WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 5, 807-820. Research Article ISSN 2277–7105

IN-VITRO EVALUATION OF ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF MALVASTRUM CORAMANDELIANUM

V. Suresh*[1], S. K. Senthil Kumar[2], S. Logeshwari[3], P. Manochithra[3], M. S. Mohammed Muaaz[3], S. Naveenkumar[3] and T. Nevethini[3]

[1]Professor, Head of the Department of Pharmacology, Arunai College of Pharmacy, Velu Nagar, Tiruvannamalai-606 603.

[2]Principal, Head of the Department of Pharmaceutics, Arunai College of Pharmacy, Velu Nagar, Tiruvannamalai-606 603.

[3]Bachelor of Pharmacy, Arunai College of Pharmacy, Velu Nagar, Tiruvannamalai-606 603.

Article Received on
07 January 2025,

Revised on 27 Jan. 2025,
Accepted on 17 Feb. 2025

DOI: 10.20959/wjpr20255-35705

***Corresponding Author**
Dr. V. Suresh
Professor, Head of the Department of Pharmacology, Arunai College of Pharmacy, Velu Nagar, Tiruvannamalai-606 603.

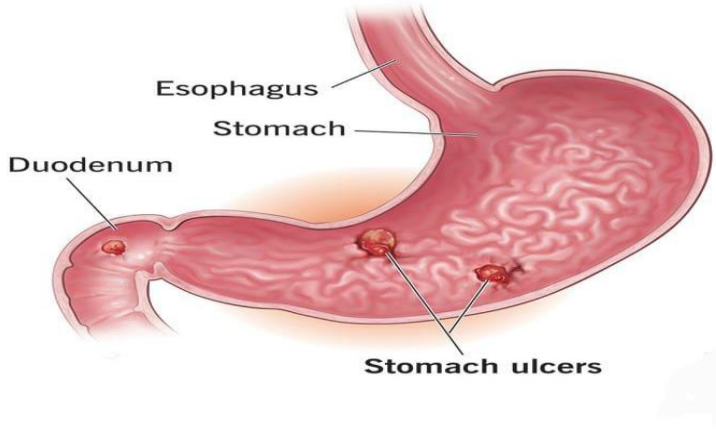
ABSTRACT

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 gastro protective properties. The *Malvastrum coromandelianum* 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, effects on artificial gastric acid neutralization and the neutralization duration capacity of a prepared preparation on artificial stomach acid, the extract significantly reduced ANC at a concentration of 1000 mg as compared with standard Aluminium hydroxide Magnesium hydroxide (500mg). While the effect on artificial gastric acid neutralization is nearly compare to the standard drug and the titration method of Fordtran's model for the determination of ANC value.

KEYWORDS: *Malvastrum coromandelianum***,** Anti-Ulcer, Aluminium hydroxide, Magnesium hydroxide.

INTRODUCTION

The word ulcer comes from the Latin word ulcers, which means ulcer or sore. Ulcer is a break on the skin, in the lining of organ, or on the surface of a tissue. An ulcer forms when the surface cells become inflamed, die, and are shed. Ulcer is a common disorder of the gastrointestinal system, which causes much discomfort to patients, disrupting their daily routines and causes mental agony. It is generally more common in those who keep themselves in hurry, become worry and consume curry. Peptic ulcer disease can be characterized by inflamed lesions or excavations of the mucosa and tissue that protect the gastrointestinal tract. Damage of mucus membrane which normally protects the esophagus, stomach and duodenum from gastric acid and pepsin causes peptic ulcer. Natural products from plants are a rich resource used for centuries to cure various ailments. The use of natural medicine in the treatment of various diseases like peptic ulcer is an absolute requirement of our time. Therefore, alternative approach in recent days is the research of medicaments from traditional medicine. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and relatively less toxic than the existing drugs. Diverse chemical compounds have been isolated from medicinal plants with antiulcer activity (Lewis and Hanson, 1991). This is an important reason to investigate antiulcer effects in medicinal plants with traditional use in gastric diseases.[1]



