber officinale* Linn and *Ferula* species on gastric ulceration and secretion in rats. *Ind. J. Exp. Biol.* 38 (10), 994ï 998.

Ahmad, B.A, Mahadeva, U.R., Abdurrazaq, M., Thant, Z., Nur, H. M., Nasir, M., and Khamsah, S.M. (2014): Reviews of herbal and their secondary metabolites in the treatment of ulcerative colitis and peptic ulcer. *J. App. Pharm. Sci.* 4 (8)**:** 080-090.

Ahmet, U., Ahmet, D., Filiz, A., Binnur, P., Mine, G., Yersu, K., Kadir, D., Sadakat, Ơ., Fatih, B., Güngör, B., Zeynel, M. and Sabahattin, K. (2011): The Etiological Factors of Duodenal and Gastric Ulcers, Peptic Ulcer Disease, Dr. Jianyuan Chai (Ed.), ISBN: 978-953-307-976-9.

Ajaikumar, K. B; Asheef, M; Babu, B. H; Padikkala, J. (2005): The inhibition of gastric mucosal injury by *punica granatum* L (pomegranate) methanolic extact. *J. Ethnopharmacol*. 96:171-176.

Ajani, E. O., Sabiu, S., Bamisaye, F. A., Adenigba, B. V., Awomoyi, D. D. and Adeyanju, M. N. (2014): Hepatoprotection and antioxidative effect of ethanolic leaf extract of *Langenaric breviflora* (bitter gourd) on Indomethacin-induced ulcerated rats. *J. Pharm. Biol. Sci*. 9 (5): 61-68.

Ajiboye, T. O.., Abdussalam, F. A., Adeleye, A. O., Iliasu, G. A., Ariyo, F. A., Adediran, Z. A., Raji, K. O. and Raji, H. O. (2013): *Bridelia ferruginea* promotes reactive oxygen species detoxification in N-nitrosodiethylamine treated rats. *J. Diet. Suppl*. 10 (3): 210-228.

Akah, P. A. and Nwafor, S. V. (1999): Studies on anti-ulcer properties of *Cissampelos mucronata* leaf extract. *Ind. J. Exp. Biol*. 37: 936-938.

Akamatsu, Y., Haga, M., Tyagi, S., Yamashita, K., Graça-Souza, A. V.,Ollinger, R., Czismadia, E., May, G. A., Ifedigbo, E., Otterbein, L. E., Bach,F. H., and Soares, M. P. (2004): Heme oxygenase-1-derived carbon monoxide protects hearts from transplant associated ischemia reperfusion injury. *FASEB J.*18: 771ï 772.

Akudor, G.C, Mbah, C.C., Anyalewechi, N.A., Idris-Usman, M., Iwuanyanwu, T.C. and Osunkwo, U.A. (2011): Pharmacological profile of aqueous extract of *Bridelia ferruginea* stem bark in the relief of pain and fever. *J.Med. Plants Res*. 5(22): 5366-5369.

Akuodor, G.C, Mbah, C.C., Essien, A. D., Akpan, J. L., Ezeokpo, B. C., Iwuanyanwu, T.C. and Osunkwo, U.A. (2011): Ulcer protective and antidiarrhoeal effects of the aqueous stem bark extract of *Bridelia ferruginea* stem bark in rodents. *Pharmacolog*. 3(11): 591-597.

Alam, S, Asad, M., Asdaq, S.M.B. and Prasad, V.S. (2009): Antiulcer activity of methanolic extract of Mormodica charantia L. in rats. *J. Ethnopharmacol*. 123(3): 464-469.

Alex, M. and Walter, K.K. (2009): Acid peptic diseases: Pharmacological approach to treatment. *Expert Rev. Clin. Pharmacol*. 2 (3): 295-314.

Al Mofley, I.A. (2010): Spices, herbal xenobiotics and the stomach: Friends or Foes? *World J. Gastrol.* 16:2710-2719.

Allen, A. and Flemström, G. (2005): Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Am. J. Physiol. Cell Physiol.* 288 (1): C1ï C19.

Almeida, E.S.S., Filho, V.C., Niero, R., Clasen, B.K., Balogun, S.O. and Martins, D.T.O. (2011): Pharmacological mechanisms underlying the anti-ulcer activity of methanol extract and canthin-6-one of *Simaba ferruginea* A. St-Hil, in animal models. *J. Ethnopharmacol.* 134 (3): 630-636.

Al-Qarawi, A.A., Abdel-Rahman, H., Ali, H. B. H., Mousa, H.M. and El-Mougy, S. A. (2005): ñThe ameliorative effect of dates (*Phoenix dactylifera* L.) on ethanol-induced gastric ulcer in rats,ò *J. Ethnopharmacol*. 98 (3): 313ï 317.

Andreo, M.A, Ballesteros, K.V.R., Hiruma-Lima, C.A., daRocha, L.R.M., Souza Brito, A.R.M., Vilegas, W. (2006): Effect of *Mouriri pusa* extracts on experimentally induced gastric lesion in rodents: Role of endogenous sulfhydryls compounds and nitricoxide in gastroprotection. J. Ethnopharmacol. 107:431-441.

American Society for Gastrointestinal Endoscopy (1999): The role of endoscopy in the management of gastroesophageal reflux disease: Guidelines for the Clinical Application. *Gastrointestinal Endoscopy*. 49(6): 834-835.

Anosike, C.A., Obidoa, O., Ezeanyika, L.U.S., Nwuba, M.M. (2009): Anti-inflammatory and anti-ulcerogenic activity of the ethanol extract of ginger (*Zingiber officinale*). *Afr. J. Biochem. Res*. 3 (12): 379ï 384.

Anosike, C.A.and Obidoa, O. (2010): Anti-inflammatory and antiulcerogenic effect of Ethanol extract of coconut (*cocos nucifera*) on experimental rats. *Afr. J. Food Agri. Nutr. Develop*. 10 (10), 4286ï 4300.

Antonio, D. and Gaetano, L. (2004): Helicobacter pylori and gastric disease: a dangerous association. *Cancer Letters* 213(1): 1-10.

Arawwawala, M., Thabrew, I., Handunnetti, S. and Arambewela, L. (2010): Anti-inflammatory activity of *Trichosanthes cucumerina* Linn. in rats. *J. Ethnopharmacol*. 131: 538-543.

Arrieta, J., Benitez, J., Flores, E., Castillo, C. and Navarrete, A. (2003): Purification of gastroprotective triterpenoids from stem bark of *Amphipterygium adstringens*; roles of prostaglandins, sulphydyls, nitric oxide and capsaicin neurons. *Planta Medica*. 69: 905-909.

Arents, N.L., Thijs, J.C., van Zwet, A. A. and Kleibeuker, J.H. (2004): Does the declining prevalence ofHelicobacter pylori unmask patients with idiopathic peptic ulcer disease? Trends over an 8 year period. *Eur. J. Gastroenterol. Hepatol.*16 (8): 779-83.

Arroyo, M.T., Forne,.M., de Argila, C.M, Feu F, Arenas J, de la Vega J, Garrigues V, Mora F.,Castro, M., Bujanda, L., Cosme, A., Castiella, A., Gisbert, J.P., Hervas, A.and Lanas, A. (2004): Prevalence of peptic ulcer not related to *Helicobacter pylori* or non-steroidal anti-inflammatory drug use is negligible in southern Europe. *Helicobacter.* 9(3):249-254.

Arun, K., Rao, Ch.V., Kumar, V.M., Ayaza, A., Naiyera, S. and Irfan, K.M. (2010): Anti-ulcerogenic and ulcer healing effects of *Zingiber officinale* (L.) On experimental ulcer models: possible mechanism for the inhibition of acid formation. *Int. J. Pharm. Res*. 1(2): 75ï 85.

Aruoma, O. I. (1998): Free radicals, oxidative stress and antioxidants in human health and diseases. *J. Am. Oil Chem. Soc*. 75: 199-212.

Arthur, C. G. and John, E. H. (2000a): The autonomic nervous system and the adrenal medulla. In: Textbook of medical physiology. 10th. Edition, W. B. Saunders, Pennsylvania. 697-708.

Arthur, C. G. and John, E. H. (2000b): Gastrointestinal Phsiology. In: Textbook of medical physiology. 10th. Edition, W. B. Saunders, Pennsylvania. 718-770.

Atherton, J.C. (2006): The pathogenesis of *Helicobacter pylori*-induced gastroduodenal diseases. *Annu. Rev. Pathol*. 1: 63ï 96.

Atsufumi, K., Hiroyuki, N., Hitomi, S., Yumiko, N., Kaori, H., Satoko, K., Minoru, N., Naoyuki, K., Ryotaro, K., Fumiko, S., Mitsuhiro, K., Kazuaki, K., Naoki, A., Hisakazu, Y. and

Kenzo, K. (2004): A protective role of protease-activatd receptor 1 in rat gastric mucosa. *Gastroenterol*. 1261 (1): 208-219.

Avila, J. R., de La Lastra, C. A., Martin, M. J., Motilva, V., Luque, I., Delgado, D., Esteban, J. and Herrerias, J. (1996): Role of endogenous sulphydryl and neutrophil infiltration in the pathogenesis of gastric mucosal injury induced by piroxicam in rats. *Inflam. Res*. 45: 83-88.

Awaad, A.S., Reham, M. E. and Gamal, A. S. (2013): Natural products in treatment of ulcerative colitis and peptic ulcer. *J. Saudi Chem. Soc*. 17: 101ï 124.

Bae, D. K., Park, D. and Lee, S.H. (2011): Different antiulcer activities of pantoprazole in stress, alcohol and pylorus ligation-induced ulcer models. Lab. *Animal Res.* 27 (1): 47ï 52.

Baena, J. M., Lopez, C., Hidalgo, A., Rame, F., Jimenez, S. and Garcia, M. (2002): Relationship between alcohol consumption and the success of Helicobacter pylori eradication therapy using Omeprazole, Clarithromycin and Amoxycillin for one week. *Eur. J. Gastroenterol*. *and Hepatol*. 14(3): 291-296.

Bailey, S. M., and Cunningham, C. C. (2002): Free Radical. *Biol. Med*. 32: 11ï 16.

Bairy, K. L, Roopa, K., Malini, S. and Rao, C.M. (2002): Protective effect of *Tinospora cardifolia* on experimentally induced gastric ulcers in rats. *J. Nat. Rem*. 24, 49-53.

Bakoma, B., Eklu-Gadegkeku, K., Agbonon, A., Aklikoku, K., Bassene, E. and Gbeassor, M. (2011): Preventive effect of *Bridelia ferruginea* against high-fructose diet induced glucose intolerance, oxidative stress and hyperlipidemia in male wistar rats. *J. Pharmacol. Toxicol*. 6(3): 249-257.

Bandyopadhyay, D. and Chattopadhyay, A. (2006): Reactive Oxygen Species induced gastric ulceration: Protection by melatonin. *Curr. Med. Chem*. 13 (10): 187-202.

Bandyopadhyay, D., Biswas, K., Bhattacharyya, M., Reiter, R.J. and Banerjee, R.K. (2001): Gastric toxicity and mucosal ulceration induced by oxygen-derived reactive species, protection by melatonin. *Curr. Mol. Med*. 1 (4): 501ï 513.

Bandyopadhyay, D., Biswas, K., Bhattacharyya, M., Reiter, R.J. and Banerjee, R.K. (2002): Involvement of reactive oxygen species in gastric ulceration, protection by melatonin. *Ind. J. Exp. Biol*. 40: 693ï 705.

Bayir, H., and Kagan, V. E. (2008): Bench to bedside review: Mitochondrial injury, oxidative stress and apoptosis- there is nothing more pratical than a good theory. *Crit. Care* 12 (1): 206.

Beckman, J. D., Chen, C., Nguyen, J., Thayanithy, V., Subramanian, S., Steer, C. J., and Vercellotti, G. M. (2011): Regulation of hemeoxygenase-1 protein expression by miR-377 in combination with miR-217. *J. Biol. Chem*. 286: 3194ï 3202.

Bekyarova, G., Tzaneva, M. and Hristova, M. (2013): Heme Oxygenase-1 Expresion in Gastric Mucosa and Liver after Burns: Preliminary Immunohistochemical Study. *J. Interdiscipl*. *Histopathol.* 1 (5): 246- 251.

Beserra, A.M., Calegari, P.I. Souza, M.D, Dos Santos, R.A., Lima, J.C., Siva, R.M., Balogun, S.O. and Martins, D.T. (2011): Gastroprotective and ulcer healing mechanisms of ellagic acid in experimental rats. *J. Agric. Food. Chem*. 13: 6957-6965.

Bhalke, R.D., Giri, A. M., Anarthe, J.S. and Pal, C.S. (2010): Antilcer activity of ethanol extract of leaves of *Sesbania grandiflora* (Linn*). Int. J. Pharm. Pharm. Sci.* 2(4):206-208.

