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Divulging the Antiulcer Potential of Muthuchippi Parpam: An *In Vitro* Approach

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Abstract: Ulcers are primarily caused by an imbalance between aggressive factors like gastric juice and protective factors such as the mucosal lining. Conventional treatments, such as proton pump inhibitors (PPIs) and antacids, have limitations due to potential side effects and recurrence of ulcers. *Muthuchippi Parpam*, a traditional Siddha medicine, is known for its therapeutic applications in gastrointestinal disorders. In this study we evaluated the *in vitro* anti-ulcer activity of *Muthuchippi Parpam* by assessing its acid-neutralizing capacity (ANC) and its inhibitory effect on the H^+/K^+ ATPase enzyme. For the evaluation of Acid Neutralizing Capacity (ANC), various concentrations (control, 1250 mg, 2500 mg, 5000 mg) of *Muthuchippi Parpam* were titrated with 0.5 N NaOH after reacting with 1.0 N HCl, and the mEq of acid consumed was calculated. For the evaluation of ATPase Inhibitory Assay, different concentrations of *Muthuchippi Parpam* (control, 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg, 1 mg) were incubated with goat parietal cell extract, followed by the addition of ATP. The release of inorganic phosphate, indicating enzyme activity, was measured spectrophotometrically at 660 nm. *Muthuchippi Parpam* showed a dose-dependent reduction in ANC, with the highest concentration (5000 mg) neutralizing 21.4 mEq of acid. The maximum ATPase inhibition observed for *Muthuchippi Parpam* was 49.56% at 1 mg. *Muthuchippi Parpam* demonstrated significant *in vitro* anti-ulcer activity through both acid-neutralizing and ATPase inhibitory mechanisms. These findings suggest that *Muthuchippi Parpam* has potential as a natural and effective anti-ulcer agent, warranting further investigation and integration into modern therapeutic regimens.

Keywords: *Muthuchippi Parpam*, ATPase, Acid Neutralizing Capacity, Ulcers

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Introduction

Anti-ulcer action refers to the ability of a substance, to prevent or alleviate the formation of ulcers in the stomach or intestines. This action typically involves reducing stomach acid production, protecting the stomach lining, or inhibiting the activity of factors that contribute to

ulcer formation, such as *Helicobacter pylori* bacteria or excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) (Woolf and Rose, 2024). *Muthuchippi Parpam* is a traditional Siddha medicine formulation renowned for its therapeutic applications, particularly in the treatment of gastrointestinal disorders such as ulcers (Choudhary *et al*., 2010; Ocasio Quinones and Woolf, 2024). *Muthuchippi Parpam* is derived from pearl oyster shell, the oyster shell contains calcium carbonate at 95% along with shell proteins 0.1–5%. This calcium-based preparation has been used historically in Siddha practice to mitigate various ailments, owing to its potent bioactive properties (Singh *et al*., 2009).

Ulcers, characterized by open sores in the lining of the stomach or duodenum, are primarily caused by an imbalance between aggressive factors like stomach acid and protective factors such as mucosal lining. Conventional treatment strategies for ulcers often involve the use of proton pump inhibitors (PPIs), H_2-receptor antagonists, and antacids, which aim to reduce gastric acidity and promote healing. However, the long-term use of these medications can lead to significant side effects and the recurrence of ulcers (Malik, 2024). This study aims to evaluate the *in vitro* anti-ulcer activity of *Muthuchippi Parpam* through two primary mechanisms: acid-neutralizing activity and ATPase inhibitory activity. By investigating these pathways, we seek to elucidate the potential of *Muthuchippi Parpam* as an effective and natural anti-ulcerogenic agent (Pandian, 2021). Understanding these mechanisms will not only affirm traditional knowledge but also pave the way for integration of these formulations into contemporary ulcer treatment.

Materials and Methods

Ingredients of Muthuchippi Parpam:

1. *Pinctada fucata*
2. *Aloe vera*
3. *Alternanthera sessilis*

Method of Preparation:

The marine drug *Muthuchippi* underwent a precise purification process followed by traditional formulation mentioned in the literature after proper identification and authentication (Veeraperumal Pillai, 1999).

