

Anti-Ulcerogenic Effect of Some Indian Medicinal Plants on Mucosal Lesion in Rats

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ABSTRACT

The antiulcer activity of the poly herbal formulation (composed of the leaf extracts from *Lantana camara*, *Annona muricata*, *Kalanchoe pinnata*) was evaluated in necrotizing agents induced ulcer model in rats. The extract at dose of 1000mg/kg produced significant inhibition of gastric lesion induced by above mentioned method. The extract reduced ulcerative lesion, gastric volume, free and total acidity and pH of gastric juice in the model. The result obtained suggesting that extract possesses significant anti-ulcer activity.

Keywords: Antiulcer, gastric lesion, *Lantana camara*, *Annona muricata*, *Kalanchoe pinnata*, free acidity, total acidity, ulcer index, gastric juice.

I. INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly for the population of non-industrialized countries (13). Peptic ulcers, also known as “ulcus pepticum” are ulcers which occur in that part of the gastrointestinal (10). Several factors are implicated in the pathogenesis of gastric ulcer including increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer (21, 16). In recent years, a powerful association between peptic ulcers and infection of *Helicobacter pylori* has been adopted. At least 70-90% of patients with gastric ulcers and 80-95% with duodenal ulcers are infected by *H.pylori* and eradication of this microorganism seems to be curative for the disease (28). There is a balance between the aggressive (*i.e.*, acid, pepsin, active oxidants, *H. pylori*) and the mucosal protective (*i.e.*, mucus, bicarbonate, prostaglandin's) factors in stomach. Thus, drug therapy of peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating defensive one (53).

Gastric hyperacidity and gastroduodenal ulcer is a very common global problem today because of unhealthy eating habits, and stress. Stress, both psychological and physical is common in everyday life and is known to induce circulatory diseases and ulceration of the digestive tract (15).

Peptic ulcer is an excoriated area of the gastric or duodenal mucosa caused by action of the gastric juice. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases (12). It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors and the mucosal defensive factors (41).

Peptic ulcer diseases comprise heterogeneous disorders, which manifest as a break in the lining of the gastrointestinal mucosa bathed by acid and pepsin. It is the most predominant of the gastrointestinal diseases (18, 25). Based on site of attack, peptic ulcer may be classified as oesophageal, duodenal, or gastric. The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermal growth factors) (57).

Prostaglandins (PGs) such PGE2 have potent effects on gastric mucosal protection (43, 30, 19). Endogenous PG synthesis also has an important role in gastric mucosal defense (44,5). Synthesis of PGs is governed by PG endoperoxide synthase, or cyclooxygenase (COX: EC 1.14.99.1), which consists of two isoforms (26). The constitutive isoform (COX-1) is dominantly expressed in platelets, prostate, and stomach. Expression of the

mitogen-inducible isoform (COX-2) is enhanced in gastric epithelial cells after growth stimulation in vitro and in gastric epithelium after acid-induced damage in vivo (56,48,51). Furthermore, COX-2-specific inhibitors delay healing of acetic acid-induced gastric ulcers in mice (31, 51), suggesting an important role for this isozyme in peptic ulcer healing.

The importance of natural phenolic compounds from plants materials is also raising interest due to their redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. In addition, they have metal chelating properties as well (37, 4). Polyphenolic compounds are secondary plant metabolites found in numerous plant species and they are reported to have multiple functions to counteract the free radicals and they also inhibit different types of oxidizing enzymes (42).

Medicinal plants represent an important source of medically important compounds. Since ancient time, medicinal plants are used to cure several types of health problems. Systemic analysis of these plants provides a variety of bioactive molecules for the development of newer pharmaceutical products. Recently, there is a growing interest in the pharmacological evaluation of various plants used in different traditional system of medicine. In last few decades, many of traditionally known plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, anti-inflammatory activity, antidiabetic activity, anthelmintic, antibacterial activity, antifungal activity, hepatoprotective activity, antioxidant activity, larvicidal activity etc (40,22,45).