Bhattacharjee, M., Bhattacharjee, S., Gupta, A. and Banerjee, R.K. (2002): Critical role of an endogenous gastric peroxidase in controlling oxidative damage in *H. pylori*-mediated and non-mediated gastric ulcer. *Free Rad. Biol. Med*. 3:731ï 43.

Bigheti, A.E., Antonio, M. A., Kohn, L.K., Rehder, V.L.G., Foglio, M.A., Possenti, A., Vilela, L. and Carvalho, J.E. (2005): Anti-Ulcerogenic activitiy of a crude hydroalcoholic extract and Coumarin Isolated from *Mikania laevigata* Schutz Bip. *Phytomed*. 12: 72-77.

Bjamason, I., Scarpignato, C., Takeuchi, K. and Rainsford, K. D. (2007): Determinants of the short-term gastric damage caused by NSAIDs in man. *Alim. Pharmacol. Therap*. 26: 95-106.

Björne, H., Govoni, M. and Törnberg, D.C. (2005): Intragastric nitric oxide is abolished in intubated patients and restored by nitrite. *Crit. Care Med*. 33 (8): 1722ï 1727.

Blandizzi, C., Tuccori, M., Colucci, R., Gori, G., Fornai, M., Antonioli, L., Ghisu, N. and Del Tacca, M. (2008): Clinical efficacy of esomeprazole in the prevention and healing of gastrointestinal toxicity associated with NSAIDs in elderly patients. *Drugs Aging.* 25(3): 197-208.

Bonacorsi, C., Raddi, M.S.G., Carlos, I.Z., Sannomiya, M. and Vilegas, W. (2009): Anti-*Helicobacter pylori* activity and immunostimulatory effect of extracts from *Byrsonima crassa* Nied (Malpighiaceae). *BMC Complemen. Altern. Med*. DOI: 10.1186/1472-6882-9-2

Bothon, F. T. D., Debiton, E., Yedomonhan, H., Avlessi, F., Teulade, J. C. and Sohounhloue, D. C. K. (2012): Alpha glucosidase inhibition, antioxidant and cytotoxicity activities of semi-ethanolic extracts of *B. ferruginea* Benth and *Ceiba pentandra* L. Gaerth from Benin. *Res. J. Chem. Sci*. 2: 31-36.

Bronislaw, L. S. and Amalia, S. (2010): Cytosolic phospholipase A2 S-nitrosylation in ghrelin protection against detrimental effect of ethanol cytotoxicity on gastric mucin synthesis - Ghrelin in gastric mucosal protection. *Health* 2 (9): 1033-1039.

Brown, L.M. (2000): Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol. Rev.* 22: 283-297.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Brzozowska, I., and Pawlik, T. (2005a): Role of prostaglandins in gastroprotection and gastricadaptation. *J. Physiol. Pharmacol*. 56 (5): 33ï 55.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Danuta, D., Pawlik, M., Sliwowski, Z., Pawlik, W. W. and Hahn, E.G. (2005b): Role of central and peripheral ghrelin in the mechanism of gastric mucosal defence. *Inflammopharmacol*.13 (1-3): 45ï 62.

Carr, Z.A., Kleinerman, R.A., Stovall, M., Weinstock, R.M., Griem, M.L and Land, C.E. (2002): Malignant neoplasms after radiation therapy for peptic ulcer. *Radiat. Res*. 157 (6): 668-77.

Carvalho, K.I.M., Fernandes, H.B., Machado, F.D.F., Oliveira, I.S., Oliveira, F.A., Nunes, P.H.M., Lima, J.T., Almeida, J.R.G.S. and Oliveira, R.C.M., (2010): Antiulcer activity of ethanolic extract of *Encholirium spectabile* Mart. ex Schult & Schult f. (Bromeliaceae) in rodents. *Biol. Res*. 43 (4): 459ï 465.

Castillo-Juarez, I., Gonzalez, V., Jaime-Aguilar, H., Martinez, G., Linares, E., Bye, R. and Romero, I. (2009): Anti-helicobacter pylori activity of plants used in Mexican traditional medicine for gastrointestinal disorders. *J. Ethnopharmacol*. 122: 402-405.

Ceranowicz, P., Warzecha, Z., Dembinski, A., Sendur, R., Cieszkowski, J., Ceranowicz, D., Pawlik, W.W., Kuwahara, A., Kato, I. and Konturek, P.C. (2009): Treatment with ghrelin accelerates the healing of acetic acid-induced gastric and duodenal ulcers in rats. *J. Physiol. Pharmacol*. 60 (1): 87-98.

Chandranath, S.I.and Bastaki, S.M., Singh J. (2002): A comparative study on the activity of lansoprazole, omeprazole and PD-136450 on acidified ethanol and indomethacin-induced gastric lesions in rat. *Clin. Exp. Pharmcol. Physiol*. 29: 173-180.

Chattopadhyay, I., Bandyopadhyay, U., Biswas, K., Maity, P., and Banerjee, R. K. (2006): Indomethacin inactivates gastric peroxidase to induce reactive oxygen- mediated gastric mucosal injury and curcumin protects it by preventing peroxidase inactivation and scavenging reactive oxygen. *Free Rad. Biol. Med.*40: 1397ï 1408.

Chatzaki, E., Lambropoulou, M., Constantinidis, T. C., Papadopoulos, N., Tache, Y., Minopoulos, G. and Grigoriads, D. E. (2006): Corticotropin-releasing factor (CRF) receptor type 2 in the human stomach: protective biological role by inhibition of apoptosis. *J.Cell Physiol.* 209 (3): 905ï 911.

Chiba, T., Sato, K., Kudara, N., Shinozaki, H., Ikeda, K., Sato, K., Endo, M., Orii, S. and Suzuki, K. (2008): Upper gastrointestinal disorders induced by non-steroidal anti-inflammatory drugs. *Inflammopharmacol.* 16: 16-20.

Chiou, S.K.,Tetsuya Tanigawa, T., Akahoshi, T., Abdelkarim, B., Jones, M. K , and Andrzej S. Tarnawski, A. S. (2005): Survivin: a novel target for indomethacin-induced gastric injury. *Gastroenterol.* 128 (1): 63ï 73.

Chowdhury, A., Mukhopadhyay, A.K., Nair, B.G., Kundu, P., De, R., Swarnakar, S. and Ramamurthy, T. (2009): Curcumin exhibits antibacterial activity against *Helicobacter pylori* infectionï Green-MedInfo summary. *Antimicrob. Agents Chemother*. 53 (4): 1592ï 1597.

Cimanga, K., Ying, L., de Bruyne, T., Appers S., Cos, P., Hermanis, N., Bakana, P., Tona, L., Kambu, K. Kalenda, D.T., Pieters, L., Vanden, B. D. and Vlietina, A. J. (2001): Radical

Scavending and Xanthine Oxidase inhibitory activity of phenolic compounds from *Bridelia ferruginea* stem bark. *J. Pharm. Pharmacol*. 53 (5): 757-761.

Cogo, L.L., Monteiro, C.L.B., Miguel, M.D., Miguel, O.G., Cunico, M.M., Ribeiro, M.L., de Camargo, E.R., Kussen, G.M.B., Nogueira, K.S. and Costa, L.M.D. (2010): Anti-*Helicobacter pylori* activity of plant extracts traditionally used for the treatment of gastrointestinal disorders. Braz. *J. Microbiol*. 41: 304ï 309.

Cola-Miranda, M., Barbastefano, V., Hiruma-Lima, C.A., Calvo, T.R., Vilegas, W. and Brito, A.R.M.S. (2006): Antiulcerogenic activity of *Indigofera truxillensis* Kunth. *Biota. Neotropica.* 6 (3), 1ï 9.

De Souza, A.E.S., Filho, V.C, Niero, R., Clasen, B.K., Balogun, S.O.and Oliveira, M.D.T (2011): Pharmacological mechanisms underlying the anti-ulcer activity of methanol extract and Canthin-6-one of Simaba ferruginea A anti st. hil in animal models. *J. Ethnopharmacol.* 134: 630-636.

de-Fariaa, F.M, Almeidab, A.C.A, Luiz-Ferreirab, A., Dunderb, R.J.,Takayamab,C., da Silva,M.S., da Silva,M.A., Vilegas, W., Rozzad, L.A., Pellizzond, C.H., Tomae, W., and Souza-Brito, A.R.M. (2012): Mechanisms of action underlying the gastric antiulcer activity of the *Rhizophora mangle* L. *J. Ethnopharmacol*. 139: 234ï 243.

Devaraj, V.C., Asad, M., and Prasad, S. (2007): Effects of leaves and fruits of *Moringa oleifera* on gastric and duodenal ulcers. *Pharm. Biol*. 45 (4): 332-338.

Dharmani, P. and Kuchibhotla, V.K. (2005): Exploring Indian Medicinal Plant for antiulcer activity. *Ind. J. Exp. Biol*. 43:517-521

Dhasan, P. B., Jegadeesan, M. and Kavimani, S. (2010): Antiulcer activity of the aqueous extract of fruits of *Mormodica cymbalaria* Hoof F. in Wistar rats. *Pharmacogn. Res.* 2(1): 58-61

Dugani, A., Auzzi, A., Naas, F.and Megwez, S. (2008): Effects of the oil and mucilage from flaxseed (Linum Usitatissimum) on gastric lesions induced by *ethanol* in rats. Lib. *J. Med.* 6 (12), 166ï 169.

Dundon, W. G., de Bernard, M. and Montecucco, C. (2001): Virulence factors of *Helicobacter pylori*. *Int. J. Med. Microbiol*. 290: 647-658.

Ekanem, J. T., Kolawale, O. M. and Abbah, O. C. (2008): Trypanocidal potential of methanolic extract of *Bridelia ferruginea* Benth bark in Rattus novergicus. *Afri. J. Biochem. Res*. 2: 45-50.

Ezike, A. C., Akah, P. A., Okoli, C. O., Ezeuchenne, N. A. and Ezeugwu, S. (2009): *Carica papaya* (paw-paw) unripe fruit may be beneficial in ulcer. J. Med. Food, 12: 1268-1273.

Ezike, A. C., Akah, P. A., Nnamani, M. E., Okoli, C. O., Ojike, F. U., Eze, F. S., Chime, C. E. and Azosiri, I. J. (2011): Studies on the antiulcer and gastrointestinal effects of stem bark extract of *Bridelia ferruginea*. *J. Complem. Integ. Med*. 8 (1): Article 27. DOI. 10.2202/1553-3840.1507.

Ezike, A. C., Akah, P. A., Okoli, C. O., Ufere, I. K., Ezeudu, E., Okoye, C. F., Ashara, C. and Igbokwe, I. N. (2014): Studies on gastrointestinal effects of *Desmodium velutinum*: a traditional remedy for diarrhea. Am. J. Pharmacol. Toxicol. 9 (2): 114-124.

Ezekwesili, C. N., Ghasi, S., Adindu, C. S. and Mefor, N. C. (2014): Evaluation of the antiulcer properties of aqueous extract of unripe *Musa paradisiaca* Linn. Peel in Wistar rats. *Afri. J. Pharm. Pharmacol*. 8 (39): 1006-1011.

Enomoto, S., Yanaoka, K., Utsunomiya, H., Niwa, T., Inada, K., Deguchi, H., Ueda, K., Mukoubayashi, C., Inoue, I., Maekita, T., Nakazawa, K., Iguchi, M., Arii, K., Tamai, H., Yoshimura, N., Fujishiro, M., Oka, M. and Ichinose, M. (2010): Inhibitory effects of Japanese apricot (*Prunus mume* Siebold et Zucc; Ume) on *Helicobacter pylori*-related chronic gastritis. *Eur. J. Clin. Nutr*. 64 (7): 714ï 719.

Evans, W. C. (1996): Trease and Evanôs Textbook of Pharmacognosy. 13th Edn. Balliere Tindal, London. 315- 679.

Feldman, M., Walker, P., Goldschmiedt, M. and Cannon, D. (1992): Role of affect and personality in gastric acid secretion and serum gastrin concentration: Comparative studies in normal men and in male duodenal ulcer patients. *Gastroenterol.* 102: 175-180.

Fereshteh, E., Farahnaz, N., Morteza, Y., Javad, H. and Nejad, E.S. (2009): Anti-*Helicobacter pylori* activity and essential oil composition of *Thymus caramanicus* from Iran. *Nat. Prod. Commun*. 4 (8), 1139ï 1142.

Fernandes, H.B., Silva, F.V., Passos, F.F.B., Bezerra, R.D.S., Chaves, M.H., Oliveira, F.A. and Oliveira, R.C.M. (2010): Gastroprotective effect of the ethanolic extract of *Parkia platycephala* Benth leaves against acute gastric lesion models in rodents. *Biol. Res*. 43 (4), 451ï 457.