Pathologically, the definition of a peptic ulcer is straightforward: it is a defect in the gastric or duodenal wall that extends through the muscularis mucosae (The lowermost limit of the mucosa) into the deeper layers of the wall (Sub mucosa or the muscularis propria).1–3 It is

within these layers that the ulcer may erode a major blood vessel to produce the complication of potentially life-threatening hemorrhage. In contrast, erosions [which are often numerous in patients taking non-steroidal anti-inflammatory drugs (NSAIDs) or aspirin] are superficial lesions confined to the mucosa and carry minimal risk of major bleeding unless they are either very extensive or fail to heal and instead deepen to involve the sub mucosal layers, and thereby become an ulcer.[2]

The development of an ulcer is often the result of an imbalance between the protective mechanisms of the stomach lining and the aggressive factors that can damage it. One of the primary causes of ulcers is infection with the bacterium Helicobacter pylori (H. pylori), which can colonize the stomach lining and produce toxins that damage the mucosa. Another common cause of ulcers is the use of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin or ibuprofen, which can reduce blood flow to the stomach lining and impair its ability to protect itself against acid.[1]

In addition to H. pylori infection and NSAID use, other factors can contribute to the development of ulcers, including excessive acid production, stress, and genetic predisposition. When the stomach lining is damaged, the underlying tissue can become exposed to stomach acid, leading to further damage and inflammation. If left untreated, ulcers can lead to complications such as bleeding, perforation, and obstruction, which can be life-threatening. Treatment for ulcers typically involves a combination of medications, including antibiotics to eradicate H. pylori, proton pump inhibitors (PPIs) to reduce acid production, and cytoprotective agents to protect the mucosal lining. Lifestyle changes, such as avoiding NSAIDs, managing stress, and quitting smoking, can also help to promote healing and prevent future ulcers.[2]

Anti- ulcer medication is taken for a large variety of complaints, such as functional snonulcer dyspepsia, stomach upset, gastro-esophageal reflux, gastritis, and gastric or duodenal ulcer. Most dyspeptic symptoms are dealt with by the patient without seeking medical advice by using antacids. These drugs act via buffing the gastric pH and inactivating the major gastric protease pepsin. The highly potent, long-acting PPIs and H2-receptor antagonists can almost totally abolish acid secretion, although antisecretory therapy increasing the gastric pH above 3.0 is not necessary for ulcer healing.[1-2]

Plant profile

Latin name: *Malvastrum coromandelianum*

Family: Malvaceae.

Common names: False mallow, Broom weed, clock plant.

Synonyms: Malva tricuspidata, Malvastrum tricuspidatum, Malva coromandeliana, Malva coromandilina Linn, Malva tricuspidata R.Br, Malva luzonica Blanco Malva tricuspidatum A

Parts used: leaves.

Malvastrum coromandelianum

Description: An erect branching herb or under shrub, 0.6-0.9 m high. Stem petiole and main nerves on the lower surface of the leaf stellately hairy, with the hairs few branched, ascending or descending; hairs on the blade often simple. Leaves up to 6.5 cm long, ovate or ovate lanceolate, irregularly toothed. 5 nerved at the base, nerves impressed above, prominent beneath; petiole up to 18 mm long, not swollen near the base, flattened or slightly channeled above, densely hairy; stipules 5 mm long linear hairy, stipules 5 mm long linear hairy. Peduncles 12 mm long. Bracteoles 3, linear about half the length of the calyx. Calyx campanulate, cleft half way down lobes 5, triangular, acute. Corolla 12 mm across, pale yellow, exceeding the calyx. Staminal tube anthriferous to the top without sterile teeth. Styles as many as carpel; stigmas capitates. Carpels 8-12, reniform with 3 projecting spines, bristly between spines.[16]

MATRIALS AND METHOD

Processing of plant

The collected plant has identified by Dr.V.Suresh kumar, Assistant Professor, Dept of Botany, Government Arts College, Tiruvannamalai (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 (figure1a), it has subjected to prepare fine powder with help of pestle and motor (figure 1b).

The fine material powder is collected and used for extraction of the crude drug in solvent by soxhlet extraction method.

Figure1a: (Dried leaves).

Figure 1b: (Fine powder).

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.

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.

