INTRODUCTION

A peptic ulcer is a mucosal lesion of the stomach or duodenum in which acid and pepsin play major pathogenic roles. Although our knowledge of the cause of peptic ulcer is incomplete, available information supports a central role for bacterium *Helicobacter pylori* and a necessary role for acid and pepsin (Friedman and Peterson, 1998). Development of gastric ulceration is due to the imbalance between aggressive factors (principally gastric acid and pepsin) and factors that participate in mucosal defense or resistance to ulceration. Peptic ulcer results when gastroduodenal mucosal defenses are unable to protect the epithelium from the corrosive effects of acid and pepsin. Richardson (1989); Friedman and Peterson (1998) describe aggressive and defensive factors and their role in peptic ulcer as follows:

1. Aggressive factors

1.1. Acid and pepsin: The gastric mucosa has an extraordinary capacity to secrete acid by a process involving oxidative phosphorylation. Multiple chemical, neural, and hormomal factors participate in the regulation of gastric acid secretion. Acid secretion is stimulated by gastrin and by

postganglionic vagal fibers via muscarinic cholinergic receptors on parietal cells.

Gastrin, the most potent known stimulant of gastric acid secretion, is contained in and released into the circulation from G cells. The released gastrin is stimulated by the neuropeptide, gastrin-releasing peptide and inhibited by somatostatin produced by D cells in the antrum. Gastrin stimulates gastric acid secretion by a direct stimulation of parietal cells and by a stimulation of histamine release from enterochromaffin-like (ECL) cells.

Vagal stimulation increases gastric acid secretion by cholinergic stimulation of parietal cell secretion, by enhancing release of gastrin from antral G cells (by both inhibition of the release of somatostatin by antral D cells and by direct stimulation of G cells).

The gastric mucosa contains large amounts of histamine contained in cytoplasmic granules of mast cells and ECL cells. Histamine is the most important stimulant of gastric acid secretion and is released from ECL cells by the action of both gastrin and cholinergic activity. The basolateral membranes of parietal cells contain receptors for histamine, gastrin, and acetylcholine that stimulate acid secretion and for prostaglandins and somatostatin that inhibit acid secretion.

Histamine stimulates gastric secretion by increasing parietal cell cyclic adenosine monophosphate (AMP), thereby activating cyclic AMP-dependent protein kinase.

The major physiologic stimulus for gastric acid secretion is ingestion of food. The regulation of gastric acid secretion has been classified into three phases: Cephalic, gastric and intestinal. The cephalic phase, which includes cortical and hypothalamic components, is mediated primarily by vagal activation, which increases gastric acid secretion principally by direct stimulation of ECL and parietal cells and to a lesser extent by promoting gastrin release. The gastric phase results from stimulation of chemical and mechanical receptors in the gastric wall by luminal contents. Mechanical distention of the stomach stimulates gastric acid secretion but results in little gastrin release. Food (principally protein and products of protein) in the stomach promotes gastric acid secretion by increasing gastrin release. Food in the proximal small intestine stimulates the intestinal phase of gastric acid secretion by producing the release of small amounts of gastrin and other peptides that stimulate gastric secretion and by a direct effect of absorbed amino acids on parietal cells.

1.2. *Helicobacter pylori* infection: *H. pylori* produces a variety of proteins that appear to mediate or

facilitate its damaging effect on the gastric mucosa. It reduces the thickness and viscosity of the mucous gel overlying the gastric mucosal epithelial cells.

1.3. Nonsteroidal anti-inflammatory drugs (NSAIDs): NSAIDs (e.g. aspirin indomethacin diclofenac, phenylbutazone, etc.) associated with an increased incidence of gastric ulcer. NSAIDs are directly toxic to the gastric mucosa and they deplete protective endogenous mucosal prostaglandin (PG) by inhibiting PG synthesis. They also may contribute to ulcer formation by interrupting the gastric mucosal barrier, permitting back-diffusion of hydrogen ions that may injure the gastric mucosa. They reduced gastric mucous and bicarbonate secretion and may increase gastric acid secretion. Depletion of mucosal prostaglandins also impairs epithelial cell replacement after injury.

2. Defensive factors

2.1. Gastric mucous: Gastric mucus secreted by mucous cells of the gastric mucosal epithelium and gastric glands is important in mucosal defense and in preventing peptic ulceration. Mucous secretion is stimulated by mechanical or chemical irritation and by cholinergic stimulation.

