CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND ANTIULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *MIRABILIS LONGIFLORA*

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Article Received: March 2022 **Accepted:** March 2022 **Published:** April 2022

Abstract
Gastroesophageal reflux disease, gastritis, peptic ulcer, duodenal ulcer, and other peptic illnesses are frequent in today's lifestyle. This could be the result of a hectic lifestyle or an unbalanced diet. The pathophysiology underlying these illnesses could be a mismatch between offensive and defensive mechanisms, either due to excessive acid and pepsin secretion or a decreased ability of the gastro-duodenal mucosal barrier to guard against acid-pepsin secretion from the stomach. Non-steroidal anti-inflammatory drugs (NSAIDs) are a type of therapy that has been shown to be beneficial in treating a variety of ailments. Some people take NSAIDs every day as a preventative measure. NSAIDs, on the other hand, can cause a wide range of serious adverse effects. Natural compounds that are edible have been found in studies to help prevent stomach ulcers. The goal of this study was to see if a hydroalcoholic extract of Mirabilis longiflora rhizomes has antiulcer efficacy in rats. The well-known test methodology was used to determine qualitative analysis of various phytochemical elements as well as quantitative analysis of total flavonoids and alkaloids. The anti-ulcer effect of hydroalcoholic extract in rats was tested in vivo against an ethanol-induced stomach ulcer. The volume and pH of stomach fluid, free acidity, total acidity, pepsin activity, ulcer index, and percent inhibition of ulcer index were all used as outcome measures, depending on the model. Phenols, flavonoids, saponins, and tannins were found in preliminary phytochemical screening. Total flavonoids (0.438 mg/100mg) were found in Mirabilis longiflora extract, followed by alkaloids (0.896 mg/100mgFurther hydroalcoholic extracts of 100 and 200 mg/kg/p.o dramatically reduced the volume of gastric content, total/free acidity, and pepsin activity, while increasing the pH of the gastric juice. The results of this investigation indicated that Mirabilis longiflora extract had anti-ulcer pharmacologic activity as a result of one or more secondary metabolites found in it. As a result, this research backs up its anti-ulcer use in Indian folk medicine. More research is needed into isolating particular phytochemicals and understanding mechanisms of action.

Keywords: *Mirabilis longiflora, Phytochemical constituents, Antiulcer, Non-steroidal anti-inflammatory drugs, Ethanol-induced gastric ulcer.*

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Please cite this article in press Suresh Kumar Yadav et al, ***Extraction, Phytochemical Investigation And Antiulcer Activity Of Hydroalcoholic Extract Of Mirabilis Longiflora.,*** *Indo Am. J. P. Sci, 2022; 09(04)*

INTRODUCTION:

The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care [1]. From 250 to 500 thousand plant species are estimated to exist on the planet, and only between 1 and 10% are used as food by humans and other animals [2]. Brazil has the world's highest biodiversity, accounting for over 20% of the total number of known species. This country presents the most diverse flora, with more than 55 thousand described species, which corresponds to 22% of the global total. Such biodiversity is followed by a wide acceptance of the medicinal plant use [3]. Most of the Brazilian population (80%) consumes only 37% of the commercially available drugs and depend almost exclusively on medicines of natural origin [4]. Thus, phytotherapics entered the market promising a shorter and cheaper production, since basic requirements to use medicinal plants do not involve strict quality control regarding safety and efficacy compared to the other types of drugs [5].

Traditional medicine is "the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness". There are many different systems of traditional medicine, and the philosophy and practices of each are influenced by the prevailing conditions, environment, and geographic area within which it first evolved, however, a common philosophy is a holistic approach to life, body, and the environment, and an emphasis on health rather than on disease. Generally, the focus is on the overall condition of the individual, rather than on the particular ailment or disease from which the patient is suffering, and the use of herbs is a core part of all systems of traditional medicine [6].

A history of heartburn, gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion for peptic ulcer. Medicines associated with ulcer include NSAIDs (non-steroid anti-inflammatory drugs) that inhibit cyclooxygenase, and most glucocorticoids (e.g. dexamethasone and prednisolone). A major causative factor (60% of gastric and up to 50-75% of duodenal ulcers) is chronic inflammation due to Helicobacter pylori that colonizes the antral mucosa. The immune system is unable to clear the infection, despite the appearance of antibodies. Thus, the bacterium can cause a chronic active gastritis (type B gastritis). Gastrin stimulates the production of gastric acid by parietal cells. In H. pylori colonization responses to increased gastrin, the increase in acid can contribute to the erosion of the mucosa and therefore ulcer formation. In Western countries the percentage of people with Helicobacter pylori infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80 etc.)[7]. Most patients with peptic ulcer disease present with abdominal discomfort, pain or nausea. The pain is located in the epigastrium and usually does not radiate. However, these symptoms are neither sensitive nor specific. Pain radiating to the back may suggest that an ulcer has penetrated posteriorly, or the pain may be pancreatic in origin. Pain radiating to the right upper quadrant may suggest disease of the gallbladder or bile ducts.