Acid Neutralizing Capacity:

Take 1250 mg, 2500 mg and 5000 mg of *Muthuchippi Parpam* were mixed separately with distilled water to make a total volume of 70 ml. Stirred each mixture for 1 min. Then, added 1.0 N HCl (2 ml HCl +25 ml distilled water) to each 70 ml mixture. Stirred the solutions for 15 min. Then add a few drops of phenolphthalein indicator and stirred. Immediately titrated the excess HCl in each mixture with 0.5 N NaOH. Continued titration until a stable pH of 3.5 was maintained for 10 to 15 sec. Recorded the volume of 0.5 N NaOH used (V NaOH). Then, using the following formula, the number of mEq of acid consumed was determined:

Acid consumed in total (mEq) = (30×N HCl) – (V NaOH×N NaOH)

Where, N HCl – Normality of HCl; N NaOH – Normality of NaOH; V NaOH - volume of NaOH used for titration.

ANC values are reported as mEq of acid consumed by 5 ml of standard as well as test preparation (Garad, 2012).

ATPase Inhibitory Assay:

To assess the inhibition of H^+/K^+-ATPase, the following experimental procedure was employed. The enzyme used in the study was derived from goat parietal cell extract, with a concentration of 300 µg per 0.1 ml. The test drug *Muthuchippi Parpam* was tested at various concentrations (control, 0.2 mg, 0.4 mg, 0.60 mg, 0.8 mg, 1 mg) to evaluate its inhibitory effects. The enzyme (0.1 ml of goat parietal cell extract, 300 µg) was pre-incubated with *Muthuchippi Parpam* at different concentrations for 60 min at 37 ºC. The reaction was initiated by adding 200 µL of 2 mM ATP to the mixture. Additionally, 200 µL of 2 mM MgCl2 and 200 µl of 10 mM KCl were added to the reaction

Table 1: Acid neutralizing capacity of *Muthuchippi Parpam*

S. No.	CONCENTRATION	VOLUME	RESULTS	ANC per Gram
1.	CONTROL	35 ml	12.00 mEq	1200
2.	1250 mg	7.2 ml	18.35 mEq	14.6
3.	2500 mg	13.3 ml	19.75 mEq	7.9
4.	5000 mg	12.5 ml	21.4 mEq	4.28

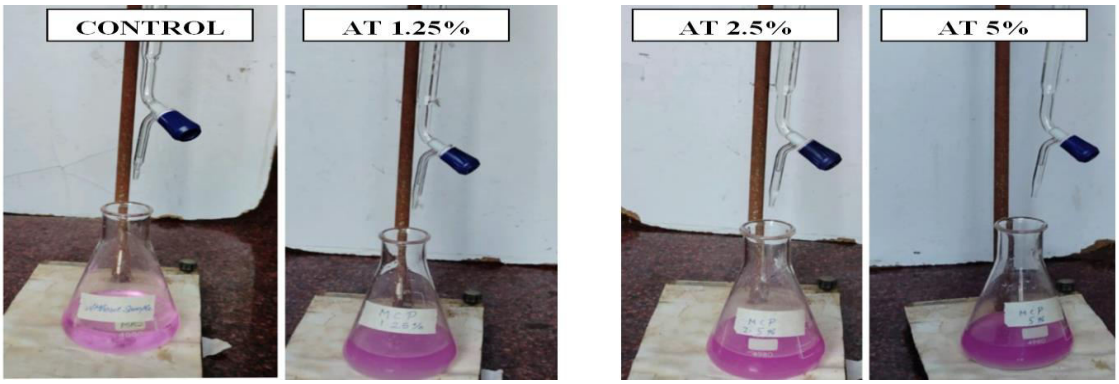

Fig. 1: Acid neutralizing capacity of *Muthuchippi Parpam.*

Table 2: ATPase Inhibitory Assay of *Muthuchippi Parpam*

Concentration	Optical Density	Results
control	0.569	0.00
0.2 mg	0.501	11.95
0.4 mg	0.452	20.56
0.6 mg	0.354	37.79
0.8 mg	0.314	44.82
1 mg	0.287	49.56

mixture. The combination underwent a 30 min incubation period at 37 ºC. The reaction was stopped by adding 0.5 ml of 4.5% ammonium molybdate. Following this, 0.5 ml of 60% perchloric acid was added. After that, the mixture was centrifuged for 10 min at 2000 rpm. The release of inorganic phosphate, which indicates enzyme activity, was measured using a spectrophotometer at 660 nm, following the Fiske and Subbarow (1925) method.