2.2. Gastric bicarbonate: Nonparietal gastric epithelial cells secrete bicarbonate ions into the mucous gel. Gastric bicarbonate secretion is stimulated by calcium, certain PGE and F series, cholinergic agents, and α-adrenergic agents.

2.3. Mucosal barrier: Normally, the luminal surfaces and intercellular tight junctions of the gastric epithelial cells create a gastric mucosal barrier that is almost completely impermeable to diffusion of hydrogen ions from the lumen. Mucosal barrier can interrupt by bile acids, salicylates, ethanol, and weak organic acids, permitting hydrogen ions to diffuse into gastric tissue. The results may be cell injury, release of histamine from mast cells, further stimulation of acid secretion, damage to small blood vessels, mucosal hemorrhage, and erosion or ulceration.

2.4. Mucosal blood flow: The maintenance of normal blood flow to the gastric mucosa is an essential component of mucosal resistance to injury. Decreased mucosal blood flow, accompanied by diffusion of luminal hydrogen ions, is thought to be important in producing gastric mucosal damage.

2.5. Prostaglandins: PGs are abundant in the gastric mucosa. Administration to animals of various PGs, particularly those of the E series, has been shown to prevent

gastric mucosal injury caused by a wide variety of agents. Endogenous PGs stimulate secretion of gastric mucus and bicarbonate. They participate in the maintenance of gastric mucosal blood flow and of the integrity of the gastric mucosal barrier and promote epithelial cell renewal in response to mucosal injury.

Physiologic regulation of gastric secretion and possible mechanisms of drugs used in the treatment of gastric ulcer are diagrammatically shown in Figure 1 (Brunton, 1996).

Three major pathways regulating parietal acid secretion include 1) neural stimulation via the vagus nerve, 2) endocrine stimulation via gastrin released from antral G cells, and 3) paracrine stimulation by release of histamine from ECL cells.

Vagal stimulation and the action of gastrin (from duodenal and antral G cells) stimulate histamine release from paracrine ECL or mast cell. Histamine in turn activates parietal cell H_2-receptor that is linked to the stimulation of adenylyl cyclase, causing activation of the cyclic AMP pathway. Gastrin and muscarinic stimuli also may act directly on the parietal cell to activate Ca^{+2}-sensitive pathways. H_2-receptor antagonists not only block the effects of histamine but also blunt responses to acetylcholine and gastrin, thus contributing to the remarkable clinical efficacy of these agents.

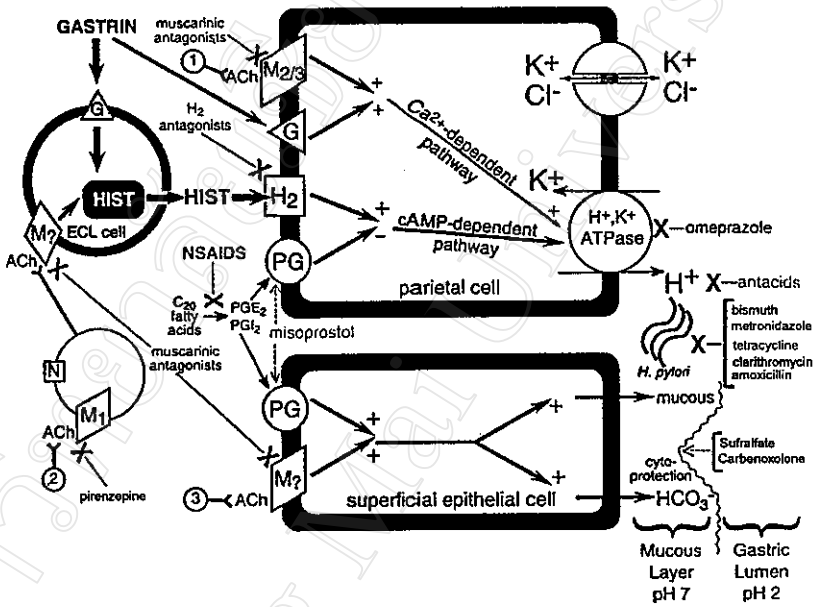

Figure 1 Physiological and pharmacological regulation of gastric secretion: The basis for therapy of peptic ulcer (from Brunton, 1996: *Goodman and Gilman's The Pharmacological basis of therapeutic*, 9th ed., New York, Macmillan Publishing Co., Inc., pp. 902)

