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A combination of *Psidium guajava* and *Aloe vera* Aqueous Extracts Prevents Ethanol-Induced Gastric Ulcer in Rats through Suppression of Oxidative Stress and Gastric Inflammation

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Abstract

Background: Gastric ulcers are a common gastrointestinal disorder for which there is a need to search for a better prevention method or treatment options. Accordingly, this study aimed to assess the protective effect of a combination of *Psidium guajava* and *Aloe vera* extracts against ethanol-induced gastric ulcers in rats.

Methods: The gastric ulcer was induced in rats using one oral dose of absolute ethanol (5 mL/kg body weight (b.w)). A combination of *P. guajava* and *A. vera* at doses of 125, 250 and 500 mg/kg b.w and omeprazole 20 mg/kg b.w were given by gavage daily for 21 days before the induction of ulcers. The stomachs of rats were analyzed both macroscopically and biochemically 24 hours following ethanol administration.

Results: Pretreatment of rats with the combination of *P. guajava* and *A. vera* extracts significantly ($P<0.05$) reduced the ulcer index induced by ethanol. Also, administration of the plant extracts reduced stomach content of malondialdehyde, and enhanced stomach antioxidant enzymes (superoxide dismutase and catalase) activities as well as glutathione content in ethanol-intoxicated rats. Furthermore, the plant extract significantly ($P<0.05$) decreased the stomach level of pro-inflammatory cytokines such as TNF-α, INF-δ, IL-1β and IL-6. The efficacy of the combination of plant extracts at 250 and 500 mg/kg b.w was comparable to that of Omeprazole.

Conclusion: Overall, findings from this study revealed that the combination of *P. guajava* and *A. vera* aqueous extracts displayed a gastro-protective effect on ethanol-induced gastric ulcer, likely through the suppression of oxidative stress and gastric inflammation.

Keywords: *Psidium guajava*; *Aloe vera;* gastric ulcer; oxidative stress; antioxidants; anti-inflammatory.

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Background

Gastric ulcers are a common gastrointestinal disorder that affects millions of people worldwide and can cause significant discomfort and even life-threatening complications [1]. In Africa, the prevalence of gastric ulcers is relatively high, with studies reporting rates ranging from 2.5% to 17% in different regions [2]. In Cameroon, a study published in 2016 found that the prevalence of gastric ulcers was 7.7%, with men being more commonly affected than women [3]. The pathogenesis of gastric ulcers is multifactorial and involves a complex interplay between multiple factors that contribute to the disruption of the balance between gastro-protective and aggressive factors in the upper gastrointestinal tract [4]. Moreover, reactive oxygen species (ROS), the primary mediators of oxidative stress, and other pro-inflammatory factors are excessively produced at the site of gastric ulcers by white blood cells, therefore exacerbating stomach injury [5]. One of the most common causes of gastric ulcers is the infection with the bacterium *Helicobacter pylori*, which can damage the protective layer of mucus that lines the stomach and duodenum, allowing acid and other digestive juices to irritate and erode the underlying tissue [6]. Other causes of gastric ulcers include long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, and excessive alcohol consumption which, can damage the protective lining of the stomach, or increase acid production and irritate the stomach lining [7]. The current therapeutic approach for gastric ulcers typically involves the use of proton pump inhibitors (PPIs), which are drugs that reduce the production of stomach acid and help promote the healing of the ulcer [8]. Other drugs, such as histamine H_2-receptor antagonists and antacids, may also be used to reduce acid production and alleviate symptoms [8]. Although these drugs can be effective in treating gastric ulcers, they have several limitations. For example, sustained administration of PPIs has been associated with headache, diarrhea, nausea, and weakness [8]. Overall, while conventional drugs remain a common approach for the treatment of gastric ulcers, their limitations and potential side effects highlight the need for alternative options that are safe, effective, and well-tolerated. Accordingly, the search for new therapies and approaches to manage gastric ulcers is necessary to improve patient outcomes and quality of life. In recent years, there has been increasing interest in the therapeutic benefits of natural remedies, particularly those derived from plants. *Psidium guajava* and *Aloe vera* are two such plants. *P. guajava*, commonly known as “guava”, is a tropical fruit tree. The fruit and leaves of the plant have been used in many cultures for their medicinal properties, including the treatment of diarrhea, dysentery, and digestive disorders [9]. *A. vera* is a succulent plant that grows in various climates, including tropical and subtropical regions of Africa. The plant has been used for centuries for the treatment of wounds, burns, and skin irritations [10]. Phytochemical investigation reported that these plants are rich sources of bioactive compounds such as polysaccharides, tannins, polyphenols, and anthraquinones, which are known for their anti-inflammatory, antioxidant, antimicrobial, and immune-modulating properties [11, 12]. In addition, *P. guajava* and *A. vera* have been found to display a gastro-protective effect through the inhibition of gastric acid secretion [13] and the promotion of mucosal healing [14], respectively. Considering the complex pathophysiology of gastric ulcers, a multi-targeting agent or a combination of agents targeting various aspects of the pathogenesis of gastric ulcers and having gastro-protective action would be beneficial for the prevention and/or treatment of the disease. Knowing that plants contain a variety of bioactive molecules acting differently, we hypothesized that the combination of *P. guajava and A. vera* could be highly effective in preventing gastric ulcers. Thus, the capacity of the combination of extracts from *P. guajava and A. vera* to protect rats against ethanol-induced gastric ulcers was examined in the present investigation.

