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BIOACTIVITY-GUIDED FRACTIONATION, MOLECULAR DOCKING, AND GASTROPROTECTIVE EVALUATION OF *MIMOSA PUDICA* AGAINST EXPERIMENTALLY INDUCED GASTRIC ULCERS

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ABSTRACT
Peptic ulcers are a common disease that occurs due to an imbalance between aggressive factors like hydrochloric acid, pepsin, oxidation, alcohol, nonsteroidal anti-inflammatory drugs, and H.pylori bacteria and defensive factors like mucus formation, bicarbonate, prostaglandins, and antioxidants. The current study evaluates the gastroprotective activity of Mimosa pudica by conducting a phytochemical screening, antioxidant analysis, antimicrobial assay, molecular docking studies, and in-vivo antiulcer tests. Various extracts of Mimosa pudica have been made; however, methanolic extract of Mimosa pudica (MEMP) showed remarkable bioactivity. The preliminary phytochemical study was positive for flavonoids, alkaloids, tannins, phenolics, terpenoids, steroids, and saponins. Three phytochemical spots of MEMP with Rf values of 0.95, 0.71, and 0.52 were seen on the TLC plate. The scavenging activity of MEMP in DPPH was 67.67±1.95% at 200 µg/ml concentration, while BEMP in hydrogen peroxide showed 58.69±0.23%. The quantitative phytochemical analysis of MEMP gave phenolics, flavonoids, tannins, saponins, and alkaloids of 2.45±0.54mg/10. Antimicrobial screening showed that there was significant antibacterial action with a maximum inhibition zone of 25mm on Pseudomonas aeruginosa. Docking studies for the drug molecule with the target protein of Gastric H+, K+-ATPase (PDB ID: 2XZB) showed significant binding activity of Mimosine (-8.2 kcal/mol), Quercetin (-7.6 kcal/mol) and Luteolin (-7.6 kcal/mol), compared to Ranitidine (-5.4 kcal/mol). Anti-ulcer screening of MEMP showed that at a dose of 500 mg/kg, it showed significant anti-ulcer activity by reducing the ulcer index and also offering significant ulcer protection with 55.60%, 64.50%, and 65.23% protection in pyloric ligation, aspirin-induced and ethanol-induced ulcers, respectively. Histopathological studies also confirmed significant gastric mucosal protection.

KEYWORDS: Mimosa pudica, Antiulcer activity, Methanolic extract, In-silico study

INTRODUCTION
Peptic ulcer disease refers to a gastrointestinal disease associated with mucosal erosion of either the stomach or the duodenum, arising from excessive gastric acid secretion, pepsin secretion, oxidative damage, alcohol ingestion, stress, extended use of non-steroidal anti-inflammatory drugs, and infection with Helicobacter pylori bacteria. Erosion of the stomach mucosa that causes ulcers is ascribed to an imbalance between the aggressive and protective factors of the gastric mucosa. Aggressive factors include hydrochloric acid, pepsin, reactive oxygen species, inflammatory cytokines, bile reflux, alcohol ingestion, and certain anti-ulcer medications such as aspirin and indomethacin [1]. On the other hand, protective factors include mucus and bicarbonate secretion, prostaglandin secretion, blood flow to the mucosa, cell regeneration, nitric oxide secretion, and antioxidative processes. Conventional methods for the treatment of peptic ulcers such as the use of proton pump inhibitors, H2-receptor blockers, antacids, antibiotics, prostaglandins, and cytoprotectants are commonly practiced [2]. These conventional medications, however, can induce unwanted side effects such as headaches, diarrhea, constipation, malabsorption of nutrients, hyper-acid secretion, modification of normal bacterial flora, and adverse drug reactions. Hence, there is increased interest in discovering natural antiulcer drugs.

Plants contain biologically active constituents, such as flavonoids, alkaloids, tannins, saponins, phenolics, terpenoids, and glycosides, with the ability to provide gastroprotection by acting as antioxidants, anti-inflammatory agents, antisecretory substances, cytoprotectors, and inhibitors of Helicobacter pylori [3]. Mimosa pudica Linn, or “touch-me-not” or “Lajjalu”, is a member of the Fabaceae family found commonly in tropical and subtropical areas of India. The traditional use of M. pudica for the treatment of ulcers, diarrhea, inflammation, wounds, piles, dysentery, and other disorders associated with the gastrointestinal tract is widespread. Leaf, root, stem, and seed extracts of M. pudica

have considerable medicinal significance because of their high amounts of flavonoids, alkaloids, tannins, terpenoids, glycosides, phenols, and mimosine [4].

