UNIVERSITI PUTRA MALAYSIA

MODE OF ACTION FOR GASTROPROTECTIVE ACTIVITY OF Muntingia calabura L. LEAVES IN RATS

TAVAMANI D/O BALAN

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By

TAVAMANI D/O BALAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2016

I would like to dedicate this thesis to:

my parents,
my dearest father, Mr. Balan S/O Swaminathan and my lovely mother, Mrs. Puspekrani D/O Muniandi

for bringing me here…
for believing in me…
for their unconditional love…

Appa and amma, this is for you….

Love you always!

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

MODE OF ACTION FOR GASTROPROTECTIVE ACTIVITY OF *Muntingia calabura* L. LEAVES IN RATS

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Chairman : Assoc. Prof. Zainul Amiruddin Zakaria, PhD
Faculty : Medicine and Health Sciences

Gastric ulcer is one of the most common gastrointestinal disorders. As current antiulcer treatments are associated with wide range of side effects, there is a need to discover an effective and safer new antiulcer agent. *Muntingia calabura* L. (family Muntingiaceae), known as Jamaican cherry or *kerukup siam* has been employed traditionally to treat various ailments including gastrointestinal disorders. The traditional use of *M. calabura* and its potential antioxidant properties lead to the present research with the hope of finding an effective gastroprotective agent. The present study aimed to investigate the antiulcer activity of *M. calabura* methanolic leaves extract (MEMC) and its fractions using rat models, determine the underlying mechanism(s) of action and identify the phytochemical constituents present in the plant. Acute toxicity study was conducted using a single oral dose of 2000 mg/kg MEMC. The antiulcer activity of MEMC was evaluated in ethanol- and indomethacin-induced gastric ulcer rat models. The rats were administered 8% Tween 80, 100 mg/kg ranitidine, and MEMC (doses 25-500 mg/kg) orally for seven days, followed by ulcer induction using absolute ethanol (5 mL/kg) or indomethacin (100 mg/kg). The rats were euthanized; macroscopic and histological observations of the stomach were done. Fractionation of MEMC yielded petroleum ether (PEF), ethyl acetate (EAF) and aqueous (AQF) fractions. Their antiulcer property was investigated using ethanol-induced gastric ulceration as described above. MEMC and its fractions were subjected to antioxidant and anti-inflammatory studies including superoxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, oxygen radical absorbance capacity (ORAC), total phenolic content (TPC), inhibition of nitric oxide (NO), lipoxygenase (LOX) and xanthine oxidase (XO) activity. Evaluation of gastric content and quantification of mucus were carried out in pylorus-ligated model. Possible involvement of endogenous NO and sulfhydryl (SH) compounds was determined in animals pre-treated with NG-nitro-L-arginine methyl esters (L-NAME) or N-ethylmaleimide (NEM) prior to MEMC or EAF treatment. Superoxide dismutase (SOD), gluthathione (GSH), catalase (CAT), malondialdehyde (MDA), prostaglandin E_2 (PGE_2) and NO

level in the stomach tissue homogenate treated with EAF was determined. Phytochemical screening and High Performance Liquid Chromatography (HPLC) analysis was conducted on MEMC, PEF and EAF. EAF was further subjected to Ultra-high-Performance Liquid Chromatography-Electrospray Ionization (UHPLC-ESI) analysis. The LD_{50} of MEMC was >2000 mg/kg. MEMC exerted significant (p<0.001) gastroprotection in both the ulcer models. PEF and EAF significantly (p<0.001) attenuated the ethanol-induced gastric lesions. MEMC and its fractions showed high antioxidant and anti-inflammatory activities. MEMC and EAF significantly (p<0.01) reduced volume of gastric content and increased the mucus production. Pre-treatment with L-NAME or NEM reversed the gastroprotection of MEMC and EAF. EAF markedly ameliorated the SOD, GSH, CAT, PGE_2 and NO level while reducing MDA level. HPLC profiling showed the presence of quercetin and gallic acid in MEMC, PEF and EAF. UHPLC-ESI confirmed the presence of these compounds in EAF. In conclusion, MEMC and EAF exert significant antiulcer activity. The underlying gastroprotective mechanisms of MEMC and EAF could be associated with the antioxidant, anti-inflammatory, antisecretory, participation of mucus, antiperoxidative, modulation of NO and SH compounds and presence of flavonoids and phenols.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

MOD TINDAKAN AKTIVITI GASTROPELINDUNG DAUN *Muntingia calabura* L. PADA TIKUS

Oleh

TAVAMANI A/P BALAN

November 2016

Pengerusi : Profesor Madya Zainul Amiruddin Zakaria, PhD
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Ulser gastrik merupakan salah satu gangguan gastrousus yang biasa terjadi. Sebagaimana diketahui rawatan antiulser semasa dikaitkan dengan pelbagai jenis kesan sampingan, terdapat keperluan untuk mencari agen antiulser yang baharu dan selamat. *Muntingia calabura* L. (keluarga Muntingiacea), dikenali sebagai ceri Jamaica atau *kerukup siam* telah digunakan secara tradisional untuk merawat pelbagai penyakit termasuklah gangguan gastrousus. Penggunaan *M. calabura* secara tradisional dan ciri-ciri antioksidanya yang berpotensi membawa kepada kajian terkini dengan harapan untuk menjumpai agen gastropelindung yang berkesan. Kajian ini bermatlamat untuk mengkaji aktiviti antiulser ekstrak daun metanol *M. calabura* (MEMC) dan cebisannya menggunakan model tikus, menentukan mekanisme dasar tindakan dan mengenal pasti juzuk fitokimia yang hadir dalam tumbuhan. Kajian ketoksikan akut dilakukan menggunakan dos oral tunggal 2000 mg/kg MEMC. Aktiviti antiulser MEMC dinilai dalam model ulser gastrik tikus teraruh indomethacin dan etanol. Tikus diberi 8% Tween, 100 mg/kg ranitidina, dan MEMC (dos-dos 25-500 mg/kg) secara oral selama tujuh hari, diikuti aruhan ulser menggunakan etanol mutlak (5 mL/kg) atau indomethacin (100 mg/kg). Tikus dieutanasia; pemerhatian makroskopik dan histologi perut dilakukan. Pemeringkatan eter petroleum terhasil MEMC (PEF), etil asetat (EAF) dan pecahan akueus (AQF). Ciri antiulsernya dikaji menggunakan pengulseran gastrik teraruh etanol seperti diterangkan di atas. Kajian antioksida dan antikeradangan bagi MEMC dan pecahannya termasuklah superoksida dan pengaut radikal 2,2-difenil-1-pikrilhidrazil (DPPH), kapasiti penyerapan radikal oksigen (ORAC), kandungan fenolik total (TPC), perencatan nitrik oksida (NO), aktiviti lipoksigenase dann xantin oksidase telah dijalankan. Penilaian kandungan gastrik dan pengkuantitian mukus dilaksanakan dalam model pilorus terligasi. Keterlibatan berkemungkinan bahan endogenus NO dan sulfuhidril ditentukan dalam haiwan yang diprarawat dngan NG-nitro-L-arginina metil ester (L-NAME) atau N-etilmaleimida (NEM) sebelum rawatan MEMC atau EAF. Superoksida dismutase (SOD), glutation (GSH) katalase (CAT), malondialdehid (MDA),

prostaglandin E_2 (PGE_2) dan tahap NO dalam tisu perut homogenat dirawat dengan EAF ditentukan. Imbasan fitokimia dan analisa Kromatografi Cecair Prestasi Tinggi (HPLC) dilakukan ke atas MEMC, PEF dan EAF. Analisa Pengionan Elektosemburan Kromatografi Cecair Prestasi Ultratinggi (UHPLC-ESI) kemudiannya dilakukan terhadap EAF. LD_{50} MEMC ialah >2000 mg/kg. MEMC mengeluarkan gastropelindung yang signifikan (p<0.001) pada kedua-dua model ulser. PEF dan EAF (p<0.001) mengecilkan lesi gastrik teraruh etanol. MEMC dan pecahannya menunjukkan aktiviti antioksida dan antikeradangan yang tinggi. MEMC dan EAF (p<0.001) menurunkan isi padu kandungan gastrik dengan signifikan dan meningkatkan pengeluaran mukus. Prarawatan dengan L-NAME atau NEM membalikkan gastropelindung MEMC dan EAF. EAF memperbaiki tahap SOD, GSH, CAT, PGE_2 dan NO dengan ketara di samping menurunkan tahap MDA. Profil HPLC menunjukkan kehadiran kuersetin dan asid galik dalam MEMC, PEF dan EAF. UHPLC-ESI mengesahkan kehadiran bahan-bahan ini dalam EAF. Sebagai kesimpulan, MEMC dan EAF mengeluarkan aktiviti antiulser yang signifikan. Mekanisma gastropelindung dasar MEMC dan EAF dapat dikaitkan dengan antioksida, antikeradangan, antirembesan, keterlibatan mukus, antiperoksidatif, modulasi bahan NO dan SH serta kehadiran flavonoid dan fenol.

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I certify that a Thesis Examination Committee has met on 2 November 2016 to conduct the final examination of Tavamani d/o Balan on her thesis entitled "Mode of Action for Gastroprotective Activity of *Muntingia calabura* L. Leaves in Rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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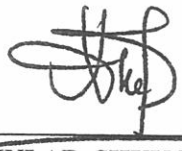

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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AA	Ascorbic acid
AAPH	2,2'-Azobis (2-methylpropionamidine) dihydrochloride
Ach	Acetylcholine
ACUC	Animal Care and Use Committee
ANOVA	Analysis of Variance
AQF	Aqueous fraction of *Muntingia calabura*
ATP	Adenosine triphosphate
AUC	Area under the curve
BSA	Bovine serum albumin
Ca^{2+}	Calcium ions
CagA	Cytotoxin-associated gene A
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CBX	Carbenoxolone
Cl^-	Chloride ions
cNOS	Constitutively expressed nitric oxide synthase
CO_2	Carbon dioxide
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
Cu^{2+}	Cuprum ions
dH_2O	Distilled water
DMEM	Dulbecco's Modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTNB	5,5'-dithio-bis-(2-nitrobenzoic acid)
EAF	Ethyl acetate fraction of *Muntingia calabura*
ECL	Enterochromaffin-like cells
EDTA	Ethylenediaminetetraacetic acid
EGF-R	Epidermal growth factor receptor
eNOS	Endogenous nitric oxide synthase
EP1	Prostaglandin E_2 receptor 1
EP2	Prostaglandin E_2 receptor 2
EP3	Prostaglandin E_2 receptor 3
EP4	Prostaglandin E_2 receptor 4
ET	Electron transfer
Fe^{2+}	Ferric ions
Fe^{3+}	Ferrous ions
GABAergic	Gamma-aminobutyric acid-ergic
GAE	Gallic acid equivalent
GSH	Glutathione
h	Hour(s)
H^+	Hydrogen ions
H^+/K^+ ATPase	Hydrogen potassium ATPase
H_2 receptor	Histamine type 2 receptor
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide

HAT	Hydrogen atom transfer
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
i.p.	Intraperitoneal injection
IFN-γ	Interferon gamma
IL-1ß	Interleukin-1ß
IL-8	Interleukin-8
iNOS	Inducible nitric oxide synthase
K^+	Potassium ions
LD_{50}	50% oral lethal dose
L-NAME	N^G-nitro-l-arginine methyl esters
LOX	Lipoxygenase
LPS	Lipopolysaccharide
LT	Leukotriene
MALT	Mucosa associated lymphoid tissue
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
MEMC	Methanolic extract of *Muntingia. calabura*
min	Minutes
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
Na^+	Sodium ion
NaOH	Sodium hydroxide
NBT	Nitro-blue tetrazolium
NDGA	Nordihydroguaiaretic acid
NEM	N-ethylmaleimide
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO/sGC/cGMP	NO/soluble guanylate cyclase/cyclic guanosine monophosphate
NOS	Nitric oxide synthase
NSAID	Non-steroidal anti-inflammatory drug
OD	Optical density
OECD	Organisation for Economic Co-operation and Development
ORAC	Oxygen radical absorbance capacity assay
p.o	Oral administration
PEF	Petroleum ether fraction of *Muntingia calabura*
PGE_2	Prostaglandin E_2
PGI_2	Prostacyclin
PPIs	Proton pump inhibitors
QR	Quinone reductase
RAW 264.7	Monocytic macrophages cell line
ROS	Reactive oxygen species
ROW	Relative organ weight
RT	Retention time
SEM	Standard Error of Mean
SH	Sulfhydryl
SOD	Superoxide dismutase
TE	Trolox equivalents
TFF	Trefoil factor family
TNF-α	Tumor necrosis factor-α

TPC	Total phenolic content
UA	Ulcer area
UHPLC-ESI	Ultra High Performance Liquid Chromatography-Electrospray Ionization
UPM	Universiti Putra Malaysia
UV	Ultraviolet
WHO	World Health Organization
XO	Xanthine oxidase

CHAPTER 1

INTRODUCTION

1.1 Introduction

Gastric ulcer is one of the major gastrointestinal disorders that affect considerable number of people around the world. The prevalence of gastrointestinal disorders varies from more than 80% in the developing countries to less than 40% in the developed world (Kusters et al., 2006; Malaty, 2007). It has been projected that 15.5 million people in the United States were affected by peptic ulcer disease in the year 2011 (Schiller et al., 2012). Some authors refer to gastric ulcers as the new "plague" of the 21st century (O'Malley, 2003). The pathophysiology of gastric ulcer is associated with the imbalance between noxious and protective factors in the stomach. Gastric mucosal damage occurs when noxious factors "overwhelm" an intact mucosal defense, or weakening of the mucosal defensive mechanisms (Laine et al., 2008). The noxious factors in this context include alcohol ingestion, acid and pepsin secretion, poor diet, stress, reactive oxygen species (ROS), the use of non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori* infection (Rang et al., 2012; Zheng et al., 2014). On the other hand, the key defense factors and mechanisms that afford mucosal defense include sufficient mucus secretion and mucosal blood flow, bicarbonate secretion, intact mucus barrier, prostaglandins, surface active phospholipids, increased levels of antioxidants, activity of anti-inflammatory compounds and adequate levels of nitric oxide (NO) (Mota et al., 2009; Amaral et al., 2013; Zheng et al., 2014).