Methods

Reagent

Trichloroacetic acid, thiobarbituric acid, naphthylethylenediamide, sulphanilamide, phosphoric acid, and Dithiobis (2-Nitrobenzoic acid) (DTNB) were purchased from Sigma Aldrich (Germany). Tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1beta (IL-1β), and interferon gamma (IFN-γ) ELISA kit were purchased from R&D Systems, USA. All additional reagents and chemicals utilized in the research are commercially accessible and were of analytical quality.

Collection and authentication of plant materials

Fresh leaves of *Psidium guajava* and *Aloe vera* were harvested in Bomaka, Buea (Cameroon) in March 2022. The identification of the species was done at the National Herbarium of Cameroon with voucher specimen numbers: HNC-Num 60041 and HNC-Num 17027 were given to *P. guajava* and *A. vera* respectively.

Animals and Ethical Consideration

Young male Wistar rats (8-10 weeks old) weighing between 150-200 g were used. They were provided by the Medical Research and Applied Biochemistry Laboratory, Faculty of Health Sciences, University of Buea; and maintained under standard conditions (clean cages placed in a well-ventilated house with photoperiod of 12 hours light and 12 hours dark cycle). The animals were fed with a conventional commercial diet and water ad libitum. All the procedures followed the ARRIVE guidelines on animal care and were approved by the Institutional Animal Care and Use Committee of the University of Buea-Cameroon (UB-IACUC N°07/2023).

Preparation of plant extracts

Fresh leaves of *P. guajava* and *A. vera* were air-dried at room temperature and ground into fine powder using an electric blender. Two hundred grams of each powder were soaked separately for 24 hours in 2 L of distilled water with regular shaking. Each mixture was then filtered using Whatman paper N° 1 and the filtrate obtained was dried in an oven at 40° C to yield a dark semi-solid extract with the weight of 81 g and 41 g respectively for *P. guajava* and *A. vera*.

Induction of gastric ulcer by oral administration of 95% ethanol

The animals were distributed into 6 groups of 5 animals each and treated orally for 21 days as follows: Group 1 was given 20 mg/kg body weight (b.w) omeprazole and served as positive control. Groups 2, 3, and 4 were given a mixture of *P. guajava* and *A. vera* extracts (1:1, weight/weight (w/w)) at the dose of 125, 250, and 500 mg/kg b.w respectively, and served as treatment groups. Groups 5 and 6 were given 10 mL/kg b.w distilled water and served as ulcer model and normal control respectively. After the last treatment, the animals were deprived of food, with free access to water for 24 hours. Then, animals of groups 1 to 5 were given 95% ethanol (5 mL/kg b.w) to induce gastric ulcers. After 24 hours, the animals were killed by decapitation under anesthesia (mixture of diazepam

2 mg/kg b.w and ketamine 100 mg/kg b.w). Blood samples were collected in dry tubes and centrifuged (3000 rpm, 10 minutes, 4◦C). The serum obtained was used to assess the level of pro-inflammatory cytokines. The stomach was then excised, rinsed with normal saline (0.9% NaCl), weighed, and used for assessing the severity of ulceration and preparation of 10% homogenates for biochemical analysis.