Various pharmacological experiments have confirmed the antioxidant, anti-inflammatory, antimicrobial, wound-healing, hepatoprotective, antidiabetic, and anti-ulcer properties of Mimosa pudica. This herb's anti-ulcer effect could be due to its ability to inhibit gastric acid secretion, increase mucus secretion, scavenging free radicals, and protection against lipid peroxidation and oxidative damage to gastric mucus membrane [5]. Studies carried out in the past had shown that there was a significant decrease in the ulcer index using experimental models created through the use of ethanol, aspirin, pylorus ligation, and NSAIDs. Bioactivity-guided fractionation is one of the scientific methodologies involved in isolating and identifying active fractions or compounds that cause a specific pharmacological property. Using this technique, plant extracts are systematically fractionated and tested for bioactivity to find the fraction with the highest biological effect [6].

In recent years, molecular docking approaches have emerged as a significant in-silico technique for the prediction of interactions of phytoconstituents with specific targets in the pathway of ulcer development. Molecular docking investigations against various target molecules like gastric H^+/K^+ ATPase, urease of H. pylori, cyclooxygenase enzymes, histamine H_2 receptors, and inflammatory mediators provide insights into the mechanism of action of phytochemicals in antiulcer activity. Various phytoconstituents from plants having medicinal value possess good binding properties with gastric proton pumps and enzymes of H. pylori bacteria, suggesting their significance as therapeutics [7]. Thus, the current study aims to conduct bioactivity-guided fractionation, antiulcer activity, and molecular docking investigation of Mimosa pudica plant.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Mimosa pudica* will be collected from an appropriate area. Authentication of the plant will be done by a taxonomist/botanist. A voucher specimen will be kept in the institutional herbarium.

Drying and Powdering

The collected leaves will be washed with distilled water to clean dust and other foreign materials. The leaves will be dried in shade at room temperature so that thermolabile compounds do not get degraded. The dried plant material will be ground with the help of a grinder and sieved. 40 g of the powder will be stored in air-tight containers [8].

Preparation of Extracts

Powdered leaves will be subjected to successive solvent extraction with increasing polarity of solvents like chloroform, methanol, butanol and distilled water. The extraction process can be done by Soxhlet extraction or cold maceration. Filtration of the extract, concentrating using rotary evaporation, and drying will be done. Yield percentage of the extract will be determined [9].

Preliminary Phytochemical screening

These extracts will be analyzed through phytochemical analysis qualitatively for the presence of major secondary metabolites. Qualitative phytochemical analysis is a very significant process where the presence of some specific types of secondary metabolites present in plants can be detected. These phytochemicals are accountable for various pharmacological actions like antioxidant, anti-inflammatory, antimicrobial, wound-healing, and anti-ulcer properties. In this present study on the Mimosa pudica plant extract, different kinds of chemical analysis could be done to detect the presence of alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, steroids, terpenoids, carbohydrates, and proteins [10].

In-Vitro Antioxidant Activity

Antioxidant activity of crude extracts and fractions of *mimosa pudica* will be tested through various in vitro methods in order to ascertain their free radical scavenging and reducing properties. Oxidative stress is a factor that is crucial in the pathogenesis of gastric ulcers because of damage to the lining membranes in the stomach, increasing lipid peroxidation and decreasing the body's antioxidant mechanisms. Hence, antioxidant activity testing becomes necessary in any anti-ulcer research [11]. The DPPH free radical scavenging test will be used in order to assess the power of the extract to donate electrons or hydrogen ions in order to reduce stable DPPH free radical. The reduction will be seen as a reduction in the intensity of the purple color produced. The ABTS radical scavenging method will also be employed in testing the scavenging of ABTS+ radicals. It is an ideal tool for measuring both hydrophilic and lipophilic antioxidants. Inhibition of nitric oxide radical formation from the sodium nitroprusside solution will be assessed. Hydrogen peroxide scavenging activity will indicate the scavenging ability of the samples against hydrogen peroxide, which produces very reactive hydroxyl radicals that cause damage to cells [12]. Ferric reducing antioxidant power activity will provide the electron donating ability of the sample in reducing ferric ions into ferrous ions. All

tests will be done at different concentrations, and comparison will be made with respect to standard antioxidants like ascorbic acid and gallic acid. Percentage inhibition and IC_{50} values will be determined. Finally, the sample which exhibits high antioxidant activity, low IC_{50} value, and good reducing power will be taken for further testing [13].

Bioactivity-Guided Fractionation

Selection of Active Extract

From all these extracts, the one that has high antioxidative as well as anti-ulcer properties shall be subjected to the fractionation process. Since *mimosa pudica* is a phytochemical, it is expected that the Methanol extract may have strong activity because of its flavonoids, phenolics, tannins, and other active polar components [14].

Fractionation of Active Extract

The active crude extract will then be diluted in distilled water and sequentially extracted with successively polar solvents such as chloroform, methanol, butanol, and aqueous solvent fractions. These fractions will be dried and subjected to phytochemical testing, antioxidant testing, and anti-ulcer testing [15]. The fraction with the most protective effects against ulcers, antioxidant effects, and phytochemicals will be isolated as the active fraction. This active fraction will then be further analyzed through chromatography via TLC and GC-MS [16].