Ferreira, A., Balla, J., Jeney, V., Balla, G., and Soares, M. P. (2008): A central role for free heme in the pathogenesis of severe malaria: the missing link. *J. Mol. Med*. 86: 1097ï 1111.

Ferreira, A.L., Almeida, A.C.A., Cola, M., Barbastefano, V., Almeida, A.B.A., Batista, L.M., Silva, E.F., Pellizzon, C.H.,Hiruma-Lima, C.A., Santos, L.C., Vilegas, W. and Brito, A.R.M.S. (2010): Mechanisms of the gastric antiulcerogenic activity of *anacardium humile* St. Hil on ethanol-induced acute gastric mucosal injury in rats. *Mol.* 15: 7153ï 7166.

Filaretova, L.P., Filaretova, L. P., Filaretov, A.A. and Makara, G. B (1998): Corticosterone increase inhibits stress-induced gastric erosions in rats. *Am*. *J. Physiol. Gastrointest*. *Physiol*.274 (6) G1024ï G1030.

Filaretova, L., Bagaeva, T. and Podvigina, T. (2001): Various ulcerogenic stimuli are potentiated by glucocorticoid deficiency in rats. *J. Physiol*. 95: 59-65.

Filaretova, L., Podvigina, T. and Bagaeva, T (2007): Gastroprotective role of glucocorticoid hormones. *J. Pharmacol. Sci.* 104: 195-201.

Finkel, T. and Holbrook, N. J. (2000): Oxidants, oxidative stress and the biology of ageing. *Natur.* 408: 239-247

Fiorucci, S., Distrutti, E., Cirino, G. and Wallace, J. L. (2006): The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. *Gastroenterol.* 131 (1): 259ï 271.

Fiske, C.H. and Sabbarow, Y. (1925): The colourimetric determination of phosphorous. *J. Biol. Chem.* 66, 375-400.

Galijasevic, S., Saed, G.M, Diamond, M.P. and Abu-Soud, H.M. (2003): Myeloperoxidase up-regulates the catalytic activity of inducible nitric oxide synthase by preventing nitric oxide feedback inhibition. *Proc. Natl. Acad. Sci* USA. 100:14766ï 71.

Ganguly, K.; Sharma, A.V.; Reiter, R.J. and Swarnakar, S. (2010). Melatonin promotes angiogenesis during protection and healing of indomethacin-induced gastric ulcer: role of matrix metaloproteinase-2. *J. Pineal Res.* 49 (2): 130-140.

Garcia-Barrantes, P. M. and Badilla, B. (2011): Anti-ulcerogenic properties of *Quassia amara* L. (Simaroubaceae) standardized extracts in rodent models. *J. Ethnopharmacol*. 134 (3): 904-110.

Gazzieri, D., Trevisani, M., Springer, J., Harrison, S., Cottrell, G. S., Andre, E., Nicoletti,P., Massi, D., Zecchi, S., Nosi, D., Santucci, M., Gerard, N. P., Lucattelli, M., Lungarella,G., Fischer, A., Grady, E. F., Bunnett, N. W. and Geppetti, P. (2007): Substance P released by TROV1-expressing neurons produces reactive oxygen species that mediate ethanol-induced gastric injury. *Free Rad. Biol. Med*. 43: 581ï 589.

Gege-Adebayo., G. I.; Igbokwe, V. U., Shafe, M. O., Akintayo, C. O. and Mbaka, D.I. (2013): Antiulcer effect of *Ocimum gratissimum* on indomethacin-induced ulcer and percentage of superoxide dismutase on Wistar rats. *J. Med. Med. Sci*. 4 (1) 8-12.

Gems, D. and Partridge, L. (2008): Stress reponse and aging: ñthat which does not kill us makes us strongerò. *Cell Metab.* 7: 200-203.

Ghana Herbal Pharmacopoeia (GHP). (1992): *Bridelia leaf*: The Advent Press.

Ghorbani, M.J., Salehi, Z., Eskafi, E.S. and Ejtehadi, F. (2014): Analysis of HSPA1BA1267G gene polymorphism in peptic ulcer. *Mol. Biol*. 48 (5): 634-637.

Ghosal, S. (1985): Steryl glycosides and acyl steryl glycosides from *Musa paradisiaca*. *Phyochem*. 24:1807ï 1810.

Ghosal, S. and Saini, K. S. (1984): Sitoindosides 1 and 11; Two new anti-ulcerogenic steryl acyl-glycosides from *Musa Paradisiaca*. *J. Chem. Res*. 23: 965ï 975.

Goel, R. K. and Sairam, K. (2002): Anti-ulcer drugs from indigenous sources with emphasis on *Musa sapientum, Tamra bhasma, Asparagus racemosus and Zingiber officinale*. *Ind. J. Pharmacol*. 34: 100-110.

Gozzelino, R., Jeney, V., and Soares, M. P. (2010): Mechanism of cell protection by hemeoxygenase-1. *Annu. Rev. Pharmacol.Toxicol*. 50: 323ï 354.

Graham, D. Y., Javed, S. U., Keihanian, S., Abudayyeh, S. and Opekun, A. R. (2010): Dual proton pump inhibitor plus amoxicillin as an empiric anti-*H. pylori* therapy: studies from the United States. *J. Gastroenterol*. 45: 816-820.

Guha, M., Kumar, S., Choubey, V., Maity, P., and Bandyopadhyay, U. (2006): Apoptosis in liver during malaria: role of oxidative stress and implication of mitochondrial pathway. *FASEB J*. 20: 1224ï 1226.

Halter, F., Tarnawski, A. S., Schmassmann, A. and Peskar, B. M (2001): Cyclooxygenase-2 implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives. *Gut.* 49 (3): 443ï 453.

Harsh, M. (2005): Gastrointestinal Tract. In: Harsh Mohan Textbook of Pathology. 5th Edition. Anshan Ltd. 550-607.

Hasebe, K., Horie, S., Komasaka, M., Yano, S. and Watanabe, K. (2001): Stimulatory effects of nitric oxide donors on gastric acid secretion in isolated mouse stomach. *Eur. J. Pharmacol*. 420: 159ï 164.

Hatakeyama, M. (2006): Helicobacter pylori CagA-a bacterial intruder conspiring gastric carcinogenesis. *Int. J. Cancer*. 119: 1217 ï 23.

Hawkey, C.J. and Langman, M.J. (2003): Non-steroidal anti-inflammatory drugs: overall risks and management. Complementary roles for COX-2 inhibitors and proton pump inhibitors. *Gut.* 52: 600ï 608.

Heeba, G.H., Hassan, M.K.A. and Amin, R. (2009): Gastroprotective effect of simvastatin against indomethacin-induced gastric ulcer in rats: role of nitric oxide and prostaglandins. *Eur. J. Pharmacol*. 607: 188ï 193.

Hiruma-Lima, C.A., Calvo, T.R., Rodrigues, C.M., Andrade, F.D.P., Vilegas, W. and Brito, A.R.M.S. (2006): Antiulcerogenic activity of *Alchornea castaneaefolia*: effects on somatostatin, gastrin and prostaglandin. *J. Ethnopharmacol*. 104: 215ï 224.

Hiruma-Lima, C. A., Batista, L. M., Almeida, A. B. A., Magri, L.P., dos Santos, L. C., Vilega, W. and Brito, A.R.M.S. (2009): Antiulcerogenic action of ethanolic extract of the resin from *Virola surinamensis* Warb. (Myristicaceae). *J. Ethnopharmacol*. 122 (2): 406ï 409.

Ho, S.B., Anway, R., Shekels, L. L., Toribara, N. W. and Ota, H. (2004): The adherent gastric mucous layer is composed of alternating layers of MUC5AC and MUC6 mucin proteins. *Dig. Dis. Sci.* 49 (10): 1598ï 1606.

Holzer, P. (2007): Role of visceral afferent neurons in mucosal inflammation and defense. *Curr. Opin. Phamacol*.7 (6): 563ï 569.

Hoye, A. T., Davoren, J. E., Wipf, P., Fink, M. P. and Kagan, V. E. (2008): Targeting mitochondria. *Acc. Chem. Res*. 41 (1): 87ï 97.

Hsu, Y. M., Lai, C. H., Su, C.H., Kuo, W. W., Kuo, C. L., Hsu, C. K., Wang, C. K., Chang, C. Y. and Chung, J. G. (2010a): *Solanum lyratum* extract inhibits *Helicobacter pylori*-mediated apoptosis in human gastric epithelial cells. *Anticaner Res*. 30 (4): 1189ï 1193.

Hsu, Y.M., Weng, J.R., Huang, T.J., Lai, C.H., Su, C.H. and Chou, C.H. (2010b): *Solanum torvum* inhibits *Helicobacter pylori* growth and mediates apoptosis in human gastric epithelial cells. *Anticancer Res*. 23 (5): 1401ï 1405.

Ibara, J-R., Elion Itou, R.D.G., Ouamba, J.M., Diatewa, M., Gbeassor, M. and Abena, A..A. (2007): Preliminary evaluation of antiulcerogenic activity of *ceiba pentandra gaertn* and *helicrysum mechowianum* klatt in rats. *J. Med. Sci*. 7 (3), 485ï 488.

Inas, Z. A.; Abdallah-Hala, H. A.; Khattab, H. and Gehan, H. H. (2011): Gastroprotective effect of *Cordia myxa* L. fruit extract against indomethacin-induced gastric ulcer in rats. *Life Sci. J.* 8 (3): 433-445.

Inoue, K., Takahashi, T., Uehara, K., Shimuzu, H., Ido, K., Morimatsu, H., Omori, E., Katayama, H., Akagi, R., and Morita, K. (2008): Protective role of hemeoxygenase-1 in the intestinal tissue injur in hemorrhagic shock in rats. *Shock.* 29: 252ï 261.

Ishihara, T., Suemasu, S., Asano, T., Tanaka, K. I., Mizushima, T. (2011): Stimulation of gastric ulcer healing by heat shock protein 70. *Biochem. Pharmacol*. 82: 728ï 736.

Israel, C., Violeta, G., Héctor, J. A., Gisela, M., Edelmira, L., Robert, B. and Irma, R. (2009): Anti-*Helicobacter pylori* activity of plants used in Mexican traditional medicine for gastrointestinal disorders. *J. Ethnopharmacol*. 122: 402ï 405.

Israel, D. A., Salama, N., Arnold, C. N., Moss, S. F., Ando, T., Wirth, H., Tham, K. T., Margorita, C., Martin, J. B., Stanley, F. and Richard, M. P. (2001): *Helicobacter pylori* strain-specific differences in genetic content, identified by microarray, influence host inflammatory responses. *J. Clin. Invest*. 5: 611-620.

Itoh T, Seno H, Kita T, Chiba T, Wakatsuki Y. (2005): The response to *Helicobacter pylori* differ between patients with gastric ulcer and duodenal ulcer. *Scand. J. Gastroenterol*. 40 (6): 641-7.

Iwu, M. M. (1993): Handbook of African Medicinal Plants.CRC Press, London. 133-135.

Jadhav, S.A. and Prasanna, S.M. (2011): Evaluation of antiulcer activityof *Zizyphus oenoplia* (L) Mill. Root in rats. *As. J. Pharmaceut.Clin. Res*. 4 (1), 92ï 95.

Jain, A. S. and Surana, S. J. (2009a): Antiulcerogenic effects of *Gymnosporia rothiana* (Celastraceae) against different experimental models. *Pharmacogn. Magaz*. 5 (19): 100ï 104.

Jain, A.S. and Surana, S.J. (2009b): Antiulcerogenic effects of S*pathodea falcata* against different experimental models. *Asi. J. Pharmac. Clin. Res*. 2 (3): 54ï 59.

Jain, K. S., Shah, A. K., Bariwal, J., Sheike, S. M., Kale, A. P., Jagtap, J. R. and Bhosale, A. V. (2007): Recent advances in proton pump inhibitors and management of acid peptic disorder. *Bioorg. Med. Chem*. 15 (3): 1181-1205.

Jain, V. and Panigrahi, M.K. (2012): Evaluation of Anti-Ulcer Activity of *Arisaema leschenaulti*i. *Intl. J. Phytomed*. 4: 437-440.

Jainu, M. and Shyamala, C.S.D. (2006): Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: possible mechanism for the inhibition of acid formation. *J. Ethnopharmacol*. 104 (1ï 2), 156ï 163.

James, M. C. and Vinay, K. (2003): The oral cavity and the GIT. In: Robbinôs Basic Pathology. 7th Edition. Saunders, India, 543-590.