Measurement of ulcer severity

The gastric tissue specimens were gently rinsed with normal saline (0.9% NaCl) to remove blood clots and then examined macroscopically. The length of the ulcers (mm) was measured using a Vernier Caliper and the gastric ulcer index (UI) and percent ulcer protection was calculated. The mean of the ulcer lengths per rat was expressed as the ulcer index.

$$\%\ \text{ulcer protection} = \frac{(\text{UI of ulcer model} - \text{UI of treated group}) \times 100}{UI\ of\ ulcer\ model}$$

Measurement of cytokine levels

A homogenate of stomach tissue was created using ice-cold phosphate buffer (0.1 M; pH 7.4; total volume of 10 mL) that contained 0.2% Halt Protease Inhibitor Cocktail EDTA-Free (Thermo Fisher Scientific). The homogenization was performed using a Potter homogenizer. Subsequently, the homogenate was centrifuged (17000×g; 20 minutes; 4 °C) and the supernatant was collected and used for the measurement of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, and IFN-γ), both in serum and stomach homogenates using the respective Quantikine ELISA kit (R&D Systems, USA), as directed by the manufacturer.

Measurement of Oxidative Stress Makers

The levels of oxidative stress markers in the stomach tissues (10% weight/volume (w/v)) were evaluated by measuring the stomach content of Malondialdehyde (MDA), as an index of lipid peroxidation. In addition, the activity of the antioxidant superoxide dismutase (SOD), and catalase (CAT) as well as the content of cellular reduced glutathione (GSH)), was also assessed

Determination of MDA content

The level of Malondialdehyde (MDA) in the stomach was assessed to evaluate lipid peroxidation, following the procedure outlined by Djeungoue et al. [15]. In summary, 0.5 mL of 20% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid were combined with 1 mL of supernatant from the tissue homogenate. This mixture was then placed in a water bath at 90◦ C for one hour. Afterward, the mixture underwent centrifugation at 3000 rpm for 10 minutes. The supernatant collected was used to measure the absorbance at 546 nm. The concentration of MDA was quantified by using its molar extinction coefficient ($\varepsilon = 1.56 \times 10^5\ M^{-1}.cm^{-1}$) expressed as µmol of MDA per gram of tissue.

Determination of Catalase Activity

Catalase activity in the stomach was determined by the method described by Njayou et al. [16] with slight modification. Briefly, a total of 250 µL of homogenate supernatant was combined with 250 µL of phosphate buffer (50 mM), followed by the sequential addition of 1 mL of hydrogen peroxide (30 mM) to the mixture. The absorbance was measured at 340 nm at intervals of 30, 60, and 90 seconds after introducing hydrogen peroxide (H_2O_2) into the reaction medium, employing a spectrophotometer. The calculation of catalase activity was performed using the designated formula.

$$\text{Activity} = \frac{\text{AA x } 10^3 \text{x Vt}}{1 \text{ x } \varepsilon \text{ x Vs}}$$

Where AA is average absorbance; ε is the extinction coefficient ($\varepsilon\ H_2O_2 = 43.6\ M^{-1}.cm^{-1}$); Vt is the total volume of the supernatant and Vs is the volume of supernatant used for assay. Catalase activity was expressed as µmol of H_2O_2 consumed per mg of protein.

Determination of reduced glutathione level

The level of reduced glutathione (GSH) in the stomach was measured using the method outlined by Kouam et al. [17]. A volume of 20 µL from the tissue homogenate supernatant was combined with 3 mL of Ellman reagent, which consists of 5 mg of DTNB (Disthio-bis (2-nitrobenzoic acid) in 250 mL of phosphate buffer). This mixture was allowed to stand at room temperature for 1 hour, after which the absorbance was measured with a spectrophotometer at a wavelength of 405 nm. The concentration of GSH was determined using the molar extinction coefficient (ƐGSH = 13,600 $M^{-1}.cm^{-1}$) and was expressed in micromoles of protein.