The percentage of enzyme inhibition was calculated using the formula:

Percentage of inhibition = (Activity (control) - Activity (test)/Activity (control))×10

By following this protocol, the inhibitory effect of various concentrations of *Muthuchippi Parpam* on H^+/K^+-ATPase activity can be accurately determined (Gupta, 2013).

Results

Acid Neutralizing Capacity:

The Acid Neutralizing Capacity of *Muthuchippi Parpam* was studied for concentration (control, 1250 mg, 2500 mg, 5000 mg) and standard Aluminium Hydroxide + Magnesium Hydroxide ($Al(OH)_3$+$Mg(OH)_2$) (500mg). The results obtained envisage that *Muthuchippi Parpam* at concentrations control, 1250 mg, 2500 mg, and 5000 mg showed a significant reduction in acid neutralizing capacity (ANC), i.e., 1200, 14.6, 7.9, and 4.28, respectively (Table 1), as compared to standard

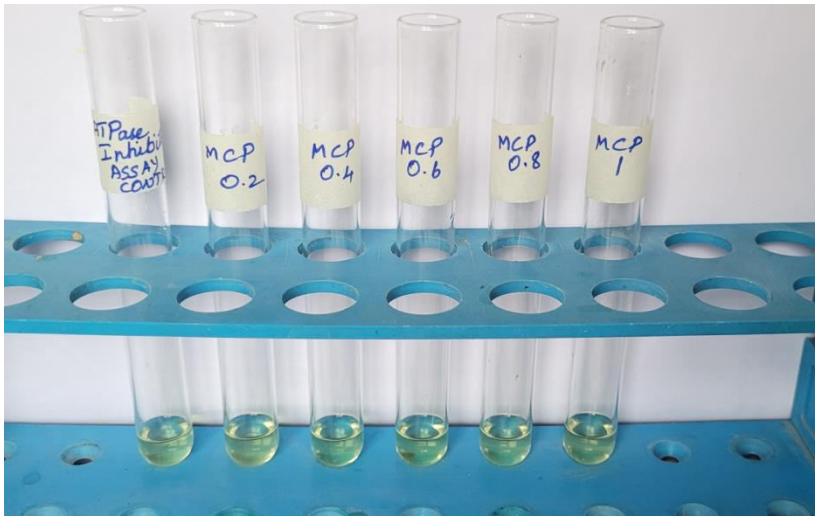

Fig. 2:- ATPase inhibiting activity of *Muthuchippi Parpam.*

$Al(OH)_3$+$Mg(OH)_2$ (500 mg) which is 15 (Carolin, 2024). Initially, the ANC value of the standard antacids mixture was determined for the comparison with ANC value (Carolin, 2024). *Muthuchippi Parpam* at a concentration of 5000 mg has been found to neutralize acid significantly as compared to standard (Fig. 1).

ATPase Inhibitory Assay of Muthuchippi Parpam:

The H^+/K^+-ATPase inhibition activity of *Muthuchippi Parpam* at a various concentration (control, 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg, 1 mg) was compared with Omeprazole at the concentration (20 µg, 40 µg, 60 µg, 80 µg, and 100 µg) considered as standard obtained from previous studies (Sumia *et al.*, 2019). Significant action was demonstrated in a dose-related way. Maximum percentage inhibition observed for *Muthuchippi Parpam* was 49.56% at a concentration of 100 µg (Table 2; Fig. 2).