Jang, H.J., Choi, M.H, Shin, W.G, Kim, K.H., Chung, Y.W., Kim, K.O., Park, C.H., Baek, I.H., Baik, K.H.,Kae, S.H. and Kim, H.Y. (2007): Has Peptic Ulcer Disease Changed During

the Past Ten Years in Korea? A Prospective Multi-center Study. *Dig. Dis. Sci.* 53: 1527-1531.

Jince, M.J., Kandhasamy, S. and Sellamuthu, M. (2010): Protective effects of methanolic extract of *Hedyotis puberula* (G. Don) R. Br. Ex Arn. Against experimentally induced gastric ulcer in rats. *J. Ethnopharmacol*. 131 (1): 216-219.

Jose, R.S., Marivane, L., Luiz, C.K.J, Rivaldo, N. and Sergio, F.A. (2010): Antiulcer effects of *Achyrocline satureoides* (Lam.) DC (Asteraceae) (Marcela), a folk medine plant, in different experimental models. *J. Ethnopharmacol*. 130 (2): 334-339.

Jung, H., Choi, J., Hwan Nam, J. and Juhn Park, H. (2007): Antiulcerogenic effects of the flavonoid-rich fraction from the extract of *orostachys japonicus* in mice. *J. Med. Food.* 10 (4): 702ï 706.

Kalra, P., Sharma, S. and Kuma, R.S. (2011): Antiulcer effect of the methanolic extract of *Tamarindus indica* seeds in different experimental models. *J. Pharm. Biol. Sci*. 3: 236-241.

Kansara, S. S. and Singhal, M. (2013): Evaluaton of antiulcer activity of *Moringa oleifera* seed extract. *J. Pharm. Sci. Biosci. Res*. 3 (1): 20-25.

Karatapanis, S., Skorda, L., Georgopoulos, S., Papantoniou, N., Papastergiou, V., Ntoutsikos, K., Komnianides, K. and Lisgos, Ph. (2009): Levofloxacin-based triple therapy versus bismuth-based quadruple therapy as a second line treatment for the eradication of *H. pylori* infection. *Ann. Gastroenterol*. 22 (4): 263-267.

Karbalay-Doust, S. and Noorafshan, A., (2009): Antiulcerogenic effects of *Matricaria chamomilla* extract in experimental gastric ulcer in mice. *Iran. J. Med. Sci*. 34 (3): 198ï 203.

Kathriarachchi, H., Hoffmann, P., Samuel, R., Wurdack, K.J. and Chase, M.W. (2005): Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid atpB, matK, 3ôndhF, rbcL, nuclear PHYC). *Mol. Phylogenet. Evol*. 36: 112 -134.

Kato, S., Aihara, E., Yoshii, K. and Takeuchi, K. (2005): Dual action of prostaglandin E2 on gastric acid secretion through different EP receptor subtypes in the rat. *Am. J. Physiol. Gastrointest. Liver Physiol*. 89(1): G64ï G69.

Kauffman, G. L. (1997): Stress, the brain and the gastric mucosa. *Am. J. Surg.* 174: 271-275.

Kawabata, A., Nishikawa, H., Saitoh, H., Nakaya, Y., Hiramatsu, K., Kubo, S., Nishida, M., Kawao, N., Kuroda, R., Sekiguchi, F., Kinoshita, M., Kakehi, K., Arizono, N., Yamagishi, H. and Kawai, K. (2004): A protective role of protease-activated receptor 1 in rat gastric mucosa. *Gastroenterol.* 126: 208-219.

Kelly, S. M., Guilheme, E. N. D., Meri, E. F. P, Anderson, L.F., Alba, R. M., Clelia, A. H., Jose, M. B. and Leonia, M. B. (2009): Flavonoids with gastro protective activity. *Mol*. 14: 979-1012.

Khare, S., Asad, M., Sunil, S. and Dhamanigi, P. V. S. (2008): Antiulcer activity of cod liver oil in rats. *Ind. J. Pharmacol*. 50: 209-214.

Kobata, A., Kotani, T., Komatsu, Y. (2007): Dual action of nitric oxide in the pathogenesis of ischemia/reperfusion-induced mucosal injury in mouse stomach. *Digest.*75: 188ï 197.

Konturek, P.C., Brzozowski, T., Pajdo, R., Ptak, A., Nikiforuk, A., Pawlik, W.W. and Hahn, E.G. (2004): Ghrelin, A new gastroprotective factor in gastric mucosa. *J. Physiol. Pharmacol*.55 (2): 325-336.

Konturek, P. C., Brzozowski, T., Burnat, G., Szlachcic, A., Koziel, J., Kwiecien, S., Konturek, S. J. and Harsch, I. A. (2010): Gastric ulcer healing and stress-lesion preventive

properties of pioglitazone are attenuated in diabetic rats. *J. Physiol. Pharmacol.* 61: 429-436.

Kuan-Yuan, W., Shui-Nin, L., Chiang-Shin, L., Daw-Shyong, P., Yu-Chung, S., Deng-Chang, W., Chang-Ming, J., Chun-Huang, L., Tsu-Nai, W. and Wen-Ming, W. (2004): Effect of ingesting *Lactobacillus* and *Bifidobacterium*-containing yoghurt in subjects with colonized Helicobacter pylori. *Am. J. Clin. Nutr*. 80 (3): 737-741.

Kushima, H., Nishijima, C. M., Rodrigues, C. M., Rinaldo, D., Sassa, M. F., Bauab, T. M., Distasi, L. C., Carlos, I. Z., Brito, A. R. M. S., Vilegas, W. and Hiruma-Lima, C. A. (2009): Antiinflammatory Immunomodulatory and anti-*Helicobacter pylori* action. *J. Ethnopharmacol*. 123: 430-438.

Kwiecien, S., Brzozowski, T., Konturek, P. C., Konturek, S. J., Pawlik, M., Pajdo, R., Drozdowicz, D., Ptak, A. and Hahn, E. J. (2001): Effect of central and peripheral actions of histamine and its metabolite N-alpha methyl histamine on gastric secretion and acute gastric lesions. *J. Physiol. Pharmacol*. 52 (4): 625-638.

Laine, L., Takeuchi, K. and Tarnawaski, A. (2008): Reviews in basic and clinical gastroenterology. Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterol*. 135: 135ï 141.

Lakshimi, V., Singh, N., Shrivasta, S., Mishra, S. K., Dharmani, P. and Palit, G. (2009): Gedunin and photogedunin of *Xilocarpus granatum* show significant anti-secretory effects and protect the gastric mucosa of peptic ulcer in rats. *Phytomed.* 17 (8-9): 569-574.

Landeira-Fernandez, J. (2004): Analysis of the cold-water restraint procedure in gastric ulceration and body temperature. *Physiol. Behav*. 82 (5): 827-833.

Lassen A, Hallas J, Schaffalitzky de Muckadell OB (2006): Complicated and uncomplicated peptic ulcers in a Danish county 1993-2002: a population-based cohort study. *Am. J. Gastroenterol.* 101 (5): 945-953.

Lay, C. S. and Lin, C. (2010): Correlation of CYP2C19 genetic polymorphisms with *Helicobacter pylori* eradication in patients with cirrhosis and peptic ulcer. Chin. *Med. Assoc.* 73: 188-193.

Levenstein, S. (2002): Psychosocial factors in peptic ulcer and inflammatory bowel disease. *J. Consul. Clin. Psychol*. 70 (3): 739-750.

Li, X., Schwacha, M. G., Chaudry, I. H., and Choudhry, M. A. (2008): Hemeoxygenase-1 protects against neutrophil-mediated intestinal damage by down regulation of neutrophil P47-phox and P67phox activity and oxygen production. *J. Immunol*.180: 6933ï 6940.

Liam, J., Murray, M. D., Athena, J., Lane, Ph.D., Lan, M., Harvey, P.D., Jenny, L., Donovan, P. D., Prakash Nair, M. D., Richard, F., and Harvey, M. D. (2002): Inverse Relationship between alcohol consumption and active *Helicobacter pylori* infection: The Bristol *Helicobacter* Project. *Am. J. Gastroenterol*. 97: 27-50.

Lichtenberger, L.M. (1999): Gastroduodenal mucosal defense. *Curr. Opin. Gastroenterol*. 15(6): 463- 472.

Lichtenberger, L.M. (2005): The hydrophobic barrier properties of gastrointestinal mucus. *Ann. Rev. Physiol.* 17 (3): 178-188.

Ligha, A.E. and Fawehinmi, H.B. (2009): Protection by liquorice in alcohol induced gastric mucosa damage. *Pak. J. Nutr*. 8 (10): 1532ï 1536.

Lim, Y. J., Lee, J. S., Ku, Y. S. and Hahm, K. B. (2009): Rescue strategies against non-steroidal antiinflammatory drug-induced gastroduodenal damage. *Gastroenterol. Hepatol*. 24: 1169-1178.

Londonkar, R. L. and Poddar, P. V. (2009): Studies on activity of various extracts of *Mentha arvensis* Linn against drug induced gastric ulcer in mammals. *World J. Gastrointest. Oncol*. (1): 82ï 88.

Londonkar, R.L. and Ranirukmini, R.K., (2010): Antiulcerogenic study of different extracts of *Butea frondosa* Roxb in albino mice. *J. Pharmacogn*. 1 (1): 6ï 9.

Lorke, D. (1983): A new approach to practical acute toxicity testing. *Arch. Toxicol*. 54, 275-289.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. N. (1951): Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem*. 193, 265-275.

Luiz-Ferreira, A., de Almeida, A. C. A., Cola, M., Barbastefano, V., de Almeida, A.B.A., Batista, L. M., Farias-Silva, E., Pellizzon, C. H., Hiruma-Lima, C.A., Santos, L. C., Vilegas, W., Brito, A. R. M. S., (2010): Mechanisms of the gastric antiulcerogenic activity of *Anacardium humile* St. Hill on ethanol-induced acute gastric mucosal injury in rats. *Molecul.* (15): 7153ï 7166.

Magaji, R. A., Okasha, M. A. M., Abubakar, M. S., Fatihu, M. Y. (2007): Anti-ulcerogenic and anti-secretory activity of the n-butanol portion of *Syzygium aromaticum* in rat. *Nig. J. Pharm. Sci*. 6 (2): 119ï 126.

Mahady, G. B., Pendland, S. L., Stoia, A. and Chadwick, L. R. (2003): *In vitro* susceptibility of *Helicobacter pylori* to isoquinoline alkaloids from *Sanguinaria canadensis* and *Hydrastis canadensis*. *Phytother. Res.* 17: 217ï 221.

Mahmood, A. A., Mariod, A. A., Al-Bayaty, F. and Abdel-Wahab, S. I. (2010): Anti-ulcerogenic activity of *Gynura procumbens* leaf extract against experimentally-induced gastric lesions in rats. *J. Med. Plants Res*. 4 (8), 685ï 691.

Mahmood, A.A., Sidik, K., Salmah, I., Suzainur, K.A.R. and Philip, K. (2005): Antiulcerogenic activity of *ageratum conyzoides* leaf extract against ethanol-induced gastric ulcer in rats as animal model. *Int. J. Molecul. Med. Adv. Sci.* 1 (4): 402ï 405.

Main, A. I. H. and Whittle, B. J. R. (1975): Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by use of Non-steroidal anti-inflammatory drugs. *Bri. J. Pharmacol*. 53: 217-224.

Maity, S., Vedasiromoni, J.R. and Ganguly, D.K. (1998): Role of glutathione in the antiulcer effect of hot water extract of black tea (*Camellia sinensis*). *Japn. J. Pharmacol*. 78: 285-292.

Maity, P., Biswas, K., Roy, S., Banerjee, R. K., and Bandyopadhyay, U. (2003): Smoking and pathogenesis of gastroduodenal ulcer- recent mechanistic update. *Mol. Cell. Biochem.* 253: 329ï 338.

Maity, P., Bindu, S., Choubey, V., Alam, A., Mitra, K., Goyal, M., Dey, S., Guha, M., Pal, C., and Bandyopadhyay, U. (2008): Lansoprazole protects and heals gastric mucosa from NSAID-induced gastropathy by inhibiting mitochondrial as well as Fas-mediated death pathways with concurrent induction of mucosal cell renewal. *J. Biol. Chem*. 283: 14391ï 14401.

Majumdar, D., Bebb, J. and Atherton, J. (2011): *Helicobacter pylori* infection and peptic ulcers. *Med*. 39: 154-161.

Malairajan, P., Gopalakrishna, G., Narasimhan, S. and Kala Veni, K. J. (2008): Evalution of anti-ulcer activity of *Polyalthia longifolia* (Sonn) Thwaites in experimental animals. *Ind. J. Pharmacol*. 40 (3): 126ï 128.

Malfertheiner, P., Chan, F. K. and McColl, K. E. (2009): Peptic ulcer disease. *Lancet.* 24: 374 (9699): 1449-1461.