Antacids neutralize gastric HCl production acidity. Covalent inhibitors of the H^+/K^+-ATPase, such as omeprazole, inhibit acid secretion, the final common pathway in gastric acid secretion. PGs, by inhibiting histamine-stimulated adenylyl cyclase activity in the parietal cell, reduce activity through the histamine-evoked cyclic AMP-dependent pathway and thereby reduce acid secretion. PGs also stimulate the secretion of mucus and bicarbonate by adjacent superficial epithelial cells, contributing the cytoprotective effects of endogenous PGs, and the protective effects of stable analog of PGE_1, such as misoprostol.

Drugs for Treatment of Peptic Ulcers

The goals of therapy for ulcers are relief from pain, promotion of healing, and prevention of recurrence. Therapeutic strategies are aimed at balancing aggressive factors (gastric acid secretion, pepsin, *H. pylori* infection) against defensive or cytoprotective factors (bicarbonate, mucus, and PG).

Medications used in peptic ulcers are grouped according to their therapeutic uses (Brunton, 1996; Altman, 1998) as follows:

1. Antacids: Antacids are weak bases that react with gastric hydrochloric acid to form salt and water. Their usefulness in peptic ulcer disease appears to lie in their ability to reduce gastric acidity. Animal studies have demonstrated mucosal protection by antacids, either through the stimulation of prostaglandin production or the binding of an unidentified injurious substance.

2. Gastric antisecretory drugs

2.1. H_2-receptor antagonists (cimetidine, ranitidine, famotidine, and nizatidine): The H_2-antagonists reduce both the volume of gastric juice secreted and its H^+ concentration.

2.2. Antimuscarinic agents (pirenzepine): Cholinergic antagonists are now rarely used and only as adjuncts to H_2-antagonists, especially in patient refractory to treatment with the latter or those with nocturnal pain.

2.3. Proton pump inhibitors (omeprazole, lansoprazole): Proton pump inhibitors irreversibly inhibit the gastric parietal cell proton pump, and they require activation in the acid environment of the secretory canaliculus of the parietal cell. Omeprazole and lansoprazole produce only small and inconsistent changes in the volume of gastric juice acid in the secretion of pepsin and intrinsic factor and do not affect gastric motility.

2.4. Octreotide: Octreotide is a long-acting synthetic somatostatin analog. It has been found to significantly inhibit the secretion of several circulating peptide hormones, and gastric and pancreatic secretion.

3. Mucosal protective agents

3.1. Sucralfate: Sucralfate is a sulfated disaccharide developed for use in peptic ulcer disease. Its mechanism of action is thought to involve polymerization and selective binding to necrotic ulcer tissue, where it may act as a barrier to acid, pepsin, and bile. In addition, sucralfate may directly adsorb bile salt. The drug may also cause stimulation of endogenous prostaglandin synthesis. Sucralfate requires an acid pH to be activated and so should not be administered simultaneously with antacids, H_2-antagonists, or proton pump inhibitors.

3.2. Colloidal bismuth compounds: Bismuth compounds have no substantial capacity to neutralize gastric acid. Their beneficial effects have been ascribed to cytoprotection (enhanced secretion of mucus and bicarbonate, inhibition of pepsin activity, and accumulation of bismuth substrate is preferentially in the craters of gastric ulcers). These effects may be secondary to the antibacterial effect of bismuth compounds against *H. pylori* in the gastrointestinal

mucosa. Bismuth has been shown to promote healing of both gastric and duodenal ulcers as effectively as cimetidine and to be effective in preventing ulcer recurrence.

3.3. Carbenoxolone: Carbenoxolone is a synthetic derivative of glycyrrhizic acid and has been shown to be effective in healing both gastric and duodenal ulcers. It appears to alter the composition and quantity of mucus, thereby enhancing the mucosal barrier to HCl. The mechanism of action is not clear but is thought to involve an increase in the production, secretion, and viscosity of intestinal mucus.

3.4. Prostaglandins: Misoprostol, a methyl analog of PGE_1, is used for the prevention of ulcer induced by the administration of NSAIDs. PGs are produced by the gastric mucosa, and have a cytoprotective effect. This effect may be mediated through inhibition of histamine stimulated cAMP production.