Lantan camara introduced in India as an ornamental plant but entirely naturalized and found throughout India. However, it is listed as one of the significant medicinal plants of the world. The plant *Lantana camara* (Verbanaceae), generally known as wild or red sage is the most widespread species of this genus and it is a woody straggling plant with various flower colors, red, pink, white, yellow and violet. It is an ever green strong smelling shrub, with stout recurved prickles, leaves opposite, ovate, acute or sub- acute, crenate -serrate, scab rid on both side (54).

Scientific classification

Kingdom: Plantae

Order : Lamiales

Family : Verbenaceae

Genus : *Lantana*

Species : *camara*

L. camara is a low erect or subscandent vigorous shrub with tetragonal stem, stout recurved prickles and a strong odour of black currents. Plant grows up to 1 to 3 meters and it can spread to 2.5 meter in width. Leaves are ovate or ovate oblong, acute or sub acute crenate serrate, rugose above, scabrid on both sides. The leaves are 3-8 cm long by 3-6 cm wide and green in colour. Leaves and stem are covered with rough hairs. Small flower held in clusters (called umbels). Colour usually orange, sometime varying from white to red in various shades and the flower usually change colours as they ages. Flowers are having a yellow throat, in axillary head almost throughout the year. The calyx is small, corolla tube slender, the limb spreading 6 to 7 mm wide and divided in to unequal lobes. Stemen four in two pairs, included and ovary two celled, two ovuled. Inflorescences are produced in pairs in the axils of opposite leaves. Inflorescences are compact, dome shaped 2-3 cm across and contain 20-40 sessile flowers. Root system is very strong and it gives out new fresh shoots even after repeated cuttings (47).

Annona muricata L. belongs to the family of Annonaceae has a widespread pantropical distribution and has been proudly known as corossol. It is a widespread small tree and has its native in Central America (1). Intensive chemical investigations of the leaves and seeds of this species have resulted in the isolation of a great number of acetogenins. The isolated compounds display some of the interesting biological or the pharmacological activities, such as antitumoral, cytotoxicity, antiparasitic and pesticidal properties. Roots of these species are used in traditional medicine due to their antiparasitical and pesticidal properties (9).

Scientific classification

Kingdom: Plantae-Plants

Class: Magnoliopsida

Order: Magnoliales

Family: Annonaceae

Genus: *Annona*

Species: muricata

The genus name 'Annona' is from the Latin word 'anon', meaning 'yearly produce', referring to the fruit production habits of the various species in this genus. *Annona muricata* is a slender, evergreen tree, 5-10 m in height and 15 cm in diameter; trunk straight; bark smooth, dull grey or grey-brown, rough and fissured with age; inner bark pinkish and tasteless; branches at first ascending with the crown forming an inverted cone, later spreading; crown at maturity spherical due to lack of apical dominance; twigs brown or grey, bearing minute raised dots (lenticels); root system extensive and superficial, spreading beyond the diameter of the crown although shallow rooted; juvenile plants have a taproot that is eventually lost. Leaves alternate, 7.6-15.2 cm long, 2.5-7.6 cm wide, leathery, obovate to elliptic, glossy on top, glabrous on underside, simple; stipules absent; blade oblanceolate, green on top, paler and dull on under side with fine lateral nerves; a strong, pungent odour; petioles short, 3-10 mm long (3).