Assessment of superoxide dismutase activity

Total superoxide dismutase was measured using the method outlined by Sun et al [18]. This approach is founded on the principle that the xanthine oxidase system serves as a superoxide generator, which inhibits the reduction of nitroblue tetrazolium. After adding a 1.0 mL mixture of ethanol and chloroform (5/3, volume/ volume (v/v)) to an equal volume of the sample, the activity was evaluated in the ethanol phase of the homogenate following centrifugation. The absorbance obtained from nitroblue tetrazolium reduction to blue formazan by superoxide was determined spectrophotometrically at 560 nm.

$$\%\ \text{Inhibition} = \frac{\text{Absorbance (reaction blank)} - \text{Absorbance (sample)}}{\text{Absorbance (reaction blank)}} \times 100$$

One unit of SOD was considered as the amount of enzyme causing 50% inhibition in the nitroblue tetrazolium reduction rate.

Statistical analysis

The data are expressed as mean ± standard deviation (n=5). To compare the mean values across different treatment groups, a one-way analysis of variance (ANOVA) was used, followed by Dunnett's post hoc test when significant differences in variances were identified. A significance level of $P < 0.05$ was used to determine meaningful differences between the groups. The analyses were conducted with Prism Version 5.03 statistical software (Graph Pad Inc., USA).

Results

Gross Evaluation of Gastric Mucosa

As illustrated by Figure 1, the administration of 95% ethanol-induced severe gastric ulcer while rats receiving distilled water (normal control) showed no signs of ulceration. However, pre-treatment of rats with the combination of *P. guajava* and *A. vera* extract significantly diminished the gastric injury and ulcer index

when compared to the ulcerated group. The inhibition percentage of the ulcer index in rats pre-treated with the combination of *P. guajava* and *A. vera* extract increased in a dose-dependent manner. The ulcerated rats produced the lowest gastric mucus content while the animal group pre-treated with the combination of *P. guajava* and *A. vera* (500 mg/kg) and omeprazole (20 mg/kg) showed a significant increase in mucus weight (g) with respect to the ulcerated group as shown in Table 1.

Effect of a combination of P. guajava and A. vera pre-treatment, on lipid peroxidation (MDA) and antioxidant enzymes

The ulcer model rats revealed a major reduction in antioxidant (SOD, CAT, and GSH) endogenous enzyme activities. Rats pre-treated with a combination of *P. guajava* and *A. vera* and pre-treatment with omeprazole demonstrated an elevation of all antioxidant activities with respect to the ulcer control rats, as shown in Table 2. The CAT activity and SOD enzyme activities as well as GSH content were significantly ($P<0.05$) higher at a dose of 500 mg/kg of a combination of *P. guajava* and *A. vera* extract and omeprazole 20 mg/kg than in ulcer model rats. Also, the MDA content was significantly ($P<0.05$) lower at a dose of 500 mg/kg of a combination of *P. guajava* and *A. vera* extract and omeprazole 20 mg/kg compared to ulcer control rats.

Effect of a Combination of P. guajava and A. vera Pre-treatment, on Gastric Inflammatory Parameters

The ulcer control rats revealed a major increase in inflammatory cytokines (TNF-α, IFN- γ, IL-1β, IL-6). Rats pre-treated with a combination of *P. guajava* and *A. vera* and pre-treatment with omeprazole demonstrated a decrease in all inflammatory cytokines with respect to the ulcer control rats, as shown in Table 3. The levels of TNF-α, IFN-Y, IL-1β and IL-6 were significantly ($P<0.05$) lower at a dose of 500 mg/kg of a combination of *P. guajava* and *A. vera* extract and omeprazole 20 mg/kg than ulcer model rats (Table 3).

Discussion

The consumption of alcohol is recognized as a major factor contributing to gastric ulcers in humans; therefore, researchers have utilized an animal model of gastric injury induced by ethanol to replicate the conditions humans may face, allowing them to investigate the anti-ulcer effects of natural products or novel therapies aimed at providing gastric protection [19]. Oral administration of absolute ethanol in the animal model is destructive to stomach tissue, since it penetrates rapidly and easily into the gastric mucosa, producing gastric lesions [19] thus, making it useful in determining the anti-ulcer capacity of drugs, as well as the likely pathways involved in this process [20]. Such lesions are characterized by extensive sub-mucosal edema, hemorrhage, desquamation of epithelial cells and infiltration of inflammatory cells, which are typical characteristics of alcohol-induced damage in humans [19].

