



Evaluation of Antiulcer Activity of Hydroalcohol Extract of Peel of *Musa balbisiana* Fruits by a New Apparatus and Model

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Abstract

Background: Ulcers, a prevalent gastrointestinal disorder, impose significant health burdens worldwide. There are lots of market antiulcer drugs to treat the ulcer, but due to lots of their side effects and high cost, the utility of herbal medicines is increasing day by day. Traditional experimental *in vivo* approaches involve animal sacrifice, raising ethical concerns, while *in vitro* models do not show the antiulcer effect directly on the tissue. In response, our study developed a non-invasive model, eliminating the need for animal sacrifice while ensuring reliable results. **Aim:** This study explored the potential antiulcer properties of *Musa balbisiana* fruit peel hydroalcohol extract, utilising a novel apparatus and an animal cruelty-free model. **Methods:** *Musa balbisiana* fruit peel hydroalcohol extract 400 mg as a test drug and marketed Digene tablets as a standard were assessed using the developed model, and ulcer parameters were evaluated through innovative apparatus measurements. **Results:** In the blue index analysis, tissue treated with Ringer-Locke's salt solution, Ringer-Locke's salt solution + HCl, Ringer-Locke's salt solution with Digene 400 mg + HCl, Ringer-Locke's salt solution with *M. balbisiana* fruit peel hydroalcohol extract 400 mg + HCl showed the blue index of respective values of 52.95, 83.42, 64.48, and 65.51. **Conclusion:** The research results demonstrated promising antiulcer effects as compared to control and standard when blue indexes are measured, suggesting the potential therapeutic value of *M. balbisiana* fruit peel in ulcer management. Furthermore, the utilisation of a non-invasive model underscores its applicability in ethical research practices, fostering.

Keywords: Antacid Properties, Antiulcer Properties, Hydroalcohol Extract, *Musa balbisiana*, Novel Apparatus and Model

1. Introduction

Plants are essential to civilization as they can be utilised for food, animal feed, and a variety of other purposes¹. Plants have been used for medicinal purposes since ancient times. Plants contain a vast reservoir of organic compounds, many of which contain medicinal property and may serve as the basis for the future development of novel medications effective against a wide range of pathological conditions². Plants are recognized for their

abundant therapeutic properties due to the existence of several secondary metabolites. Now day's herbal medicines are proven to be more efficient due to their lower cost and side effects than synthetic medicines.

Ulcers are wounds of skin or mucous membrane with inflamed dead tissue. Ulceration occurs when the natural balance is disrupted by either increased aggressive factors or decreased mucosal endurance or protective factors. The stomach mucosa is constantly exposed to potentially harmful substances such as acid,

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pepsin, *Helicobacter pylori* bacteria, bile acids, alcohol consumption, food ingredients, some medicines (e.g. Analgesic), free radicals, stressful circumstances, prostaglandin production inhibition, and so on. These agents are linked to the formation of gastric ulcers³.

There are various conventional *in vivo* and *in vitro* models for antiulcer evaluation. There are certain drawbacks of these models. *In vivo* conventional models require animal sacrifice, whereas *in vitro* conventional models do not show the effect of acid on stomach tissue directly^{3,4}. This novel *in vitro* apparatus and model allows for direct observation of the effect of gastric acid on stomach tissue without the need for animal sacrifice as the stomach can be acquired from a butcher shop.

Peptic Ulcer Disease (PUD) affects 4 million individuals globally each year, with a prevalence of 5-10% in the overall population⁵. In 2015, around 87.4 million people worldwide developed a new peptic ulcer⁶. The global incidence of peptic ulcers in 2019 was around 8.09 million, which indicates an increase by 25.82% from 1990⁷. Peptic ulcers caused 327,000 deaths in 1990 and 267,500 in 2015^{8,9}.

Treatment of ulcer consists of quit smoking and alcohol, stop using NSAIDs, and use medications that decrease stomach acid. Usually Proton Pump Inhibitor (PPI) or H2 blocker are used to inhibit acid secretion and antacids are used to neutralize the secreted acid¹⁰.