Several factors are also associated in the occurrence of peptic ulcer including stressful lifestyle, alcohol consumption, use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDS), *Helicobacter pylori* infections, smoking, lower socio-economic status and family history. Although ulcer is not a deadly disease, it can lead to more serious complications like gastrointestinal bleeding, perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction. Medications are used to relieve the pain, heal ulcerations and delay recurrence of ulcerations. These include antibiotics, antacids and proton pump inhibitors. Several drugs are available in the market for gastric ulcer therapy; however, most of these drugs are associated with unwanted side effects. In this context, this research aims to evaluate the anti-ulcer properties of medicinal plant *Mirabilis longiflora*.

MATERIAL AND METHODS:

Material

All the drugs, solvents and chemicals used in the study were of analytical grade. Ranitidine was obtained as a gift sample from Scan Research Lab, Bhopal, MP, India. All other chemicals e.g. Methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India. Tris buffer, Topfer's reagent, Folin's Reagent and Phenolphthalein were purchased from Hi-Media Pvt. Ltd., Mumbai, India.

Methods

Collection of plant material

Leaves of *Mirabilis longiflora* was collected from local area of Bhopal in the month of May, 2021.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs [8-9]:

Defatting of plant material

Leaves of *Mirabilis longiflora* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlet extraction

60.5 gm of dried powdered leaves of *Mirabilis longiflora* has been extracted with hydroalcoholic solvent (ethanol : water, 70:30 v/v) using soxhlet extraction process for 24-48 hrs, filtered and dried using vacuum evaporator at 40°C[10].

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Take}} x100$$

Phytochemical Screening

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods[11].

1. **Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins
Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes
Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative estimation of bioactive compound
Estimation of total phenolic content
The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method[12].

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol.

Preparation of Extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol.

Procedure: 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content
Determination of total flavonoids content was based on aluminium chloride method[13]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

In vivo* antiulcer activity of hydroalcoholic extract of *Mirabilis longiflora
Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study
Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the hydroalcoholic extract of leaves of *Mirabilis longiflora* were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425[14]. The hydroalcoholic extract of leaves of *Mirabilis longiflora* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively.

Experimental designs
Ulcer induced by absolute ethanol
The rats were divided into four groups of six each.
Group I (toxicant control) received absolute ethanol (1 ml/animal)
Group II was treated with ranitidine (50 mg/kg)
Groups III was treated with hydroalcoholic extract of leaves of *Mirabilis longiflora* 100 mg/kg/p.o.
Groups IV was treated with hydroalcoholic extract of leaves of *Mirabilis longiflora* 200 mg/kg/p.o.

The animals were treated with ranitidine (100 mg/kg), dose of hydroalcoholic extract of leaves of *Mirabilis longiflora* 100 and 200 mg/kg (once daily) for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were fasted for 24 h and they received 1 ml of absolute ethanol orally. The animals were sacrificed after 1 h of ulcerogen administration, and their stomachs were excised and the gastric contents were aspirated. The contents were subjected to centrifugation at 1000 rpm for 10 min and then analyzed for pH (digital pH meter), pepsin activity, total and free acidity[15].

Antiulcer Screening

The ulcer index was determined using the formula:
Ulcer index = 10/X
Where X = Total mucosal area/Total ulcerated area.
Based on their intensity, the ulcers were given scores as follows:
0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,
3 = perforated or penetrated ulcer.

RESULTS AND DISCUSSION:

The current study was designed for the first time, to study gastroprotective effect of hydroalcoholic extract of leaves of *Mirabilis longiflora* against ethanol-induced gastric ulcer in comparison to ranitidine, which is widely approved and used for gastric ulcer treatment. This study is based on our phytochemical screening of this extract which revealed the presence of different chemical constituents. These phytoconstituents, particularly flavonoids and tannins, were previously established to be among the possible cytoprotective agents involved in reducing gastric ulcer. Treatment of rats with hydroalcoholic extract of leaves of *Mirabilis longiflora* significantly reduced the ulcer index at both doses compared to ulcerated group. Moreover, ulcerated animals treated with hydroalcoholic extract of leaves of *Mirabilis longiflora* showed a better reduction in ulcer index than the standard drug ranitidine, indicating that *C. ignea* could be valuable in healing gastric ulcer. This result is in line with the study of Abebaw *et al*., [16], who reported similar effect for Osyris quadripartite Decne extract as compared to ranitidine. Lullmann *et al*., [17] stated that elevated concentration of the hydrogen ion is an aggressive factor facilitating gastric damage via decreasing pH in gastric juice.