The knowledge of traditional medicine and medicinal plants and their study of scientific chemical principles may lead to the discovery of newer and cheaper drugs. *Kalanchoe pinnata* (Lam., syn. *Bryophyllum pinnatum*, *B. calycinum*; Local name: Pathorkuchi, Coughpatha; English name: Air plant; Family: Crassulaceae) is an herb found ubiquitously in Bangladesh. It has tall hollow stems, fleshy dark green leaves that are distinctly scalloped and trimmed in red, and bell-like pendulous flowers (14). *Kalanchoe pinnata* (*K. pinnata*) has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawaii. It is also widely distributed in the Philippines, where it is known as katakataka or katakataka which means astonishing or remarkable (14). The leaves of *K. pinnata* have a variety of uses in the traditional system of medicine in Bangladesh. They are eaten for diabetes, diuresis, dissolving kidney stones, respiratory tract infections, as well as applied to wounds, boils, and insect bites (14). It is useful for preventing alcoholic, viral and toxic liver damages. The aqueous extract of this plant have shown anti-inflammatory, anti-diabetic, anti-tumor and cutaneous leishmanicidal activities (49,50,55,32).

Scientific classification

Kingdom: Plantae-Plants

Class: Magnoliopsida

Order: Saxifragales

Family: Crassulaceae stonecrop family

Genus: *Kalanchoe*

Species: *pinnata*

Kalanchoe pinnata (Family: Crassulaceae) is an important plant which has many traditional medicinal uses. *Kalanchoe pinnata* (Family: Crassulaceae) is an erect, succulent, perennial shrub that grows about 1.5 m tall and reproduces through seeds and also vegetatively from leaf buds. It has a tall hollow stems, freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers. This plant can easily be propagated through stems or leaf cutting. It is an introduced ornamental plant that is now growing as a weed around plantation crop. *K. pinnata* is used in ethnomedicine for the treatment of earache, burns, abscesses, ulcers, insect bites, whitlow, diarrhoea and cithiasis (36). In traditional medicine, *Kalanchoe* species have been used to treat ailments such as infections, rheumatism, and inflammation (33) and have immunosuppressive effect as well (27).

II. METHODS AND MATERIAL

Collection And Extraction Of The Plant

The leaves of *L.camara*, *A.muricata* and *K.pinnatum* were collected around Vellore district. After washing the plant with running water, the leaves were separated and dried in shade for 20 days at room temperature. After shade drying, the leaves were grinded through blender and converted into coarse of powder. The powder was extracted by continuous hot extraction using the Soxhlet apparatus. The extracts were collected and preserved in a desiccator until used for further studies.

Test animal

Adult healthy wistar rats weighting 150 g were used and kept in the animal house. The animals were kept in plastic cages (34 × 47 × 18 cm³) at animal house, in an air conditioned environment with four rats in each cage and maintained at room temperature of (25 ± 2) °C with relative humidity (60% ± 10%) under 12 h night and

light cycle. The animals used for the experiment were approved by animal ethics committee.

Preparation and Dose of the Test Drug

The dose of the test drug was calculated by the method of Miller and Tainter (1944) (29), found to be 1000mg/kg the dose of the extract was calculated with reference, the aqueous extract of the drug was used in the dose of 150mg/kg. Standard drug, Rabeprazole (Manufactured in India by Cipla Laboratories Ltd.) was used in the dose of 20mg/kg.

Phytochemical analysis

The preliminary phytochemical analysis of *L.camara*, *A.muricata*, *K.pinnata* leaves aqueous extract was carried out for carbohydrate, saponins, flavonoids, triterpenoids, tanins and alkaloids.

Acute Toxicity Study

The oral acute toxicity study of aqueous extract of *L.camara*, *A.muricata*, *K.pinnatum* Were evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 420 on wistar rats, where the limit test dose of 1000 mg/kg was used. All the animals were kept at overnight fasting before to every experiment with free excess to water. The test drug was administered and observed for 14 days to determine urea, creatinine, SGOT, SGPT level.

EXPERIMENTAL DESIGN

The rats were randomly divided into 6 groups, of 4 rats each as follows:

- Group-I: Control group animals received no treatment.
- Group-II: animals were administrated with Necrotizing agents (Negative control).
- Group-III: animals received 1000 mg/kg body weight of freshly prepared *L.camara*.
- Group-IV: animals received 1000 mg/kg bodyweight of freshly prepared *A.muricata*.
- Group-V: animals received 1000 mg/kg body weight of freshly prepared *K.pinnatum*.
- Group-VI: animals received 20mg/kg body weight of Rabeprazole.

