UNIVERSITI PUTRA MALAYSIA

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

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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

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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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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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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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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.

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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.

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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.

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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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