4. Eradication of *Helicobacter pylori:* *H. pylori* is a gram negative rod that colonizes the mucus on the luminal surface of the gastric epithelium. *H. pylori* infection causes an inflammatory gastritis and is putative contributor to peptic ulcer disease, gastric lymphoma and adenocarcinoma. Single agent therapy of *H. pylori* infections has proven relatively ineffective. Double or triple antimicrobial therapies, in combination with

antisecretory drugs, are being used successfully to treat peptic ulcers that are due at least in part to *H. pylori* infection.

Unfortunately, most of the currently used anti-ulcer drugs have no limiting side effects (Barrowman and Pfeiffer, 1992), and no single anti-ulcer agent has been shown to have absolute healing property without relapse (Howden *et al.*, 1988). Thus, efforts have been directed towards finding suitable anti-ulcer drugs from natural products. In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcer. The first drug effective against gastric ulcer was carbenoxolone, discovered as a result of research on a commonly used indigenous plant, *Glycyrrhixa glabra*, family Leguminosae (Brown *et al.*, 1959). The medicinal plants were reported to have anti-ulcer activity are listed in Table 1.

Quite a number of medicinal plants exhibit promising anti-ulcer activity. Banana (*M. sapientum*, Musaceae) showed a significant anti-ulcer activity in different experimental models (Sanyal *et al.*, 1961, 1964, 1965). Various preparations of dried unripe plantain banana were found to be anti-ulcerogenic against aspirin-induced ulceration in the rat, and were effective both as a prophylactic treatment and in healing ulcers induced

Table 1 List of medicinal plants reported to have anti-ulcer activity

Plants	References
Compositae	
Sanssurea lappa Clark	Yamahara *et al.*, 1985
Euphorbiaceae	
Croton subylratus Kuze.	Ogiso *et al.*, 1978
	Kitazawa *et al.*, 1980
	Kobayashi and Tabata, 1992
Labiatae	
Ocimum tenuiflorum Linn.	Singh *et al.*, 1970
	Mokkhasamit *et al.*, 1971
	Singh, 1986
Leguminosae	
Glycyrrhiza glabra Linn.	Hong *et al.*, 1982
	Dai *et al.*, 1992
Liliaceae	
Allium sativum Linn.	Chang, 1985
	Joshi *et al.*, 1987

Plants	References
Musaceae	
Musa sapientum Linn.	Sanyal *et al.*, 1961, 1964, 1965;
	Best *et al.*, 1984;
	Goel *et al.*, 1986, 1989;
	Mukhopadhyaya *et al.*, 1987
Umbelliferae	
Centella asiatica (Linn.)	Cho *et al.*, 1981;
Urban	Rhee *et al.*, 1981;
	Shin *et al.*, 1982;
	Chatterje *et al.*, 1992
Zingiberaceae	
Alpinia galanga (Linn.)	Mitsui *et al.*, 1976;
	Al-Yahya *et al.*, 1990
Curcuma longa Linn.	Shinha *et al.*, 1975;
	Prucksunand *et al.*, 1986;
	Rafatullah *et al.*, 1990
Zingiber officinale Roce.	Yamahara *et al.*, 1988;
	Al-Yahya *et al.*, 1989;
	Rafatullah *et al.*, 1990;
	Yoshikawa *et al.*, 1992

by aspirin (Best *et al.*, 1984). Pulp powder of plantain banana (*M. sapientum* var. *paradisiaca*) was shown to have significant anti-ulcerogenic activity in rats subjected to aspirin, indomethacin, phenylbutazone and prednisolone treatment and in guinea-pigs treated with histamine (Goel *et al.*, 1986). The anti-ulcerogenic action of banana preparations appears to be due to their ability to stimulate the growth of gastric mucosa (Best *et al.*, 1984). Banana powder not only increased cellular mucus, mucosal thickness, and strengthened mucosal resistance against ulcerogens but also promoted healing by inducing cellular proliferation (Goel *et al.*, 1986; Mukhopadhyaya *et al.*, 1987). Incubation of ethanolic extract of banana with human gastric and colonic mucosa was found to cause a concentration-dependent increase in the eicosanoid accumulation (Goel *et al.*, 1989).