Antacids are of two types systemic antacids and non-systemic antacids. Systemic antacids are bases of sodium and non-systemic antacids are bases of aluminium, magnesium, or calcium. They neutralize stomach acid and produce water and a neutral salt. Systemic antacids are absorbed in the systemic circulation thus not used that much. Non-systemic antacids are mostly used as they are not absorbed systemically. Antacids may aid patients by inhibiting pepsin action, binding to bile acids in the stomach, and increasing local prostaglandin (PGE₁). Over-The-Counter (OTC) antacid medications (Digene, Mylanta, DiGel, Maalox) combine aluminium hydroxide and magnesium hydroxide to maximize each compound's buffering qualities. Combinations balance the constipation action of aluminium hydroxide with the laxative action of magnesium hydroxide¹¹.

M. balbisiana colla is a robust herb from the Musaceae family that grows both in wild and farmed in various states of India like Tamil Nadu, West Bengal, Assam etc. Every component of the plant is traditionally

used by Indians for food, religious rituals, and social gatherings. This banana is used for nourishment and as a nutritional supplement since ancient times¹². Different portions of this plant have medical purposes including gastritis, gout, jaundice, blood production, ulcer, health tonic, cough, pinworm infection, diarrhoea, treatment of infertility in women, morning sickness, surgical dressing, and stress associated illnesses¹³.

Due to the extensive presence of potassium and chloride, *M. balbisiana* is highly alkaline in nature¹⁴. Peels of *M. balbisiana* contain several minerals, including magnesium and potassium¹⁵. It is already known that bases of magnesium such as Mg(OH)₂ works as an antacid. According to a study, the phytochemical composition of the peel of *M. balbisiana* includes: Trigonelline, rutin, ferulic acid, vanillin, isovanillic acid¹⁶. Among them, trigonelline is an alkaloid that may show antacid properties due to its basic nature^{17,18}, and rutin is a flavonoid that has antioxidant properties, which can help to improve blood circulation and reduce inflammation¹⁹⁻²¹.

Other plants that contain alkaloids and flavonoids are already proven to have antacid properties. That's why the peel of *M. balbisiana* has a high chance of showing antacid properties.

2. Materials and Methods

2.1 Plant Materials

In the month of february 2024, fresh peels of *M. balbisiana* were collected from the local area of Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, Nadia.

2.2 Preparation of Extract

The fruits of *M. balbisiana* were collected, and the peels were separated from them. Then the peels were cleaned with tap water and manually cut into small pieces. After that, they were dried under the shed for 7 consecutive days. After that, 40 gm dried sample was macerated into 70% hydroethanolic solution for 72 hours. Then the extract was dried by the air-drying method. Then the extract was sealed in aluminium foil and stored at 4°C^{22,23}.

2.3 Phytochemical Screening

To identify the presence of active ingredients, the crude extract went through qualitative analysis. Standard practices were used to conduct phytochemicals tests^{22,24}.

2.4 Animal Tissue

The domestic goat, or *Capra hircus*, is a prevalent animal in India's rural areas and many other developing nations. The abomasum or true stomach of *C. hircus* was collected from a local butcher shop near Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, Nadia.

2.5 Standard Drug

Marketed Digene 400 mg tablet.

2.6 Acid Solution Preparation

To prepare 1 liter of 0.1N HCl with a pH of 1, 10 ml of HCl (35% w/w) was added into 990 ml of distilled water.

2.7 Ringer-locke Salt Solution Preparation

To prepare 1 liter of isotonic Ringer-Locke salt solution, the listed amounts of chemicals were dissolved in distilled water and make up the volume up to 1 liter (Table 1)²⁵.

Table 1. Composition of Ringer-Locke salt solution

Solution Composition	Amount [gm/l]
Sodium Chloride	9
Calcium Chloride	0.24
Potassium Chloride	0.42
Sodium Bicarbonate	0.5
Glucose	1

2.8 Digene Solution Preparation

To prepare 1 mg/ml standard solution one Digene 400 mg tablet is powdered with mortar and pestle and dissolved in 400 ml of Ringer-Locke salt solution.

2.9 Test Solution Preparation

To prepare the test solution, 400 mg of hydroalcohol extract of dried *M. balbisiana* fruit peel is dissolved in

400 ml of Ringer-Locke salt solution and placed into the sample chamber.

2.10 Measurement of pH

The pH of each solution was measured independently as well as the mixture of acid and different sample (1:1) were also measured with the help of pH meter and a neutral buffer solution.