Approximately 30 g of the powder of plant material from which the extract has to take into packed into Soxhlet main jar. The solvent is poured in the round bottom flask and extract condensation under reduced pressure, and a controlled temperature of 60-80 ^{o}C has set to boil through the regulated heating mantle (figure 2). 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.[14]

Figure 2: Soxhlet apparatus extraction.

The condensed solvent falls back on 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 coloring 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.

Preparation of test solutions

MCE was prepared at concentration of 100, 500 and 1000mg/ml in ethanol were used for the experiment. These test solution were termed as MCE100, MCE500 and MCE1000, respectively. $Al(OH)_3Mg(OH)_2$ was dissolved in distilled water to make a 5% w/v solution and used as the active control.

Non-standard abbreviation

MCE- *Malvastrum coromandelianum* Extract

MCE100- MCE100mg/ml

MCE500-MCE500mg/ml

MCE1000-MCE1000mg/ml

$Al(OH)_3Mg(OH)_2$(500mg)- Aluminium hydroxide Magnesium hydroxide (1:1)

Conformation tests for crude extract

Chemical Constituents	Tests	Pet. Ether Extract	Chloroform extract	Hydro alcoholic extracts
Carbohydrates	Molish's test Benedicts test Feeling's test	-ve -ve -ve	-ve -ve -ve	-ve -ve - ve
Alkaloids	Mayers test	-ve	-ve	-ve

	Wagner's test Dragendroff's test Hager's test	-ve -ve -ve	-ve -ve -ve	-ve -ve -ve
Glycosides	Modified borntragger's test Legal's test	-ve -ve	+ve +ve	+ve +ve
Saponins	Froth test	+ve	+ve	+ve
Phytosterols and triterpenoids	Liberman Burchard test Salkowski's test (Steroid)	+ve +ve	+ve +ve	+ve +ve
Fats and oil	Stain test	+ve	+ve	+ve
Phenolic and Tannins	Ferric chloride test Lead acetate test	-ve -ve	-ve -ve	+ve +ve
Proteins and amino acids	Milon's test Biuret test Ninhydrin test	+ve -ve -ve	-ve -ve -ve	-ve -ve -ve
Test for flavonoids	Shinoda test	-ve	-ve	+ve

List of in-vitro preclinical models for evaluating anti-ulcer efficacy

1. Effect on pH at temperatures ranging from 25°C to 37°C
2. Effects on artificial gastric acid neutralisation
3. The neutralisation duration capacity of a prepared preparation on artificial stomach acid.
4. A titration method was used to assess neutralising capacity in vitro.

1. PH determination of test sample

The pH of MCE100, MCE500, MCE1000, $Al(OH)_3Mg(OH)_2$ and water was determined at temperature ranging from 25^o to 37^oC.**(**Graph 1)

2. Effects on artificial gastric acid neutralization

The artificial acid is created in the lab in this experimental model and its pH is set to be similar to that of genuine stomach acid, ranging from 1.2 to 3.2.

Procedure: 2 grams of sodium chloride and 3milligrams of pepsin-powder are dissolved in 500millilitres of distilled H2O to make artificial stomach acid juice. Prepare an HCl solution as well as enough water. The pH of the freshly prepared solution was modified to 1.2, which is a very acidic state. The formation of gastric ulcers is caused by a very high acidic condition, which damages the mucosal layer. If this pH is not managed, it will develop into more severe ulcers in the stomach.[8]

For assessing MCE100, MCE500, MCE1000, $Al(OH)_3Mg(OH)_2$ and water the medication for their neutralizing action on gastric acidity, both drugs were added individually to prepare

gastric juice with a low pH value of 1 to 2.4, and the neutralization impact was assessed using artificial gastric juice titration (Table 1).