Currently, the prevention and treatment of gastric ulcers has gained lots of interest and became an important challenge confronting the medicine world. To date, there are a few approaches used to prevent gastric ulceration, which include potentiation of the mucosal defense together with reduction of acid secretion and its neutralization, stimulation of gastric mucin synthesis, enhancement of antioxidant levels in the stomach, and inhibition of the *H. pylori* growth (Panda and Khambat, 2014). Secretion of gastric acid is believed to be the central component of gastric ulcers despite the presence of many causative factors (Mota et al., 2009) and therefore, inhibition of gastric acid secretion tend to be the key therapeutic target for ulcer diseases (Jain et al., 2007). On the other hand, another key factor in the pathogenesis of gastric ulcers is the production of reactive oxygen species (ROS). According to Amaral et al. (2013), the production of ROS and a concomitant reduction of antioxidant capacity are responsible for cell damage and death due to their extreme reactivity. Moreover, Kahraman et al. (2003) said that the ROS causes damage to the essential cell constituents, which are proteins, lipids and nucleic acids, resulting in the formation of toxic compounds. Therefore, controlling the ROS

formation and gastric acid secretion as well as enhancing antioxidant capacity are essential for the treatment of these pathologies (Boligon et al., 2014).

The current medicinal treatment of gastric ulcers include acid blockers that reduce acid secretion, proton pump inhibitors, antibiotics to eradicate *H. pylori* and tissue lining protecting agents such as sucralfate and bismuth cholinergics (Bighetti et al., 2005; Wallace, 2005). Although advances have been made in the treatment of peptic ulcers, the morbidity and mortality toll is still very high (Akah et al., 2009). The research and development of new antiulcer remedies is necessary due to several key points that include: 1. The available drugs for the management of ulcers faces a major drawback as they are associated with undesirable side effects such as increased susceptibility to pneumonia and bone fractures, deficiency of iron and vitamin B12, hypergastrinemia, thrombocytopenia and cancer (Dacha et al., 2015); 2. Existence of drug-drug interactions (Sheen and Triadafilopoulos, 2011); and 3. Frequent ulcer recurrence has been observed in patients following treatment (Kangwan et al., 2014). Hence, there is a pressing need to discover effective and safe alternative therapies to prevent and treat gastric ulcers and the search has also been extended to herbal drugs for their easy and abundant availability, improved protection, cost effective and reduced toxicity (Mohod and Bodhankar, 2013). Validation of the efficacy and harnessing of medicinal plants used in folk medicine for the treatment of peptic ulcer diseases is a very promising approach to overcome the limitations of orthodox medicines (Akah et al., 2009). Plant extracts can be valuable and serve as a new source of therapeutics in the treatment of gastric ulcers whereby antisecretory, cytoprotective and antioxidant activities, isolated or in combination, are the three main functions of a gastroprotective agent, which play the key role in gastric mucosal protection (Al Mofleh, 2010).

Muntingia calabura L. (family Muntingiaceae), commonly known as Jamaican cherry or *kerukup siam* in Malaysia, is widely cultivated in warm areas of Asian region, including Malaysia (Chin, 1989). In Asia and tropical America, various parts of this tree have been documented for several medicinal uses. *M. calabura*'s leaves, flowers, barks and roots have been used as a folk remedy to treat headaches, fever and incipient cold. According to Peruvian folklore, the leaves are used to provide relief from gastric ulcers and to reduce swelling of the prostate gland (Morton, 1987). Besides, they are also employed as antiseptic, antispasmodic, and antidyspeptic agent (Kaneda et al., 1991; Nshimo et al., 1993).

Scientific evaluations of *M. calabura* have revealed several pharmacological activities possessed by the plant. This include antitumor (Kaneda et al., 1991; Su et al., 2003), antibacterial (Zakaria et al., 2006a), antinociception (Zakaria et al., 2006b, 2007a, 2007b), anti-inflammatory, antipyretic (Zakaria et al., 2007b), antioxidant and antiproliferative (Zakaria et al., 2011) activities exhibited by the leaves of *M. calabura*, while several types of flavonoids have been isolated and identified from the leaves, roots and stem barks of *M. calabura* (Kaneda et al.,

1991; Nshimo et al., 1993; Su et al., 2003; Chen et al., 2005; Sufian et al., 2013).

M. calabura was chosen in the present study based on the fact that it has been traditionally used as an antidyspeptic agent and to provide relieve from gastric pain in certain part of the world. However, in Malaysia, the medicinal values of *M. calabura* are not well documented and it is considered as a negelected plant as it received lack of attention among the community eventhough it is abundantly available (Mahmood et al., 2014). Moreover, *M. calabura* has been reported to contain high total phenolic content and to exert high antioxidant activities, which are important in the mechanisms of antiulcer of any compounds/extracts. It is well known that gastric ulcer is associated with generation of free radicals that causes oxidative stress in the tissues. Hence, extracts/compounds with antioxidant activity play a very important role in scavenging those free radicals. In lieu of this, *M. calabura* has been reported to exert a remarkable antioxidant activity, and therefore, is believed to exert effective gastroprotection against gastric ulcers. On the other hand, it is also worth to note that the leaves of *M. calabura* are either boiled or steeped in water to provide relieve from gastric ulcers traditionally, as well as the direct intake of the leaves as a tea-like beverage in Peru (Mahmood et al., 2014) indicate that the leaves of *M. calabura* are fairly safe for human consumption. The traditional use of the plant for the treatment of gastric ulcer together with its potential antioxidant properties justified the present research with hope of finding an alternative/natural gastroprotective agent as a replacement to the currently available side effect-baring drugs used in the second-line treatment.

Despite the extensive literature review on this plant and to the best of our knowledge, there was no scientific report available in support of the traditional claim of the gastric-ulcer protective activity of *M. calabura*. In an attempt to fulfil this lack, the present study was designed to evaluate the gastroprotective effect and the possible mechanistic activity of *M. calabura* methanolic leaves extract and its fractions. In addition, based on the phytochemical analyses of *M. calabura* crude extract and fractions, the present work also identifies the compounds that may well contribute to the gastroprotective activity of the plant.

1.2 Problem statement

Gastric ulcer is a serious gastrointestinal disease that affects a considerable number of people in the world, accounting for morbidity and mortality as well as causing significant impact on the quality of life. Eventhough there are a number of synthetic drugs available, gastric ulcer therapy faces a major drawback as most of the drugs available in the market are often associated with adverse effects, existence of drug-drug interactions and frequent ulcer recurrence in patients following treatment. Therefore, there is a necessity to discover an alternative gastroprotective agent as a replacement to the currently available side effect-baring drugs used in the second-line treatment. An alternative therapy for ulcer prevention and treatment would be the use of plant derived

preparations. *M. calabura* has been traditionally used in treatment of gastrointestinal disorders. Besides, *M. calabura* also found to exert significant antioxidant activity, contain high amount of phenol as well as flavonoid and possess excellent radical scavenging effect, which could make this plant a promising alternative to the conventional antiulcer treatment. However, there was no scientific finding that has been reported to validate the traditional use of *M. calabura* in prevention or treatment of gastric ulcers. In line with this, the current study was designed to evaluate the potential antiulcer activity the mechanism(s) of action of *M. calabura* leaves as the candidate of future antiulcer agent as well as to place the ethnomedicinal claim of *M. calabura* leaves in gastroprotection on a solid scientific footing.

1.3 Hypothesis

In this study, it is hypothesized that the methanol extract of *M. calabura* leaves (MEMC) will reduce gastric mucosal injury in ethanol- and indomethacin-induced gastric ulceration in the rat models while the fractions of MEMC will show gastroprotective effect against ethanol-induced ulceration in rat model. Besides, it is also hypothesized that the mechanism of action underlying the gastroprotective activity of MEMC and its most effective fraction will involve strengthening of the protective factors such as antioxidant and anti-inflammatory activities, mucus secretion, prostaglandin production and modulation of nitric oxide and sulfhydryl compounds as well as weakening of the noxious factors, which include gastric acid secretion and oxidative stress. Furthermore, it is hypothesized that several phytochemical constituents identified in MEMC and its fraction are partly responsible for the gastroprotection exerted by the extract and the fraction.

1.4 Research objectives

The general objectives of the present study are:

1. To evaluate the antiulcer activity of MEMC and its fractions against gastric ulceration using rat models.
2. To investigate the mechanim(s) of action of MEMC and its most effective fraction against gastric ulceration.

The specific objectives of the present study are:

1. To assess the toxicity of MEMC by performing the acute toxicity evaluation.
2. To investigate the antiulcer activity of MEMC using rats on gastric ulcer models, which include:
   a) ethanol-induced ulcer model with macroscopic (gross examination) and histopathological evaluation.
   b) indomethacin-induced ulcer model with macroscopic (gross examination) and histopathological evaluation.

3. To evaluate the gastroprotective effect of the fractions obtained from MEMC using ethanol-induced ulcer model with macroscopic (gross examination) and histopathological evaluation.
4. To investigate the *in-vitro* antioxidant and anti-inflammatory activities of MEMC and its fractions by determining the total phenolic content, radical scavenging activity and the nitric oxide inhibitory effect and cytotoxicity in inflammatory-induced RAW 264.7 cell line, xanthine oxidase (XO) and lipoxygenase (LOX) inhibiton of MEMC and its fractions.
5. To elucidate the mechanisms of action underlying the gastroprotective activity of MEMC and the most effective fraction using the pylorus-ligation model, gastric content analysis, determination of gastric mucus secretion, assessing the involvement of nitic oxide and sulfhydryl compound in the mechanism of gastroprotection and carry out biochemical analysis using the stomach homogenate for the most effective fraction of MEMC to determine the levels of SOD, catalase (CAT), GSH, MDA, PGE_2 and NO.
6. To identify the phytochemical compounds present in MEMC and its fractions using HPLC and UHPLC-ESI analysis.

REFERENCES

Aasen, S. and Lundin, K.E.A. 2013. Stomach duodenum normal anatomy, function and congenital anomalies. In: *Abdominal Imaging,* ed. B. Hamm, and P.R. Ros, pp. 367-382. New York: Springer-Verlag Berlin Heidelberg.

Abdelwahab, S. 2013. Protective mechanism of gallic acid and its novel derivative against ethanol-induced gastric ulcerogenesis: involvement of immunomodulation markers, Hsp70 and Bcl-2-associated X protein. *International Immunopharmacology* 16: 296-305.

Abdelwahab, S.I., Mohan, S., Abdulla, M.A., Sukari, M.A., Abdul, A.B., Taha, M.M., Syam, S., Ahmad, S. and Lee, K.H. 2011. The methanolic extract of *Boesenbergia rotunda* (L.) Mansf. and its major compound pinostrobin induces anti-ulcerogenic property in vivo: possible involvement of indirect antioxidant action. *Journal of Ethnopharmacology* 137: 963-970.

Abdulla, M.A., Ahmed, K.A.A., AL-Bayaty, F.H. and Masood, Y. 2010. Gastroprotective effect of *Phyllanthus niruri* leaf extract against ethanol-induced gastric mucosal injury in rats. *African Journal of Pharmacy and Pharmacology* 4: 226-230

Abraham, N.S. 2012. Proton pump inhibitors: potential adverse effects. *Current Opinion in Gastroenterology* 28: 615-20.