In-silico study of active compounds

To assess the antiulcer activity of the selected active phytochemicals from Mimosa pudica on H(+), K(+)-ATPase proton pump receptor (Protein Data Bank ID: 2XZB) of the stomach, molecular docking study was done. The 3D structure of the target protein was taken from the Protein Data Bank. Bioactive compounds such as mimosine, quercetin, and luteolin were collected from PubChem database and subjected to docking analysis. Molecular docking was conducted using AutoDock software with MGL tools. Grid box parameters were defined by setting the values to x = 20.7075, y = 38.7045, and z = -33.7057 with grid spacing of 0.375 Å. Binding energy was predicted through Lamarckian Genetic Algorithm (LGA). The ligand–receptor complexes having least binding energy were analyzed for interactions using Discovery Studio software.

Experimental Animals

Healthy Wistar albino rats of either sex of weight 150-200g will be used for the experiment. Experimental animals will be maintained in standard laboratory conditions, where environmental factors like temperature, humidity and light will be kept at 12 hours per day. Animals will be provided with standard pellet food and water ad libitum. The experiment will be approved by Institutional Animal Ethics Committee and performed based on CPCSEA guidelines [17].

Acute Toxicity Test

Acute oral toxicity test will be carried out based on OECD guidelines 423 and 425. Acute oral toxicity will be done for extract by giving orally to animals and observing animals for death, changes in behavior, tremors, convulsions, salivation, diarrhea, lethargy, sleep and body weight changes. As per the toxicity study, appropriate doses like 100, 200 and 400mg/kg body weight will be selected for antiulcer activity. [18]

Antiulcer Activity

Experimental Design

The animals will be segregated into seven categories in such a way that there will be six animals in each group. While group one will be the control group, group two will be the ulcerated control group, and group three will be treated with the conventional medicine like omeprazole, groups four and five will be treated with low and high doses of the crude extract, respectively [19].

Antiulcer Models

Pylorus Ligation-Induced Gastric Ulcer

The pylorus ligation model is a well-known technique used in testing anti-secretory action. This technique involves ligating the pyloric end of the stomach, resulting in the collection of gastric juice and hence increased acidity, resulting in ulcer development. Animals will be deprived of food for a period of 24 hours prior to the experiment with access to drinking water [20]. The test extract, fraction, and standard drugs will be given orally. After one hour, animals will be put to anesthesia and ligation of the pylorus done. Four hours later, animals will be sacrificed, and their stomachs will be dissected out. The amount of gastric juice will be collected and analyzed for volume, pH, free acid, and total acid content [21].

Ethanol-Induced Gastric Ulcer

Ethanol-induced ulcer model is ideal for studying cytoprotection and antioxidant activities. This model involves gastric mucosal damage as a result of direct necrotic effects, oxidative stress, lipids peroxidation, inflammation, and reduced mucus secretion. The animals will be starved for 24 hours. The test extract, active fraction, and standard drug will be given orally to animals. After one hour, absolute ethanol will be applied to cause the formation of a gastric ulcer. After one hour, the animals will be sacrificed, and their stomachs will be extracted and observed for the presence of ulcers [22].

Anti-ulcer activity will be determined based on the level of ulcer index, percent ulcer protection, and gastric mucus content. The oxidative stress will be determined using the measurement of lipid peroxidation, superoxide dismutase, catalase, and reduced glutathione levels [23].

NSAID-Induced Gastric Ulcer

The NSAID-induced gastric ulcer model will be used to evaluate the gastroprotective effect of *Mimosa pudica* extract and active fraction. Aspirin or indomethacin induces gastric ulceration by inhibiting cyclooxygenase enzymes, reducing prostaglandin synthesis, decreasing mucus and bicarbonate secretion, and increasing acid back-diffusion, resulting in mucosal injury. Animals will be pretreated orally with crude extract, active fraction, or standard drug such as omeprazole. [24] After the treatment period, aspirin or indomethacin will be administered to induce ulcers. Animals will be sacrificed after the required time, and stomach tissues will be examined for ulcer index, percentage protection, gastric mucus, prostaglandin-related cytoprotection, oxidative stress markers, and histopathological changes. [25]

RESULTS AND DISCUSSION

This study aimed at analyzing the potential of Mimosa pudica extracts concerning their phytochemical composition, antioxidant activity, antimicrobial activity, antacid activity, and anti-ulcer activity. Various extracts were prepared and tested to determine the bioactive fraction. From all the fractions, the methanolic extract of Mimosa pudica (BTPP) showed significant biological activity and was further investigated.

Extractive Yield and Phytochemical Analysis

Among all the tested solvent types – namely butanol, chloroform, methanol, and water – the butanolic extract exhibited the highest percentage yield (16% w/w). The butanolic extract had a dark orange color and a dried powdered texture, demonstrating the successful extraction of the phytoconstituents in butanol. Preliminary phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolic compounds, proteins, triterpenoids, steroids, anthraquinone glycosides, and saponin glycosides in the methanolic extract of Mimosa pudica.