Discussion

Antacids are alkaline medications that counteract the acidic nature of the stomach and work as a buffer to keep the pH of the gastric juice stable. They are classified into two groups: non-absorbable chemicals like magnesium hydroxide ($Mg(OH)_2$), aluminum phosphate ($AlPO_4$), and aluminum hydroxide ($Al(OH)_3$), and absorbable compounds like sodium bicarbonate, calcium carbonate ($CaCO_3$), and magnesium carbonate ($MgCO_3$) (Sumiaa *et al.*, 2018). The *in vitro* method to determine the acid-neutralizing capacity (ANC) of an antacid involves measuring the amount of acid that the antacid can neutralize. This is typically done through back titration, a process where the antacid is first reacted with an excess amount of acid, and then the remaining acid is titrated with a base to determine how much acid was neutralized by the antacid (Yafout *et al.*, 2022). For many years, antacids have been proven to be beneficial in treating GERD and gastric and duodenal ulcers. They have not been shown to directly affect erosive lesions, but they can neutralize the excess of hydrochloric acid in the gastric juice, which lowers pepsin activity, speeds up the healing process, and provides quick relief from acid reflux and heartburn. In this study, *Muthuchippi Parpam* at a concentration of 5000 mg demonstrated a significant reduction in ANC by 21.4 units, indicating its effectiveness in neutralizing excessive stomach acid.

The enzyme H^+/K^+-ATPase, also known as the proton pump, plays a critical role in producing gastric acid. This enzyme is located on the apical membrane of the parietal cells in the stomach lining. It works by exchanging potassium ions (K^+) from the stomach with hydrogen ions (H^+) from the parietal cells, thereby secreting hydrochloric

acid (HCl) into the stomach (Ahmed and Clarke, 2024). In this study, we assessed the ability of *Muthuchippi Parpam* to inhibit the H^+/K^+-ATPase enzyme. Omeprazole, a well-known proton pump inhibitor (PPI), was used as a reference drug (Ahmed and Clarke, 2024). Based on previous studies, omeprazole at 20 µg, the result was 52.09; for 40 µg, the result was 58.62; at concentration of 60 µg yielded a result of 46.89; and at 80 µg, it was 60.89. Finally, at 100 µg, the result was 66.36 (Sumia *et al.f*, 2019).

Previous research demonstrated that *Muthuchippi* Shell contains 95% calcium carbonate (Ulagesan *et al*., 2022). Calcium carbonate, commonly used as an antacid which not only neutralizes stomach acid but also has an impact on gastrointestinal motility. When calcium carbonate is chewed and partially digested, the released calcium ions can stimulate peristalsis, which is the coordinated contraction and relaxation of muscles in the oesophagus and gastrointestinal tract. This increased peristaltic activity helps move the acid back into the stomach, thus alleviating heartburn symptoms by preventing acid reflux. The dual action of neutralizing excessive gastric acid and promoting motility makes calcium carbonate effective in managing symptoms of heartburn (Fritz *et al*., 2024). The study found that *Muthuchippi Parpam* inhibited the enzyme in a dose-dependent manner (control, 0.2 mg, 0.4 mg, 0.60 mg, 0.8 mg, 1 mg), which was compared with omeprazole at different concentrations in the previous study meaning that higher concentrations of the substances led to greater inhibition (Sumia *et al.*, 2019). This inhibition of H^+/K^+-ATPase by *Muthuchippi Parpam* suggests that it can effectively reduce gastric acid secretion, similar to omeprazole. This implies that *Muthuchippi Parpam* could be a potential treatment for conditions associated with excessive stomach acid, such as peptic ulcers duodenal ulcers, and gastroesophageal reflux disease (GERD).

Muthuchippi Parpam offers a promising alternative, leveraging its calcium content to neutralize gastric acid and its potential inhibitory effects on ATPase activity, a critical enzyme involved in acid secretion. The neutralization of stomach acid by calcium compounds in *Muthuchippi Parpam* can provide immediate symptomatic relief by reducing acidity. Moreover, the inhibition of ATPase activity can decrease the overall production of gastric acid, contributing to a sustained therapeutic effect.

Conclusion

Muthuchippi Parpam, a traditional Siddha medicine, exhibited notable *in vitro* anti-ulcer activity through acid-neutralizing and ATPase inhibitory mechanisms. These findings suggest that *Muthuchippi Parpam* will be a promising natural anti-ulcer agent, meriting further research and potential integration into modern therapeutic regimens for ulcer management.

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