Adinortey, M.B., Ansah, C., Galyuon, I. and Nyarko, A. 2013. *In vivo* models used for evaluation of potential antigastroduodenal ulcer agents. *Ulcers* 2013: 1-12.

Ahmad, A., Gupta, G., Afzal, M., Kazmi, I. and Anwar, F. 2013. Antiulcer and antioxidant activities of a new steroid from *Morus alba*. *Life Sciences* 92: 202-210.

Akah, P.A., Onyirioha, C.A., Nworu, C.S. and Ndu, O.O. 2009. Gastroprotective effects of the leaf extract and fractions of *Fleurya aestuans* L. (Urticaceae). *International Journal of Health Research* 2: 65-73.

Al Mofleh, I.A. 2010. Spices, herbal xenobiotics and the stomach: Friends or foes? *World Journal of Gastroenterology* 16: 2710-2719.

Allen, A. and Flemstrom, G. 2005. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *American Journal of Physiology-Cell Physiology* 288: C1-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. *Journal of Ethnopharmacology* 134:630-6.

Amaral, G.P., de Carvalho, N.R., Barcelos, R.P., Dobrachinski, F., Portella, R. de L., da Silva, M.H., Lugokenski, T.H., Dias, G.R., da Luz, S.C., Boligon, A.A., Athayde, M.L., Villetti, M.A., Antunes Soares, F.A. and Fachinetto, R. 2013. Protective action of ethanolic extract of *Rosmarinus officinalis* L. in gastric ulcer prevention induced by ethanol in rats. *Food and Chemical Toxicology* 55: 48-55.

Andersen, I.B., Jorgensen, T., Bonnevie O, Gronbaek, M. and Sorensen, T. 2000. Smoking and alcohol intake as risk factors for bleeding and perforated peptic ulcers: a population-based cohort study. *Epidemiology* 11: 434-439.

Andreo, M.A., Ballesteros, K.V., Hiruma-Lima, C.A., Machado da Rocha, L.R., Souza Brito, A.R. and Vilegas, W. 2006. Effect of *Mouriri pusa* extracts on experimentally induced gastric lesions in rodents: role of endogenous sulfhydryls compounds and nitric oxide in gastroprotection. *Journal of Ethnopharmacology* 107:431-441.

Arab,H.H., Salama, S.A., Omar, H.A., Arafa, E.A. and Maghrabi, I.A. 2015. Diosmin protects against ethanol-induced gastric injury in rats: novel anti-ulcer actions. *PLoS One* 10: e0122417.

Araki, H., Ukawa, H., Sugawa, Y., Yagi, K., Suzuki, K. and Takeuchi, K. 2000. The roles of prostaglandin E receptor subtypes in the cytoprotective action of prostaglandin E_2 in rat stomach. *Alimentary Pharmacology & Therapeutics* 14:116-124.

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. *International Journal of Pharmaceutical Research* 1: 75-85.

Awaad, A.S., El-Meligy, R.M. and Soliman, G.A. 2013. Natural products in treatment of ulcerative colitis and peptic ulcer. *Journal of Saudi Chemical Society* 17: 101-124.

Azhar-Ul-Haq, Malik, A., Anis, I., Khan, S.B., Ahmed, E., Ahmed, Z., Nawaz, S.A. and Choudhary, M.I. 2004. Enzymes inhibiting lignans from *Vitex negundo*. *Chemical and Pharmaceutical Bulletin* 52: 1269-1272.

Bagchi, D., Carryl, O.R., Tran, M.X., Krohn, R.L., Bagchi, D.J., Garg, A., Bagchi, M., Mitra, S. and Stohs, S.J. 1998. Stress, diet and alcohol induced oxidative gastrointestinal mucosal injury in rats and protection by bismuth subsalicylate. *Journal of Applied Toxicology* 18: 3-13.

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. *Indian Journal of Experimental Biology*. 40: 693-705.

Bansil, R. and Turner, B.S. 2006. Mucin structure, aggregation, physiological functions and biomedical applications. *Current Opinion in Colloid & Interface Science* 11:164-170.

Barnett, K., Bell, C.J., McKnight, W., Dicay, M., Sharkey, K.A. and Wallace, J.L. 2000. Role of cyclooxygenase-2 in modulating gastric acid secretion in the normal and inflamed rat stomach. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 279: G1292–G1297.

Barrachina, M.D., Panés, J. and Esplugues, J.V. 2001. Role of nitric oxide in gastrointestinal inflammatory and ulcerative diseases: Perspective for drugs development. *Current Pharmaceutical Design* 7: 31-48.

Barros, M.P., Lemos, M., Maistro, E.L., Leite, M.F., Sousa, J.P., Bastos, J.K. and Andrade, S.F. 2008. Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. *Journal of Ethnopharmacology* 120:372-377.

Batista, L.M., de Almeida, A.B., Lima, G.R., Falcão Hde, S., Magri Lde, P., Luiz-Ferreira, A., dos Santos, L.C., Hiruma-Lima, C.A., Vilegas, W. and Brito, A.R. 2014. Gastroprotective effects (in rodents) of a flavonoid rich fraction obtained from *Syngonanthus macrolepsis*. *Journal of Pharmacy and Pharmacology* 66: 445-452.

Baumgartner, H.K., Starodub, O.T., Joehl, J.S., Tackett, L. and Montrose, M.H. 2004. Cyclooxygenase 1 is required for pH control at the mouse gastric surface. *Gut* 53: 1751-1757.

Beck, P.L., Xavier, R. and Won, J. 2004. Paradoxical roles of different nitric oxide synthase isoforms in colonic injury. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 286: G137-G1347.

Beil, W., Birkhoiz, C. and Sewing, K.F. 1995. Effects of flavonoids on parietal cell acid secretion, gastricmucosal prostaglandin production and *Helicobacter pylori* growth. *Arzneimittel-Forschung-Drug Research* 45: 697-700.

Ben-Arye, E., Goldin, E., Wengrower, D., Stamper, A., Kohn, R. and Berry, E. 2002. Wheat grass juice in the treatment of active distal ulcerative colitis: a randomized double-blind placebo-controlled trial. *Scandinavian Journal of Gastroenterology* 37: 444-449.

Bi, W.P., Man, H.B. and Man, M.Q. 2014. Efficacy and safety of herbal medicines in treating gastric ulcer: A review. *World Journal of Gastroenterology* 20: 17020-17028.

Bighetti, A.E., Antonio, M.A., Kohn, L.K., Rehder, V.L., Foglio ,M.A., Possenti, A., Vilela, L. and Carvalho, J.E. 2005. Antiulcerogenic activity of a crude hydroalcoholic extract and coumarin isolated from *Mikania laevigata* Schultz Bip. *Phytomedicine* 13: 72-77.

Blix, H.S., Viktil, K.K., Moger, T.A. and Reikvam, A. 2010. Drugs with narrow therapeutic index as indicators in the risk management of hospitalised patients. *Pharmacy Practice* 8: 50-55.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature* 181: 1199-1200.

Boegh, M and Nielsen, H.M. 2015. Mucus as a barrier to drug delivery–understanding and mimicking the barrier properties. *Basic and Clinical Pharmacology and Toxicology* 116: 179-186.

Boligon, A.A., Freitasa, R.B., de Brum, T.F., Waczuk, E.P., Klimaczewski, C.V., Ávila, D.S., Athayde, M.L. and Bauermann, L.F. 2014. Antiulcerogenic activity of *Scutia buxifolia* on gastric ulcers induced by ethanol in rats. *Acta Pharmaceutica Sinica B* 4: 358-367.

Borrelli, F. and Izzo, A.A. 2000. The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy Research* 14: 581-591.

Bors, W. and Michel, C. 2002. Chemistry of the antioxidant effect of polyphenols. *Annals of the New York Academy of Sciences* 957: 57-69.

Boughton-Smith, N.K. 1989. Involvement of leukotrienes in acute gastric damage. *Methods and Findings in Experimental and Clinical Pharmacology* 11: 53-59.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Brunton, L.L. 2001. *The Pharmacological Basis of Therapeutic's 10th Edition*. New York: McGraw-Hill Medical Publishing Division.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Brzozowska, I. and Pawlik, T. 2005. Role of prostaglandins in gastroprotection and gastric adaptation. *Journal of Physiology and Pharmacology* 56: 33-55.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Kwiecién, S., Pajdo, R., Brzozowska, I. and Hahn, E.G. 1998. Involvement of endogenous cholecystokinin and somatostatin in gastroprotection induced by intraduodenal fat. *Journal of Clinical Gastroenterology* 27: 125-137.

Brzozowski, T., Konturek, P.C., Sliwowski, Z., Kwiecień, S., Drozdowicz, D., Pawlik, M., Mach, K., Konturek, S.J. and Pawlik, W.W. 2006. Interaction of non-steroidal anti-inflammatory drugs (NSAID) with *Helicobacter pylori* in the stomach of humans and experimental animals. *Journal of Physiology and Pharmacology* 57: 67-79.

Bujanda, L. 2000. The effects of alcohol consumption upon the gastrointestinal tract. *American Journal of Gastroenterology* 95: 3374-3382.

Calatayud, S., Barrachina, D. and Esplugues, J.V. 2001. Nitric oxide: relation to integrity, injury, and healing of the gastric mucosa. *Microscopy Research and Technique* 53: 325-335.

Calvino Fernandez, M. and Parra Cid. 2010. *H. pylori* and mitochondrial changes in epithelial cells. The role of oxidative stress. *Revista Espanola de Enfermedades Digestivas* 102: 41-50.

Carroll, W. 2016. *Gastroenterology and Nutrition: Prepare for the MRCPCH*. Edinburgh: Elsevier Ltd.

Chan, F.K.L. 2009. *Peptic Ulcer Disease*. Philadelphia: Saunders.

Chang, W.S., Lin, C.C. and Chiang, H.C. 1996. Superoxide anion scavenging effect of coumarins. *American Journal of Chinese Medicine* 24: 11-17.

Chattopadhyay, I., Nandi, B., Chatterjee, R., Biswas, K., Bandyopadhyay, U. and Banerjee, R.K. 2004. Mechanism of antiulcer effect of Neem (*Azadirachta indica*) leaf extract: effect on H+-K+-ATPase, oxidative damage and apoptosis. *Inflammopharmacology* 12: 153-76.

Chen, H., Liao, H., Liu, Y., Zheng, Y., Wu, X., Su, Z., Zhang, X., Lai, Z., Lai, X., Lin, Z.X. and Su, Z. 2015. Protective effects of pogostone from Pogostemonis Herba against ethanol-induced gastric ulcer in rats. *Fitoterapia* 100: 110-117.

Chen, J.J., Lee, H.H., Duh, C.Y. and Chen, I.S. 2005. Cytotoxic chalcones and flavonoids from the leaves of *Muntingia calabura*. *Planta Medica* 71: 970-973.

Chen, J.J., Lee, H.H., Shih, C.D., Liao, C.H., Chen, I.S. and Chou, T.H. 2007. New dihydrochalcones and anti-platelet aggregation constituents from the leaves of *Muntingia calabura*. *Planta Medica* 73: 572-577.

Chen, J.J., Lin, R.W., Duh, C.Y., Huang, H.Y. and Chen, J.J. 2004. Flavones and cytotoxic constituents from the stem bark of *Muntingia calabura*. *Journal- Chinese Chemical Society Taipei* 51: 665-670.

Chin WY. 1989. *A Guide to the Wayside Trees of Singapore*. Singapore: BP Singapore Science Centre.

Choudhary, M.K., Bodakhe, S.H. and Gupta, S.K. 2013. Assessment of the antiulcer potential of *Moringa oleifera* root-bark extract in rats. *Journal of Acupuncture & Meridian Studies* 6: 214-220.

Chueca, E., Lanas, A. and Piazuelo, E. 2012. Role of gastrin-peptides in Barrett's and colorectal carcinogenesis. *World Journal of Gastroenterology* 18: 6560-6570.

Cook, S. 2016. *The Forest of the Lacandon Maya: An Ethnobotanical Guide*. Springer US: Springer Science+Business Media New York.

Corne, S.J., Morrisey, S.M. and Woods, R.J. 1974. A method for the quantitative estimation of gastric barrier mucus. *Journal of Physiology* 242: 116-117.

Coskun, O., Kanter, M., Armutcu, F., Cetin, K., Kaybolmaz, B. and Yazgan, O. 2004. Protective effects of quercetin, a flavonoid antioxidant, in absolute ethanol-induced acute gastric ulcer. *European Journal of General Medicine* 1: 37-42.

Cragg, G.M. and Newman, D.J. 2013. Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta* 1830: 3670-3695.

Cristians, S., Bye, R., Navarrete, A., Mata, R. 2013. Gastroprotective effect of *Hintonia latiflora* and *Hintonia standleyana* aqueous extracts and compounds. *Journal of Ethnopharmacology* 145: 530-535.