2.11 The Novel Apparatus and Model

Two solution chambers were clamped to two stands. The solution chambers each had a channel pipe linked to them so that the solution could flow through. Each channel had a flow regulator attached to it to control the solution's flow. At the ends of both channel pipes, there were needles with flow regulators. Under the two compartments, there was a mesh that was fixed to a three-legged stand. The solutions fall on the mesh drop wise through the needles, which were positioned slightly above it. The excess solution passes through the mesh and is collected in the collecting chamber underneath it.

Initially, the stomach tissue was positioned on the mesh that was fastened to a three-legged stand. In one chamber, an acid solution was placed, which was prepared according to the similar pH and normality of the gastric acid, and in another chamber, 400 ml of Ringer-Locke salt solution was placed. Every channel pipe is adjusted such that, for a continuous 1 hour, the solutions fall on the tissue drop by drop. Each drop had a volume of 0.05 ml, and the solution was flowing at a rate of 133 drops per minute. The tissue was removed after an hour, dyed with a methylene blue strainer, and the blue index (RGB colour system model) was calculated by using "ImageJ" software. In this software we can measure the blue index through the RGB colour model system, where the maximum colour index of each colour (red, blue, green) is 255 when checked individually.

A new tissue was then put on the mesh, and 400 ml of acid solution were prepared once again and added to the first chamber, while a solution of 400 ml of Ringer-Locke salt solution and one Digene 400 mg tablet were prepared and added to the second chamber. Every step of the process stayed the same.

Subsequently, a new tissue was positioned on the mesh, and 400 ml of acid solution were made and positioned in a chamber. Meanwhile, 400 ml of Ringer-Locke solution, together with 400 mg of *M. balbisiana* fruit peel hydroalcohol extract, were prepared and positioned in another chamber. Everything about the procedure remained the same.

3. Result and Discussion

3.1 Extract Preparation

After maceration with a 70% hydroalcohol solution for 72 hours, *M. balbisiana* fruit peels produced a yield of 2.81%.

3.2 Phytochemical Investigation

Through the different phytochemical investigation, the hydroalcohol extract of *M. balbisiana* peels showed the presence of different phytochemicals (Table 2).

Table 2. Phytochemical investigation results of hydroalcohol extract of *M. balbisiana* fruit peels

Phytoconstituent	Test	Observation	Result
Alkaloid	Hager's test	Yellow Colour	Present
Carbohydrate	Molish's test	Purple Colour	Present
Flavonoid	Alkaline reagent test	Deep Yellow Colour turns Colourless	Present
Tannin	Lead Acetate test	White Precipitate	Present
Saponin	Foam test	Formation of Stable Foam	Present
Glycoside	Kellar-Killiani test	No colour change	Absent
Polyphenol	Ferric Chloride test	No colour change	Absent

3.3 Result of pH Measurement

Through this comparative study of the pH values of control, standard, and test solutions, it has been seen that both the standard and test solutions were able to increase the pH. The pH-neutralizing capacity of *M. balbisiana* peel was seen to be as good as that of

marketed tablet Digene at the same dose of 1 mg/ml (Table 3, Figure 1).

Table 3. Result of comparative study of pH value

Sample	pH
Hydrochloric acid solution (0.1N)	1
Ringer Locke salt solution	8.33
Salt Solution + HCl	1.23
Salt Solution with Digene + HCl	2.45
Salt Solution with peel extract + HCl	2.08

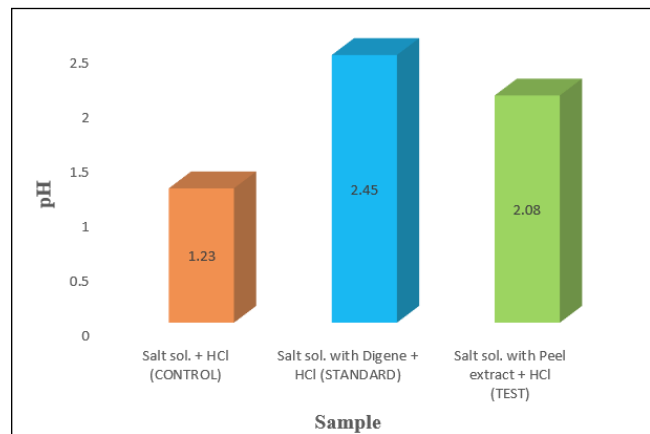


Figure 1. Graphical representation of comparative study of pH value.