Table 1 : *Mimosa pudica* extracts obtained with their appearance and % yield (gm)

SN	Extracts	Colour	Consistency	% Yield (W/W)
1	Chloroform extracts of ***Mimosa pudica***	Dark Green	Dried powdered	10 %
2	Methanolic extracts of ***Mimosa pudica***	Dark Green	Dried	15 %
3	Butanolic extracts of ***Mimosa pudica***	Dark Orange	Dried powdered	12 %
4	Water extracts of ***Mimosa pudica***	Dark Brown	Sticky	13 %

Thin Layer Chromatography (TLC)

The TLC analysis of MEMP using a mobile phase of petroleum ether: dichloromethane (6:4) showed three distinct spots with Rf values of 0.95, 0.71, and 0.52, indicating the presence of multiple phytoconstituents with varying polarities. The separation pattern suggests that the extract contains both non-polar and moderately polar compounds, demonstrating effective resolution of constituents in the selected solvent system.

Fig 1 TLC of Methanolic extracts of *Mimosa pudica*

Antioxidant Activity

DPPH Radical Scavenging Assay

The DPPH radical scavenging assay demonstrated that all extracts exhibited concentration-dependent antioxidant activity. Among the tested samples, MEMP showed the highest free radical scavenging potential, with percentage inhibition increasing from 0.96±0.23% at 10 µg/ml to 67.67±1.95% at 200 µg/ml, indicating strong antioxidant activity. BEMP and WEMP exhibited moderate activity, while CEMP showed minimal inhibition at all concentrations tested. The standard, Ascorbic Acid, demonstrated maximum antioxidant activity with 71.83±1.6% inhibition at 200 µg/ml. The significant antioxidant potential of MEMP may be attributed to the presence of flavonoids and phenolic compounds capable of scavenging free radicals effectively.

Table 1 Percent inhibition of extracts by using DPPH radical scavenging assay

Sr. No.	Samples	Percent Inhibition					
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
1	CEMP	1.28±0.42	1.87±0.45	2.13±0.57	2.40±0.54	2.32±2.08	2.93±0.27
2	MEMP	0.96±0.23	15.25±1.50	19.22±1.20	27.40±1.89	37.75±1.98	67.67±1.95
3	BEMP	0.87±0.36	2.63±0.50	5.50±0.49	17.25±1.02	20.13±1.23	21.12±1.23
4	WEMP	1.53±0.31	3.60±0.49	7.65±1.43	13.78±1.57	17.6±2.22	19.12±1.36
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
25	Ascorbic Acid	28.76±0.23	38.54±0.28	51.61±4.31	58.28±0.15	61.52±1.17	71.83±1.6

Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay revealed that all extracts exhibited concentration-dependent antioxidant activity. Among the tested fractions, BEMP showed the highest hydrogen peroxide scavenging potential, with percentage inhibition increasing from 15.26±0.98% at 10 µg/ml to 58.69±0.23% at 200 µg/ml, indicating significant antioxidant activity. MEMP demonstrated moderate scavenging activity, whereas CEMP and WEMP showed comparatively lower inhibition values. The standard, Ascorbic Acid, exhibited maximum antioxidant activity with 71.83±1.6% inhibition at 200 µg/ml. The notable antioxidant effect of BEMP may be attributed to the presence of flavonoids, phenolic compounds, and other reducing phytoconstituents capable of neutralizing hydrogen peroxide radicals effectively.

Table 2 Percent inhibition of extracts by using Hydroxyl peroxide method

Sr. No.	Samples	Percent Inhibition					
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
1	CEMP	1.98±0.41	3.78±0.59	6.68±0.56	9.74±0.65	12.68±1.09	17.26±1.85
2	MEMP	4.12±2.03	9.35±0.36	11.76±0.36	14.25±1.36	17.64±1.23	20.42±0.98
3	BEMP	15.26±0.98	24.45±0.7	32.26±0.98	37.85±0.36	45.23±1.15	58.69±0.23
4	WEMP	2.15±1.45	4.68±1.34	5.66±1.26	8.95±1.56	15.26±1.56	20.62±0.56
		Drug Concentration (µg/ml)					
		10	20	40	80	100	200
25	Ascorbic Acid	28.76±0.23	38.54±0.28	51.61±4.31	58.28±0.15	61.52±1.17	71.83±1.6

