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# *IN-VITRO* EVALUATION OF ANTIULCER ACTIVITY OF AQUEOUS EXTRACT OF *MALVASTRUM TRICUSPIDATUM*

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**Keywords:**

*Malvastrum tricuspidatum*, Anti-ulcer, Aqueous extract, Aluminium hydroxide

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**ABSTRACT:** There are several factors that may induce ulcers in human beings such as stress, chronic use of anti-inflammatory drugs, etc. Though in most cases, the etiology of ulcer is unknown, it has generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism. Thus, the search for a safe anti-ulcer drug that optimizes these properties is continuing, and part of the search is the evaluation of medicinal plants for gastroprotective properties. The *Malvastrum tricuspidatum* has used in folk medicine for the treatment of inflammation and gastrointestinal diseases. In this study, we assessed for anti-ulcer activities with aqueous extract and *in-vitro* method as the acid-neutralizing capacity and H+/K+ - ATPase inhibition activity method and in acid-neutralizing capacity (ANC), the extract significantly reduced ANC to 9.33 at a concentration of 1500 mg as compared to 15.7 with standard Aluminium hydroxide + Magnesium hydroxide (500mg). While in $H^+$ /$K^+$ - ATPase inhibition activity, the extract showed maximum percentage inhibition of 62.18% at the concentration 100µg as compared to 69.56% with standard Omeprazole.

**INTRODUCTION:** An ulcer is one of the common causes of hospital consultation with an increasing prevalence worldwide [1]. Peptic ulcer manifest as a non-fatal disease, majorly represented by recurrent symptoms of epigastric pain, which are often relieved by food or alkali, besides to trigger much discomfort to patients, disrupting their daily routines and also causing mental agony [2]. Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a substantial burden for health care resources [3].

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The mucosal injury occurs when these noxious factors destroy an intact mucosal layer or when it gets impaired [4]. The endogenous destructive elements in the stomach are HCl, pepsin, biliary reflux, lipid peroxidation [5]. Although the anti-ulcer drugs are available, which has many disadvantages; thus, we need a new search for anti-ulcer drugs with minimum side effects or no side effects [6]. This disease arises from acid peptic injury of the digestive tract, which results in mucosal breaks that reach the submucosal epithelium [7]. Ulcers were open wounds in the transparent skin or mucus membrane, or they may present internally in the digestive systems which have inflamed dead tissues in and around the ulcer [8] the peptic ulcers are erosion of the lining of the stomach or the duodenum [9]. The ulcers range from superficial epithelial damage to deeper abrasions, causing organ bleeding and perforation [10, 11].

The *H. pylori* infection and the use of non-steroidal anti-inflammatory drugs (NSAIDs) are the predominant causes of peptic ulcer disease [12]. There are two types of ulcers, namely peptic ulcers and duodenal ulcers. According to the site of presence, the adults sometimes have both abscess, the gastric ulcer is present in the stomach, characterized by pain, in older age people, it has shared. Eating spicy food substances increases the symptoms of ulcers; the other symptoms are nausea, vomiting, and weight loss. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur even in the complete absence of acid [13]. The prevalence of Peptic ulcers is now a day increasing among the population due to the unhealthy food habits of the people.

Peptic ulcer exists in two primary forms. First, the acute peptic ulcer does not extend when it penetrates to the lamina muscularis mucosa when compared to the submucosa. It is also occurring due to stress in the form of severe burns, curling's ulcer, and brain damage. In the chronic peptic ulcer, the muscularis propria has a full-thickness, and its base is in the serosal layer, which involves the organ to out with the gut altogether it includes the gastric and duodenal ulcers [14].

## MATERIALS AND METHODS:

**Processing of Plant Material:** The collected plant material has identified by Dr. V. Chelladurai, Research Officer (Retd.), Survey of Medicinal Plants Unit, CCRAS (Siddha), Govt. Siddha Medical College campus, Palayamkottai. A voucher specimen (Voucher No. ATC27/08/2005) has been deposited at the herbarium unit of the Department of Botany, Siddha Medical College, Palayamkottai, Tirunelveli (District), Tamilnadu, India. The plant was washed with tap water 3 times and sterilized by spraying with 70% alcohol.