In this study, a gastric ulcer model was created using 95% ethanol (5 ml/kg). Ethanol is a common cause of gastric ulcers and induces gastric damage through multiple mechanisms, including dehydration, which impairs the mucosal cell barriers and has cytotoxic effects. This cytotoxicity leads to the activation of leukocytes that release reactive oxygen species (ROS) and inflammatory cytokines, both of which can contribute to cell death.

P. guajava, and *A. vera* are natural plants that have shown protective, antioxidant, and anti-inflammatory properties in various studies conducted in different in vivo models over periods of 2 weeks or 21 days; hence, a duration of 21 days was chosen for this investigation [13]. Three treatment doses were chosen based on prior studies, and the dose-response relationships for anti-gastric ulcer effects were evaluated in rats with ethanol-induced gastritis that received oral treatment with 125, 250, and 500 mg/kg of a blend of *P. guajava* and *A. vera* over 21 days. Our findings indicated that among the three dosage levels of the *P. guajava and A. vera* combination, 500 mg/kg exhibited the strongest protective effect against gastric ulcers. The elevated gastric ulcer index (GUI) in rats exposed to ethanol underscores the significant ulcerogenic potential of ethanol, which aligns with existing research [21]. The marked decrease in the gastric ulcer index (GUI) and the improved protective index (PI) resulting from the combination of *P. guajava* and *A. vera* indicate a strong anti-ulcer effect that rivals that of omeprazole, a widely recognized anti-ulcer medication. Additionally, pre-treating with a combination of *P. guajava, A. vera*, and omeprazole led to fewer gastric lesions in the gastric wall mucosa; it also resulted in increased mucus levels in ethanol-treated rats, likely due to heightened production of prostaglandin E2 in the gastric mucosa. Moreover, we investigated the role of inflammatory cytokines, lipid peroxidation, and antioxidant enzyme pathways to understand the mechanisms behind the anti-ulcer effects of the *P. guajava* and *A. vera* combination. The administration of ethanol prompts the infiltration of neutrophils; It triggers the immune response of T lymphocytes, leading to a chain reaction of inflammation, oxidative stress, and the death of epithelial cells. A significant increase in the levels of inflammatory cytokines, such as TNF-α, IL-6, and IL-1β can cause considerable damage to the gastrointestinal mucosa, worsening the level of inflammation in the human body [22]. In this study, we observed that pre-treatment with a combination of *P. guajava, A. vera,* and omeprazole resulted in reduced production of inflammatory cytokines. During the occurrence of gastric ulcers, macrophages produce TNF-α, hindering the healing process in various ways, which include reducing gastric microcirculation, promoting neutrophil infiltration, and triggering inflammatory signaling pathways, leading to the production of additional inflammatory cytokines that further amplify its production [19]. IL-6, IFN-γ, and IL-1β are pro-inflammatory cytokines that play a crucial role in the process of acute inflammation. Elevated levels of these cytokines activate neutrophils, macrophages, and lymphocytes at the inflammation site, enhancing the production of inflammatory mediators and worsening gastric mucosal injury [23]. In line with earlier studies, [24] our findings demonstrated that the oral intake of ethanol initiates a localized inflammatory response, raising the levels of inflammatory cytokines in the gastric tissue. Nevertheless, the pre-treatment with a combination of *P. guajava* and *A. vera* along with omeprazole mitigated this detrimental effect, resulting in decreased levels of the inflammatory markers TNF-α, IL-6, IL-1β, and IFN-γ.