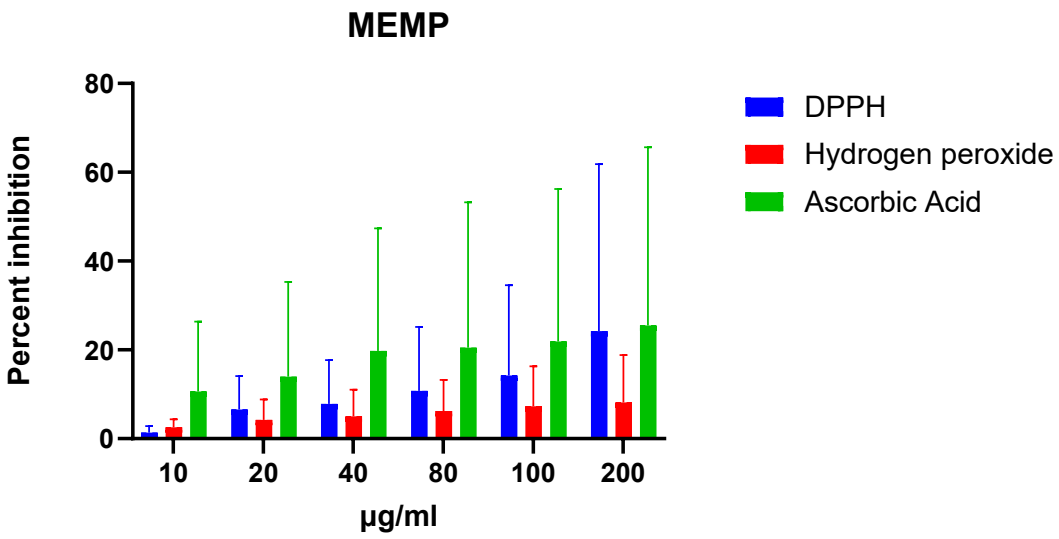


Fig 2 Percent inhibition of methanolic extracts of *Mimosa pudica* by DPPH & HP

Quantitative Phytochemical Estimation

Phytochemical assessment of MEMP showed that there is the existence of considerable amounts of secondary metabolites responsible for medicinal properties. These included phenols, which were at the level of 2.45±0.54 mg/100g. Flavonoids were also present at a concentration of 4.58±1.22 mg/100g. This suggests that it has a good antioxidant property. The tannins were also determined to be 1.25±0.75% w/w, which may assist in providing mucosal protection and astringency. The crude saponins were at 1.45±1.09% w/w, possibly conferring cytoprotection and anti-inflammatory action. Alkaloid content was found to be considerably high, at 10.24±1.46

Table 3 Quantitative phytochemical estimation from the extract of the Plant

Plant extract	Total Phenolic content mg/100 g	Total flavonoid content mg/100 gm	Tannin content %w/w	Crude saponin content %w/w	Alkaloid content %w/w
MEMP	2.45±0.54	4.58±1.22	1.25±0.75	1.45±1.09	10.24±1.46

Antimicrobial Activity

Zone of Inhibition

The antibacterial study of MEMP showed that the extract displayed a strong antimicrobial effect against all the tested bacteria in a dose-dependent fashion. Maximum activity against Pseudomonas aeruginosa (MTCC 1688) yielded an inhibition zone of 25 mm for a concentration of 250 µg/ml, followed by Escherichia coli (MTCC 443) which showed an inhibition of 24 mm for the same dose. Moderate inhibition activity was noted against Proteus vulgaris (MTCC 8427) and Salmonella typhi (MTCC 98) where the inhibition zone of 21 mm and 20 mm respectively, were obtained for 250 µg/ml. At the lowest concentration (5 µg/ml) no inhibition was observed. Although the antibacterial activity of MEMP was less compared to the standard antibiotic ampicillin and chloramphenicol, the extract showed considerable antibacterial properties. The reason behind the antimicrobial activity could be due to the presence of various plant bioconstituents like flavonoids, alkaloids, tannins, phenolics and more.

Table 4 : Zone of inhibition of extracts and standard antibiotics against Gram-negative organisms and Gram-positive organism

SN	Extr. code	Zone of Inhibition (mm)																			
		E. coli (MTCC 443)					*P. aeruginosa* (MTCC 1688)					*P. vulgaris* (MTCC 8427)					*S. typhi* (MTCC 98)				
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
	Various extracts																				

1	MEMP	-	13	16	21	24	-	11	17	20	25	-	9	15	18	21	-	10	13	18	20
Standard Antibiotics																					
7	AMP	20	23	28	28	28	20	23	24	26	27	17	20	23	28	31	16	19	22	25	29
8	CMP	14	15	16	19	23	14	15	15	18	24	15	19	20	24	28	12	15	19	24	27

Extracts: MEMP: -Methanolic extract of mimosa pudica **Standard antibiotics**-AMP: Ampicillin; CMP: Chloramphenicol;

Minimum Inhibitory Concentration (MIC)

The results of MIC assay indicated that the MEMP had significant antibacterial properties against Gram negative as well as Gram positive bacteria. MIC values for E.coli (MTCC 443), P.aeruginosa (MTCC 1688), Proteus vulgaris (MTCC 8427), S.typhi (MTCC 98), and B.cereus (MTCC 7278) ranged from 250 µg/ml, which indicated that MEMP had promising antibacterial properties. However, higher MIC values were noted for B.subtilis (MTCC 441), S.aureus (MTCC 96), and Micrococcus luteus (MTCC 106) at 500 µg/ml, which showed comparatively lesser effectiveness. Though MEMP was not as effective as commercial antibiotics such as Ampicillin and Chloramphenicol, its antimicrobial properties

Table 5 : Minimum inhibitory concentration of extracts and standard antibiotics against Gram positive and Gram-negative organism.