Dacha, S., Razvi, M., Massaad, J., Cai, Q and Wehbi, M. 2015. Hypergastrinemia. *Gastroenterology* 3: 201-208.

Dahanukar, S.A., Kulkarni, R.A. and Rege, N.N. 2000. Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology* 32: S81-S118.

Daniels, I.R. and Allum, W.H. 2005. The anatomy and physiology of the stomach. In: *Upper Gastrointestinal Surgery*, ed. J.W.L. Fielding, and M.T. Hallissey, pp, 17-37. London: Springer-Verlag London Limited.

de la Lastra, C.A., Martin, M.J. and Motilva, V. 1994. Antiulcer and gastroprotective effects of quercetin, a gross and histologic study. *Pharmacology* 48: 56-62.

de-Faria, F.M., Almeida, A.C.A., ALuiz-Ferreira, A., Takayama, C., Dunder, R.J., da Silva, M.A., Salvador, M.J., Abdelnur, P.V., Eberlin, M.N., Vilegas, W., Toma, W. and Souza-Brito, A.R.M. 2012. Antioxidant action of mangrove polyphenols against gastric damage induced by absolute ethanol and ischemia-reperfusion in the rat. *The Scientific World Journal* 2012: 1-9.

Deakin, M. and Williams, J.G. Histamine H_2-receptor antagonists in peptic ulcer disease. Efficacy in healing peptic ulcers. *Drugs*. 1992 44: 709-719.

Debbab, A., Aly, A.H., Lin, W.H. and Proksch, P. 2010. Bioactive compounds from marine bacteria and fungi. *Microbial Biotechnology* 3: 544-563.

Dias, A., Garcia, C., Majewski, M., Wallner, G., McCallum, R.W., Poplawski, C. and Sarosieket, J. 2011. Gastric juice prostaglandins and peptide growth factors as potential markers of chronic atrophic gastritis, intestinal metaplasia and gastric cancer: their potential clinical implications based on this pilot study. *Digestive Diseases and Sciences* 56: 3220-3225.

Dias, P.C., Foglio, M.A., Possenti, A. and de Carvalho, J.E. 2000. Antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis* L. *Journal of Ethnopharmacology* 69: 57-62.

Ding, S., Minohara, Y., Fan, X.J., Wang, J., Reyes, V.E., Patel, J., Dirden-Kramer, B., Boldogh, I., Ernst, P.B. and Crowe, S.E. 2007. *Helicobacter pylori* infection induces oxidative stress and programmed cell death in human gastric epithelial cells. *Infection and Immunity* 75: 4030-4039.

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M. and Mérillon, J. 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry* 57: 1768-1774.

Ellis, H. 2011. Anatomy of the stomach. *Surgery* 29: 541-543.

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. *Molecules* 15: 7153-7166.

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

Ford, A.C., Delaney, B.C., Forman, D. and Moayyedi, P. 2004. Eradication therapy in *Helicobacter pylori* positive peptic ulcer disease: systematic review and economic analysis. *American Journal of Gastroenterology* 99: 1833-1855.

Fornai, M., Antonioli, L., Colucci, R., Tuccori, M. and Blandizz, C. 2011. Pathophysiology of gastric ulcer development and healing: molecular mechanisms and novel therapeutic options. In: *Peptic Ulcer Disease*, ed. J. Chai, pp. 113-142. Crotia: InTech.

Forte, J.G. and Zhu, L. 2010. Apical recycling of the gastric parietal cell H,K-ATPase. *Annual Review of Physiology* 72: 273-96.

Fujino, S., Suzuki, Y. and Tanaka, T. 1985. Cost-benefit analysis of medicinal treatment for gastric ulcers. Long-term model including healing and recurrence. *Health Policy* 5: 45-72.

Geibel, J.P. and Wagner, C. 2006. An update on acid secretion. *Reviews of Physiology, Biochemistry and Pharmacology* 156: 45-60.

Glavin, G.B. and Szabo, S. 1992. Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. *The FASEB Journal* 6: 825-831.

Goh, K.L., Wong, H.T., Lim, C.H. and Rosaida, M.S. Time trends in peptic ulcer, erosive reflux oesophagitis, gastric and oesophageal cancers in a multiracial Asian population. *Alimentary Pharmacology and Therapeutics* 29: 774-780.

Gomathi, R., Anusuya, N. and Manian, S. A dietary antioxidant supplementation of Jamaican cherries (*Muntingia calabura* L.) attenuates inflammatory related disorders. *Food Science and Biotechnology* 22: 787-794.

Groenen, M.J.M., Kuipers, E.J., Hansen, B.E. and Ouwendijk, R.J.T. 2009. Incidence of duodenal ulcers and gastric ulcers in a Western population: Back to where it started. *Canadian Journal of Gastroenterology* 23: 604-608.

Gülçin, I., Küfrevioglu, O., Oktay, M. and Büyükokuroglu, M.E. 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *Journal of Ethnopharmacology* 90: 205-215.

Gupta, M. and Eisen, G.M. 2009. NSAIDs and the gastrointestinal tract. *Current Gastroenterology Reports* 11: 345-353.

Hajrezaie, M., Golbabapour, S., Hassandarvish, P., Gwaram, N.S., Hadi, A.H.A., Ali, H.M., Majid, N. and Abdulla, M.A. 2012. Acute toxicity and gastroprotection studies of a new schiff base derived copper (II) complex against ethanol-induced acute gastric lesions in rats. *PLoS One* 7: e51537.

Halliwell, B. 2001. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs and Aging*. 18: 685-716.

Halliwell, B. and Gutteridge, J.M.C. 2001. *Free Radicals in Biology and Medicine, 3rd ed*. United Kingdom: Oxford University Press.

Hanai, H., Kanauchi, O., Mitsuyama, K., Andoh, A., Takeuchi, K., Takayuki, I., Araki, Y., Fujiyama, Y., Toyonaga, A., Sata, M., Kojima, A., Fukuda, M. and Bamba, T. 2004. Germinated barley foodstuff prolongs remission in patients with ulcerative colitis. *International Journal of Molecular Medicine* 13: 643-647.

Handa, O., Naito, Y. and Yoshikawa, T. 2010. *Helicobacter pylori*: a ROS-inducing bacterial species in the stomach. *Inflammation Research* 59: 997-1003.

Haschek, W.M. and Rousseaux, C.G. 1991. *Handbook of Toxicologic Pathology*. California: Academic Press Inc San Diego.

Hatazawa, R., Tanaka, A., Tanigami, M., Amagase, K., Kato, S., Ashida, Y. and Takeuchi, K. 2007. Cyclooxygenase-2/prostaglandin E_2 accelerates the healing of gastric ulcers via EP_4 receptors. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 293: G788-G797.

Hatler, F., 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: 443-453.

Hayllar, J. and Bjarnason L. 1995. NSAIDs, Cox-2 inhibitors and the gut. *Lancet* 346: 521-522.

Heeba, G.H., Hassan, M.K. and Amin, R.S. 2009. Gastroprotective effect of simvastatin against indomethacin-induced gastric ulcer in rats: role of nitric oxide and prostaglandins. *European Journal of Pharmacology* 607: 188-193.

Holzer, P. 2006. Neural regulation of gastrointestinal blood flow. In: *Physiology of the Gastrointestinal Tract*, ed. L.R. Johnson, pp. 817-839. New York: Academic Press.

Hu, X.T., Ding, C., Zhou, N. and Xu, C. 2015. Quercetin protects gastric epithelial cell from oxidative damage in vitro and in vivo. *European Journal of Pharmacology* 754, 115-124.

Huang, C.C., Chen, Y.M., Wang, D.C., Chiu, C.C., Lin, W.T., Huang, C.Y and Hsu,M.C. 2013. Cytoprotective effect of American ginseng in a rat ethanol gastric ulcer model. *Molecules* 19: 316-326.

Huang, D., Ou, B. and Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* 53: 1841-1856.

Huang, D., Ou, B., Hampch-Woodill, M., Flanagan, J.A. and Prior, R.L. 2002. High throughput assay of oxygen radical absorbance capacity (ORAC) using a multi- channel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of.Agriculture and Food Chemistry* 5: 4437-4444.

Huh, K., Kwon, T.H., Shin, U.S., Kim, W.B., Ahn, B.O., Oh, T.Y. and Kim, J. 2003. Inhibitory effects of DA-9601 on ethanol-induced gastrohemorrhagic lesions and gastric xanthine oxidase activity in rats. *Journal of Ethnopharmacology* 88: 269-273.

Huh, K., Shin, U.S. and Lee, S.H. 1996. The effect of rebamipide on gastric xanthine oxidase activity and type conversion in ethanol-treated rats. *Free Radical Biology & Medicine* 20: 967-971.

Hussein, S.A., El-Senosy, Y.A. and Hassan, M.F. 2014. Gastroprotective, antiapoptotic and anti-inflammatory effect of alpha-lipoic acid on ethanol induced gastric mucosal lesions in rats. *American Journal of Biochemistry and Molecular Biology* 4: 48-63.

Izzo, A.A., Di Carlo, G., Mascolo, N. and Capasso, F. 1994. Antiulcer effect of flavonoids: role of endogenous PAF. *Phytotherapy Research* 8: 179-181.

Jain, K.S., Shah, A.K., Bariwal, J., Shelke, S.M., Kale, A.P., Jagtap, J.R. and Bhosale, A.V. 2007. Recent advances in proton pump inhibitors and management of acid-peptic disorders. *Bioorganic & Medicinal Chemistry* 15: 1181-1205.

Jalilzadeh-Amin, G., Najarnezhad, V., Anassori, E., Mostafavi, M. and Keshipour, H. 2015. Antiulcer properties of *Glycyrrhiza glabra* L. extract on experimental models of gastric ulcer in mice. *Iranian Journal of Pharmaceutical Research* 14: 1163-1170.

Jamal, A., Javed, K., Aslam, M. and Jafri, M. 2006. Gastroprotective effect of cardamom, *Elettaria cardamomum* Maton. fruits in rats. *Journal of Ethnopharmacology* 103:149-153.

Jayakumari, S., Anbu, J., Ravichandran, V., Anjana, A., Kumar, G.M.S. and Singh, M. 2012. Antiulcerogenic and free radical scavenging activity of flavonoid fraction of *Psidium Guajava* Linn. leaves. *International Journal of Pharmacy and Pharmaceutical Sciences* 4: 170-174.

Jensen, M. 1999. *Trees Commonly Cultivated in Southeast Asia: An Illustrated Field Guide. (2nd ed.)*. Bangkok: Craftsman Press.

Joish, V.N., Donaldson, G., Stockdale, W., Oderda, G.M., Crawley, J., Sasane, R., Joshua-Gotlib, S. and Brixner, D.I. 2005. The economic impact of GERD and PUD: examination of direct and indirect costs using a large integrated employer claims database. *Current Medical Research and Opinionv* 5: 21:535-544.

Jones, A.W. 2016. Perspectives in rug development and clinical pharmacology: The discovery of histamine H_1 and H_2 antagonists. *Clinical Pharmacology in Drug Development* 5: 5-12.

Kahraman, A., Çakar, H. and Köken, T. 2012. The protective effect of quercetin on long-term alcohol consumption-induced oxidative stress. *Molecular Biology Reports* 39: 2789-2794.

Kahraman, A., Erkasap, N., Koken, T., Serteser, M., Aktepe, F. and Erkasap, S. 2003. The antioxidative and antihistaminic properties of quercetin in ethanol-induced gastric lesions. *Toxicology* 183: 133-142.

Kaneda, N., Pezzuto, J.M., Soejarto, D.D., Kinghorn, A.D., Farnwort, N.R., Santisuk, T., Tuchinda, P., Udchachon, J. and Reutrakul, V. 1991. Plant anticancer agents, XLVII. New cytotoxic flavonoids from *Muntingia calabura* roots. *Journal of Natural Products* 54: 196-206.

Kangwan, N., Park, J.M., Kim, E.H. and Hahm, K.B. 2014. Quality of healing of gastric ulcers: natural products beyond acid suppression. *World Journal of Gastrointestinal Pathophysiology* 5: 40-47.

Karthyaini and Suresh, K. 2012. Pharmacognostic evaluation, *in vitro* antioxidant and *in vivo* anti-inflammatory studies of *Muntingia calabura* Linn. *Journal of Global Trends in Pharmaceutical Sciences* 3: 805-811.

Kato, S., Aihara, E., Yoshii, K. and Takeuchi, K. 2005. Dual action of prostaglandin E_2 on gastric acid secretion through different EP-receptor subtypes in the rat. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 289: G64-G69.

Kaur, A., Robin, S., Sharma, R. and Kumar, S. 2012. Peptic ulcer: a review on etiology and pathogenesis. *International Research Journal of Pharmacy* 3: 2230-8407.

Khan, W.I. and Ghia, J.E. 2010. Gut hormones: ermerging role in immune activation and inflammation. *Clinical and Experimental Immunology* 161: 19-27.