The purified plant material was shade dried at room temperature to avoid chemical changes and frequently observed for any fungal contamination as the plant material rich in water content. When the plant material was dried entirely, it has subjected to prepare fine powder with the help of a pestle and motor. The fine material powder is collected and used for extraction of the crude drug in aqueous solvents by Soxhlet extraction method.

**Extraction by Soxhlet Apparatus:** Extraction by Soxhlet apparatus the extraction procedure for the isolation of crude drug from plants has been practiced for a long time. The mode of extraction process depends on the presence of water content of the plant materials that have been extracted by the type of substance that has been isolated. Usually, the crude extract has taken from the Soxhlet apparatus with the aqueous solvent. This apparatus mainly consists of three parts, a round bottom flask in which the solvent has taken, the main jar in which the material from which the compounds to been extracted has kept loaded, and a condenser in which condensation of vapours of solvents takes place. 100 g of the powder of plant material from which the extract has to take into packed into Soxhlet main jar. The solvent is poured into the round bottom flask and extract condensation under reduced pressure, and a controlled temperature of 60-80 °C has set to boil through the regulated heating mantle. The vapour of the solvent pass-through drive tubes enters the condenser through the main jar and gets condensed where there is a continuous flow of water in the condenser.

The condensed solvent falls back on the packed material in the main jar before collecting in a jar itself. The collection and extraction of material take place simultaneously in the main jar, as seen by the colouring of the solvent as a compound of material gets dissolved in the solvent. Thus, the crude plant material extract has been obtained, and it usually takes 7-8 h to complete an extraction. The solvent has evaporated, and finally, it yields green extract; this has been stored in the refrigerator for further usage.

### *In-vitro* Evaluation of Antiulcer Activity:

**Acid Neutralizing Capacity:** The aqueous extract of acid-neutralizing capacity value are 100mg, 500mg, 1000mg, 1500mg. The aluminium hydroxide and magnesium hydroxide (500mg) have compared for the standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1.0 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed. With 0.5N Sodium hydroxide, the excess HCl was

immediately titrated until the pink colour is attained [15]. The moles of acid neutralized is calculated by, Moles of acid neutralized = (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH) Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided by Grams of Antacid/Extract.

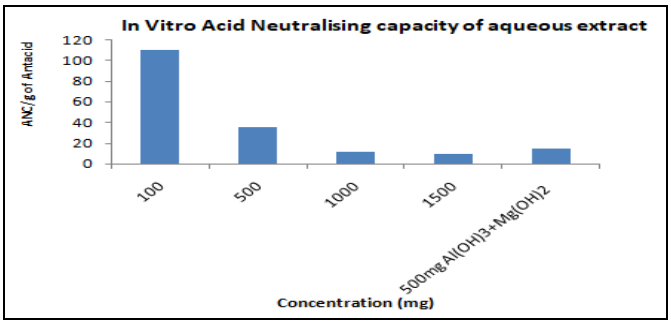
**H+/K+ - ATPase Inhibition Activity:** Preparation of H+/K+ - ATPase Enzyme: To prepare H+/K+ - ATPase enzyme sample the fresh goat stomach has purchased from the local slaughterhouse, the gastric mucosa of the fundus was cut-off and opened, the inner layer of the stomach has scrapped out for the parietal cell. The parietal cell obtained from the stomach has homogenized in 16mM Tris buffer with PH of 7.4, which has 10% Triton X-100 and centrifuged at 6000 rpm for 10mins after centrifuged the supernatant solution has used for the H+/K+- ATPase inhibition Protein content are used to find out according to Bradford's method were BSA are used for standard. Assessment of H+/K+ ATPase inhibition: Per-incubated for 60 min at 37 ºC for the reaction mixture of the sample containing 0.1ml of enzyme extract (300µg) and plant extract with different concentration (20µg, 40µg, 60µg, 80µg, 100µg).