3. Acid neutralizing capacity

The extract of acid-neutralizing capacity MCE100, MCE500, MCE1000. 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.[8]

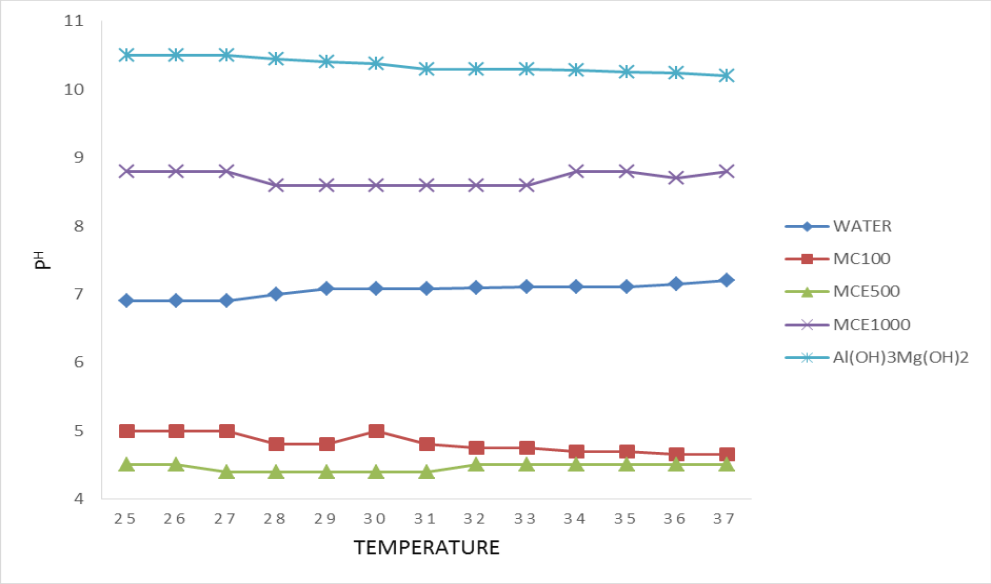
4. Using a Titration Method of Fordtran's Model for the Determination of Neutralizing Capacity In-vitro

250 ml beaker is taken and 90 ml of MCE100, MCE500, MCE1000, $Al(OH)_3Mg(OH)_2$ and water in different beakers are placed at 37˚C, then continuous stirring is done with the help of magnetic stirrer at 30 rpm for creating stomach movements or stomach environment. Then these MCE100, MCE500, MCE1000, Al(OH)3Mg(OH)2 and water are titrated separately with artificial gastric juice to the endpoint of pH 3. The consumed volume of artificial gastric juice is measured as a reference drug. We can use the combination of magnesium hydroxide and aluminium hydroxide.[8]

RESULT

1. Effect on pH at temperatures ranging from 25°C to 37°C

The pH values of MCE100, MCE500, MCE1000, $Al(OH)_3Mg(OH)_2$ and water at temperatures ranging from 25 °C to 37 °C were fairly constant indicating that temperature did not affect pH significantly and that sample was a good candidate for antacid activity (Graph 1).



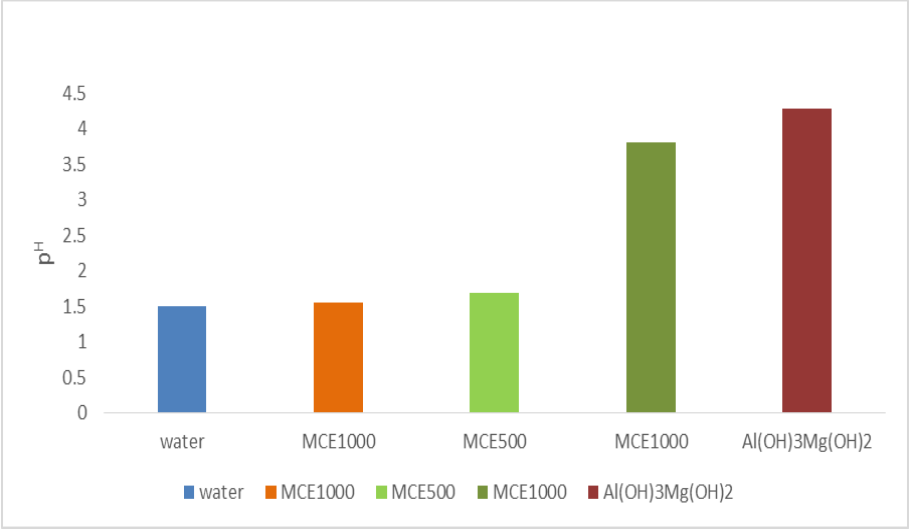
Graph 1: Effect on pH at temperatures ranging from 25 °C to 37 °C.