All treatments were administered orally for 11 days. Score of mucosal damage were microscopically observed.

Histological observation

In the 11th day, after 24 h fasting the animals were sacrificed and stomach of each animal was opened along the greater curvature. Specimens of the gastric tissue were fixed in 10% buffered formalin and were processed in the paraffin tissue-processing machine. Sections of the stomach were sectioned at 5µm and stained with hematoxylin and eosin for histological evaluation (20). Paraffin sections were stained with toluidine blue. The effect of drugs was evaluated through assessment of inflammatory and necrotic changes in the mucosal tissue.

Necrotizing agents-induced Gastric Ulceration and Its Protection Studies

Before ulcer induction animals of both control and experimental groups kept separately in standard controlled conditions were fasted for 24 h with free access to water. Acute gastric ulcers were induced by necrotizing agents (90% ethanol, 0.1N NaoH and NaCl). The control group received the vehicle only, whereas the experimental group administered with necrotizing agents for gastric ulceration. After 1 h, the animals were sacrificed, and gastric lesions in the fundic stomach were scored and expressed as ulcer index. *L.camara*, *A.muricata*, *K.pinnata* leaves aqueous extract was administered orally 30 min prior to necrotizing agents treatment to see the gastroprotective effect. Rabeprazole were administered orally at a dose of 20 mg/ kg body weight respectively.

Assessment of gross mucosal damage

The lesion in the glandular portion was examined under a 10 x magnifying glass and length was measured using a divider and scale and gastric lesion was scored as follows:

Scoring of ulcer was made as follows:

- Normal stomach..... (00)
- Red coloration..... (0.5)
- Spot ulcer..... (01)
- Hemorrhagic streak... (1.5)
- Ulcers..... (02)

Perforation..... (03)

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1 \text{ N}} \times 100$$

Ulcer index of each animal was calculated by adding the values and their mean values were determined and percentage inhibition was calculated (24).

Formula for Ulcer Protection

$$\% \text{ Protection} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test})}{\text{No. of Animals}} \times 100$$

Determination of pH and volume of gastric juice

Gastric juice (1 mL) was diluted with 1 mL distilled water and was measured using a pH meter and the volume of gastric juice also measured by measuring tubes.

Free and Total Acidity

Free and total acidity were determined by titrating with 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicator. The free and total acidity were expressed as $\mu\text{equiv}/100 \text{ g}$.

Table:1 Effect of *L.camara*, *A.muricata*, *K.pinnatum* leaves aqueous extract gastric juice volume, pH, total acidity, free acidity, total ulcer index and ulcer protection.

Group	Gastric juice volume in ml	Gastric juice pH	Free acidity (mEq/dl)	Total acidity (mEq/dl)	Total Ulcer index	Ulcer protection(%)
Control	3.78±0.12	3.1±0.30	54.6±0.04	61.35±0.06	0.01±0.00	99
Disease control	1.3±0.04	1.4±0.05	97.87±0.44	119.2±0.37	3.65±0.11	9
<i>L.camara</i> 150mg/kg	1.82±0.05	2.25±0.06	51.22±0.28	59.25±0.06	2.55±0.17	36.5
<i>A.muricata</i> 150mg/kg	2.27±0.07	2.47±0.04	47.72±0.07	56.49±0.30	2.25±0.06	44.2
<i>K.pinnata</i> 150mg/kg	2.82±0.05	2.87±0.08	44.82±0.04	53.72±0.08	1.77±0.07	56.5
Rabeprazole 20mg/kg	3.27±0.08	3.05±0.13	39.82±0.04	50.67±0.37	0.8±0.13	80.4

Values are expressed as mean \pm SEM. $P > 0.05$ when compared to normal control group by Statistical analysis by One-way ANOVA followed by Dunnett's method.