The reaction was initiated by adding substrate 2 mM ATP (200µL), in addition to this 2mM $MgCl_2$ (200µL) and 10mM KCl (200µL) has added. After 30 min of incubation at 37 ºC the reaction was stopped by 4.5% ammonium molybdate, and 60% perchloric acid was added and centrifuged at 2000rpm for 10 min, and in spectrophotometrically inorganic phosphate was released and measured at 660nm by following the Fiske-Subbarow method. Briefly, at 10 min at room temperature, 1ml of supernatant 4ml of Millipore water, 1ml of 2.5% of ammonium molybdate, 0.4ml of ANSA was added. At 660nm inorganic phosphate, absorbance has been measured at various doses of the extract; the enzyme activity has been calculated as micromoles of Pi released per hour. Results were compared with the known anti-ulcer PPA inhibitor Omeprazole and expressed as Mean ± SEM [16] % enzyme inhibition has calculated using the formula:

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100

## RESULTS AND DISCUSSION:

**Acid Neutralizing Capacity:** The neutralizing effect of the aqueous extract was studied for four concentration (100mg, 500mg, 1000mg, 1500mg) and standard Aluminium Hydroxide + Magnesium Hydroxide [$Al(OH)_3$+$Mg(OH)_2$](500mg). The results obtained envisage that the extract at concentration 100mg, 500mg, 1000mg, and 1500mg showed a significant reduction in acid-neutralizing capacity (ANC), *i.e.*, 110.5, 35.5, 11.75, and 9.3, respectively, as compared to standard $Al(OH)_3$+$Mg(OH)_2$ (500 mg) which is 15.7. The extract at a concentration of 1500 mg has been found to neutralize acid more significantly as compared to standard. The results have tabulated in **Table** & **Graph 1**.




**GRAPH 1: EFFECT OF AQUEOUS EXTRACT OF ON ACID NEUTRALIZING CAPACITY**

**TABLE 1: EFFECT OF AQUEOUS EXTRACT OF ON ACID NEUTRALIZING CAPACITY**

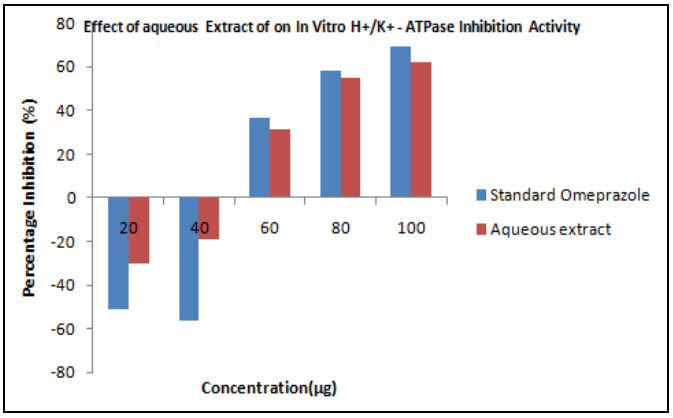
| S. no. | Concentration (mg) | Volume of NaOH consumed (ml) | mEq of Acid Consumed | ANC per gram of Antacid |
|---|---|---|---|---|
| 1 | 100 | 37.9 | 13.05 | 110.5 |
| 2 | 500 | 29.5 | 17.25 | 35.5 |
| 3 | 1000 | 39.5 | 9.75 | 11.75 |
| 4 | 1500 | 42 | 12 | 9.33 |
| 5 | 500mg $Al(OH)_3$+$Mg(OH)_2$ | 45.3 | 7.85 | 15.7 |

**H+/K+ - ATPase Inhibition Activity:** The H+/ K+ - ATPase inhibition activity of aqueous extract at a various concentration (20µg, 40µg, 60µg, 80µg, 100µg) has compared with Omeprazole as standard. The extract significantly showed activity in a dose-dependent manner. Maximum percentage inhibition of 62.18±0.54% has been observed for extract at a concentration of 100µg, and standard Omeprazole

showed 69.56±1.72%. The results have been tabulated in **Table 2** and **Graph 2**.