Khattab, M.M., Gad, M.Z. and Abdallah, D. 2001. Protective role of nitric oxide in indomethacin-induced gastric ulceration by a mechanism independent of gastric acid secretion. *Pharmacological Research* 43: 463-467.

Khled khodja, N., Boulekbache-Makhlouf, L. and Madani, K. 2014. Antioxidant capacity of crude extracts and their solvent fractions of selected Algerian Lamiaceae. *Industrial Crops and Products* 52: 177-182.

Ko, J.K. and Cho, C.H. 2000. Alcohol drinking and cigarette smoking: a "partner" for gastric ulceration. *Zhonghua Yi Xue Za Zhi (Taipei) = Chinese Medical Journal* 63: 845-854.

Kochar, N.I., Chandewal, A.V., Bakal, R.L. and Kochar, P.N. 2011. Nitric oxide and the gastrointestinal tract. *International Journal of Pharmacology* 7: 31-39.

Komoike, Y., Nakashima, M., Nakagiri, A. and Takeuchi, K. 2003. Prostaglandin E receptor EP_1 subtype but not prostacyclin IP receptor involved in mucosal blood flow response of mouse stomachs following barrier disruption. *Digestion* 67: 186-194.

Konda, Y., Kamimura, H., Yokota, H., Hayashi, N., Sugano, K. and Takeuchi, T. 1999. Gastrin stimulates the growth of gastric pit with less-differentiated features. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 277: G773-G784.

Konig, G.M., Kehraus, S., Seibert, S.F., Abdel-Lateff, A. and Müller, D. 2006. Natural products from marine organisms and their associated microbes.*Chembiochem: a European Journal of Chemical Biology* 7: 229-238.

Konturek, S.J., Konturek, P.C. and Brzozowski, T. 2005. Prostaglandins and ulcer healing. *Journal of Physiology and Pharmacology* 56: 5-31.

Konturek, S.J., Konturek, P.C., Brzozowski, T. and Bubenik, G.A. 2007. Role of melatonin in upper gastrointestinal tract. *Journal of Physiology and Pharmacology* 58: 23-52.

Konturek, S.J., Konturek, P.C., Konturek, J.W., Plonka, M., Czesnikiewicz-Guzik, M., Brzozowski, T. and Bielanski, W. 2006. *Helicobacter pylori* and its involvement in gastritis and peptic ulcer formation. *Journal of Physiology and Pharmacology* 57: 29-50.

Kopic, S. and Geibel, J.P. 2010. Update on the mechanisms of gastric acid secretion. *Current Gastroenterology Reports* 12: 458-64.

Kubes, P. and Wallace, J.L. 1995. Nitric oxide as a mediator of gastrointestinal mucosal injury?—Say it ain't so. *Mediators of Inflammation* 4: 397-405.

Kumar, A., Singh, V. and Chaudhary, A.K. 2011. Gastric antisecretory and antiulcer activities of *Cedrus deodara* (Roxb.) Loud. in Wistar rats. *Journal of Ethnopharmacology* 134: 294-297.

Kusters, J.G., van Vliet, A.H.M. and Kuipers, E.J. 2006. Pathogenesis of *Helicobacter pylor*i infection. *Clinical Microbiology Reviews* 19: 449-490.

Kwiecień, S., Brzozowski, T. and Konturek, S.J. 2002. Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. *Journal of Physiology and Pharmacology* 53: 39-50.

Laine, L. and McQuaid, K.R. 2009. Endoscopic therapy for bleeding ulcers: an evidence-based approach based on meta-analyses of randomized controlled trials. *Clinical Gastroenterology and Hepatology* 7: 33-47.

Laine, L., Takeuchi, K. and Tarnawski, A. 2008. Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology* 135: 41-60.

Lakhanpal, P. and Rai, D.K. 2007. Quercetin: a versatile flavonoid. *Internet Journal of Medical Update* 2: 22-37.

Laloo, D., Prasad, S.K., Krishnamurth, S. and Hemalatha, S. 2013. Gastroprotective activity of ethanolic root extract of *Potentilla fulgens* Wall. ex Hook. *Journal of Ethnopharmacology* 146: 505-514.

Lanas, A. 2008. Role of nitric oxide in the gastrointestinal tract. *Arthritis Research & Therapy* 10: S4 PMC2582807.

Lanas, A. and Scheiman, J. 2007. Low-dose aspirin and upper gastrointestinal damage: epidemiology, prevention and treatment. *Current Medical Research and Opinion* 23: 163-173.

Langmead, L and Rampton, D.S. 2006. Review article: complementary and alternative therapies for inflammatory bowel disease. *Alimentary Pharmacology and Therapeutics* 23: 341-349.

Lee, K.H., Padzil, A.M., Syahida, A., Abdullah, N., Zuhainis, S.W., Maziah, M., Sulaiman, M.R., Israf, D.A., Shaari, K. and Lajis, N.H. 2011. Evaluation of antiinflammatory, antioxidant and antinociceptive activities of six Malaysian medicinal plants. *Journal of Medicinal Plant Research* 5: 5555-5563.

Lemos, L.M., Martins, T.B., Tanajura, G.H., Gazoni, V.F., Bonaldo, J., Strada, C.L., Silva, M.G., Dall'oglio, E.L., de Sousa Júnior, P.T. and Martins, D.T. 2012. Evaluation of antiulcer activity of chromanone fraction from *Calophyllum brasiliesnse* Camb. *Journal of Ethnopharmacology* 141: 432-439.

Leth, R., Elander, B., Fellenius, E., Olbe, L. and Haglund, U. 1985. Acid secretion in isolated gastric glands from healthy subjects and ulcer patients. Scandinavian Journal of Gastroenterology 20: 641-646.

Lewin, M.J. 1999. Cellular mechanisms and inhibitors of gastric acid secretion. *Drugs Today (Barc)* 35: 743-52.

Lewis, D.A. and Hanson, P.J. 1991. Antiulcer drugs of plant origin. *Progress in Medicinal Chemistry* 28:201-31.

Li, W.F., Hao, D.J., Fan, T., Huang, H.M., Yao, H. and Niu, X.F. 2014. Protective effect of chelerythrine against ethanol-induced gastric ulcer in mice. *Chemico-Biological Interactions* 208, 18-27.

Lichtenberger, L.M. 1999. Gastroduodenal mucosal defense. *Current Opinion in Gastroenterolgy* 15: 463-472.

Lichtenberger, L.M., Zhou, Y., Dial, E.J. and Raphael, R.M. 2006. NSAID injury to the gastrointestinal tract: evidence that NSAIDs interact with phospholipids to weaken the hydrophobic surface barrier and induce the formation of unstable pores in membranes. *Journal of Pharmacy and Pharmacology* 58: 1421-1428.

Lim, J.H., Kim,J.H., Kim, N., Lee, B.H., Seo, B.J., Kang, J.M., Jo, S.Y., Park, J.H., Nam, R.H., Chang, H., Kwon, J.W. and Lee, D.H. 2014. Gastroprotective effect of *Cochinchina momordica* seed extract in nonsteroidal anti-inflammatory drug-induced acute gastric damage in a rat model. *Gut and Liver* 8: 49-57.

Linden, S.K., Sutton, P., Karlsson, N.G., Korolik, V. and McGuckin, M.A. 2008. Mucins in the mucosal barrier to infection. *Mucosal Immunology* 1: 183-197.

Liu, Y., Zhang, Y., Dong, P., An, R., Xue, C., Ge, Y., Wei, L. and Liang, X. 2015. Digestion of nucleic acids starts in the stomach. *Scientific Report* 5:11936.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Review* 4: 118-126.

Lowry, O.H., Rosenborough, N.J., Farr, A.L. and Randal, R.J. 1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* 193: 265-275.

Lu, H. and Graham, D.Y. 2006. New development in the mechanistic understanding of peptic ulcer diseases. *Drug Discovery Today: Disease Mechanisms* 3: 431-437.

Mahadeva, S., Yadav, H., Everett, S.M. and Goh, K.L. 2012. Economic impact of dyspepsia in rural and urban Malaysia: a population-based study. *Journal of Neurogastroenterology and Motility* 18: 43-57.

Mahendran, P., Sabitha, K.E. and Shyamala Devi, C.S. 2002. Prevention of HCl-ethanol induced gastric mucosal injury in rats by *Garcinia cambogia* extract and its possible mechanism of action. *Indian Journal of Experimantal Biology* 40: 58-62.

Mahmood, A.A., Al-Bayaty, F.H., Salmah, I., Nor Syuhada, A.B., Harita, H. and Mughrabi, F.F. 2011. Enhancement of gastric ulcer by *Areca catechu* nut in ethanol-induced gastric mucosal injuries in rats. *Journal of Medicinal Plant Research* 5: 2562-2569.

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. *Journal of Medicinal Plant Research* 4: 685-691.

Mahmood, N.D., Nasir, N.L.M., Rofiee, M.S., Tohid, S.F.M., Ching, S.M., Teh, L.K., Salleh, M.Z. and Zakaria, Z.A. 2014. *Muntingia calabura*: A review on its traditional uses, chemical properties, and pharmacological observations. *Pharmaceutical Biology* 52: 1598-1623.

Maity, P., Biswas, K., Roy, S., Banerjee, R.K. and Bandyopadhyay U. 2003. Smoking and the pathogenesis of gastroduodenal ulcer-recent mechanistic update. *Molecular and Cellular Biochemistry* 253: 329-338.

Makola, D., Peura, D.A. and Crowe, S.E. 2007. *Helicobacter pylori* infection and related gastrointestinal diseases. *Journal of Clinical Gastroenterology* 41: 548-558.

Malaty, H.M. 2007. Epidemiology of *Helicobacter pylori* infection. *Best Practice & Research Clinical Gastroenterology* 21:205-214.

Marieb, E.N. and Hoehn K. 2007. *Human Anatomy & Physiology 7th ed*. San Francisco: Pearson/Benjamin Cummings.

Martin, M.J., La-Casa, C., Alarcon De La Lastra, C., Cabeza, J., Villegas, I. and Motilva, V. 1998. Anti-oxidant mechanisms involved in gastroprotective effects of quercetin. *Zeitschriftfür Naturforschung C - A Journal of Biosciences* 53: 82-88.

Martins, J.L., Rodrigues, O.R., da Silva, D.M., Galdino, P.M., de Paula, J.R., Romão, W., da Costa, H.B., Vaz, B.G., Ghedini, P.C. and Costa, E.A. 2014. Mechanisms involved in the gastroprotective activity of *Celtis iguanaea* (Jacq.) Sargent on gastric lesions in mice. *Journal of Ethnopharmacology* 155: 1616-1624.

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. *Phytotherapy Research* 23: 1355-1360.

Mason, J., Axon, A.T., Forman, D., Duffett, S., Drummond, M., Crocombe, W., Feltbower, R., Mason, S., Brown, J. and Moayyedi, P. 2002. The cost-effectiveness of population *Helicobacter pylori* screening and treatment: a Markov model using economic data from a randomized controlled trial. *Alimentary Pharmacology and Therapeutics* 16: 559-568.

Masuda, E., Kawano, S., Nagano, K., Tsuji, S., Ishigami, Y., Hayashi, N., Tsujii, M., Sasayama, Y., Michida, T. and Fusamoto, H. 1991. Effect of ethanol on endothelin-1 release from gastric vasculature. *Gastroenterology Japan* 26: 81-82.

Mejia, A. and Kraft, W.K. 2009. Acid peptic diseases: pharmacological approach to treatment. *Expert Review of Clinical Pharmacology* 2: 295-314.

Mishra, C.S.K. 2009. *Biotechnology Applications*. Bangalore: I.K. International Publishing House Pvt. Ltd.

Miwa, H., Sakaki, N., Sugano, K., Sekine, H., Higuchi, K., Uemura, N., Kato, M., Murakami, K., Kato, C., Shiotani, A., Ohkusa, T., Takagi, A., Aoyama, N., Haruma, K., Okazaki, K., Kusugami, K., Suzuki, M., Joh, T., Azuma, T., Yanaka, A., Suzuki, H., Hashimoto, H., Kawai, T. and Sugiyama, T. Recurrent peptic ulcers in patients following successful *Helicobacter pylori* eradication: a multicenter study of 4940 patients. *Helicobacter* 9: 9-16.

Mohamad Yusof, M.I., Salleh, M.Z., Lay Kek, T., Ahmat, N., Nik Azmin, N.F. and Zakaria, Z.A. 2013. Activity-guided isolation of bioactive constituents with antinociceptive activity from *Muntingia calabura* L. leaves using the formalin test. Evidence-Based Complementary and Alternative Medicine 2013:715074.

Mohd. Sani, M.H., Zakaria, Z.A., Balan, T., Teh, L.K. and Salleh, M.Z. 2012. Antinociceptive activity of methanol extract of *Muntingia calabura* leaves and the mechanisms of action involved. *Evidence-Based Complementary and Alternative Medicine* 2012: Article ID 890361. doi:10.1155/2012/890361.