Alcohol administration leads to the degradation of the gastric wall mucosal barrier through the action of reactive oxygen species (ROS) and the up-regulation of cytokines, which causes damage due to oxidative stress [25]. In this research, intubation with ethanol resulted in significant oxidative-stress-related damage in rats suffering from ethanol-induced ulcers; this finding aligns with prior studies [26]. An imbalance in the redox state between the generation of reactive oxygen species (ROS) and the scavenging action of antioxidants can lead to oxidative stress, primarily driven by infiltrating inflammatory cells as the major source of ROS [20]. The superoxide radical anions ($O2-$), produced by neutrophils, react with lipids, leading to lipid peroxidation [20]. This investigation

showed that the redox balance was disrupted due to elevated lipid peroxidation (measured as MDA), along with a reduction in antioxidant enzymes like GSH, SOD, and CAT in the gastric tissues with ulcers. The rise in MDA levels was linked to an increase in reactive oxygen species (ROS) alongside a reduction in antioxidant enzymes [20]. The combination of *P. guajava* and *A. vera*, along with omeprazole pre-treatment, notably reduced lipid peroxidation (measured as MDA) and restored the diminished levels of GSH, SOD, and catalase activity in the ulcerated gastric mucosa. The pairing of *P. guajava* and *A. vera* exhibits significant anti-lipid peroxidation properties and the ability to scavenge free radicals [20]. This finding suggests that plant extracts can boost antioxidant activity, likely due to the presence of antioxidant phytochemicals in these plants. Both *P. guajava* and *A. vera* are abundant in phenolic compounds, which may contribute to the improved antioxidant effects. Research indicates that intake of antioxidant-rich phenolic compounds is linked to the prevention of chronic illnesses such as cancer, diabetes, and cardiovascular diseases. This study indicates that phenolic compounds have positive effects on gastro-protective activity [26]. This research has potential limitations. While the study observed the gastro-protective effects of a combination of *P. guajava* and *A. vera,* the underlying mechanism of action was not explored in depth. Additional studies, such as mechanistic investigations are necessary to understand the specific pathways. Also, the study utilized a rat model to simulate ethanol-induced gastric ulcers. While animal models provide valuable insights, there may be differences in the response to treatment between rats and humans, limiting the direct translation of findings to clinical applications.