III. RESULTS AND DISCUSSION

Preliminary phytochemical screening

The phytochemical screening of the plant extract revealed the presence of various bioactive constituents like alkaloids, flavonoids, saponins and tanins.

Acute Toxicity Study

The oral acute toxicity study of aqueous extract of *L.camara*, *A.muricata*, *K.pinnatum* Were evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 420 on wistar rats, where the limit test dose of 1000 mg/kg was used. No mortality observed for 14 days.

Necrotizing agents- Induced Gastric Ulceration

In the present study the anti-ulcer activity of leaves of *L.camara*, *A.muricata*, *K.pinnatum*. Revealed that the minimum ulcer index was observed with Rabeprazole.



a. Normal control



b. Experimental Control



c. L.camara 1000mg/kg



d. A.muricata 1000mg/kg



e. K.pinnata 1000mg/kg

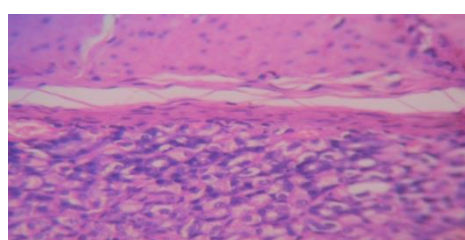


f. Rabeprazole 20mg/kg

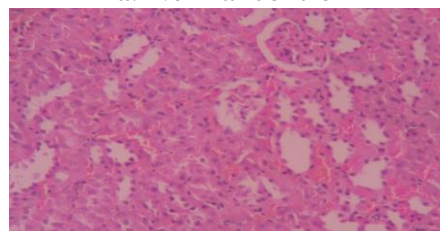
Figure 1. Morphological Features of Stomach in Stress Induced Ulcer

Morphological study of stomach

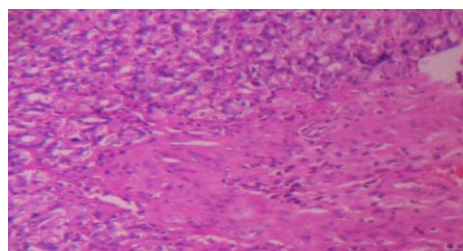
In normal group stomach integrity was maintained and appeared normal. In control group severe bleeding, perforation, spot ulcer were observed but, in standard group and extract treated groups, animal showed less ulceration and stomach integrity was maintained.



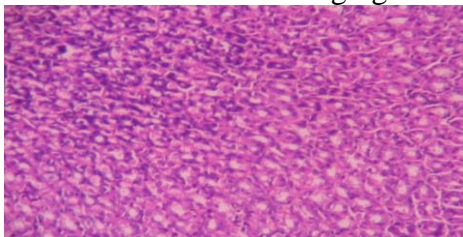
a. Normal control



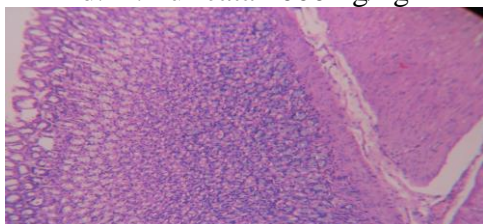
b. Experimental Control



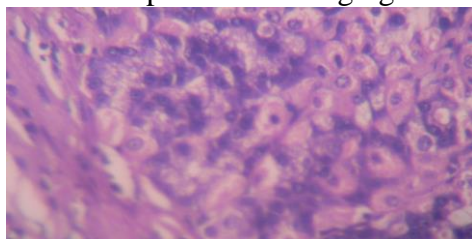
c. *L.camara* 1000mg/kg



d. *A.muricata* 1000mg/kg



e. *K.pinnata* 1000mg/kg



f. Rabeprazole 20mg/kg

Figure 2. Histology of Stomach in Stress induced Ulcer

Histopathological study

Histopathological examination of gastric mucosa in the normal control group showed intact gastric mucosa and continuous epithelial surface. Experimental control revealed mucosal ulceration. In *L.camara* (1000mg/kg) group, superficial erosions and few ulcers accompanied with mild inflammatory was observed. In *A.muricata* (1000mg/kg) group, slight ulcer with inflammatory infiltrate and congestion in few areas was observed. In *K.pinnata* (1000mg/kg) group, section revealed intact mucosa with no inflammation. In Rabeprazole (20mg/kg) group, showed intact gastric mucosa without any inflammatory.