3.4 Antiulcer Activity by Novel Apparatus and Model

The purpose of the study was to evaluate the hydroalcohol extract of *M. balbisiana* fruits peels antiulcer properties against the commercially available Digene (marketed antacid tablet). When compared to the market standard of Digene tablets and control, the dosage of 1 mg/ml of *M. balbisiana* peel exhibits strong antiulcer action on the stomach tissue (Table 4, Figures 2-6).

Colours are simply light waves. Magazines, pictures, and other natural objects, such as oranges, generate colour by eliminating or absorbing some wavelengths of light and reflecting others back to the viewer. The colour spectrum is composed of many frequencies and wavelengths. Each colour belongs to a unique part of the spectrum.

The human eyes determine the main colour palettes. If the visible portion of the light spectrum is divided into thirds, the dominant colours are red, green, and

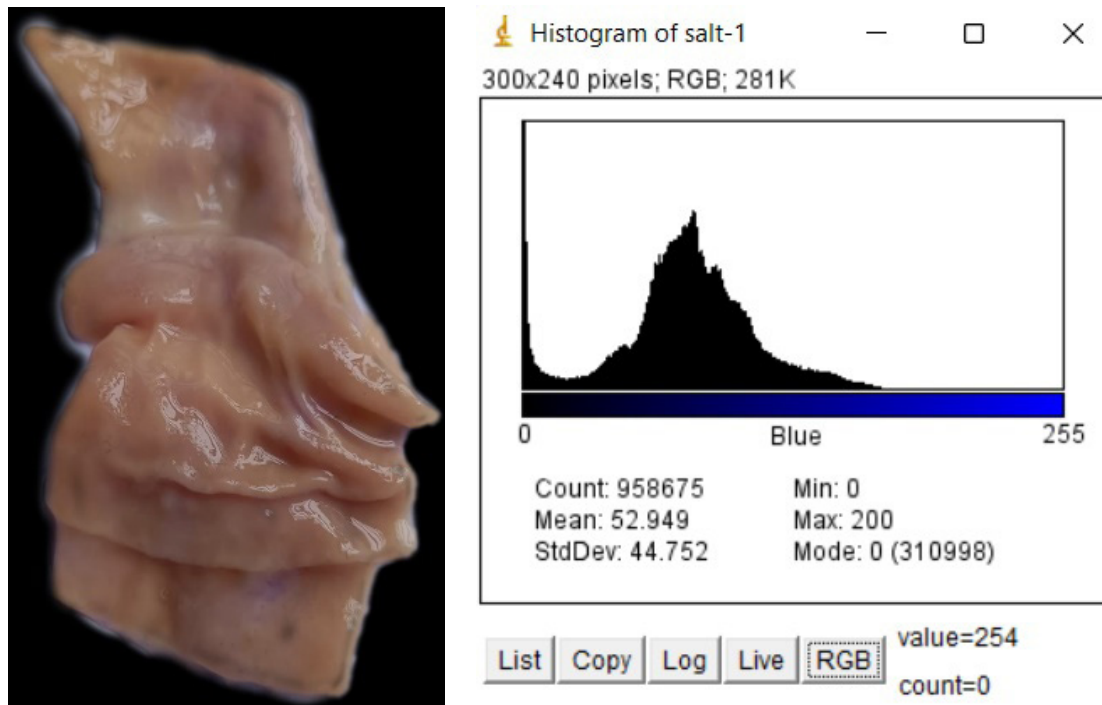


Figure 2. Tissue treated with Ringer-Locke's salt solution and its blue index.