Manjusha, C., Vipin, K. and Surender, S. (2014): Gastric antisecretory and cytoprotective effects of hydroalcoholic extracts of *Plumeria alba* Linn. leaves in rats. *J. Integ. Med*. 12 (1): 42-51.

Marhuenda, E., Martin, M. J. and Alarcon de la Lastra, C. (1993): Anti-ulcerogenic activity of aescine in different experimental models. *Phytother. Res*. 7: 13-16.

Maria, S. D., de Almeida, A. C. A., de Faria, F. M., Luiz-Ferreira, A., da Silva, M. A., Vilegas, W., Pellizzon, C. H. and Brito, A. R. M. S. (2011): *Abarema cochliacarpos*: Gastroprotective and ulcer healing activities. *J. Ethnopharmacol*. 132 (1): 134-142.

Marrotta, F., Tajiri, H., Safran, P., Fesce, E. and Ideo, G. (1999): Ethanol related gastric mucosal damage: evidence of a free radical-mediated mechanism and beneficial effect of oral supplementation with bionormalizer, a novel natural antioxidant. *Diges.* 60: 538-543.

Martin, G. R. and Wallace, J. L. (2006): Gastrointestinal inflammation: a central component of mucosal defense and repair. *Exp. Biol. Med*. 231: 130-137.

Martyn, P., Leen-Jan, V. D., Silvia, F., Bernhard, K., Federico, C., Jorge, V., Gladys, L., Didier, C., Nubia, M.and Ikuko, K. (2007): *Helicobacter pylori* Cytotoxin-Associated Genotype and Gastric Precancerous Lesions. *J. Natl. Cancer Inst*. 99: 1328ï 34.

Massignani, J. J., Lemos, M., Maistro, E. L., Schaphauser, H. P., Jorge, R. F., Sousa, J. P. B., Bastos, J. K. and Andrade, S. F. (2009): Antiulcerogenic activity of the essential oil of *Baccharis dracunculifolia* on different experimental models in rats. *Phytother. Res*. 23: 1355-1360.

Matteo, F., Luca, A., Rocchina, C., Marco, T. and Corrado, B. (2011): Pathophysiology of gastric ulcer development and healing: Molecular mechanisms and novel therapeutic options, peptic ulcer disease, Dr. Jianyuan Chai (Ed.), ISBN: 978- 953-307-976-9.

Mayty P. (2003): Studies focused merely on a single oxygen species through the induction of cytochrome. Smoking and pathogenesis of gastroduodenal ulcer. *Recent machanistic update.* 97: 450.

Mazumder, P. M., Sasmal, D. and Nambi, R. A. (2008): Antiulcerogenic and antioxidant effects of *Coccinia granids* (Linn) Voigt leaves on aspirin-induced gastric ulcer in rats. *Nat. Prod. Rad*. 7 (1), 15ï 18.

McQuaid, K. R. (2004): Drugs used in gastrointestinal diseases. In: Basic & Clinical Pharmacology. 19th Edition, McGraw-Hill, New York, 1034-1045.

Middleton, E., Kandaswany, C. and Theoharides, T. C. (2000): The effects of plant flavonoids on mammalian cells: Implications for inflammation. *Heart Dis. Cancer Pharmacol. Rev*. 52: 673-751.

Miguel, G., Faleiro, L., Cavaleiro, C., Salgueiro, L. and Casanova, J. (2008): Susceptibility of *Helicobacter pylori* to essential oil of dittrichia viscosa subsp. revoluta. *Phytother. Res.* 22: 259ï 263.

Milani, S. and Calabrò, A. (2001): Role of growth factors and their receptors in gastric ulcer healing. *Microsc. Res. Tech*. 53 (5): 360-371.

Minaiyan, M., Ghannadi, A. and Salehi, E. (2005): Antiulcerogenic effect of *Zataria multiflora* Boiss. on cysteamine induced duodenal ulcer in rats. *Iran. J. Pharmaceut. Sci*. 1 (4): 223ï 229.

Minaiyan, M., Ghassemi-Dehkordi, N. and Mohammadzadeh, B., (2006): Anti-ulcer effect of *Tripleurospermum disciforme* (C.A. Mey) Shultz Bip on pylorus ligated (Shay) rats. *Res*. *Pharmaceut. Sci*. 1, 15ï 21.

Mizushima, T. (2010): Protective role of HSP70 against various gastrointestinal diseases. *Curr. Opin. Pharmacol*. 16:1190-1196.

Molna´ r, P., Deli, J., Tanaka, T., Kann, Y., Tani, S., Gye´ma´ nt, N. Molna´ r, J. and Kawase, M. (2010): Carotenoids with anti-*Helicobacter pylori* activity from golden delicious apple. *Phytother. Res.* 24, 644ï 648.

Montrose, M.H. (2006): Gastroduodenal mucosal defense, In: Physiology of the gastrointestinal tract, Johnson LR. 1259-1291. Academic Press, New York.

Moon, B. Y., Sa-Ouk, K. and Boon, P. C. (2000): Enzyme-like activity of glycated cross-linked proteins in free radical generation. *Ann. New York Acad. Sci.* 899: 168-181.

Moon, J.K., Kim, J. R., Ahn, Y. J., Shibamoto, T. (2010): Analysis and anti-*Helicobacter* activity of sulforaphane and related compounds present in broccoli (*Brassica oleracea* L.) sprouts. *J. Agri. Food Chem*. 58 (11), 6672ï 6677.

Morsy, M. A.; Ashour, O. M.; Fouad, A. A. and Abdel-Gaber, S. A. (2010): Gastroprotective effects of the insulin sensitizers rosiglitazone and metformin against indomethacin-induced gastric ulcers in Type 2 diabetic rats. *Clin. Exp. Pharmacol. Physiol*. 37: 173-177.

Morsy, M. and Azza, E. (2011): Prevention of gastric ulcers, peptic ulcer disease, Dr. Jianyuan Chai (Ed.), ISBN: 978-953-307-976-9, InTech, Available from: http//www.intechopen.com/books/peptic-ulcer disease/prevention of gastric ulcer.

Morsy, M.; Ashour, O. Amin, E. and Rofaeil, R. (2009). Gastroprotective effects of telmisartan on experimentally-induced gastric ulcers in rats. *Pharmazie.* 64: 590-594.

Moshen, M., Alireza, G. and Alireza, K. (2006): Anti-ulcerogenic effect of ginger (rhizome of *Zingiber officinale* (Roscoe) on cystemine induced duodenal ulcer in rats. *DARU* 14: 97ï 101.

Motawi, T. K., Abd-Elgawad, H. M. and Shahin, N. N. (2008): Gastroprotective effect of leptin in indomethacin - induced gastric injury. *J. Biomed. Sci.* 15: 405ï 412.

Mózsik, G., Karadi, O., Kiraly, A., Debreceni, A., Figler, M., Nagy, L., Par, A., Par, G., Suto, G. and Vincze, A. (2001): The key-role of vagal nerve and adrenals in the cytoprotection and general gastric mucosal integrity. *J. Physiol*. (Paris). 95: 229ï 237.

Muralidhar, T. S., Balaji, A., Bandopadhyay, C. and Shantha, S. L. (2009): Cytoprotective effect of ulgen, a polyherbal formulation against physical and chemical factor induced gastric ulcers in Wister albino rats. *Am. J. Pharmacol. Toxicol*. 4 (3), 80ï 84.

Murray, C. D., Kamm, M. A., Bloom, S. R., and Emmanuel, A. V. (2003): Ghrelin for the gastroenterologist, history and potential. *Am. J. Gastroenterol*. 68: 202.

Nassini, R., Andre, E., Gazzieri, D., De Siena, G., Zanasi, A., Geppetti, P. and Materazzi, S. (2010): A bicarbonate-alkaline mineral water protects from ethanol-induced hemorrhagic gastric lesions in mice. *Biol. Pharmaceut. Bull*. 33: 1319ï 1323.

Navarrete, A., Trejo-Miranda, J. L. and Reyes-Trejo, L. (2002): Principles of root bark of *Hippocratea excelsa* (Hippocrataceae) with gastroprotective activity. *J. Ethnopharmacol*. 79:383ï 388.

Nayeb-Hashemi, H. and Kaunitz, J. D. (2009): Gastroduodenal mucosal defense. *Curr. Opin. Gastroenterol.* 25: 537-543.

Ndip, R.N., Malange, Tarkang, A.E., Mbullah, S. M., Luma, H. N., Malongue, A., Ndip, L. M., Wirmum, C. and Efange, S. M. N. (2007): *In vitro* anti-*Helicobacter pylori* activity of extracts of selected medicinal plants from North West Cameroon. *J. Ethnopharmacol*. 114 (3): 452ï 457.

Ndip, R.N., Ajonglefac, A.N., Mbullah, S.M., Tanih, N.F., Akoachere, J.-F.T.K., Ndip, L.M., Luma, H.N., Wirmum, C., Ngwa, F. and Efange, S.M.N. (2008): *In vitro* anti-*Helicobacter pylori* activity of *Lycopodium cernuum* (Linn) Pic. Serm. *Afr. J. Biotechnol*. 7 (22): 3989ï 3994.

Ndububa, D. A., Agbakwuru, A. E. and Adebayo, R. A. (2001): Upper gastrointestinal findings and incidence of *Helicobacter pylori* infection among Nigerian patients with dyspepsia. *West Afr. J. Med*. 20 (2): 140-145.

Nene-Bi, S. A., Zahoui, O. S., Soro, T. Y. and Traore, F. (2012): Diuretic and natriuretic activity of an aqueous extract of *Bridelia ferruginea* Benth in normal rats. *J. Physio. Pharmacol.* 2(10): 349-353.

Nene-Bi, S.A., Zahoui, O.S., Soro, T.Y. and Traore, F. (2010): Evaluation de leffet dun extrait aqueux de *Bridelia ferruginea* Benth (Euphorbiaceae) sur les activites spontanee et locomotrice chez le rat. *Intl. J. Biol. Chem. Sci*. 04(1): 34-41.

Newton, J. (2000): The human trefoil peptide, TFFl, is present in different molecular forms that are intimately associated with the adherent mucus gel in normal stomach. *Gut.* 46(3): 312ï 320.

Nguemfo, E. L., Dimo, T., Dongmo, A. B., Azebaze, A. G., Alaoui, K., Asongalem, A. E., Cherrah, Y. and Kamtchouing, P. (2009): Antioxidative and anti-inflammatory activities of some isolated constituents from the stem bark of *Allanblackia monticola* Staner. L. C. (Guttiferae). *Inflmmopharmacol*. 17: 37-41.

Ngueyem, T., Chai, J., Li, A., Akahoshi, T., Tanigawa, T. and Tarnawski, A. S. (2007): Novel roles of local IGF-1 activation in rat gastric ulcer healing: promotes actin polymerization, cell proliferation, re-epithelialization and induces COX-2 in a PI3K-dependent manner. *Am. J. Pathol.* 170 (4): 1219-1228

Ngueyem, T.A., Brusotti, G., Caccialanza, G. and Vitafinzi, P. (2009): The genus *Bridelia*: A phytochemical and ethnopharmacological review. *J. Ethnopharmacol*. 124: 339-349.

Nishida, K., Ohta, Y. and Ishiguro I. (1998): Contribution of NO synthases to neutrophil infiltration in the gastric mucosal lesions in rats with water immersion restraint stress. *FEBS Lett.* 425: 243ï 248.

Nordmann, G.N. (2000): Anticarcinogenic effect of selenium. Cell Mol. Life Sci. 57: 1864-1873.

Nunes, P.H.M., Cavalcanti, P.M.S., Galvao, S.M.P. and Martins, M.C.C. (2009): Antiulcerogenic activity of *Combretum leprosum*. *Pharmazie* 64 (1): 58ï 62.

Nwafor, P. A., Jacks, T. W., Ekanem, A. U. and Poh, C. F. (2005): Antiulcerogenic and antidiarrhoeal potential of methanolic extract of *Pausinystalia macroceras* stem bark in rats. Nig. *J. Nat. Prod. Med.* 9: 63-67.

Nwokediuko, S. C., Uchenna, I., Olive, O. and Neri, P. (2012): Time trends of upper gastrointestinal diseases in Nigeria. *Ann. Gastroenterol*. 25 (1): 52-56.

OÕMahony, R., Al-Khtheeri, H., Weerasekera, D., Fernando, N., Vaira, D. and Holton, J. (2005): Bactericidal and anti-adhesive properties of culinary and medicinal plants against *Helicobacter pylori*. *W. J. Gastroenterol.* 11: 7499ï 7507.

Ochi, T., Shibata, H., Higuti, T., Kodama, K., Kusumi, T. and Takaishi, Y. (2005): Anti-*Helicobacter pylori* compounds from *santalum album*. *J. Nat. Prod*. 68 (6): 819ï 824.