DISCUSSION

The peptic ulcer results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms (11). To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal protection, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid (17).

In the present study, which could be attributed to the endogenous generation of PG, was responsible for maintaining the cellular integrity of the gastric epithelium; therefore, such endogenous release of PG would play a physiological role in protecting the gastric mucosa.(43) On the other hand, it has been reported that plants and/or spices sometimes exhibit their cytoprotective action through their mild irritant property.(5,38,39) Furthermore, Robert *et al.*,(44) have also described the ability of a mild irritant in protecting gastric mucosa against strong irritants. This protection is called adaptive cytoprotection..

Phytochemical analysis on the leaves aqueous extract gave positive results for flavonoids, alkaloids, saponins, carbohydrate, tanins and triterpens. The obtained results strongly suggest that flavonoids and alkaloids are the major components of the extract and therefore some of the pharmacological effects could be attributed.

Flavonoids are among the cytoprotective materials able to increase mucus, bicarbonate and prostaglandin secretion, strengthening of gastric mucosal barrier and scavenging of free radicals which are very important in preventing ulcerative and erosive lesions of gastrointestinal tract (46).

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones.

The pharmacological activities of flavonoids were closely related to their functional group. In addition, there may be some interference rising from other chemical components present in the extract. Moreover, flavonoids may exert their cell structure protection through a variety of mechanisms; one of their potent effects may be through their ability to increase levels of glutathione, a powerful antioxidant (35).

Flavonoids have anti-inflammatory activity and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species (50). Hence, many studies have examined the antiulcerogenic activities of plants containing flavonoids. Plants containing flavonoids were found to be effective in preventing this kind of lesion, mainly because of their antioxidant properties. Recently, the antioxidant activity of flavonoids has attracted interest because of the strong evidence that oxidation processes are involved in the mechanisms of several gastric disorders, including ulcerogenesis (23).

The phyto-constituents like flavonoids, tannins, terpenoids, and Saponin have been reported in several anti-ulcer literatures as possible gastro protective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed (7). Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants (6,34).

Oral administration of RAB1 (Rabeprazole) significantly reduced ulcer index, gastric juice free and total acidity and pepsin activity. However, the drug has not produced any significant quantitative change in the mucin content. Rabeprazole was reported to significantly increase the production of mucin (a defense factor) in rats. It prevented or reduced the size of gastric ulcers. Rabeprazole caused dose-dependent inhibition. Rabeprazole is an inhibitor of the gastric proton pump. It causes dose-dependent inhibition of acid secretion and has a more rapid onset of indomethacin-induced ulceration. R(+)-

rabeprazole appears to be the major isomer having anti-ulcer activity (8).

The anti-ulcer activity of the leaves aqueous extract of *L.camara*, *A.muricata*, *K.pinnatum* was evaluated against gastric lesions induced by necrotizing agents.

Treatment with *L.camara*, *A.muricata*, *K.pinnatum* protected the gastric mucosa from damage by increasing the mucin content significantly. Apparently, the free radicals scavenging property of *L.camara*, *A.muricata*, *K.pinnatum* might contribute in protecting the oxidative damage to gastric mucosa.

IV. CONCLUSION

Herbal products are well thought-out to be symbols of safeguard in comparison to the synthetic product that are regarded as unsafe to human life and environment. While herbs had been prized for their medicinal significance. The three plants extracts and anti-ulcer drug RAB1 compared. Among these, the anti-ulcer drug Rabeprazole and *K.pinnatum* were more effective than the *L.camara*, *A.muricata*.

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