The present study showed significant reduction in gastric pH level in ethanol treated rats comparing to normal control group. Hydroalcoholic extract of leaves of *Mirabilis longiflora* treatment in ethanol-ulcerated groups significantly improved gastric pH levels with simultaneous decreases in gastric secretion in comparison to ethanol group. The efficiency of hydroalcoholic extract of leaves of *Mirabilis longiflora* in increasing gastric pH could be attributed to the presence of phytoconstituents in the extract. According to Zhao *et al.*, [81] and Liu *et al.*, [82], phytoconstituents have a main role in the mechanism of gastro-protection by rising pH of gastric juice.

Moreover, our results showed that pre-treatment with hydroalcoholic extract of leaves of *Mirabilis longiflora* had similar effects on gastric pH as the reference ranitidine drug, which has a great ability to decrease stomach acid production and neutralize stomach acidic environment. Furthermore, our study showed that, ethanol ulcerated rats have significant reduction in pepsin activity in comparing to normal group and this is in agreement with Puurunen, [83] who clarified that, high concentrations of ethanol can reduce peptic activity due to its ability to inhibit pepsinogen activation to pepsin. On the other hand, hydroalcoholic extract of leaves of *Mirabilis longiflora* treatment improved pepsin activity in gastric secretion in dose dependent manner, indicating that hydroalcoholic extract of leaves of *Mirabilis longiflora* has the ability to regulate ethanol effect on peptic activity.

Table 1: % Yield of leaves extract of *Mirabilis longiflora*

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	7.86%

Table 2: Phytochemical screening of extract *of Mirabilis longiflora*

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids**		
	Mayer's Test:	-ve	Green coloured
	Wagner's Test:	-ve	Green coloured
	Dragendroff's Test:	-ve	Light Green coloured
	Hager's Test:	-ve	Yellow coloured
2.	**Glycosides**		
	Legal's test	-ve	Green coloured
3.	**Flavonoids**		
	Lead acetate	+ve	Yellow coloured precipitate
	Alkaline Reagent Test:	-ve	Yellow coloured

4.	**Phenolics** Ferric Chloride Test	+ve	Black coloured
5.	**Proteins** Xanthoproteic test	-ve	Green coloured
6.	**Carbohydrates** Molisch's Test: Benedict's Test: Fehling's Test:	 -ve -ve +ve	 Yellow coloured Yellow coloured Red precipitate
7.	**Saponins** Froth Test: Foam Test:	 +ve -ve	 Layer of foam No foam
8.	**Diterpins** Copper acetate test	-ve	Green coloured
9.	**Tannins** Gelatin Test:	+ve	White precipitate

Table 3: Total phenolic and total flavonoid content of *Mirabilis longiflora*

S. No.	Total Phenol content	Total flavonoid content
1.	0.438 mg/100mg	0.896 mg/100mg

Table 4: Effect of hydroalcoholic extract of leaves of *Mirabilis longiflora* on ulcer index by ethanol induced ulcers in rats

Treatment and dose	Ulcer Index	pH	Total acidity (mEq/lt)	Free acidity (mEq/lt)	Pepsin activity (Per ml/h)
Control	7.50 ±0.50	2.85±0.30	75.10±0.30	59.98±0.35	3.25±0.25
Ranitidine (50 mg/kg, p.o.)	2.65±0.10***	4.75±0.15***	45.01±0.20 ***	25.35±0.25 ***	2.45±0.15 ***
Hydroalcoholic extract of leaves of *Mirabilis longiflora* (100 mg/kg, p.o.)	3.75±0.20**	3.98±0.20**	60.35±0.30*	39.85±0.35**	3.45±0.31**
Hydroalcoholic extract of leaves of *Mirabilis longiflora* (200 mg/kg, p.o.)	3.15±0.18***	4.20±0.15***	43.25±0.30 ***	36.85±0.40 ***	2.65±0.32***

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

CONCLUSION:

In conclusion, the results of the present study demonstrated that the hydroalcoholic extract of leaves of *Mirabilis longiflora* at both doses attenuated ethanol-induced gastric ulcer through its antioxidant and anti-inflammatory effects. This gastroprotective efficiency of hydroalcoholic extract of leaves of *Mirabilis longiflora* could be possibly attributed to the presence of wealthy phytoconstituents. Therefore hydroalcoholic extract of leaves of *Mirabilis longiflora* could be used as a promising anti-ulcer agent in the treatment of gastric ulcers due to its comparable anti-ulcer effect to that of ranitidine. However, further researches should be taken to further explore the underlying mechanisms of action.

CONCLUSION:

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