Minimal inhibition concentration (µg/ml)									
SN	**Extract Code**	***E. coli* MTCC 443**	***P. aeruginosa* MTCC 1688**	***P. vulgaris* MTCC 8427**	***S. typhi* MTCC 98**	***B. subtilis* MTCC 441**	***S. aureus* MTCC 96**	***M. luteus* MTCC 106**	***B. cereus* MTCC 7278**
Various extracts									
1	MEMP	250	250	250	250	500	500	500	250
Standard Antibiotics									
7	AMP	100	100	100	100	200	250	100	100
8	CMP	50	50	100	50	50	50	100	50

In silico anti-ulcerative activity evaluation of some bioactive compounds from *Mimosa pudica,* through molecular docking approach

The in-silico molecular docking of chosen phytoconstituents of Mimosa pudica with gastric H(+), K(+)-ATPase (proton pump) receptor (PDB ID: 2XZB) was found to have excellent antiulcer activity. The analysis showed that the phytocompounds of Mimosa pudica had favorable binding energy towards the binding site of proton pump, which could be indicative of their probable role as inhibitors of gastric acid secretion. The interaction of these compounds with vital amino acid residues of the receptor indicates the stability of ligand-receptor complex formation. From the compounds docked with the target protein, the flavonoids and phenols had relatively lesser values for binding energy compared to others, showing that they had greater interaction energy. This finding indicates that their binding affinity towards the target receptor is comparatively high. The docking scores were similar to the reference antiulcer compound Ranitidine, thus proving the potential gastroprotective activity.

Table 6: Analysis of inhibiting potency of bioactive compounds from *Mimosa pudica,* against the 2XZB receptor with an in-silico approach

Drug/ phytocompounds	**Binding Affinity (kcal/mol)**	**Amino acid Interaction**
Ranitidine (3001055)	-5.4	ASN87 GLY271 PRO209 ARG207 GLN177 ALA191 PHE254
Mimosa pudica		
Mimosine (155385)	-8.2	LEU307 ALA860 ALA859 PHE311 TYR340 LEU796 ILE793 TYR863
Quercetin (72537)	-7.6	ASN475 GLU470 MET477 ALA476 ARG454
Luteolin (101252)	-7.6	TYR340 TYR863 LEU307 ALA859 ALA860 PHE311

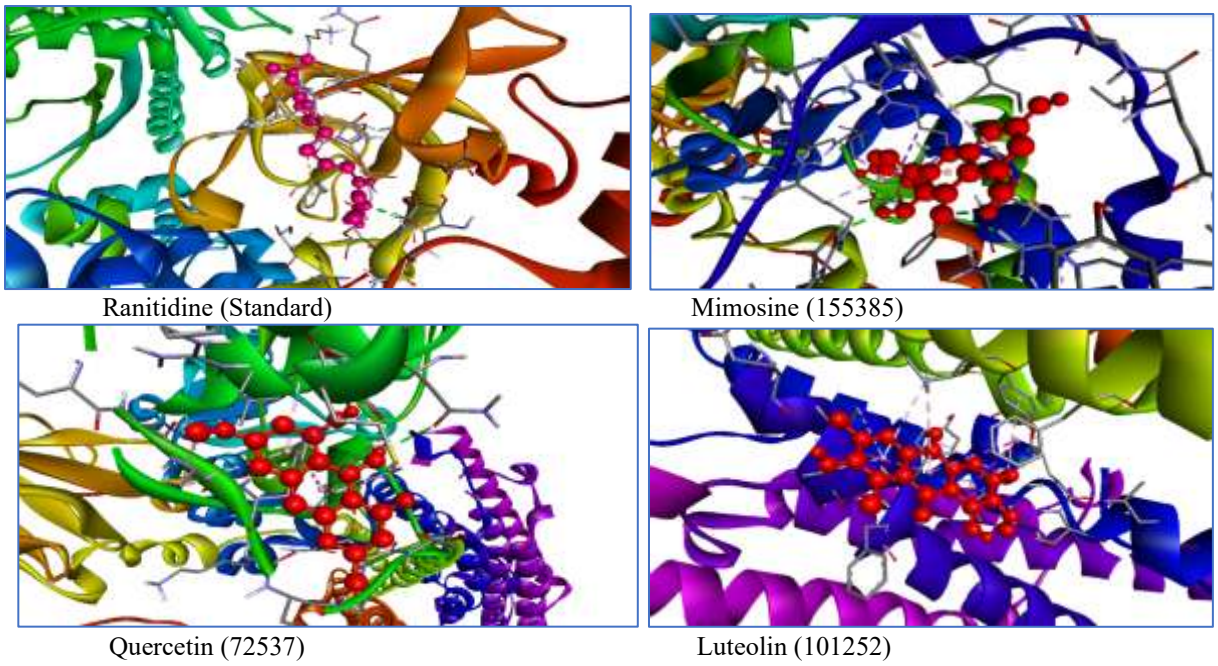


Figure 3: Docked poses of ligands with targeted protein pocket

In-vivo antiulcer activity of alcohol induced ulcer model of Butanolic extract of Mimosa pudica