**TABLE 2: EFFECT OF AQUEOUS EXTRACT OF ON *IN-VITRO* H+/K+ - ATPase INHIBITION ACTIVITY**

| S. no. | Concentration (µg) | Percentage Inhibition (%) (Mean ± SEM) | |
|---|---|---|---|
| | | Standard Omeprazole | Aqueous extract |
| 1 | 20 | -51.25±0.78 | -30.12±0.26 |
| 2 | 40 | -56.32±1.24 | -18.84±1.86 |
| 3 | 60 | 36.58±1.58 | 31.64±0.68 |
| 4 | 80 | 58.62±0.24 | 55.36±1.54 |
| 5 | 100 | 69.56±1.72 | 62.18±0.54 |




**GRAPH 2: EFFECT OF AQUEOUS EXTRACT OF ON *IN VITRO* H+/K+ - ATPase INHIBITION ACTIVITY**

**DISCUSSION:** The peptic ulcer etiology was unknown in many cases, and its generally accepted due to the results from an imbalance aggressive between the factor and the mucosal integrity maintain through the endogenous defense mechanism [17]. Acidity is a common gastrointestinal problem attributed to a functional disorder that can result from a variety of reasons [18]. Excessive secretion of gastric acid or stomach acid (*i.e.*, HCl), inflames the stomach lining and produces ulceration [19]. Antacids act by neutralizing gastric acid and thereby reduce the gastric pH. The regain balance is maintained for the use of therapeutic agents differently for the use of gastric acid secretion inhibition or by increasing the mucosal production to boost the mucosal defense mechanism by stabilizing the surface epithelial cells or inhibition of prostaglandin synthesis [20]. The acid-neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize, and it has been measured by a process known as back titration. In ANC, the aqueous extract at 1500mg concentration showed a significant reduction in ANC of 9.33.

Hyperchlorhydria is a problem characterized by uncontrolled hypersecretion of hydrochloric acid from parietal cells of gastric mucosa through proton pump. H+/K+ - ATPase is a key enzyme in inducing acidity; it has located on the apical secretory membrane of parietal cells [21]. In H+/K+-ATPase inhibition activity, the extract showed maximum percentage inhibition of 62.18% at 100µg concentration.

The data reported here is indicative that the aqueous extract may possess an antacid, antisecretory, antiulcer property which may be due to the presence of compounds in the mixture. However, further studies are required to establish its exact mode of action and the active principles involved in its antiulcer effect.

**CONCLUSION:** On the basis of the results, we may conclude that the aqueous extract of the species may be considered as a sole source of novel antiulcer drugs. However, a detailed study on the isolation of active constituents from this species and its underlying mechanism of action responsible for its antiulcer effect is to be studied in the future.

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**CONFLICTS OF INTEREST:** The authors declare that there are no conflicts of interest.

**REFERENCES:**

1. Onoja SO, Chinagorom O, Ikpa CBC, Madubuike KG, Ezeigbo II, Ijioma SN, Anaga AO and Ezeja MI: Gastroprotective effects of methanol extract of *Eremomastax speciosa* leaf harvested in Southern part of Nigeria in rat. The EuroBiotech Journal 2018; 2(4): 200-08.
2. Hamedi S, Arian AA and Farzaei MH: Gastroprotective effect of aqueous stem bark extract of *Ziziphus jujuba* L. Against HCl/ethanol-induced gastric mucosal injury in rats. Journal of Traditional Chinese Medicine 2015; 35: 666-70.
3. Tanih NF, Ndip LM, Clarke AM and Ndip RN: An overview of pathogenesis and epidemiology of *Helicobacter pylori* infection. African Journal of Microbiology Research 2010; 4(6): 426-36.
4. Alauddin, Chaturvedi S, Azmi L, Shukla I, Naseem Z, Rao CV and Agarwal NK: Gastroprotective effect of formononetin against ethanol-induced gastric ulceration in rats via augmentation of cytoprotective markers and curtailing apoptotic gene expression. Pharmacognosy Magazine 2018; 14(59): 605-12.