Odabasoglu, F., Cakir, A., Suleyman, H., Bayir, Y., Halici, M. and Aslam, A. (2006): Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *J. Ethnopharmacol*. 103: 59-65.

Okokon, J. E., Antia, B. S., Umoh, E. E. (2009): Antiulcerogenic activity of ethanolic leaf extract of *lasianthera Africana*. *Afr. J. Trad. Compl. Altern. Med*. 6 (2): 150ï 154.

Okoli, C. O., Akah, P. A., Ezike, A. C., Udegbulam, S. O., Okoye, T. C., Mbanu, T. P. and Ugwu, E. (2009): Studies on wound healing and antiulcer activities of extract of aerial parts of *Phyllantus niruri* L. (Euphorbiaceae). *Am. J. Pharmacol. Toxicol*. 4 (4): 118-126.

Okpo, S. O., Ching, F. P., Ayinde, B. A., Udi, O. O., Alonge, P. O. and Eze, G. O. (2011): Gastroprotective effects of the ethylacetate fraction of *Anchomanes difformis*. *Intl. J. Health Res*. 4 (4): 155-161.

Okunji, O. C. (1982): Chemical Constituents of *Bridelia ferruginea* Benth. Leaves, M. Sc. Dissertation. University of Nigeria, Nsukka.

Okuyama, E. and Yamazaki, M. (1983): The principles of *Tetragonia tetragonoides* having an antiulcerogenic activity. Isolation and identification of sterylglucoside mixture (compound A) *Yakugaku Zasshi.* 103:43ï 48.

Olajide, D. A., Makinde, J. M., Okpako, D. T. and Awe, S. O. (2000): Studies on the anti-inflammatory and related pharmacological properties of the aqueous extract of *Bridelia feruginea* stem bark. *J. Ethnopharmacol*. 71(1-2): 153-60.

Olajide, O.A., Okpako, D.T. and Makinde, J. M. (2003): Anti-inflammatory properties of *Bridelia ferruginea* bark. Inhibition of lipopolysaccharideï induced septic shock and vascular permeability. *J. Ethnopharmacol.* 88 (2-3): 221-224.

Olatunji, M. K. and Taofik, O. S. (2010): Effect of waste water treated with methanolic bark extract of Bridelia ferruginea Benth on rat kidney and liver. *J. App. Sci. Envirn. San*. 14-23.

Olbe, L., Carlsson, E. and Lindberg, P. (2003): A proton-pump inhibitor expedition: the case histories of omeprazole and esomeprazole. *Nat. Rev. Drug Disc*. (2): 132ï 139.

Oliveira, C.P., Kassab, P., Lopasso, F.P., Souza, H.P., Janiszews, K.M., Laurindo, F.R., Iriya, K. and Lavdanna, A.A. (2003): Protective effect f ascorbic acid in experimental gastric cancer: reduction of oxidative stress. *World J. Gastroenterol*. 9: 446-448.

Oliveira, G.L., Soares, A.R.P., da Silva, G.Q., Colares, A.V., Galvao, F.F.R., Martins, J.G.C., Herzog, A.L.C. and Rolim, A.C. (2009): Gastroprotective effect of medicinal plants from *Chapada do araripe*. *Braz. J. Yng. Pharm*. 1 (1): 54ï 56.

Ologundudu, A., Lawal, A.O., Ololade, I.A., Omonkhua, A.A. and Obi, F.O. (2008): The anti-ulcerogenic activity of aqueous extract of *carica papaya* fruit on aspirinï induced ulcer in rats. *The Intl. J. Toxicol*. 5(2).

Olumayokun, A. O. (1999): Investigation of the effects of selected medicinal plants on experimental thrombosis. *Phytother. Res*. 13 (3): 231-232.

Olumayokun, A.O., Mutalib, A.A., Uchechukwu, P. O. and Bernd, L. F. (2012): *Bridelia ferruginea* produces antineuroinflammatory activity through inhibition of nuclear factor-KappaB and P38 MAPK signaling. *Evid. Complem. Altern. Med.* (10):1155-1163.

Omotade, I. O and Seun, O. B. (2012): *In vitro* antioxidant activity of ethanolic extract of *B. ferruginea* (stem bark). *Acad. Res. Internl*. 2:2 246-251.

Onasanwo, S. A., Neetu, S., Saba, A. B., Oyagbemi, A. A., Oridupa, O. A. and Gautam, P. (2011): Antiulcerogenic and invitro anti-oxidant activities of *Lagenaria breviflora* (LB) whole fruit ethanolic extract in laboratory animals. *Pharmacogn. Res*. 3:2-8.

Onoruvwe, O., Olayinka, A. O., Lot, T. Y. and Udo, F. V. (2001): Effects of the stem bark and leaf extracts of *Bridelia Ferruginea* on rat bladder smooth muscle. *Fitoterap*. 72(3): 230-235(6).

Onunkwo, G. C., Akah, P. A. and Udeala, O. K. (1996): Studies on *Bridelia ferruginea* leaves (1) stability and hypoglycemic actions of the leaf extracts tablets. *Phytother. Res*. 10: 418-420.

Ott, M., Gogvadze, V., Orrenius, S., and Zhivotovsky, B. (2007): Mitochondria, oxidative stress and cell death. *Apoptosis* 12: 913ï 922.

Pai, R., Soreghan, B., Szabo, L., Akahoshi, T., Tanigawa, T. and Tarnawski, A.S. (2002): Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat. Med*. 8 (3): 289ï 293.

Park, B. S., Lee, H. K., Lee, S. E., Piao, X. L., Takeoka, G.R., Wong, R.Y., Ahn, Y. J. and Kim, J. H. (2006): Antibacterial activity of *Tabebuia impetiginosa* Martius ex DC (Taheebo) against *Helicobacter pylori*. *J. Ethnopharmacol*. 105 (1ï 2): 255ï 262.

Park, S., Hahm, K., Oh, T., Jin, J. and Choue, R. (2004): Preventive effect of the flavonoids, wogonin against ethanol-induced gastric mucosal damage in rats. *Dig. Dis. Sci*. 49: 384-394.

Patel, A., Patel, A. Patel, A., Patel, N. M. (2010): Determination of polyohenols and free radical scavenging activity of *Tephrosia purpurea* Linn. leaves (Leguminosae). *Pharmacogn. Res.* 2: 152-8.

Pereira, F. H., Guimaraĕ es, L. A. F., Cerutti, S. M., Rodrigues, R. F. O. and Araujo, C. E. P. (2005): Preliminary anti-ulcerogenic and chemical analysis of the aerial parts of *trixis divaricata* sprengel. *Acta. Farm. Bonaerense* 24 (1): 80ï 84.

Perez-Perez, G.I., Rothenbacher, D. and Brenner, H. (2004): Epidemiology of *Helicobacter pylori* infection. Helicobacter 9 (Suppl. 1): 1ï 6.

Peskar, B.M. (2001): Role of cyclooxygenase isoforms in gastric mucosal defense. *J. Physiol.* (Paris). 95 (1-6): 3-9.

Pillai, S. I., Kandaswamy, M. and Subramanian, S. (2010): Antiulcerogenic and ulcer healing effects of *Indian propolis* in experimental rat ulcer models. *J. ApiPro. ApiMed. Sci*. 2 (1): 21-28.

Prabha, P., Karpagam, T., Varalakshmi, B., and Chandra, A.S. (2011): Indigenous anti-ulcer activity of *Musa sapientum* on peptic ulcer. *Pharmacogn. Res*. 3(4): 232-238.

Rafatullah, S., Tariq, M., Al-Yahya, M. A., Mossa, J. S. and Ageel, A. M. (1990): Evaluation of turmeric (*Curcuma Longa*) for gastric and duodenal antiulcer activity in rats. *J. Ethnopharmacol*. 29, 25ï 34.

Rajesh, K., Prakash, G. and Majunatha, H. (2013): *In vitro* antioxidant and H^+K^+ATPase inhibition activities of *Acalypha wilkesiana* foliage extract. *J. Pharm. Bioall. Sci.* 5 (3): 178-188.

Raju, D., Ilango, K., Hitra, V. and Ashish, K. (2009): Evaluation of antiulcer activity of methanolic extrat of *Terminalia chebula* fruits in experimental rats. *J. Pharm. Sci. Res.* 1(3): 101-107.

Rao, C. V., Ojha, S. K., Radhakrishnan, K., Govindarajan, R., Rastogi, S., Mehrotra, S. and Pushpangadan, P. (2003): Antiulcer activity of *Utleria salicifolia* rhizome extract. *J. Ethnopharmacol.* 91: 243-249.

Rao, C. V., Sairam, K. and Goel, R. K. (2000): Experimental evaluation of *Bacopa monniera* on rat gastric ulceration and secretion. *Ind. J. Physiol. Pharmacol*. 44: 35-41.

Rashid, M. A., Gustafson, K. R., Cardellina, J. H. and Boyd, M. R. (2000): A new podophyllotoxin derivatitive from *Bridelia ferruginea*. *Nat. Prod. Let*. 14: 285-292.

Rifat-uz-Zaman, M., Muhammad, S. A. and Muhammad, S. K. (2013): In vitro gastric H^+ K^+ -ATPase, anti-*Helicobacter pylori*, antimuscarinic and antioxidant activities of a gastroprotective purified fraction of *Phyllanthus emblica* fruit. *J. Med. Plant. Res*. 7 (32): 2414-2419.

Robert, A., Nezamis, J. E., Lancaster, C. and Hanchar, A. J. (1979): Cytoprotection by prostaglandins in rats: Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterol.* 77: 433-443.

Roldao, E.D.F., Witaicenisa, A., Seitoa, L.N., Hirumalimab, C.A. and Satasi, L. C. D. (2008): Evaluation of the antiulcerogenicand analgesic activity of *Cardia verbenacea* DC. (Boraginaceae). *J. Ethnopharmacol*. 119: 94-98.

Romero, J. J. and Lichtenberger, L. M. (1990): Sterol-dependence of gastric protective activity of unsaturated phospholipids. *Dig. Dis. Sci*.35: 1231ï 1238.

Roslida, A.H., Siti, N.H. and Fezah, O. (2010): Gastroprotective effect of *Acanthopanax trifoliatus* on experimentally induced acute ulcer in rats. *Pharmacologyonline*. 2: 828-841.

Sabiu, S., Taofeeq, G., Taofik, S., Emmanuel, A., Abdulhakeem, S., Ismaila, N. and Abdulazeez, B. (2015): Indomethacin-induced gastric ulceration in rats: protective roles of *Spandias mombin* and *Ficus exasperate*. *Toxicol. Reports*. 2: 261-267.

Sachin, S. S. and Archana, J. R., (2009): Antiulcer activity of methanol extract of *erythrina indica* lam. leaves in experimental animals. *Pharmacogn.* 1 (6): 396ï 401.

Sachs, G., Spenney, J. G. and Lewin, M. H. (1978): H^+ transport: regulation and mechanism in gastric mucosa and membrane vesicles. *Physiol. Rev*. 58: 106-173.

Sajjad, K.M., Khanam, S., Deepak, M. and Shivananda, B.G. (2006): Antioxidant activity of a new diary heptanoid from *Zingiber officinale*. *Pharmacogn. Mag*. 2: 254-7.

Samik, B., Chinmay, P., Sumanta, D., Manish, G., Athar, A., Mohd, S. I., Shubham, D., Souvik, S., Rahul, K., Pallab, M. and Uday, B. (2011): Translocation of Heme Oxygenase-1 to Mitochondria is a novel cytoprotective mechanism against Non-steroidal anti-inflammatory drug-induced mitochondrial oxidative stress, apoptosis and gastric mucosal injury. *J. Biol. Chem*. 286 (45): 39387-39402.

Sa´nchez, L. M. P., Ruedas, D. and Gomez, B. C. (2001): Gastric antiulcer effects of *Rhizophora mangle* L. *J. Ethnopharmacol*. 77: 1-3.

Sannomiya, M., Fonseca, V.B., Silva, M.A., Rocha, L.R.M., Santos, L.C., dos, Hiruma-Lima, C.A., Brito, A.R.M.S. and Vilegas, W. (2005): Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. *J. Ethnopharmacol*. 97: 1ï 6.

Santhosh, K. B., Tiwari, S. K., Saikant, R., Manoj, G., Kunwar, A., Sivaram, G., Abid, Z., Ahmad, A., Priyadarsini, K. I. and Khan, A. A. (2010): Antibacterial and ulcer healing effects of organoselenium compounds in naproxen-induced and *Helicobacter Pylori* infected wistar rat model. *J. Trace Elem. Med. Biol.* 24: 263-270.

Sanmugapriya, E. and Venkataraman, S., (2007): Antiulcerogenic potential of *Strychnos potatorum* Linn seeds on aspirin plus pyloric ligation-induced ulcers in experimental rats. *Intl. J. Phytother. Phytopharmacol*. 14 (5): 360ï 366.