Anti-ulcer results revealed a significant degree of gastroprotection from MEMP in a dose-dependent manner. The ulcer index was high in the control group (9.23 ± 0.51), while the normal group presented a low ulcer index of 0.60 ± 0.10 due to a minimum presence of gastric lesions. MEMP, when administered in doses of 250 mg/kg and 500 mg/kg, significantly decreased the ulcer index to 5.60 ± 0.32 and 3.21 ± 0.24, respectively, thus showing a percentage of inhibition of 31.20% and 55.60%. The latter is equivalent to the percentage of inhibition caused by the reference drug Ranitidine (59.30%).

Table 7 : Comparison of *Mimosa pudica* treated pylorus ligated rat

Group	Dose (mg/kg)	Ulcer index	Percentage inhibition
Normal	-	0.60 ± 0.10	-
Control	-	9.23 ± 0.51[a]	-
MEMP	250	5.60 ± 0.32[b]	31.20
MEMP	500	3.21 ± 0.24[c]	55.60
Ranitidine	50	3.13 ± 0.32[b]	59.30

*** P < 0.05 when compared with control

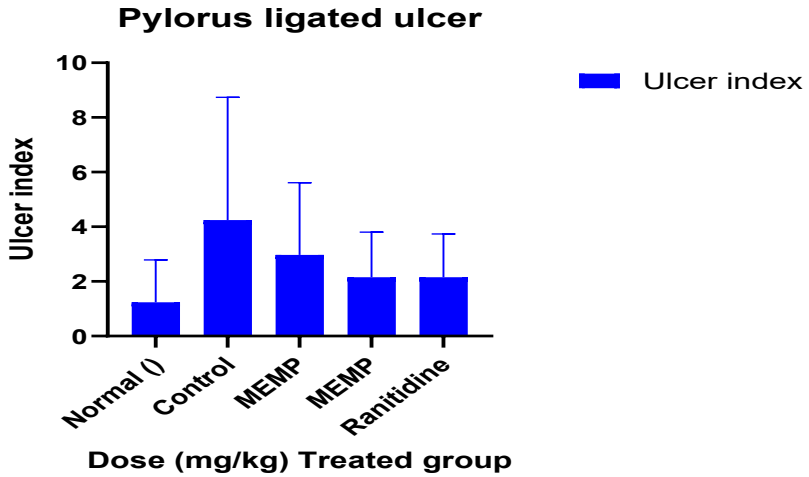


Fig 4 Graphical comparison of *Mimosa pudica* in Pylorus ligated rats.

Aspirin-Induced Ulcer Model

According to the data presented on Table no 8, there was a significant activity of aspirin-induced ulcers for 500 mg/kg of Mimosa pudica extract. In the group where aspirin was used as a standard, there was a high ulcer index value of 4.016 ± 0.22, which shows that the aspirin application resulted in high gastric mucosa damage. In the case when Mimosa pudica was used, the ulcer index decreased sharply and became 1.83 ± 0.12, which corresponds to 64.50% ulcer protection. The results obtained showed that there was a statistically highly significant ($P < 0.001$) reduction of the ulcer index in comparison to the control group.

In turn, the standard antiulcer drug called Ranitidine demonstrated even higher ulcer protective effect with 1.20 ± 0.18 ulcer index and 74.05% ulcer protection. While the activity of Mimosa pudica was a little lower than that of the antiulcer drug, the extract still showed a strong protective action against aspirin-induced ulcers. It can be assumed that the gastroprotective action of the drug is due to the content of flavonoids, tannins, alkaloids, and phenols that have antioxidant and cytoprotective properties.

Table 8: Comparison of *mimosa pudica* treated in Aspirin Induced rats

Sr. No.	Treatment Group	Mean Body Weight (gm)	Ulcer Index (Mean ± SEM)	% Ulcer Protection
1.	Control (Aspirin)	~179.67	4.016 ± 0.22	0.00%
2.	Ranitidine	~195.50	1.20 ± 0.18*	74.05%
3.	Mimosa pudica (500 mg/kg)	~177.50	1.83 ± 0.12**	64.50%
4.	P value		<0.001	