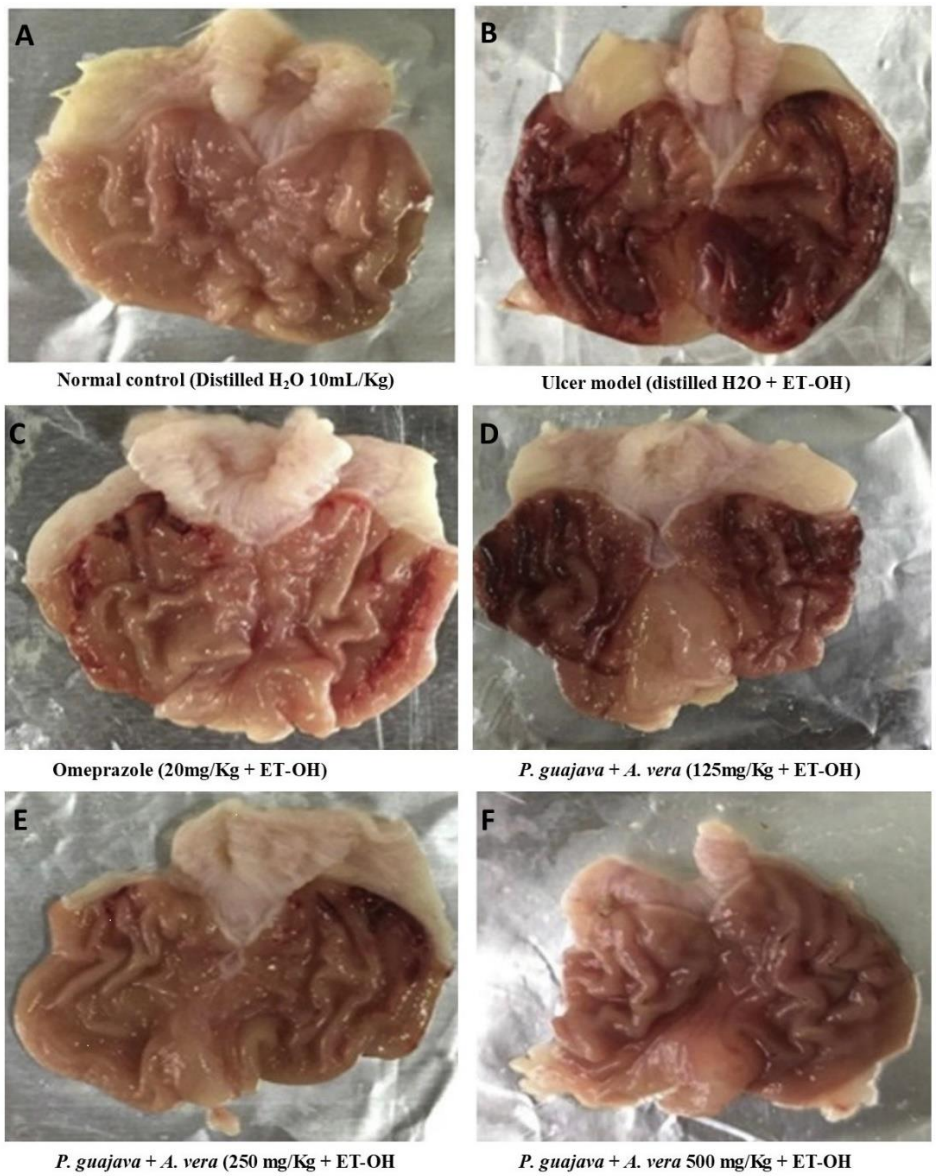

Figure 1. Effect of the combination of P. guajava and A. vera on morphological aspect of the stomach

Table 1. Effect of a combination of *P. guajava* and *A. vera* on Gastric ulcer index, Gastric protective index and Gastric mucus weight.

Groups	Parameters				
	Gastric Ulcer Index		Gastric Protective Index	Gastric Mucus (g)	
	Mean ± SD	**P-value (Dunnett)**		**Mean** ± SD	**P-value (Dunnett)**
Omeprazole 20mg/Kg + ET-OH	1.1±0.53	0.29	67±7.3	1.8±0.84*	0.024
P. guajava+*A. vera* 125mg/Kg + ET-OH	3.2±2.78	0.97	2.6±49	1.2±0.45	0.368
P. guajava+*A. vera* 250mg/Kg + ET-OH	2.53±2.95	0.96	24±27	1.4±0.55	0.168
P. guajava+*A. vera* 500mg/Kg + ET-OH	1±0.0	0.22	68±9.7	1.8±0.84*	0.024
Ulcer model (distilled H2O +ET-OH)	3.3±2.06	/	0.0±32	0.46±0.11	/
Normal control (Distilled H_2O 10mL/Kg)	0±0.0*	0.039	100±0.0	2.4±1.14*	0.0007

Data are expressed as mean ± SD, n=5; *denotes significant difference with ethanol treated group, p<0.05 using ANOVA followed by Dunnett's *post hoc* test. GUI: Gastric ulcer index, GPI: Gastric protective index and GM: Gastric mucus weight, ET-OH: Ethanol.

Table 2. Effect of the combination of *P. guajava* and *A. vera* on oxidative stress

Groups	Oxidative Stress Parameters							
	MDA (μmol/g tissue)		GSH (U/mg tissue		CAT (U/mg tissue)		SOD (U/mg tissue)	
	Mean ± SD	**P-value (Dunnett)**	**Mean** ± SD	**P-value (Dunnett)**	**Mean** ± SD	**P-value (Dunnett)**	**Mean** ± SD	**p-value (Dunnett)**
Omeprazole 20mg/Kg+ ET-OH.	11.52±1.81*	<0.0001	3.53±0.47*	<0.0001	0.427±0.05*	<0.0001	25.39±0.77*	0.0037
P. guajava+*A. vera* 125mg/Kg+ ET-OH.	20.44±2.98	0.08	1.4±0.15	0.90	0.295±0.02	0.106	19.13±1.29	0.976
P. guajava+*A. vera* 250mg/Kg+ ET-OH.	17.6±2.20	0.0007	2.15±0.10	0.003	0.323±0.08	0.016	21.56±2.02	0.294
P. guajava+*A. vera* 500mg/Kg+ ET-OH.	11.38±2.54*	<0.0001	3.43±0.48*	<0.0001	0.403±0.03*	<0.0001	25.93±4.56*	0.0018
Ulcer model (distilled H2O +ET-OH)	24.13±1.09	/	1.19±0.12	/	0.213±0.05	/	17.91±0.90	/
Normal control (Distilled H_2O 10mL/Kg)	11.87±0.72*	<0.0001	3.33±0.49*	<0.0001	0.433±0.091*	<0.0001	25.86±7.24*	0.0019

Data are expressed as mean ± SD, n=5; *denotes significant difference with ethanol treated group, p<0.05 using ANOVA followed by Dunnett's *post hoc* test. MDA: Malondialdehyde, GSH: Reduced glutathione, SOD: Superoxide dismutase and CAT: catalase, ET-OH: Ethanol.