Mohod, S.M. and Bodhankar, S.L. 2013. Antiulcer activity of aqueous extract of leaves of *Madhuca indica* J. F. Gmel against naproxen induced gastric mucosal injury in rats. *Journal of Acute Disease* 2: 127-133.

Morton, J.F. 1987. Jamaica cherry. In: *Fruits of Warm Climates,* ed. J.F. Morton, pp. 65-69. Miami: Florida Flair Books.

Mota, K.S., Dias, G.E., Pinto, M.E., Luiz-Ferreira, A., Souza-Brito, A.R., Hiruma-Lima, C.A., Barbosa-Filho, J.M. and Batista, L.M. 2009. Flavonoids with gastroprotective activity. *Molecules* 14: 979-1012.

Mukherjee, M., Bhaskaran, N., Srinath, R., Shivaprasad, H.N., Allan, J.J., Shekhar, D. and Agarwal, A. 2010. Anti-ulcer and antioxidant activity of GutGard. *Indian Journal of Experimental Biology* 48: 269-274.

Musumba, C., Pritchard, D.M. and Pirmohamed, M. 2009. Review article: cellular and molecular mechanisms of NSAID-induced peptic ulcers. *Alimentary Pharmacology & Therapeutics* 30: 517-531.

Newton, J., Allen, A., Westley, B.M. and May, F.E.B. 2000. The human trefoil peptide, TFF1, is present in different molecular forms that are intimately associated with the adherent mucus gel in normal stomach. *Gut* 46: 312-320.

Nguyen, 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, reepithelialization and induces COX-2 in a PI3K-dependent manner. *American Journal of Pathology* 170: 1219-1228

Nishio, H., Terashima, S., Nakashima, M., Aihara, E. and Takeuchi, K.2007. Involvement of prostaglandin E receptor EP_2 subtype and prostacyclin IP receptor in decreased acid response in damaged stomach. *Journal of Physiology and Pharmacology* 58: 407-421.

Nivethetha, M., Jayasari, J., and Brindha, P. 2009. Effects of *Muntingia calabura* L. on isoproterenol-induced myocardial infarction. *Singapore Medical Journal* 50: 300-302.

Noor, S.M., Mahmood, A.A., Salmah, I. and Philip, K. 2006. Prevention of acute gastric mucosal lesions by *R. hasseltii* in rats. *Journal of Animal and Veterinary Advances* 5: 161-164.

Nordin, N., Salama, S.M., Golbabapour, S., Hajrezaie, M., Hassandarvish, P., Kamalidehghan, B., Majid, N.A., Hashim, N.M., Omar, H., Fadaienasab, M., Karimian, H., Taha, H., Ali, H.M. and Abdulla, M.A. 2014. Anti-ulcerogenic effect of methanolic extracts from *Enicosanthellum pulchrum* (King) Heusden against ethanol-induced acute gastric lesion in animal models. *PLoS One* 9: e111925.

Noro, T., Miyase, T. and Kuroyanagi, M., 1983. Monoamine oxidase inhibitor from the rhizomes of *Kaempferia galanga* L.. *Chemical and Pharmaceutical Bulletin* 31: 2708-2711.

Nshimo, C.M., Pezzuto, J.M., Kinghorn, A.D. and Farnsworth, N.R. 1993. Cytotoxic constituents of *Muntingia calabura* leaves and stems collected in Thailand. *International Journal of Pharmacology* 31: 77-81.

Nwafor, P.A., Okwuasaba, F.B. and Bind, L.G. 2000. Antidiarrhoeal and antiulcerogenic effects of methanolic extract of *Asparagus pubescens* root in rats. *Journal of Ethnopharmacology* 72: 421-427.

Nwidu, L.L. and Nwafor, P.A. 2009. Gastroprotective effects of leaf extracts of *Carpolobia lutea* (polygalaceae) G. Don. in rats. *African Journal of Biotechnology* 8: 15-19.

O'Malley, P. 2003. Gastric ulcers and GERD: the new ''plagues" of the 21st century update for the clinical nurse specialist. *Clinical Nurse Specialist* 17: 286-289.

Oates, P.J. and Hakkinen, J.P. 1988. Studies on the mechanism of ethanol-induced gastric damage in rats. *Gastroenterology* 94: 10-21.

OECD. 2001. Test No. 420: Acute Oral Toxicity-Fixed Dose Procedure, OECD Guidelines for the Testing of Chemicals, Section 4.2. France: OECD Publishing.

Oh, T.Y., Ahn, G.J., Choi, S.M., Ahn, B.O., and Kim, W.B. 2005. Increased susceptibility of ethanol-treated gastric mucosa to naproxen and its inhibition by DA-9601, an *Artemisia asiatica* extract. *World Journal of Gastroenterology* 11: 7450-7456.

Okado-Matsumoto, A. and Fridovich, I., 2001. Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu,Zn-SOD in mitochondria. *The Journal of Biological Chemistry* 276: 38388-38393.

Oliveira, F.A., Andrade, L.N., de Sousa, E.B. and de Sousa, D.P. 2014. Anti-ulcer activity of essential oil constituents. *Molecules* 19: 5717-5747.

Omar, M.H., Mullen, W. and Crozier, A. 2011. Identification of proanthocyanidin dimers and trimers, flavone C-glyciosides, and antioxidants in *Ficus deltoidea*, a Malaysian herbal tea. *Journal of Agriculture and Food Chemistry* 59: 1363-1369.

Orrenius, S. 2007. Reactive oxygen species in mitochondria-mediated cell death. *Drug Metabolism Reviews* 39: 443-455.

Pai, R., Soreghan, B., Szabo, I.L., Pavelka, M., Baatar, D. and Tarnawski, A.S. 2002. Prostaglandin E_2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nature Medicine* 8: 289-293.

Pal, C., Bindu, S., Dey, S., Alam, A., Goyal, M., Iqbal, M.S., Maity, P., Adhikari, S.S. and Bandyopadhyay, U. 2010. Gallic acid prevents nonsteroidal anti-inflammatory drug-induced gastropathy in rat by blocking oxidative stress and apoptosis. *Free Radical Biology and Medicine* 49: 258-267.

Panda, V.S. and Khambat, P.D. 2014. Antiulcer activity of *Garcinia indica* fruit rind (kokum berry) in rats. *Biomedicine & Aging Pathology* 4: 309-316.

Parra-Cid, T., Calvino-Fernández, M. and Gisbert, J.P. 2011. *Helicobacter pylori* and peptic ulcer role of reactive oxygen species and apoptosis. In *Peptic Ulcer Disease*, ed. J. Chai, pp. 165-186. Crotia: InTech.

Parsons, M.E. and Ganellin, C.R. 2006. Histamine and its receptors. *British Journal of Pharmacology* 147: S127-S135.

Pérez-Fontan, M., Lopes, D.M., Enríquez, A.G., López-Calviño, B., López-Muñiz, A., Falcón, T.G. and Rodríguez-Carmona, A. 2016. Inhibition of gastric acid secretion by H_2 receptor antagonists associates a definite risk of enteric peritonitis and infectious mortality in patients treated with peritoneal dialysis. *PLoS One* 11: e0148806.

Perry, L.M. 1895. *Medicinal Plants of East and Southeast Asia.* Cambridge MA: The Massachusetts Institute of Technology.

Preethi, K., Premasudha, P. and Keerthana, K. 2012. Anti-inflammatory activity of *Muntingia calabura* fruits. *Pharmacognosy Journal* 4:51-56.

Preethi, K., Vijayalakshmi, N., Shamna, R. and Sasikumar, J.M. 2010. *In vitro* antioxidant activity of extracts from fruits of *Muntingia calabura* Linn. from India. *Pharmacognosy Journal* 2:11-18.

Prior, R.L. and Cao, G. 1999. *In vivo* total antioxidant capacity: comparison of different analytical methods. *Free Radical Biology and Medicine* 27: 1173-1181.

Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B. and Jacob, R. 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry* 51: 3273-3279.

Priya, G., Parminder, N. and Jaspreet, S. 2012. Oxidative stress induced ulcer protected by natural antioxidants: a review. *International Journal of Pharmacy* 3: 76-81

Qvigstad, G. and Waldum, H. 2004. Rebound hypersecretion after inhibition of gastric acid secretion. *Basic and Clinical Pharmacology and Toxicology* 94: 202-208.

Rachchh, M.A. and Jain, S.M. 2008. Gastroprotective effect of *Benincasa hispida* fruit extract. *Indian Journal of Pharmacology* 40: 271-275.

Radmark, O. and Samuelsson, B. 2009. 5-Lipoxygenase: mechanisms of regulation. *The Journal of Lipid Research* 50: S40-S45.

Rahim, N.A., Hassandarvish, P., Golbabapour, S., Ismail, S., Tayyab, S. and Abdulla. M.A. 2014. Gastroprotective effect of ethanolic extract of *Curcuma xanthorrhiza* leaf against ethanol-induced gastric mucosal lesions in Sprague-dawley rats. *BioMed Research International* 2014: 416409.

Raju, D., Ilango, K., Chitram, V.I. and Ashish, K. 2009. Evaluation of anti-ulcer activity of methanolic extract of *Terminalia chebula* fruits in experimental rats. *Journal of Pharmaceutical Sciences and Research* 1: 101-107.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.J. and Henderson, G. 2012. *Rang and Dale's Pharmacology, 7th Ed.* Edinburgh: Elsevier Churchill Livingstone.

Rangachari, P.K. 2007. Horace Davenport, carbonic anhydrase, and gastric acid secretion. *Advances in Physiology Education* 31: 140-144.

Linga Rao, M. and Savithramma, N. 2014. Isolation and identification of phenolic compounds by HPLC and Electrospray Ionization Mass Spectrometry of *Svensonia Hyderobadensis* - A rare medicinal plant taxon. *International Journal of Drug Development and Research* 6: 199-207.

Rao, J.N. and Wang, J.Y. 2010. *Regulation of Gastrointestinal Mucosal Growth*. California: Morgan & Claypool Publishers.

Rask-Madsen, J., Bukhave, K., Laursen, L.S and Lauritsen, K. 1992. 5-Lipoxygenase inhibitors for the treatment of inflammatory bowel disease. *Agents and Actions* Spec No: C37-46.

Repetto, M.G. and Llesuy, S.F. 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian Journal of Medical and Biological Research* 35: 523-534.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acid. *Free Radical Biology and Medicine* 20: 933-956.

Roberts, A.T., Martin, C.K., Liu, Z., Amen, R.J., Woltering, E.A., Rood, J.C., Caruso, M.K., Yu, Y., Xie, H. and Greenway, F.L. 2007. The safety and efficacy of a dietary herbal supplement and gallic acid for weight loss. *Journal of Medicinal Food* 10: 184-188.

Rouhollahi, E., Moghadamtousi, S.Z., Hamdi, O.A.A., Fadaeinasab, M., Hajrezaie, M., Awang, K., Looi, C.Y., Abdulla, M.A. and Mohamed, Z. 2014. Evaluation of acute toxicity and gastroprotective activity of *Curcuma purpurascens* BI. rhizome against ethanol-induced gastric mucosal injury in rats. *BMC Complementary and Alternative Medicine* 14: 378.

Russell, R. 2001. Non-steroidal anti-inflammatory drugs and gastrointestinal damage-problems and solutions. *Postgraduate Medical Journal* 77: 82-88.

Saladin, K.S. 2012. *Anatomy & Physiology: the unity of form and function*. New York: McGraw-Hill.

Sanchez-Mendoza, M.E., Cruz-Antonio, L., Cupido-Sánchez, M.G., García-Castillo, G. and Arrieta, J. 2014. Gastroprotective activity of caryophyllene oxide: the role of nitric oxide, prostaglandins and sulfhydryls. *Journal of Applied Pharmaceutical Science* 4: 001-005.

Sanders, S.W. 1996. Pathogenesis and treatment of acid peptic disorders: comparison of proton pump inhibitors with other antiulcer agents. *Clinical Therapeutics* 18: 2-34

Schiller, J.S., Lucas, J.W. and Peregoy, J.A. 2012. Summary health statistics for U.S. adults: national health interview survey. *Vital Health Statistics* 10 256:1-218.

Schmeda-Hirschmann, G. and Rojas de Arias, A.A. 1992. Screening method for natural products on triatomine bugs. *Phytotheraphy Research* 6: 68-73.

Schmeda-Hirschmann, G. and Yesilada, E. 2005. Traditional medicine and gastroprotective crude drugs. *Journal of Ethnopharmacology* 100: 61-66.

Schubert, M.L. and Peura, D.A. 2008. Control of gastric acid secretion in health and disease. *Gastroenterology* 134: 1842-1860.