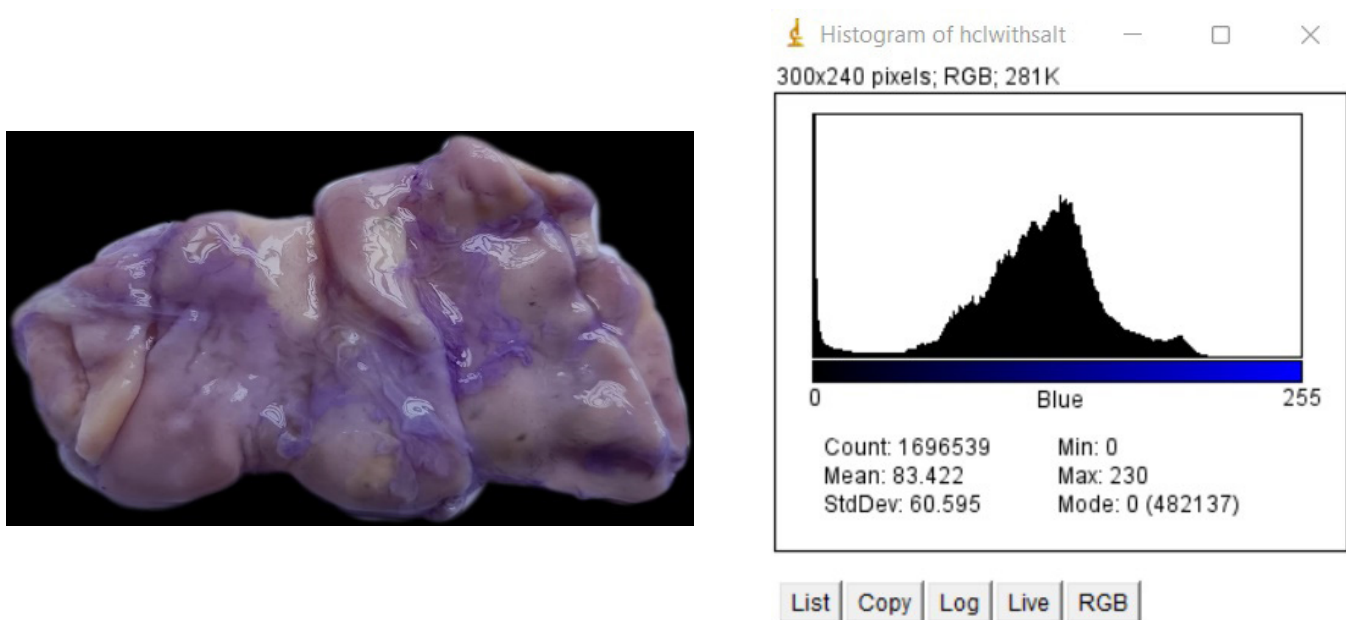


Figure 3. Tissue treated with Ringer-Locke's salt solution + HCl and its blue index.

blue. The RGB colour model is an additive colour model that uses red, green, and blue light in a variety of ways to produce a wide spectrum of colours. The RGB colour model's help us to identify different red, blue and green colour index²⁶.

The blue index indicated the amount of ulceration occurred on the tissue as we used methylene blue for staining purpose. It showed that highest ulceration occurred on the tissue treated with the control solution (Ringer-Locke's Salt Solution + HCl). Both the standard