Ginger (*Zingiber officinale* Rosc.) has cytoprotective and anti-ulcerogenic effect (Al-Yahya *et al.*, 1989). The extract of ginger at the dose of 500 mg/kg orally exerted highly significant cytoprotection against 80% ethanol, 0.6M HCl, 0.2 M NaOH and 25% NaCl-induced gastric lesions. The extract also prevented the occurrence of gastric ulcers induced by NSAIDs and hypothermic restraint stress. In addition, *Z. officinale* (100 mg/kg) per orally could prevent peptic ulcer from HCl/EtOH

(Rafatullah *et al.*, 1990). It was also found that terpeniod and 6-gingerol, the constituents from *Z. officinale*, showed anti-ulcer activity when tested in HCl/EtOH-induced gastric lesions in rats (Yamahara *et al.*, 1988). A new anti-ulcer principle named 6-gingesulfonic acid, isolated from *Zingiber* showed anti-ulcer activity, which was more potent than 6-gingerol and 6-shogaol when tested on HCl/EtOH-induced gastric lesions in rats (Yoshikawa *et al.*, 1992).

An oral dose of 500 mg/kg of the ethanolic extract of turmeric (*Curcuma longa*, Zingiberaceae) produced significant anti-ulcerogenic activity in rats subjected to hypothermic restraint stress, pyloric ligation, indomethacin and reserpine administration (Rafatullah *et al.*, 1990). In addition, it was found that turmeric extract not only increased the gastric wall mucus significantly but also increased the non-protein sulfhydryl (NP-SH) content in the glandular stomachs of the rats. Clinical trials of turmeric were performed by a group of Vietnamese and Swedish scientists (Dau *et al.*, 1998). The effect of turmeric (6 g daily as suggested in the Vietnamese pharmacopoeia) on the healing of duodenal ulcer was compared with an equal amount of placebo, suffering from duodenal ulcer. The study disclosed that turmeric was not superior to placebo in healing duodenal ulcer either after four or eight weeks of treatment.

Trans-Dehydrocrotonin (DHC), the major diterpene isolated from *Croton cajucara* Benth (Euphorbiaceae), at an oral dose of 100 mg/kg showed a significant anti-ulcerogenic effect on ulcer induced by hypothermic restraint stress, ethanol, and pylorus ligature (Brito *et al.*, 1998). However, no significant changes in indomethacin-induced gastric lesions or modifications in gastric parameters such as wall mucus, secretory rate, pH, and total acid content were found after DHC treatment.

***Kaempferia galanga* Linn.**

K. galanga Linn. (Zingiberaceae), known commonly as Phroh hom, Homproh, Wannteendin, Wann phaen din yen (Figure 2). Key (1996) has described botanical characteristics of the plant as follows: it is a perential, acaulescent herb. Leaves 2-3, resting on the ground, roundish, abruptly acuminate, base attenuate, margin thin, reddish, 8-10 cm long by 6-7 cm wide. Inflorescence sessile, included within the leaf sheaths; bracts lanceolate-acute, 2.5-4.5 cm long. Flower 6-12 in an extended rosette, white with violet patch in the center; calyx matching the bracts; corolla tube 2.0-2.5 cm long; anther nearly sessile; stamens obvoal-cuneiform: labium deeply divided into 2 obviod lobes. The rhizomes occur in slices 2.5 cm in diameter, the