Sen, S., Asokkumar, K., Umamaheswari, M., Sivashanmugam, A.T. and Subhadradevi, V. 2013. Antiulcerogenic effect of gallic acid in rats and its effect on oxidant and antioxidant parameters in stomach tissue. *Indian Journal of Pharmaceutical Sciences* 75: 149-155.

Shah, V., Lyford, G., Gores, G. and Farrugia, G. 2004. Nitric oxide in gastrointestinal health and disease. *Gastroenterology* 126: 903-913.

Shakeerabanu, M., Sujatha, K., Praveen, Rajneesh, C.P. and Manimaran, A. 2011. The defensive effect of quercetin on indomethacin induced gastric damage in rats. *Advances in Biological Research* 5: 64-70.

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. *Gastroenterology* 5: 43-61.

Sheen, E. and Triadafilopoulos, G. 2011. Adverse effects of long-term proton pump inhibitor therapy. *Digestive Diseases and Sciences* 56: 931-950.

Shih, C.D. 2009. Activation of nitric oxide/cGMP/PKG signaling cascade mediates antihypertensive effects of *Muntingia calabura* in anesthetized spontaneously hypertensive rats. *American Journal of Chinese Medicine* 37: 1045-1058.

Shih, C.D., Chen, J.J. and Lee, H.H. 2006. Activation of nitric oxide signaling pathway mediates hypotensive effect of *Muntingia calabura* L. (Tiliaceae) leaf extract. *American Journal of Chinese Medicine* 34: 857-72.

Shine, V.J., Latha, P.G., Shyamal, S., Suja, S.R., Anuja, G.I., Sini, Pradeep, S. and Rajasekharan, S. 2009. Gastric antisecretory and antiulcer activities of *Cyclea peltata* (Lam.) Hook. f. & Thoms. in rats. *Journal of Ethnopharmacology* 125: 350-355.

Sibi, G., Naveen, R., Dhananjaya, K., Ravikumar, K.R. and Mallesha, H. 2012. Potential use of *Muntingia calabura* L. extracts against human and plant pathogens. *Pharmacognosy Journal* 4: 44-47.

Siddiqua, A., Premakumari, K.B. and Sultana, R. 2010. Antioxidant activity and estimation of total phenolic content of *Muntingia calabura* by colorimetry. *International Journal of ChemTech Research* 2:205-208.

Silva, M.I.G. and de Sousa, F.C.F. 2011. Gastric ulcer etiology. In: *Peptic Ulcer Disease*, ed. J. Chai, pp. 3-28. Crotia: InTech.

Sindhe, M.A., Bodke, Y.D. and Chandrashekar, A. 2013. Antioxidant and in vivo anti-hyperglycemic activity of *Muntingia calabura* leaves extracts. *Scholars Research Library Der Pharmacia Lettre* 5: 427-435.

Singleton, V.L. and Rossi Jr, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144-158.

Smoot, D.T., Elliott, T.B., Verspaget, H.W., Jones, D., Allen, C.R., Vernon, K.G., Bremner, T., Kidd, L.C., Kim, K.S., Groupman, J.D. and Ashktorab, H. 2000. Influence of *Helicobacter pylori* on reactive oxygen-induced gastric epithelial cell injury. *Carcinogenesis* 21: 2091-2095.

Solomon, S.D., McMurray, J.J., Pfeffer, M.A., Wittes, J., Fowler, R., Finn, P., Anderson, W.F., Zauber, A., Hawk, E. and Bertagnolli, M. 2005. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *The New England Journal of Medicine* 35: 1071-1080.

Sooriakumaran, P. 2005. COX-2 inhibitors and the heart: are all coxibs the same? *Postgraduate Medical Journal* 82: 242-245.

Sostres, C., Gargallo, C.J., Arroyo, M.T. and Lanas, A. 2010. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Practice & Research Clinical Gastroenterology* 24: 121-132.

Sowndhararajan, K. and Chin, N.L. 2014. Antioxidant and anti-ulcer effects of ethyl acetate fraction of *Merremia tridentata* (L.) Hallier F. root. *Agriculture and Agricultural Science Procedia* 2: 406-414.

Sridhar, M., Thirupathi, K., Chaitanya, G. Ravikumar, B. 2011. Antidiabetic effect of leaves of *Muntingia calabura* L., in normal and alloxan-induced diabetic rats. *Pharmacologyonline* 2: 626-632.

Srivastava, V., Viswanathaswamy, A.H. and Mohan, G. 2010. Determination of the antiulcer properties of sodium cromoglycate in pylorus-ligated albino rats. *Indian Journal of Pharmacology* 42: 185-188.

Stanek, A., Gadowska-Cicha, A., Gawron, K., Wielkoszyński, T., Adamek, B., Cieślar, G., Wiczkowski, A. and Sieroń, A. 2008. Role of nitric oxide in physiology and pathology of the gastrointestinal tract. *Mini Reviews in Medicinal Chemistry* 8: 1549-1560.

Stermer, E. 2002. Alcohol consumption and the gastrointestinal tract. *Israel Medical Association Journal* 4: 200-202.

Strohl, W.R. 2000. The role of natural products in a modern drug discovery program. *Drug Discov*ery *Today* 5: 39-41.

Su,B.N., Park, E.J., Vigo, J.S., Graham, J.G., Cabieses, F., Fong, H.H., Pezzuto, J.M. and Kinghorn, A.D. 2003. Activity-guided isolation of the chemical constituents of *Muntingia calabura* using a quinonereductase induction assay. *Phytochemistry* 63: 335-341.

Sufian, A.S., Ramasamy, K., Ahmat, N., Zakaria, Z.A., Yusof, M.I. 2013. Isolation and identification of antibacterial and cytotoxic compounds from the leaves of *Muntingia calabura* L.. *Journal of Ethnopharmacology* 146: 198-204.

Sulekha, S., Madhavi, J., Venkateshwari, A., Yasmeen, S. and Prathiba, N. 2006. Superoxide dismutase phenotypes in duodenal ulcers: A genetic marker? *Indian Journal of Human Genetics* 12: 125-128.

Sung, J.J.Y., Kuipers, E.J. and El-Serag, E.B. 2009. Systematic review: the global incidence and prevalence of peptic ulcer disease *Alimentary Pharmacology and Therapeutics* 29: 938-946.

Suzuki, H., Matsuzaki, J. and Hibi, T. 2011. Ghrelin and oxidative stress in gastrointestinal tract. *Journal of Clinical Biochemistry and Nutrition* 48: 122-125.

Suzuki, K., Araki, H., Mizoguchi, H., Furukawa, O. and Takeuchi, K. 2001. Prostaglandin E inhibitors indomethacin-induced gastric lesions through EP-1 receptors. *Digestion* 63: 92-101.

Szabo, S. 1987. Mechanisms of mucosal injury in the stomach and duodenum: timesequence analysis of morphologic, functional, biochemical and histochemical studies. *Scandinavian Journal of Gastroenterology* 22: 21-28.

Szabo, S. and Vattay, P. 1990. Experimental gastric and duodenal ulcers. Advances in pathogenesis. *Gastroenterology Clinics of North America* 19: 67-85.

Szabo, S. and Tache, Y. 1995. *Neuroendocrinology of Gastrointestinal Ulceration.* New York: Plenum Press.

Szabo, S., Vincze, A., Sandor, Z., Jadus, M., Gombos, Z., Pedram, A., Levin, E., Hagar, J. and Iaquinto, G. 1998. Vascular approach to gastroduodenal ulceration: new studies with endothelins and VEGF. *Digestive Diseases and Sciences* 43: 40S-45S.

Takahashi, S., Takeuchi, K. and Okabe, S. 1999. EP_4 receptor mediation of prostaglandin E_2-stimulated mucus secretion by rabbit gastric epithelial cells. *Biochemical Pharmacology* 58: 1997-2002.

Takeuchi, K. 2010. Prostaglandin EP receptors and their roles in mucosal protection and ulcer healing in the gastrointestinal tract. *Advances in Clinical Chemistry* 51: 121-144.

Takeuchi, K., Aihara, E., Sasaki, Y., Nomura, Y. and Ise, F. 2006. Involvement of cyclooxygenase-1, prostaglandin E_2 and EP_1 receptors in acid-induced HCO_3^- secretion in the stomach. *Journal of Physiology and Pharmacology* 57: 661-676.

Tanaka, K., Tsutsumi, S., Arai, Y., Hoshino, T., Suzuki, K., Takaki, E., Ito, T., Takeuchi, K., Nakai, A. and Mizushima, T. 2007. Genetic evidence for a protective role of heat shock factor 1 against irritant-induced gastric lesions. *Molecular Pharmacology* 71: 985-993.

Tandon, R., Khanna, H.D., Dorababu. M. and Goel, R.K. 2004. Oxidative stress and antioxidants status in peptic ulcer and gastric carcinoma. *Indian Journal of Physiology and Pharmacology* 48: 115-118.

Taupin, D. and Podolsky, D.K. 2003. Trefoil factors initiators of mucosal healing. *Nature Reviews Molecular Cell Biology* 4: 721.

Testerman, T.L. and Morris, J. 2014. Beyond the stomach: an updated view of *Helicobacter pylori* pathogenesis, diagnosis, and treatment. *World Journal of Gastroenterology* 20: 12781-12808.

Tripathi, K.D. 2001. *Essentials of Medical Pharmacology, 4th ed*. New Delhi: Jaypee Brothers Medical Publishers.

Tsimogiannis, D., Samiotaki, M., Panayotou, G. and Oreopoulou, V. 2007. Characterization of flavonoid subgroups and hydroxy substitution by HPLC-MS/MS. *Molecules* 12: 593-606.

Tuorkey, M.J. and Abdul-Aziz, K.K. 2011. Gastric ulcer's diseases pathogenesis, complications and strategies for prevention. *Gastroenterology* 2: WMC001684.

Tumer, T.B., Rojas-Silva, P., Poulev, A., Raskin, I. and Waterman, C. 2015. Direct and indirect antioxidant activity of polyphenol- and isothiocyanate-enriched fractions from *Moringa oleifera*. *Journal of Agriculture and Food Chemistry* 63: 1505-1513.

Tuteja, N., Chandra, M., Tuteja,R. and Misra, M.K. Nitric oxide as a unique bioactive signaling messenger in physiology and pathophysiology. Journal of Biomedicine and Biotechnology 2004: 227-237.

Ukwuani, A.N., Ihebunna, O., Samuel, R.M. and Peni, I.J. 2012. Acute oral toxicity and antiulcer activity of *Piliostigma thonningii* leaf fraction in albino rats. *Bulletin of Environment, Pharmacology and Life Sciences* 2: 41-45.

Ulgheri, C., Paganini, B. and Rossi, F. 2010. Antisecretory factor as a potential health-promoting molecule in man and animals. *Nutrition Research Reviews* 23: 300-313.

Ulleberg, E.K., Comi, I., Holm, H., Herud, E.B., Jacobsen, M. and Vegarud, G.E. 2011. Human gastrointestinal juices intended for use in *in vitro* digestion models. *Food Digestion* 2: 52-61.

Umamaheswari, M., Asokkumar, K., Rathidevi, R., Sivashanmugam, A.T., Subhadradevi, V. and Ravi, T.K. 2007. Antiulcer and in vitro antioxidant activities of *Jasminum grandiflorum* L. *Journal of Ethnopharmacology* 110: 464-470.

Valle, J.D., Chey, W.D. and Scheiman, J.M. 2003. Acid peptic disorders, In: *Textbook of gastroenterology, 4th ed*, ed. T. Yamada, pp: 1322-1376. Philadelphia: Lippincott Williams & Wilkins.

Venables, C.W. 1986. Mucus, pepsin and peptic ulcer. *Gut* 27: 233-238.

Verheij, E.W.M. 1992. *Muntingia calabura* L. In: *Edible Fruits and Nuts. Plant Resources of South East Asia No. 2*, ed. E.W.M. Verheij, and R.E. Coronel, pp. 223-225. Bogor, Indonesia: PROSEA Foundation.

Viana, A.F., Fernandes, H.B., Silva, F.V., Oliveira, I.S., Freitas, F.F., Machado, F.D., Costa, C.L., Arcanjo, D.D., Chaves, M.H., Oliveira, F.A. and Oliveira, R.C. 2013. Gastroprotective activity of *Cenostigma macrophyllum* Tul. var. *acuminata* Teles Freire leaves on experimental ulcer models. models. *Journal of Ethnopharmacology* 150: 316-323.

Vidya, S., Ramesh, A., Alekhya, N. and Lohitha, L. 2012. Antiulcer activity of *Eugenia jambolana* leaves against ethanol-induced gastric ulcer in albino rats. *International Journal of Pharmaceutical Research and Development* 3: 106-112.

Subramaniam, V., Adenan, M.I., Ahmad, A.R. and Sahdan, R. 2003. Natural antioxidants: *Piper sarmentosum* (Kadok) and *Morinda elliptica* (Mengkudu). *Malaysian Journal of Nutrition* 9: 41-51.