2. Effects on artificial gastric acid neutralization

The pH values of MCE100, MCE500, MCE1000, $Al(OH)_3Mg(OH)_2$ and water were found to be significantly higher than water indicating a significantly better neutralizing effect than water (Table 1)(graph 2).

Table 1: Effects on artificial gastric acid neutralization.

Drug	P^H
Water	1.5±0.1
MCE100	1.55±0.1
MCE500	1.69±0.1
MCE1000	3.81±0.1
Aluminum hydroxide Magnesium hydroxide	4.28±0.1



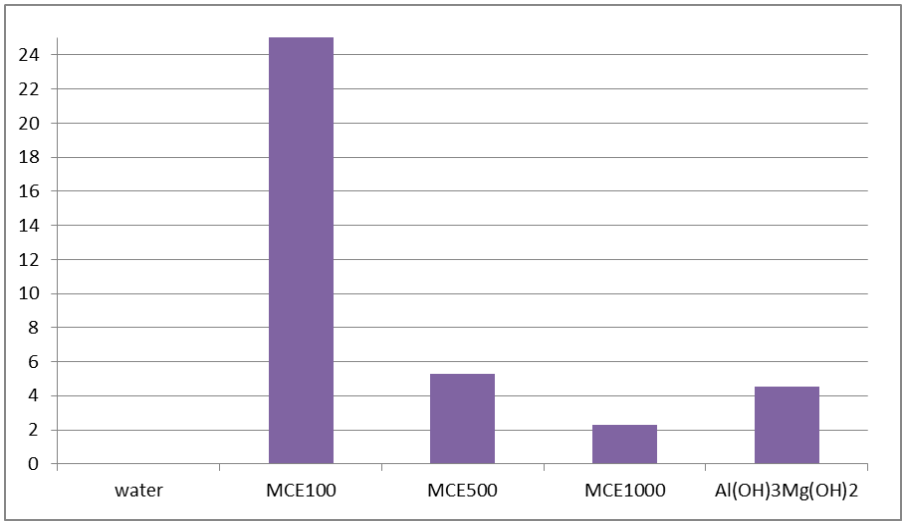
Graph 2: Effects on artificial gastric acid neutralization.

3. Acid neutralizing capacity

Acid neutralizing capacity: The neutralizing effect of the aqueous extract was studied for four concentration MCE100, MCE500, MCE1000 and water and standard Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2](500mg). The results obtained envisage that the extract at concentration MCE100, MCE500, MCE1000, showed a significant reduction in acidneutralizing 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 (Table2 & Graph 3).

Table 2: Acid neutralizing capacity.

S. No	Concentration(mg)	Volume of NaOH consumed (ml)	mEq of acid Consumed	ANC per gram of Antacid
1	Water(Blank)	5.55±0.01	2.25	-
2	MCE100	5.5±0.01	2.75	27.5
3	MCE500	5.29±0.01	2.64	5.28
4	MCE1000	4.6±0.01	2.3	2.3
5	$Al(OH)_3Mg(OH)_2$	4.53±0.01	2.265	4.53



Graph 3: Acid neutralizing capacity.

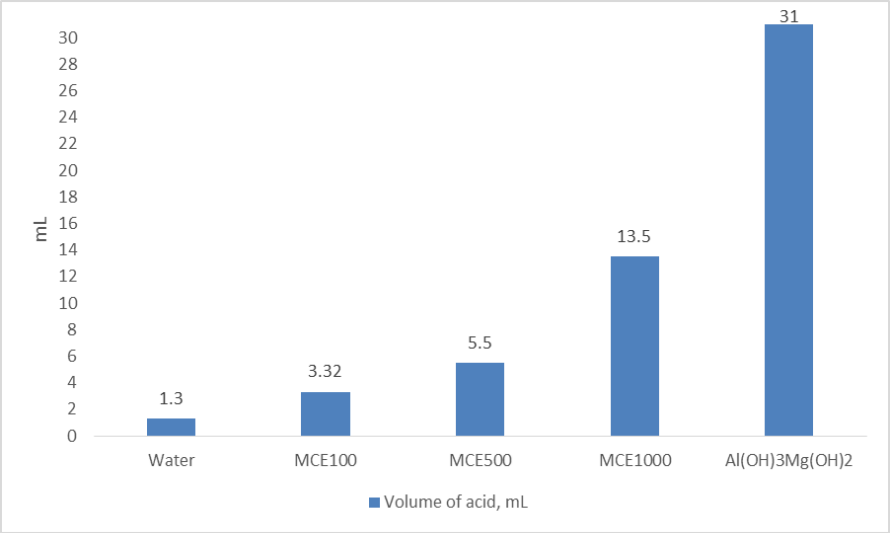
4. Using a Titration Method of Fordtran's Model for the Determination of Neutralizing Capacity In-vitro