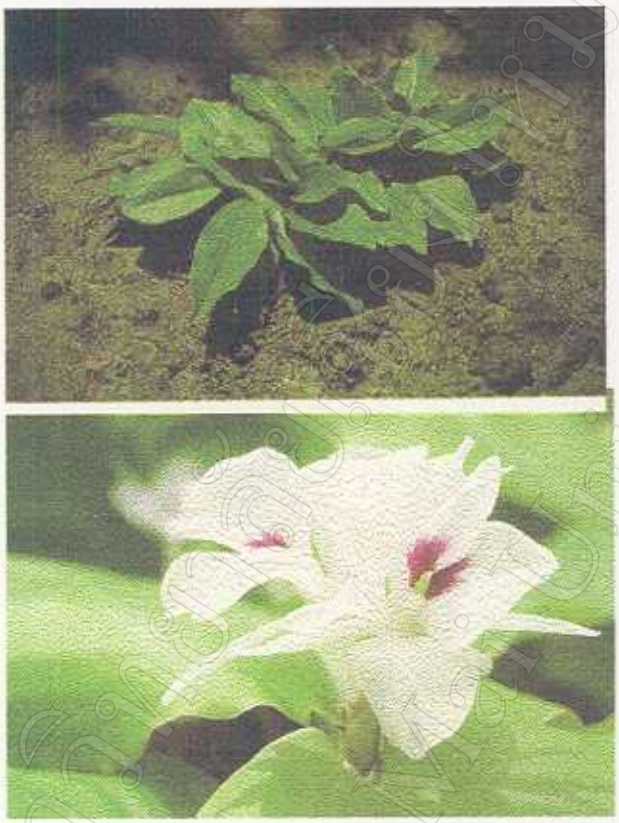

Figure 2 *Kaempferia galanga* Linn. Family Zingiberaceae

epidermis reddish, the interior white. The odor is aromatic, and the taste pungent. It is widely distributed in Southern China, Indochina, Malaysia and India.

Phytochemical study revealed that the compounds in *K. galanga* are benzenoid (para-benzoic acid), monoterpene, phenylpropanoid, ethyl ester of cinnamic acid, para-meth of cinnamic acid ethyl ester and paramethoxy cinnamic acid (Merh *et al.*, 1986; Kiuchi *et al.*, 1987, 1989; Kosuge *et al.*, 1985).

K. galanga is widely used in traditional medicines in many countries. In Indonesia, *K. galanga* rhizome is used for abdominal pain in women by applied externally and used as an ointment for swelling and rheumatism (Hirschhorn, 1983). The rhizome is used in Papua-New Guinea as an abortifacient (Paijmans, 1976). Indian used a mixture consisting of *K. galanga*, *Terminalia arjuna*, *T. chebla*, *Piper longum*, *Inula racemosa* and *Sida cordifolia* per orally for heart disease (Kumar and Prabhahar, 1987). In the Philippines, rhizome of *K. galanga* is used to treat itching/pruritis by placing slices of rhizome to the opposite side of the affected area (Madulid *et al.*, 1989). According to Thai folk medicine, dried rhizome of *K. galanga* is used as a cardiotonic, CNS stimulant, antipyretic and for fainting (Mokkhasmit *et al.*, 1971).

Others reported pharmacological activities of *K. galanga* include nematocidal (Kiuchi *et al.*, 1989), antitumor (Itokawa *et al.*, 1983), histaminergic, hypertensive and smooth muscle stimulant (Mokkhamit *et al.*, 1971), and antibacterial activities (Jeorge and Pandalai, 1949).

Extensive toxicity study of the ethanolic extract of *K. galanga* was carried out by Kanjanapothi *et. al.*, (1997). When tested by the Hippocratic screening in rats (given intraperitoneally), the extract demonstrated signs indicating CNS depression such as decrease in motor activity and respiratory rate, a loss of screen grip and analgesia. In acute toxicity test, and oral administration of 5 g/kg of the ethanolic extract produced neither mortality nor significant changes of the body and organ weights. In addition, no gross abnormalities and histopathological changes were detectable. In subacute toxicity study, an oral administration of the ethanolic extract of *K. galanga* at doses of 25, 50 or 100 mg/kg daily for 28 days appeared not to exert any significant changes in the body and organ weights. Hematological and blood chemistry analysis showed no significant changes in any of the parameters examined. Pathologically, neither gross nor microscopic abnormalities were observed.

An ethanolic extract of *K. galanga* was found to be an effective larvicide having LC_{50} value of 50.54 ppm (Tookyang, 1997). The hexane fraction yielded from fractionation of the ethanolic extract of *K. galanga* exhibited the highest larvicidal effect with the LC_{50} of 42.33 ppm. In addition, the hexane fraction showed a repellent activity and did not cause dermal irritation when applied on human skin or tested in rabbits.

Purposes of the Study

The present study was undertaken to

a) evaluate the anti-gastric ulcer activity of the methanolic extract of *K. galanga* on experimentally induced gastric lesions in rats.

b) compare the anti-gastric ulcer effect of the methanolic extract of *K. galanga* to those of available reference drugs.

c) study the possible the mechanism of action mediating anti-ulcer activity of the methanolic extract of *K. galanga*.