5. Brito SA, de Almeida CLF and de Santana TI: Antiulcer activity and potential mechanism of action of the leaves of *spondias mombin* L. Hindawi. Oxidative Medicine and Cellular Longevity 2018; 1-20.
6. Lavnya A, Kumar MP, Anbu J, Anjana A and Ayyasay S: Antiulcer activity of *Canavalia virosa* (ROXB) W & A leaves in animal model. International Journal of Life Science Pharma Research 2012; 2(4): 39-43.
7. Lanas A and Chan FKL: Peptic ulcer disease. Lancet 2017; 390: 613-24.
8. Chan FKL and Graham DY: Prevention of non-steroidal anti-inflammatory drug gastrointestinal complications - review and recommendations based on risk assessment. Alimentary Pharmacology and Therapeutics 2004; 19(10): 1051-61.
9. Debjit B, Chiranjib C, Tripathi KK, Pankaj and Kumar KPS: Recent trends of treatment and medication peptic ulcerative disorder. International Journal of Pharm Tech Research 2010; 2(1): 970-80.
10. Lanas A and Chan FK: Peptic ulcer disease. Lancet 2017; 6736: 1-12.
11. Malfertheiner P and Schulz C: Peptic Ulcer: Chapter Closed? Digestive Disease 2020; 38: 112-16.
12. Pal A, Chinnaiyan SK, Mallik A and Bhattacharjee C: Anti-ulcer activity of leaves of *Averrhoa carambola* Linn. International Journal of Pharmacological Research 2019; 09(05): e5209.
13. Vyawahare NS, Deshmukh VV, Godkari MR and Kagathara VG: Plants with anti-ulcer activity. Pharmacognosy Review 2009; 3: 108-15.
14. McSween RMN and Whaley K: Muir's Textbook of Pathology, Ed 13, (ELBS Arnold, London,) 1992: 695-96.
15. Garad MC, Upadhya MA, Kokare DM and Itankar PR: Aerial parts of *Enicostemma littorale* Blume serve as antipyretic and antacid: *in-vivo* and *in-vitro* evaluations. Pharmacognosy Communication 2012; 2(3): 42-45.
16. Gupta RKP, Pradeepa and Hanumanthappa M: *In-vitro* antioxidant and H+, K+ - ATPase inhibition activities of *Acalypha wilkesiana* foliage extract. Journal of Pharmacy and BioAllied Sciences 2013; 5(3): 214-23.
17. Prasad K: Evaluation of antiulcer and *in-vitro* antioxidant activity of methanolic extract of *Psidium guajava* root in albino Wistar rats. International Journal of Phyto-pharmacology 2014; 5(1): 59-67.
18. Thabrew MI and Arawwawala LDAM: An overview of *in-vivo and in-vitro* models that can be used for evaluating Anti-Gastric ulcer potential of medicinal plants - Review Article. Austin Biology 2016; 1(2): 1-9.
19. Houshia OJ, Eid MA, Zaid O, Zaid M and Al-daqqa N: Assessment of the value of the antacid contents of selected Palestinian plants. American Journal of Chemistry 2012; 2(6): 322-25.
20. Sharath SS, Preethy J and Kumar GS: Screening for anti-ulcer activity of *Convolvulus pluricaulis* using pyloric ligation method in Wister rats. International Journal of Pharm Science Research 2015; 6(1): 89-99.
21. Yadav P, Ganeshpurkar A and Rai G: *In-vitro* H+/K+ - ATPase inhibitory potential of methanolic extract of *Cissus quadrangularis* Linn. Pharmacognosy Research 2012; 4(2): 123-26.


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