The volume of artificial gastric juice consumed MCE100, MCE500, MCE1000, $Al(OH)_3Mg(OH)_2$ and water to titrate to pH 3.0 was significantly higher than water. All test solutions consumed higher H+ ions than water, thus exhibiting significant antacid activity.

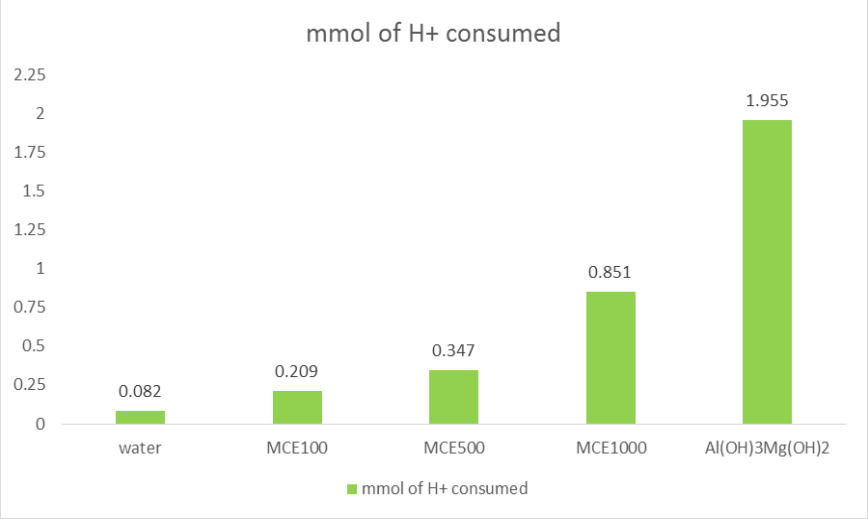
However, the neutralization capacity of MCE was lesser than $Al(OH)_3Mg(OH)_2$ (table 3 & graph 4a,4b).

Table 3: Titration Method of Fordtran's Model.

Drug	Consumed volume of gastric acid, mL	mmol of H+ consumed
Water	1.30±0.03	0.08202±0.0016
MCE100	3.32±0.03	0.20947±0.002
MCE500	5.50±0.01	0.34702±0.002
MCE1000	13.5±0.01	0.85179±0.002
$Al(OH)_3Mg(OH)_2$(500mg)	31.0±0.01	1.95597±0.002



Graph 4a: Consumed volume of gastric acid, mL, Titration Method of Fordtran's Model.



Graph 4b: mmol of acid consumed, Titration Method of Fordtran's Model.

DISCUSSION

The ulcer etiology was unknown in many cases, and it's generally accepted due to the results from an imbalance aggressive between the factor and the mucosal integrity maintain through the endogenous defense mechanism. 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. 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.

There are multiple phytoconstituents are present such as saponins, Phytosterols and triterpenoids, fats and oils, glycosides, tannins and flavonoids.

The acid-neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize. In ANC, the aqueous extract at MCE1000 concentration showed a significant reduction in ANC of 2.3.

In the Fordtran's method, the lag times before reaching selected pH values after introduction of the test solutions have been converted into antacid capacities (mmol H+) taking into account the amount of acid present in the simulated stomach at time 0 and the amount of acid introduced thereafter. All test solutions consumed significantly higher volumes of gastric acid and higher mmol of H+ than water, indicating good neutralizing capacities. $Al(OH)_3Mg(OH)_2$ due to their high alkalinity showed the best results.

The data reported here is indicative that the extract may possess an antacid, 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 ethanolic 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.