Sathar, S.A., Sojan, G.K., Srijaya, S., Premaletha, N. and Kattoor, R. V. (2014): *Helicobacter pylori* infection in patients with liver cirrhosis: prevalence and association with portal hypertensive gastropathy. *Ann. Gastroenterol*. 27 (1): 48-52

Schmeda-Hirschmann, G. and Yesilada, E. (2005): Traditional medicine and gastroprotective crude drugs. *J. Ethnopharmacol*. 100: 1-60.

Sen, S., Raja, C., Sridhar, C., Reddy, Y. S. R. and Biplab, D. (2010): Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. *Intl. J. Pharma. Sci. Rev. Res.*

Sener, G., Goren, O. F., Wusoy, B. N., Ersoy, Y., Arbak, S. and Dolger, A. G. (2005): Protective effect of Melatonin and omeprazole against Alendronat-induced gastric damage. *Dig. Dis. Sci.* 50: 1506-1512.

Sertie, J. A. A, Wiezel, G. and Woisky, R. G. (2001): Anti-ulcer activity of the ethanol extract of *Sesbania grantiflora*. *Br. J. Pharm. Sci*. 37(1):107-112.

Shankakara, S. and Sriram, N. (2014): Antiulcer activity of *Simarouba glauca* against ethanol and indomethacin-induced ulcer in rats. *Intl. J. Res. Pharmacol. Pharmacother*. 3 (2): 85-89.

Shanthi, G., Vijay kanth, G., Hitesh, L., Ganesan, M., (2010): Antiulcerogenic activities of the methanolic extract of *Cissus quadrangularis* in wistar. *Intl. J. Toxicol.* 7(2), ISSN: 1559ï 3916.

Shayne, P. (2002): Gastritis and Peptic ulcer disease. *Med. J*. 3: 7.

Shay, H., Komarov, S. A., Fels, S.S., Meranze, D., Gruenstein, M. and Siplet, H. (1945): A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterol.* 5: 43ï 61.

Sheen, E. and Tridafilopoulus, G. (2011): Adverse effects of long-term proton pump inhibitor therapy. *Dig. Dis. Sci*. 56: 931ï 950.

Siddaraju, M. N. and Dharmesh, S. M. (2007): Inhibition of gastric HKATPase and *Helicobacter pylori* growth by phenolic antioxidants of *Curcuma amada*. *J. Agric. Food Chem*. 55: 7377-7386.

Shrivastava, N., Srivastava, A., Banerjee, A. and Nivsarkar, M. (2006): Anti-ulcer activity of *adhatoda vasica* Nees. *J. Herb. Pharmacother*. 6 (2): 43ï 49.

Sibilia, V., Pagani, F., Rindi, G., Lattauda, N., Rapetti, D., De Luca, V., Campanini, N., Bulgarelli, I., Locatelli, V., Guidobono, F. and Netti, C. (2008): Central ghrelin gastroprotection involves nitric oxide/prostaglandin cross-talk. *Bri. J. Pharmacol*. 154(3): 688-697.

Singh, S. and Majumdar, D. K. (1999): Evaluation of the gastric antiulcer activity of fixed oil of *Ocimum sanctum* (Holy Basil). *J. Ethnopharmacol*. 65: 13ï 19.

Shobha, V. H. and Jamadar, M. G. (2013): Gastroprotective role of bioflavonoid silymarin in animal model of acute cold-restraint stress-induced gastric ulceration. *Al. Ameen. J. Med. Sci.* 6(1): 40-43.

Solanki h. D. and Shah N. J. (2013): Ulcer preventive (antiulcer) activity of *helicteres isora* fruit extracts. *Intl. J. Pharm. Res. Biosc*. 2(6): 594-610.

Spirt, M. J. (2004): Stress-related mucosal disease: risk factors and prophylactic therapy. *Clin Ther.* 26: 197ï 213.

Subramanian, S., Kumar, D.S., Arulselvan, P., Senthilkumar, G.P. and Rao, U.S.M. (2007): Evaluation of anti-ulcerogenic potential of *aloe vera* leaf gel extract studied in experimental rats. *J. Pharmacol. Toxicol*. 2: 85ï 97.

Sun, L. P., Guo, X. L., Zhang, Y., Chen, W., Bai, X. L., Liu, J. and Yuan, Y. (2009): Impact of pepsinogen C polymorphism on individual susceptibility to gastric cancer and its precancerous conditions in a Northeast Chinese population. *J. Cancer Res. Clin. Oncol.* 135: 1033-1039.

Sutar, N., Garai, R., Sharma, U.S., Singh, N. and Roy, S.D. (2011): Antiulcerogenic activity of *Saussurea lappa* root. *Intl. J. Pharm. Life Sci.* 2(1): 516ï 520.

Suziki, Y. M., Hayachi, M. I. and Yamagani, T. (1976): Anti-ulcer effect of *cetrexate* on various experimental gastric ulcers in rats. *Japn. J. Pharmacol*. 26: 471.

Tahara, T., Arisawa, T., Shibata, T., Yamashite, H., Nakamura, M., Yoshioka, D., Okubo, M., Maruyama, N., Kamano, T., Kamiya, Y., Fujita, H., Nakagawa, Y., Nagasaka, M., Iwata, M., Takahama, K., Watanabe, M., Nakano, H. and Hirata, I. (2012): Role of heat shock

protein (HSP) 70-2 genotypye in peptic ulcer in Japanese population. *Hepatogastrol.* 59(114): 426-429.

Tahara, T., Shibata, T., Yamashita, H., Hirata, I. and Arisawa, T. (2011): Influence of MDRI polymorphism on H. pylori-related chronic gastritis. *Dig. Dis. Sci*. 56: 103-108.

Takayama, C., de-Faria, F.M., Almeida, A.C.A., Valim-Araújo, D.A.O., Rehen, C.S., Dunder, R.J., Socca, E.A.R., Manzo, L.P., Rozza, A.L., Salvador, M.J., Pellizzon, C.H., Hiruma-Lima, C.A., Luiz-Ferreira, Souza-Brito, A.R.M. (2011): Gastroprotective and ulcer healing effects of essential oil from *Hyptis spicigera* Lam. (Lamiaceae). *J. Ethnopharmacol.* 135: 147ï 155.

Takeeda, M., Hayashi, Y., Yamato, M., Murakami, M. and Takeuchi, K. (2004): Roles of endogenous prostaglandins and cyclooxygenase isozymes in mucosal defense of inlamed rat stomach. *J. Physiol. Pharmacol*. 55: 193-205.

Takeuchi, K. (2010): Prostaglandin EP receptors and their roles in mucosal protection and ulcer healing in gastrointestinal tract. *Adv. Clin. Chem*. 51: 121-144.

Takeuchi, K., Ogawa, Y., Kagawa, S. and Ukawa, H. (2002): Gastric mucosal ulcerogenic responses following barrier disruption in knockout mice lacking prostaglandin EP1 receptors. *Aliment. Pharmacol Ther.* 16 (2): 74ï 82.

Tamashiro, P. F., Olaitan, B. S., Almeida, D. A. T., Lima, J. C. S., Marson-Ascencio, P. G., Ascencio, S. D., Rios-Santos, E. and Martins, D. T. O. (2012): Evaluation of antiulcer activity and mechanism of action of methanol stem bark extract of *Lafoensia parari* A. Sr. Hil (Lytraceae) in experimental animals. *J. Ethnopharmacol*. 144: 497-505.

Tan, P.V., Boda, M. and Etoa, F.X. (2010): In vitro and in vivo anti-*Helicobacter/Campylobacter* activity of the aqueous extract of *Enantia chlorantha*. *Pharm. Biol*. 48(3), 349ï 356.

Tanaka, A., Hatazawa, R. and Takahira, Y. (2007): Preconditioning stress prevents cold restraint stress-induced gastric lesions in rats: roles of COX-1, COX-2 and PLA_2. *Dig Dis Sci*. 52: 478ï 487.

Tarek, M. K, Abd Elgawad, H. M and Shahin N. N. (2008): Gastroprotective effect of leptin in indomethacin-induced gastric injury. *J. Biomed. Sci*. 15:405ï 12.

Tatsuguchi, A., Miyake, K., Gudis, K. and Futagami, S. (2004): Effect of *Helicobacter pylori* infection on ghrelin expression in human gastric mucosa. *Am. J. Gastroenterol*. 19 (11): 2121-2127.

Taupin, D. and Podolsky, D.K. (2003): Trefoil factors initiators of mucosal healing. *Nat. Rev. Mol. Cell Biol.* 4(9): 721-732.

Thim, L., Madsen, F. and Poulsen, S. S. (2002): Effect of trefoil factors on the viscoelastic properties of mucus gels. *Eur. J. Clin. Invest.* 32 (7): 519ï 527.

Thirunavukkarasu, P., Ramkumar, L. and Ramanathan, T. (2009): Antiulcer activity of *excoecaria agallocha* bark on NSAID-induced gastric ulcer in albino rats. *Glob. J. Pharmacol*. 3 (3): 123ï 126.

Tovey, F. I. (2015): Role of dietary phospholipids and phytosterols in protection against peptic ulceration as shown by experiments on rats. *W. J. Gastroenterol*. 21 (5): 1377ï 1384.

Toyoda, N., Gabazza, E. C., Inoue, H., Araki, K., Nakashima, S., Oka, S., Taguchi, Y., Nakamura M, Suzuki, Y., Taguchi, O., Imoto, I., Suzuki, K. and Adachi, Y. (2003):

Expression and cytoprotective effect of protease-activated receptor-1 in gastric epithelial cells. *Scand. J. Gastroenterol*. 38: 253-259.

Trease, G. E and Evans, W. C. (1992): Text Book of Pharmacognosy Bailliere Tindall Publication, Thirteenth edition.

Tripathi, K.D. (2008): Drugs for peptic ulcer. Essential of medical pharmacology. 6 th ed. New Delhi, India: Jaypee Brothers Medical Publishers. 627.

Tulassay, Z. and Herszenyi, L. (2010): Gastric mucosal defense and cytoprotective. *Bes. Pract. Res. Clin. Gastroenterol*. 24: 99-108.

Ueda, K., Ueyama, T., Yoshida, K., Kimura, H., Ito, T., Shimizu, Y., Oka, M., Tsuruo, Y. and Ichinose, M. (2008): *Am. J. Physiol. Gastrointest. Liver Physiol*. 295: G460ï G469.

University of Michigan Health System: Peptic ulcer guide (2005): 1-7

Vaibhav, M., Manali, A., Samuel, A. O., Gaurev, M., Preeti, R., Haushile, P. P., Gautam, P. and Tadigoppula, N. (2013): Antisecretory and cytoprotective effects of chebulinic acid isolated from the fruit of *Terminalia chebula* on gastric ulcers. *Phytomed.* 20 (6): 506-511.

Valle, D. L. (2005): Peptic ulcer disease and related disorders. In: Brawn, W.C., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L. and Jameson, J.L. Editors. Harrisonôs Principles of Internal medicine. 16thed. New York: McGraw Hill: 1746-1762.

Venkataranganna, M. V., Gopumadhavan, S., Sundaram, R. and Mitra, S. K. (1998): Evaluation of possible mechanism of anti-ulcerogenic activity of UL-409, herbal preparation. *J. Ethnopharmacol*. 63: 187-192.

Viana, A. F. S. C., Fernandes, H. B., Silva, F. V., Oliveira, I. S., Freitas, F. F. B. P., Chaves, M. H., Olivera, F.A., Costa, C.L.S., Arcanjo, D. D. R, Machado, F.D.F., and Oliveira,

R.C.M. (2013): Gastroprotective activity of *Cenostigma macrophllum* Tull. Var *acuminate Teles Freire* leaves on experimental ulcer models. *J. Ethnopharmacol*. 150: 316-323

Vijayakumar, M, Eswaram, M.B., Ojha, S.K., Rao, C.H.V. and Rawal, A.K.S. (2011): Antiulcer activity of hydroalcoholic extract of Mormodica dioica roxb fruit. *Ind. J. Pharm. Sci*. 73 (5): 572-577.

Wahida, B., Abderrahman, B., Nabil, C. (2007): Antiulcerogenic activity of *Zizyphus lotus* (L.) extracts. *J. Ethnopharmacol*. 112 (2): 228ï 231.

Wang G. Z. and Wang J. F. (2011): Effects of *Helicobacter pylori* and Non-Steroidal Anti-Inflammatory Drugs on Peptic Ulcer, Peptic Ulcer Disease, Dr. Jianyuan Chai (Ed.), ISBN: 978-953-307-976-9.

Wang, Y. R., Richter, J. E. and Dempsey, D.T. (2010): Trends and outcomes of hospitalizations for peptic ulcer disease in the United States, 1993 to 2006. *Ann. Surg.* 251 (1): 51-58.