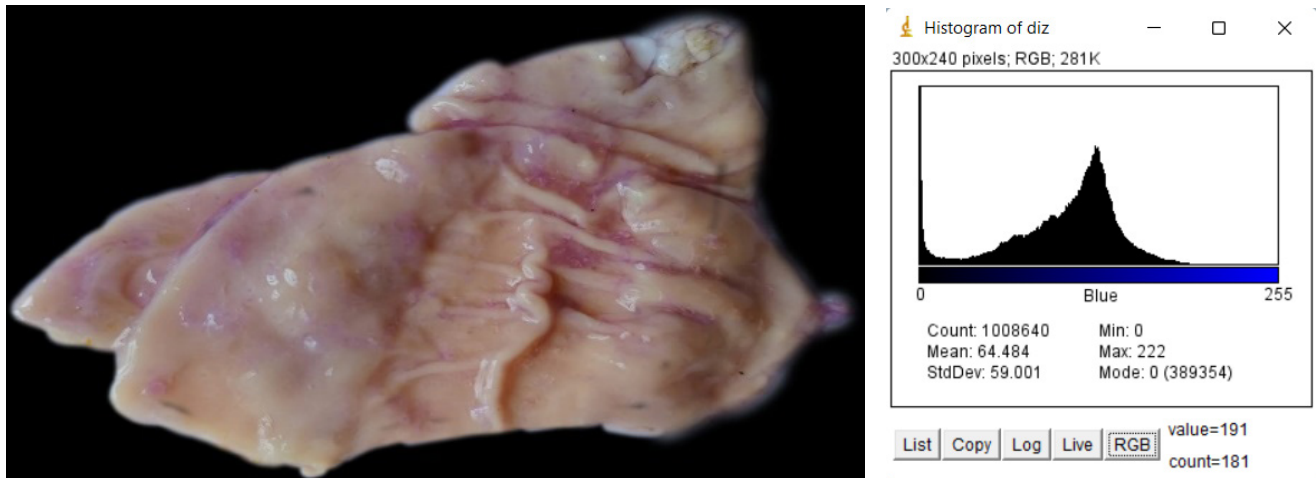


Figure 4. Tissue treated with Ringer-Locke's salt solution with Digene + HCl and its blue index.

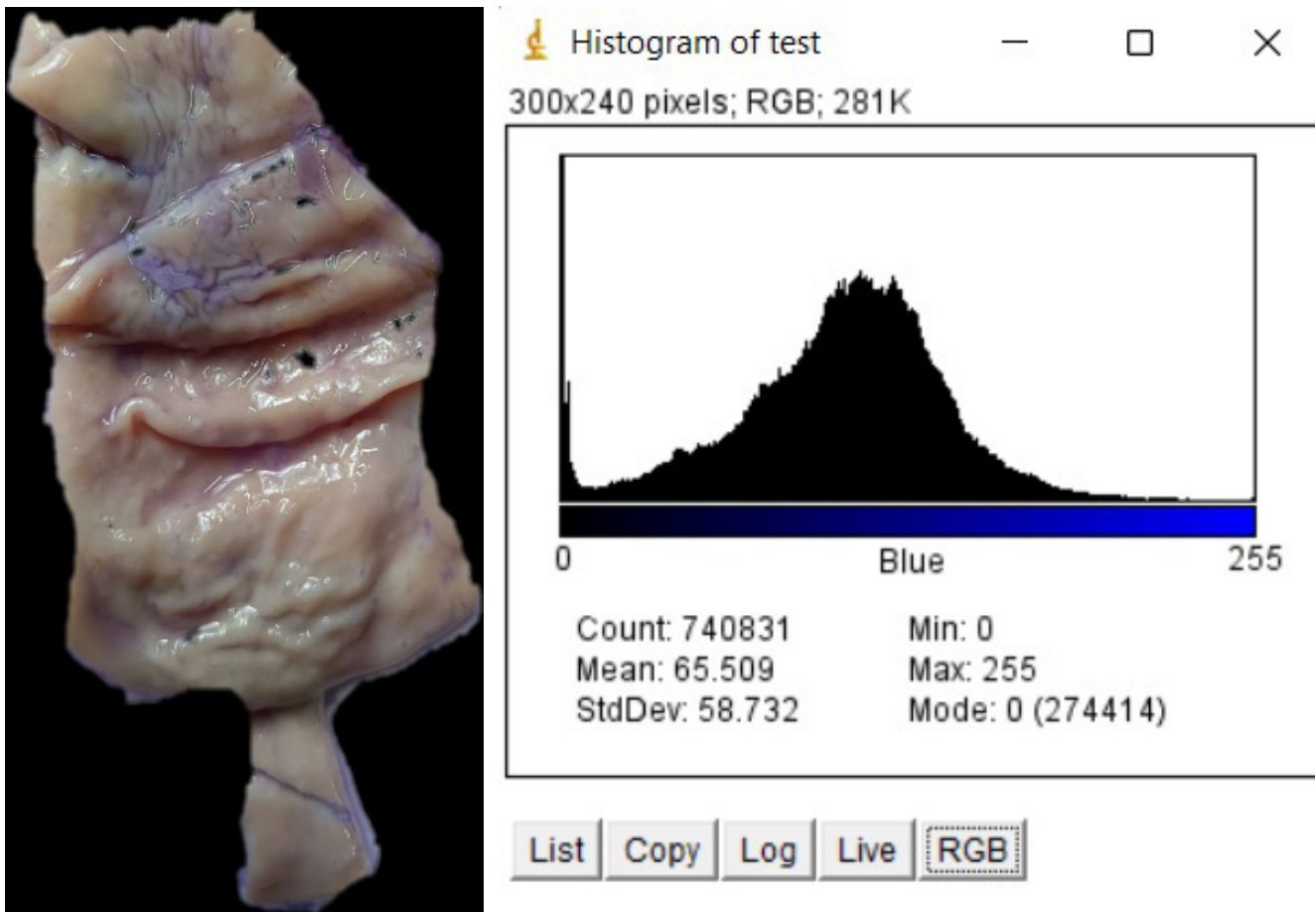


Figure 5. Tissue treated with Ringer-Locke's salt solution with *M. balbisiana* fruit peel hydroalcohol extract + HCl and its blue index.