REFERENCE

1. Humphrey Rang, British pharmacological society, London, UK, on described what is pharmacology?, 2023; 07(1).
2. O.W. Holmes, Annual Address before the Massachusetts Medical Society, 1860.
3. H.P. Rang, B. Pharmacol, 2006; 147(2).
4. Goodman and Gilman's The Pharmacological basis of therapeutics, ed. L. Brunton, B.A Chaber and B. Knollman, MCGraw-Hill Education, 2011; 12(3).
5. Geoffrey M.currie, Faculty of Science, Charles Sturt University, Wagga wagga, New south Wales described as pharmacology, Part 1: Introduction to Pharmacodynamics.
6. Rang H, Dale M, Ritter J, Flower R. Rang and Dale's Pharmacology. London, U.K: Churchill Livingston, 2008; 6: 3-112.
7. Paul j. white, Margaret Cunningham, Steve Tucker described as Defining and unpacking the core concepts of pharmacology: A Global initative, 2023; 8.
8. Tayum Yana, Keserla Bhavani, and Savi Biswakarma described as A Review On Recent Invivo And Invitro Screening Methods For Antiulcer Activity.
9. Mishra, A. P., Bajpai, A., & Chandra, S. A Comprehensive Review on the Screening Models for the Pharmacological Assessment of Antiulcer Drugs. Current clinical pharmacology, 2019; 14(3): 175–196.
10. Sai Datri & Arige LRA. A review on pharmacological screening of anti-ulcer agents. International Journal of Medical Laboratory Research, 2017; 2(2456): 44–54.
11. Pahwa, R, N., Kumar, V, & Kohli, K. Clinical manifestations, causes and management strategies of peptic ulcer disease. International Journal of Pharmaceutical Sciences and Drug Research, 2010; 2(2): 99-106. Retrieved from
12. Lanas, A., & Chan, F. Peptic ulcer disease. Lancet (London, England), 2017; 390(10094): 613–624.
13. Adinortey MB, Ansah C, Galyuon I, Nyarko A. In vivo models used for evaluation of potential antigastroduodenal ulcer agents ulcers, 2013.
14. P. Pandian published described as invitro evaluation of antiulcer activity of aqueous extract of Malvastrum tricuspidatum, 2021; 1.
15. Abhinav Prasoon Mishra*, Ankit Bajpai and Suresh Chandra described as A Comprehensive Review on the Screening Models for the Pharmacological Assessment of Antiulcer Drugs.
16. http://www.hear.org/pier/species/malvastrum_coromandelianum.htm

17. Akshay R. Yadav*, Shrinivas K. Mohite described as Antioxidant Activity of *Malvastrum coromandelianum* Leaf extracts.
18. A Yadav, S Mohite - International Journal of Pharma Sciences and, 2020.
19. Haiphali Saxena, Dharmendra Singh Rawat, Pasumarti Bhaskara Rao Int. J. Pharmacog. Phytochem, 2020.
20. G Uddin, A Rashid - Fitoterapia, described as the genus *Malvastrum*, from the family Malvaceae, is a small genus of twenty four species, distributed worldwide, 2023.
21. Vandana Sanjeev Panda and Priyanka Mangesh Shinde described as A comparative study of the antacid effect of raw spinach juices and spinach extract in an artificial stomach model.
22. Gupta, M. B., Tangri, K. K., & Bhargava, K. P. Mechanism of ulcerogenic activity of reserpine in albino rats. European journal of pharmacology, 1974; 27(2): 269–271.
23. Akram M, et.al. Peptic ulcer and Helicobacter pylori eradication: A review article. International Journal of Medical Sciences, 2010; 370 -375. 31.
24. Senay, E. C., & Levine, R. J. Synergism between cold and restraint for rapid production of stress ulcers in rats. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.), 1967; 124(4): 1221–1223.
25. Vincent, G., Glavin, G., Rutkowski, J., & Paré, W. Body orientation, food deprivation and potentiation of restraint induced gastric lesions, 1977.
26. Tamaki, H., Onoda, Y., & Kashida, T. Gastric secretion and duodenal ulcer formation induced by cysteamine in rats. Japanese journal of pharmacology, 1978; 28(4): 647–649.