Vomero, N.D. and Colpo, E. 2014. Nutritional care in peptic ulcer. *Arquivos Brasileiros de Cirurgia Digestiva : ABCD = Brazilian Archives of Digestive Surgery* 27: 298-302.

Walker, R. and Whittlesea, C. 2011. *Clinical Pharmacy and Therapeutics, 5th Edition*. London: Churchill Livingstone.

Wallace, J.L. 1989. *Endogenous Mediators of Gastrointestinal Damage*. Boca Raton: CRC Press.

Wallace, J.L. 2000. How do NSAIDs cause ulcer disease? *Bailliere's Best Practical and Research. Clinical Gastroenterology* 14: 147-159.

Wallace, J.L. 2001. Mechanisms of protection and healing: current knowledge and future research. *American Journal of Medicine* 110: 19S-22S.

Wallace, J.L. 2005. Recent advances in gastric ulcer therapeutics. *Current Opinion in Pharmacology* 5: 573-577.

Wallace, J.L. 2008. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiological Reviews* 88: 1547-1565.

Wallace, J.L. and Granger, D.N. 1996. The cellular and molecular basis of gastric mucosal defense. *FASEB Journal* 10: 731-740.

Wallace, J.L., McKnight, W., Reuter, B.K. and Vergnolle, N. 2000. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 119: 706-714.

Whittle, B.J.R. 2003. Gastrointestinal effects of non-steroidal anti-inflammatory drugs. *Fundamental and Clinical Pharmacology* 17: 301-313.

Williams, J.M., Duckworth, C.A., Burkitt, M.D., Watson, A.J.M., Campbell, B.J. and Pritchard, D.M. 2015. Epithelial cell shedding and barrier function. A matter of life and death at the small intestinal villus tip. *Veterinary Pathology* 52: 445-455.

Wong, J.Y., Abdulla, M.A., Raman, J., Phan, C.W., Kuppusamy, U.R., Golbabapour, S. and Sabaratnam, V. 2013. Gastroprotective effects of Lion's Mane Mushroom *Hericium erinaceus* (Bull.:Fr.) Pers. (Aphyllophoromycetideae) extract against ethanol-induce ulcer in rats. *Evidence Based Complementary and Alternative Medicine* 2013: 492976.

World Health Organisation. *Global Status Report on Alcohol and Health, 2014 Ed*.; WHO Library Cataloguing-in-Publication Data: Geneva, 2014.

Yan, X.M., Joo, M.J., Lim, J.C., Whang, W.K., Sim, S.S., Im, C., Kim, H.R., Lee, S.Y., Kim, I.K. and Sohn, U.D. 2011. The effect of quercetin-3-O-β-D-glucuronopyranoside on indomethacin-induced gastric damage in rats via induction of mucus secretion and down-regulation of ICAM-1 expression. *Archive of Pharmacal Research* 34: 1527-1534.

Yandrapu, H. and Sarosiek, J. 2015. Protective factors of the gastric and duodenal mucosa: An overview. *Current Gastroenterology Reports* 17: 24.

Yao, X. and Forte, J.G. 2003. Cell biology of acid secretion by the parietal cell. *Annual Review of Physiology* 65: 103-131.

Yasunaka, K., Abe, F., Nagayama, A., Okabe, H., Lozada, L., López, E., Estrada, E., Aguilar, A. and Reyes, R. 2005. Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarines and xanthones. *Journal of Ethnopharmacology* 97: 293-299.

Yen, G., Duh, P. and Tsai, H. 2002. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chemistry* 79: 307-313.

Yoshikawa, T. and Naito, Y. 2002. What is oxidative stress? *Journal of the Japan Medical Association* 45: 271–276.

Yuan, Y., Padol, I.T. and Hunt, R.H. 2006. Peptic ulcer disease today. *Nature Clinical Practice Gastroenterology and Hepatology* 3: 80-89.

Yusof, M.M., Teh, L.K., Zakaria, Z.A. and Ahmat, N. 2011. Antinociceptive activity of the fractionated extracts of *Muntingia calabura*. *Planta Medica* 77: PF21.

Zakaria, Z.A. 2007. Free radical scavenging activity of some plants available in Malaysia*. Iranian Journal of Pharmacology & Therapeutics* 6: 87-91.

Zakaria, Z.A., Fatimah, C.A., Mat Jais, A.M., Zaiton, H., Henie, E.F.P., Sulaiman, M.R., Somchit, M.N., Thenamutha, M. and Kasthuri, D. 2006a. The in vitro antibacterial activity of *Muntingia calabura* extracts. *International Journal of Pharmacology* 2: 290-293.

Zakaria, Z.A., Hassan, M.H., Nurul Aqmar, M.N., Abd Ghani, M., Mohd Zaid, S.N., Sulaiman, M.R., Hanan Kumar, G. and Fatimah, C.A. 2007c. Effects of various nonopioid receptor antagonist on the antinociceptive activity of *Muntingia calabura* extracts in mice. *Methods and Findings in Experimantal and Clinical Pharmacology* 29: 515-520.

Zakaria, Z.A., Kumar, G.H., Zaid, S.N.H., Ghani, M.A., Hassan, M.H., Nor Hazalin, N.A.M. 2007d. Analgesic and antipyretic actions of *Muntingia calabura* leaves chloroform extract in animal models. *Oriental Pharmacy and Experimental Medicine* 7: 34-40.

Zakaria, Z.A., Mat Jais, A.M., Mastura, M., Mat Jusoh, S.H., Mohamed, A.M., Mohd. Jamil, N.S., Rofiee, M.S. and Sulaiman, M.R. 2007e. *In vitro* antistaphylococcal activity of the extracts of several neglected plants in Malaysia. *International Journal of Pharmacology* 3: 428-31.

Zakaria, Z.A., Mohamed, A.M., Jamil, N.S., Rofiee, M.S., Hussain, M.K., Sulaiman, M.R., Teh, L.K. and Salleh, M.Z. 2011. *In vitro* antiproliferative and antioxidant activities of the extractsof *Muntingia calabura* leaves. *American Journal of Chinese Medicine* 39: 183-200.

Zakaria, Z.A., Mustapha, S., Sulaiman, M.R., Mat Jais, A.M., Somchit, M.N. and Abdullah, F.C. 2007a. The antinociceptive action of aqueous extract from *Muntingia calabura* leaves: the role of opioid receptors. *Medical Principles and Practice* 16: 130-136.

Zakaria, Z.A., Nor Hazalin, N., Zaid, S., Ghani, M., Hassan, M., Gopalan, H. and Sulaiman, M. 2007b. Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models. *Journal of Natural Medicines* 61: 443-448.

Zakaria, Z.A., Somchit, M.N., Sulaiman, M.R., Mat Jais, A.M. and Fatimah, C.A. 2008. Effects of various receptor antagonists, pH and enzymes on *Muntingia calabura* antinociception in mice. *Research Journal of Pharmacology* 2: 31-37.

Zakaria, Z.A., Sulaiman, M.R., Jais, A.M., Somchit, M.N., Jayaraman, K.V., Balakhrisnan, G. and Abdullah, F.C. 2006b. The antinociceptive activity of *Muntingia calabura* aqueous extract and the involvement of L-arginine/nitric oxide/cyclic guanosine monophosphate pathway in its observed activity in mice. *Fundamental Clinical Pharmacology* 20: 365-372.

Zakaria, Z.A., Abdul Hisam, E.E., Norhafizah, M., Rofiee, M.S., Othman, F., Hasiah, A.H. and Vasudevan, M. 2012. Methanol extract of *Bauhinia purpurea* leaf possesses anti-ulcer activity. *Medical Principles and Practice* 21: 476-482.

Zheng, Y.F., Xie, J.H., Xu, Y.F., Liang, Y.Z., Mo, Z.Z., Jiang, W.W., Chen, X.Y., Liu, Y.H., Yu, X.D., Huang, P. and Su, Z.R. 2014. Gastroprotective effect and mechanism of patchouli alcohol against ethanol, indomethacin and stress-induced ulcer in rats. *Chemico-Biological Interactions* 222: 27-36.

Zima, T., Fialova, L., Mestek, O., Janebova, M., Crkovska, J., Malbohan, I., Stípek, S., Mikulíková, L. and Popov, P. 2001. Oxidative stress, metabolism of ethanol and alcohol-related diseases. *Journal of Biomedical Science* 8: 59-70.

BIODATA OF STUDENT

Tavamani D/O Balan was born on the 6th of March 1985 in Ipoh, Perak, Malaysia. She obtained her primary education at Sekolah Kebangsaan Tarcisian Convent, Ipoh followed by her secondary education in Sekolah Menengah Kebangsaan Tarcisian Convent, Ipoh and later obtained her Pre-University education at Sekolah Menengah Kebangsaan Anderson, Ipoh. She joined Universiti Putra Malaysia in the year 2004 and was awarded Bachelor of Science Bioindustry, Honours First Class in the year 2009. In December 2009, she enrolled as a PhD candidate in Universiti Putra Malaysia under the supervision of Associate Prof. Dr. Zainul Amiruddin Zakaria.

LIST OF PUBLICATIONS

Zakaria, Z.A., Balan, T., Azemi, A.K., Omar, M.H., Mohtarruddin, N., Ahmad, Z., Abdullah, M.N.H., Mohd. Desa, M.N., Teh, L.K. and Salleh, M.Z. 2016. Mechanism(s) of action underlying the gastroprotective effect of ethyl acetate fraction obtained from the crude methanolic leaves extract of *Muntingia calabura*. *BMC Complementary and Alternative Medicine* 16:78.

Balan, T., Sani, M.H.M., Ahmad, S.H.M., Suppaiah, V., Mohtaruddin, N. and Zakaria, Z.A. 2015. Antioxidant and anti-inflammatory activities contribute to the prophylactic effect of semi-purified fractions obtained from the crude methanol extract of *Muntingia calabura* leaves against gastric ulceration in rats. *Journal of Ethnopharmacology* 164: 1-15.

Zakaria, Z.A., Balan, T., Suppaiah, V., Ahmad, S. and Jamaludin, F. 2014. Mechanism(s) of action involved in the gastroprotective activity of *Muntingia calabura*. *Journal of Ethnopharmacology* 151: 1184-1193.

Balan, T., Sani, M.H.M., Suppaiah, V., Mohtarrudin, N., Suhaili, Z., Ahmad Z. and Zakaria, Z.A. 2013. Antiulcer activity of methanol extract of *Muntingia calabura* leaves involves the modulation of endogenous nitric oxide and non-protein sulfhydryl compounds. *Pharmaceutical Biology* Early Online: 1-9.

Other publications

Zakaria, Z.A., Balan, T., Mamat, S.S., Mohtaruddin, N., Teh, L.K. and Salleh, M.Z. 2015. Gastroprotective mechanisms of methanol extract of *Melastoma malabathricum* leaves involved modulation of the nitric oxide, sulfhydryl groups and antioxidant effects. *BMC Complementary and Alternative Medicine* 15: 135.

Zakaria, Z.A., Yahya, F., Balan, T., Mamat, S.S., Rodzi, R., Kamisan, F.H., Fatimah, C.A. and Ibrahim, A.L. 2014. Pharmacological activities of some of the neglected and underutilized tropical plants in Malaysia. In *Novel Plant Bioresources: Applications in Food, Medicine and Cosmetics,* ed. A. Gurib-Fakim. Chichester, UK: John Wiley & Sons, Ltd. doi: 10.1002/9781118460566.ch17.

Sani, M.H.M., Zakaria, Z.A., Balan, T., Teh, L.K. and Salleh, M.Z. 2012. Antinociceptive activity of methanol extract of Muntingia calabura leaves and the mechanisms of action involved. *Evidence-based Complementary and Alternative Medicine* 2012: 890361.

Awards

Silver
ARA Tea: Gastroprotection and antioxidant activities of *Muntingia calabura* leaves in International Innovation, Design and Articulation (I-IDEA 2013), Perlis, Malaysia.

Bronze
In-vivo antiulcer activity of *Muntingia calabura* methanolic extract in Invention, Research and Innovation Exhibition (PRPI 2011), UPM Serdang, Selangor, Malaysia.

Poster

In Vivo anti-ulcer activity of *Muntingia calabura* leaves extract in 26th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology (MSPP 2012), Penang, Malaysia.

Antiulcer potential of *Sapium indicum* aqueous extract: towards the development of Halal pharmaceutical ingredient with gastroprotective property in Malaysia International Halal Research and Education Conference 2014 (MIHREC 2014), Putrajaya, Malaysia.

Gastroprotective activity of *Muntingia calabura* and *Melastoma malabathricum* chloroform leaf extracts in 29th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology (MSPP 2015), Setia City Convention Centre (SCCC), Shah Alam, Malaysia.

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