solution (Ringer-Locke's Salt Solution with Digene + HCl) and the test solution (Ringer-Locke's Salt Solution with *M. balbisiana* Peel's extract + HCl) decreased the amount of ulceration on the tissue at the same dose

of 1 mg/ml. The test sample, *M. balbisiana* fruit peel hydroalcohol extract showed potent antiulcer activity in comparison to the control as well as standard sample.

Table 4. Result of comparative study of ulcer index (blue index)

Sample	Blue Index
Ringer-Locke's Salt Solution	52.95
Salt Solution + HCl	83.42
Salt Solution with Digene + HCl	64.48
Salt Solution with fruit Peel extract + HCl	65.51

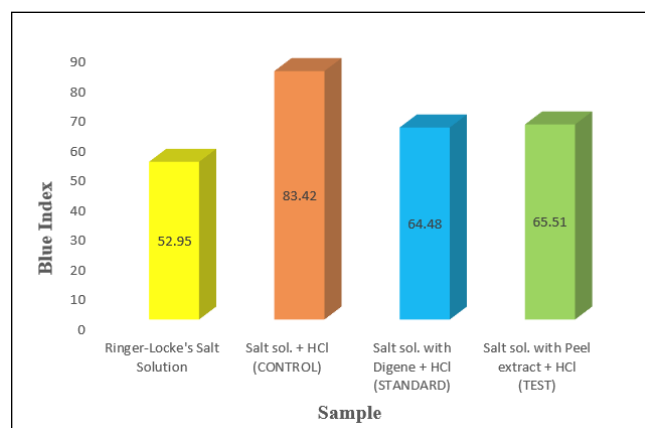


Figure 6. Graphical representation of comparative study of ulcer index (blue index).

Plants exhibit their medicinal and therapeutic qualities because of a variety of phytochemicals. There aren't many phytochemicals in this plant that give it its antiulcer characteristics. The phytochemical analysis of the peel of *M. balbisiana* reveals the presence of tannin, terpenoid, flavonoid, and alkaloid compounds.

Alkaloids exhibit their characteristics through increased mucus formation, prostaglandin synthesis, antioxidant capacity, and acid secretion inhibition. In this way, the mucus's protective layer provides protection from acid damage²⁷.

Within the inner lining of the stomach, tannin forms a tannin-protein complex layer that enhances the stomach acid's protective effects²⁸.

However, flavonoids have anti-inflammatory and antioxidant properties which help to prevent ulcer formation²⁹.

The aggressive elements, such as acid secretion and stomach volume, are reduced by terpenoid. It can lower the ulcer index in a dose-dependent way because of these antisecretory properties³⁰.

The development of *M. balbisiana* as an antiulcer agent was significantly aided by these phytochemicals.

Evaluating the plant extract's antiulcer properties was made much easier with the help of this newly created model and methodology. Developing additional medications and researching their antiulcer properties on stomach tissue will be aided by this novel approach. However, the discovery of *M. balbisiana* peel's antiulcer properties will be useful in the creation of novel antiulcer medications.

4. Conclusion

Musa balbisiana colla has long been used as an antiulcer agent; no one has investigated any of its fruit peels for their antiulcer properties. In this research, its fruit peel hydroalcohol extract was evaluated and compared to a commercially available antacid (antiulcer) medication.

Using a novel apparatus and model, the hydroalcohol extract of *M. balbisiana* fruit peel shown remarkable antiulcer efficacy in the study. This innovative device and model will be useful in the future in order to evaluate the antacid properties of various substances without sacrificing or hurting any animal. According to the research, *M. balbisiana* peel's hydroalcohol extract may be used to create a natural antacid. Although it is necessary to isolate the active chemical components individually and assess their properties for safety and effectiveness studies in order to create those new medicines from the peels.

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