Wallace, J. L. (2001): Non-steroidal anti-inflammatory drugs and the gastrointestinal tract. Mechanism of protection and healing: Current knowledge and future research. *Am. J. Med.* 110 (1A): 195-235.

Wallace, J. L. (2005): Recent advances in gastric ulcers therapeutics. *Curr. Opin. Pharmacol*. 5: 573ï 577.

Wallace, J. L. (2006): Nitric oxide-aspirin triggered lipoxins and nitric oxide-aspirin in gastric protection. *Inflamm. Alle. Drug Targ*. 5: 133-137.

Wallace, J. L. (2008): Prostaglandins, NSAIDs, and gastric mucosal protection: why doesnôt the stomach digest itself? *Physiol. Rev*. 88: 1547ï 1565.

Wasman, S. Q., Mahmood, A. A., Salehuddin, H, Zatra, A. A and, Salamah, J. (2010): Cytoprotective activites of *Polygonum minus* aqueous leaf extract on ethanol-induced gastric ulcer in rats. *J. Med. Plant. Res*. 4 (24): 2658-26665.

Willemijntje, A. and Pankaj, J.P. (2006): Pharmacotherapy of gastric acidity, peptic ulcers and gastroesophageal reflux disease. In: Goodman & Gilmanôs The Pharmacological Basis of Therapeutics. 11th Edition. McGraw-Hill, New York, 967-981.

Wolle, K. and Malfertheiner, P. (2007): Treatment of *Helicobacter pylori*. *Best Pract. Res. Clin*. *Gastroenterol*. 21: 315ï 324.

Wong, S. L., Lan, V. Z., Amy, H. Y. T., Zhijian, L., Debra, S. G., Oliver, D. K., Guillauma, L., Marc, V., Brenda, A., Howard, B., Charles, B. and Frederick, P. R. (2004): Combining biological networks to predict genetic interactions. *PNAS.* 101 (44): 15682-15687.

Wu, M. L., Ho, Y. C., and Ye, S. F. (2011): Review Article Heme oxygenase-1 in inflammation and cardiovascular disease. *Am. J. Cardiovasc. Dis.* 1(2): 150-158.

Xiao, M., Yang, Z., Jiu, M., You, J. and Xiao, R. (1992): The antigastroulcerative activity of beta-sitosterol-beta-D-glucoside and its aglycone in rats. *Hua Xi Yi Ke Da Xue Xue Bao*. 23:98ï 101.

Yadav, P., Aditiya, G. and Gopal, R. (2012): *In vitro* H^+K^+ATPase inhibitory potential of methanolic extract of Cissus quadrangularis Linn. *Pharmacogn. Res*. 4 (2): 123-126.

Yadav, S. K., Sah, A. K., Jha, R. K., Sah, P. and Shah, D.K. (2013): Tumeric (curcumin) remedies gastroprotective action. *Pharmacogn. Rev*. 7: 42-46.

Yang, Y. H., Wu, W. K., Tai, E. K., Wong, H. P., Lam, E. K., So, W. H., Shin, V. Y. and Cho, C. H. (2006): The cationic host defense peptide rCRAMP promotes gastric ulcer healing in rats. *J. Pharmacol. Exp. Ther*. 318 (2): 547ï 554.

Yoshida, N. and Yoshikawa, T. (2008): Basic and translational research on proteinase-activated receptors: implication of proteinase/proteinase-activated receptor in gastrointestinal inflammation. *J. Pharmacol. Sci.* 108: 415-421.

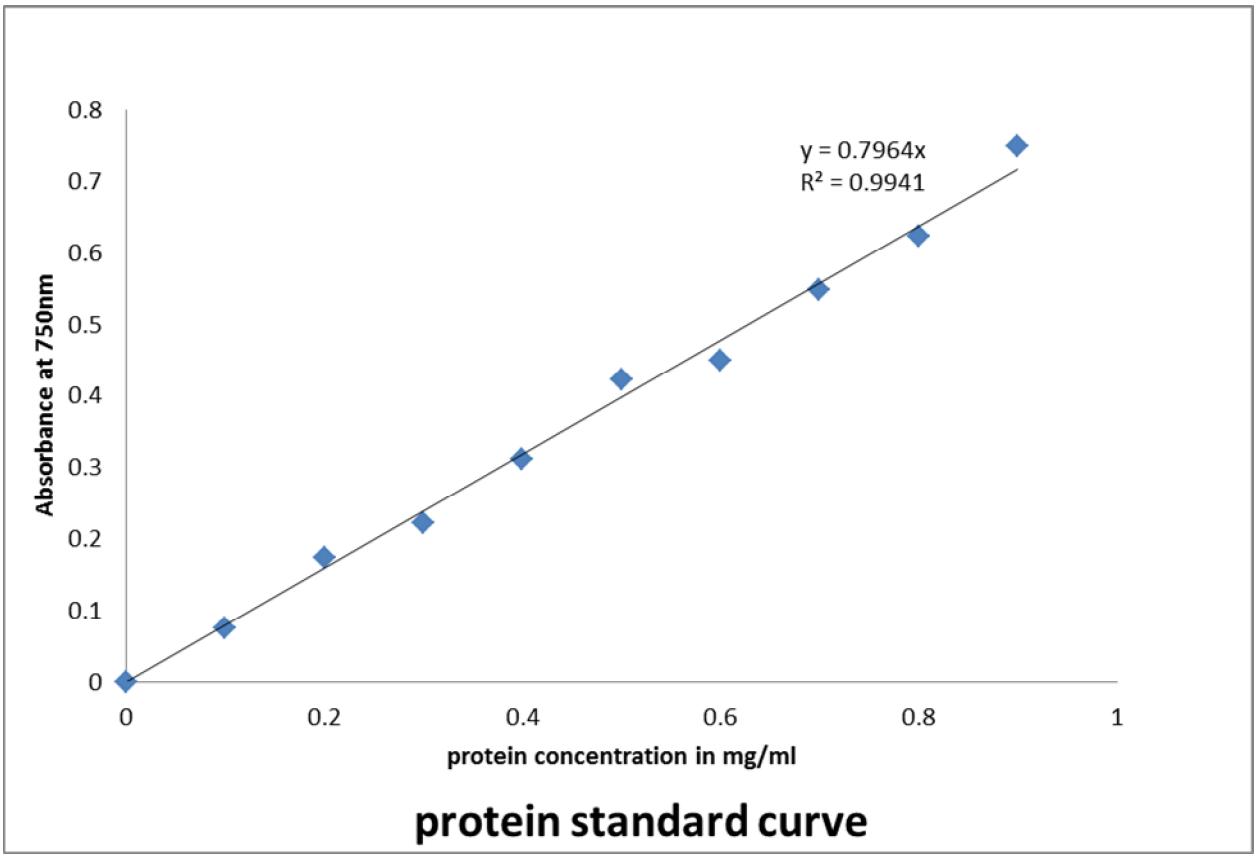
Yuan-Chuen, W., Wan-Yu, L., Deng-Chyang, W., Jeh-Jeng, W., Cheng-Hsun, W., Jyun-Ji, L., Cheng-Kun, L. (2011): *In vitro* activity of 2-methoxy-1, 4-naphthoquinone and Stigmasta-7,2,2-diene-3b-ol from *Impatiens balsamina* L. against Multiple Antibiotic-resistant *Helicobacter pylori*. *Evid. Complem. Altern. Med. Article* ID 704721. 8.

Zhaoshen, L., Duowu, Z., Xiuqiang, M, Jie, C., Xingang, S., Yanfang, G., Xiaohua, M., Li, G., Yanfang, Z., Rui, W., Xiaoyan, Y., John, D., Joseph, J.S., Börje,W., Saga, J., Wenbin, L., and Jia, H.(2010): Epidemiology of Peptic Ulcer Disease:Endoscopic Results of the Systematic Investigation of Gastrointestinal Disease in China. *Am. J. Gastroenterol*. 105: 2570ï 2577.

Zheng, Q., Chen, W. J., Lu, H., Sun, Q. J. and Xiao, S. D. (2010): Comparison of the efficacy of triple versus quadruple therapy on the eradication of *Helicobacter pylori* and antibiotic resistance. *J. Dig. Dis*. 11: 313-318.

APPENDIX

Appendix I



Appendix II

Table 11: Effect of fractions and isolated compounds on H^+K^+ATPase activity *in vitro*

Treatment	Dose (µg/ml)	Absorbance X 10^{-3}	Activity (µMPi/hour)	Inhibition of enzyme (%)	IC_{50} (µg/ml)
Fraction III	100	140.70 ± 0.58	1.31 ± 0.01[a]	50.75	98.52
	200	25.70 ± 0.58	0.24 ± 0.01[a]	90.98	
	300	21.67 ± 1.16	0.20 ± 0.01	92.59	
Fraction VI	100	101.30 ± 1.53	0.93 ± 0.02[a]	64.92	77.02
	200	50.30 ± 0.58	0.46 ± 0.01[a]	82.59	
	300	48.30 ± 0.58	0.44 ± 0.01	83.46	
BF1	100	76.30 ± 1.53	0.70 ± 0.02[a]	73.57	67.96
	200	69.30 ± 0.58	0.64 ± 0.01[a]	75.83	
	300	61.00 ± 1.00	0.56 ± 0.01	78.83	
BF2	100	93.00 ± 2.65	0.86 ± 0.03[a]	67.67	73.89
	200	48.30 ± 0.58	0.44 ± 0.01[a]	83.35	
	300	38.67 ± 1.16	0.36 ± 0.01	86.58	
Omeprazole	100	74.30 ± 0.58	0.69 ± 0.00[a]	74.1	67.48
	200	52.67 ± 0.58	0.49 ± 0.01[a]	81.69	
	300	51.00 ± 0	0.47 ± 6.8	82.22	
Control	-	286.70 ± 0.58	2.66 ± 0.006	-	-

[a]P<0.05 compared to the control (One way ANOVA; Bonferroni's post hoc); BF1 = Isolated compound 1; BF2 = Isolated compound 2.

.

Appendix III

Table 14: Effect of extract and fractions on DPPH free radical scavenging activity

Treatment	Concentration (µg/ml)	Inhibition of oxidant activity (%)	IC_{50} (µg/ml)
ME	10	2.90 ± 0.07	135.90
	20	6.70 ± 0.07	
	40	7.80 ± 0.13	
	60	21.50 ± 0.00	
	80	25.70 ± 0.07	
	100	36.80 ± 0.07	
	200	78.70 ± 0.00	
	300	79.60 ± 0.19	
Fraction III	10	12.70 ± 0.03	199.20
	20	21.70 ± 0.03	
	40	22.80 ± 0.03	
	60	30.90 ± 0.03	
	80	38.20 ± 0.03	
	100	42.60 ± 0.00	
	200	50.20 ± 0.03	
	300	53.80 ± 0.06	
Fraction VI	10	51.60 ± 0.03	9.70
	20	55.40 ± 0.03	
	40	90.80 ± 0.03	
	60	91.70 ± 0.03	
	80	91.90 ± 0.00	
	100	92.10 ± 0.06	
	200	92.30 ± 0.03	
	300	93.70 ± 0.03	
Ascorbic acid	10	53.00 ± 0.23	9.40
	20	60.00 ± 0.13	
	40	67.00 ± 0.23	
	60	74.00 ± 0.23	
	80	77.00 ± 0.13	
	100	80.00 ± 0.13	
	200	83.00 ± 0.13	
	300	88.00 ± 0.23	

ME = Methanol extract

Appendix IV

Table 15: Effect of isolated compounds on DPPH free radical scavenging activity

Treatment	Concentration (µg/ml)	Inhibition of oxidant activity (%)	IC_{50} (µg/ml)
BF1	10	22.40 ± 0.00	406.50
	20	25.10 ± 0.06	
	40	27.20 ± 0.06	
	60	31.10 ± 0.03	
	80	32.50 ± 0.06	
	100	32.80 ± 0.06	
	200	34.00 ± 0.03	
	300	36.90 ± 0.12	
BF2	10	49.82 ± 0.03	10.20
	20	52.03 ± 0.03	
	40	75.92 ± 0.06	
	60	76.68 ± 0.00	
	80	77.36 ± 0.03	
	100	81.30 ± 0.03	
	200	85.40 ± 0.03	
	300	89.45 ± 0.06	
Ascorbic acid	10	53.00 ± 0.23	9.40
	20	60.00 ± 0.13	
	40	67.00 ± 0.23	
	60	74.00 ± 0.23	
	80	77.00 ± 0.13	
	100	80.00 ± 0.13	
	200	83.00 ± 0.13	
	300	88.00 ± 0.23	

ME = Methanol extract; BF1 = Isolated compound 1; BF2 = Isolated compound 2.