Table 3. Effect of the combination of *P. guajava* and *A. vera* pre-treatment on gastric tissue cytokine profile

Groups	Cytokines Profile							
	TNF-α (pg/mL)		IFN-γ (pg/mL)		IL-1β (pg/mL)		IL-6 (pg/mL)	
	Mean ± SD	**P-value (Dunnett)**	**Mean** ± SD	**P-value (Dunnett)**	**Mean** ± SD	**P-value (Dunnett)**	**Mean** ± SD	**P-value (Dunnett)**
Omeprazole 20mg/Kg+ET-OH.	134.1±7.50*	<0.0001	121.2±19.62*	<0.0001	124.6±7.67*	<0.0001	283.3±5.66*	<0.0001
P. guajava+*A. Vera* 125mg/Kg+ET-OH.	210.5±15.77	<0.0001	269.8±22.31	0.0002	232.3±9.62	<0.0001	324.4±25.64	<0.0001
P. guajava+*A. vera* 250mg/Kg+ET-OH.	200.3±31.41	<0.0001	223.4±54.41	<0.0001	166.9±14.74	<0.0001	305.3±23.11	<0.0001
P. guajava+*A. vera* 500mg/Kg+ET-OH.	135.3±8.81*	<0.0001	109.9±17.39*	<0.0001	124.4±11.76*	<0.0001	280.3±6.66*	<0.0001
Ulcer model (distilled H2O +ET-OH)	326.7±17.97	/	341.0±9.58	/	284.2±23.75	/	443.9±12.87	/
Normal control (Distilled H_2O 10mL/Kg)	134.5±24.03*	<0.0001	114.7±11.87*	<0.0001	123.4±9.18	<0.0001	281.4±16.94 *	<0.0001

Data are expressed as mean ± SD, n=5; *denotes significant difference with ethanol treated group, p<0.05 using ANOVA followed by Dunnett's *post hoc* test. TNF-α: Tumor necrosis factor alpha, IL-6: interleukin 6, IL-1β: interleukin 1beta, IFN-γ: interferon gamma, ET-OH: Ethanol.

Conclusion

The findings of the current research indicated that the combination of *P. guajava* and *A. vera* at a dosage of 500 mg/kg reduced ethanol-induced gastric ulcers through its antioxidant and anti-inflammatory properties. The gastro-protective capability of *P. guajava* and *A. vera* may be linked to the rich presence of phytochemical compounds such as total polyphenols, flavonoids, and tannins. Consequently, *P. guajava* and *A. vera* may serve as a promising anti-ulcer agent for treating gastric ulcers, demonstrating an anti-ulcer effect similar to that of omeprazole. Nevertheless, additional studies are necessary to further investigate the underlying mechanisms of action.

Abbreviations

A. vera: Aloe vera
CAT: Catalase
GSH: Reduced Glutathione
H. pylori: Helicobacter pylori
MDA: Malondialdehyde
NSAIDs: Non-steroidal anti-inflammatory drugs
P. guajava: Psidium guajava,
PGs: Prostaglandins
PPIs: Proton pump inhibitors
PI: Protective index
SOD: Superoxide Dimutase
UI: Ulcer index

Authors' Contribution

TTM: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing; JTK: Conceptualization, supervision, validation, resources, Visualization, Project administration, Writing – original draft, Writing – review & editing; FDTB: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing; EN: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing; AFK: Conceptualization, Investigation, Methodology, Resources, Formal analysis, Validation, Supervision, Visualization, Project administration, Writing – original draft, Writing – review & editing; EAA: Conceptualization, supervision, validation, resources, Visualization, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

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