**P < 0.001 when compared with control

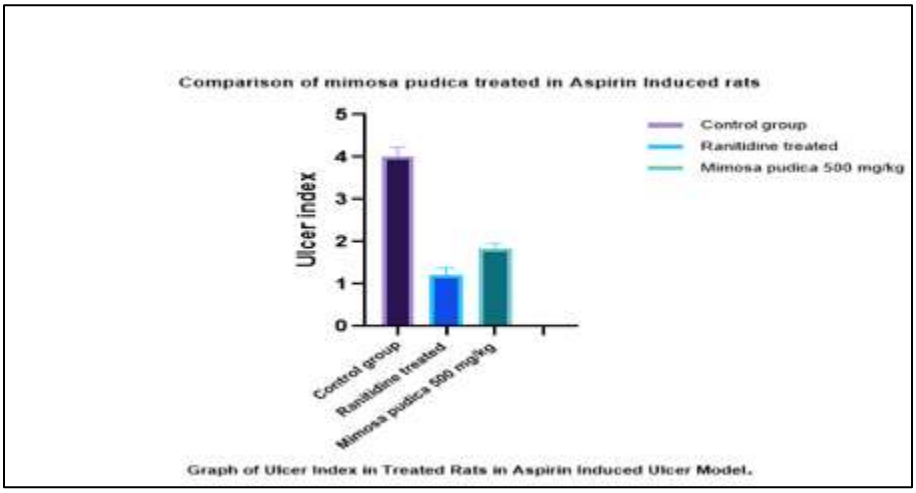


Fig 4 Graphical comparison of *Mimosa pudica* in Asprin Induced Model

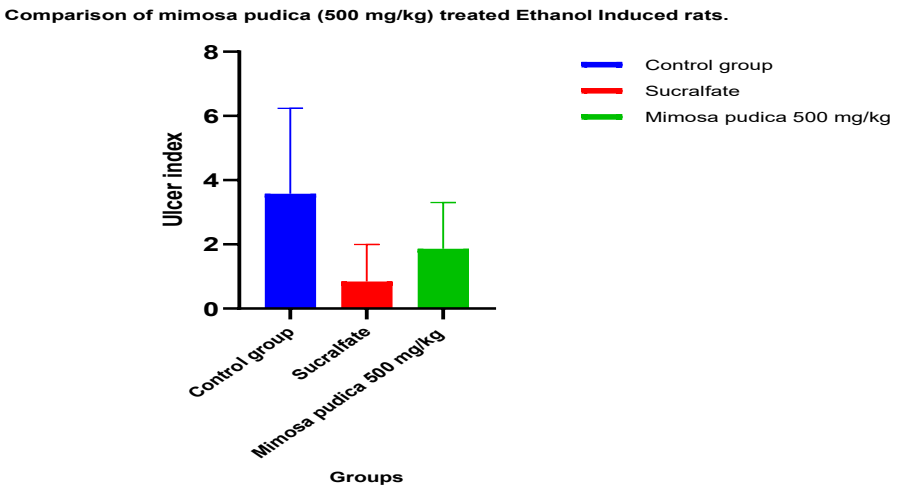
Alcohol induced ulcer model

As indicated from Table 9, the control group that was treated with ethanol had a very high ulcer index value of 6.48 ± 1.25, demonstrating serious gastric mucosa damage due to the use of ethanol. From the standard drug sucralfate treatment, the ulcer index significantly decreased to 1.66 ± 0.025 with 74.84% ulcer protection, suggesting that sucralfate is an effective gastroprotective agent. From Mimosa pudica extract treatment at 500 mg/kg as indicated in , the ulcer index significantly decreased to 2.35 ± 0.25, with 65.23% ulcer protection. According to the above data, both sucralfate and Mimosa pudica significantly protect the gastric mucosa when compared to the control group ($P<0.05$). Graph 7.22 illustrates that the ulcer index was very high in the control group, lowest in the sucralfate group, and moderately low in the Mimosa pudica group.

Table 9 : Comparison of *mimosa pudica* treated in Alcohol Induced rats

Sr. No.	Treatment Group	Dose (mg/kg)	Mean Body Weight (g)	Ulcer Index (Mean ± SEM)	% Ulcer Protection
1	Control (Vehicle)	—	176.67	6.48 ± 1.25	0.00%
2	Sucralfate	Standard	172.33	1.66 ± 0.025	74.84%
3	Mimosa pudica	500	182.50	2.35 ± 0.25	65.23%

4.	F, df value		F=480.5,		
5.	P value		<0.05		



Graph 5 Comparison of mimosa pudica (500 mg/kg) treated ethanol induced rats

DISCUSSION

This study was conducted to assess the antioxidant, antimicrobial, molecular docking, and anti-ulcer activities of the plant extracts of Mimosa pudica using in vitro, in silico, and in vivo experiments. Peptic ulcers are mainly caused by an imbalance between the aggressive and protective factors. The aggressive factors are responsible for peptic ulcers and include gastric juice, oxidative stress, NSAIDs, alcohol, and bacterial infection while the protective factors include mucus, bicarbonate, prostaglandins, and antioxidants.

Out of several different extracts isolated from Mimosa pudica, methanolic extract of M. pudica (MEMP) showed remarkable biological potential. Phytochemical investigation revealed the presence of flavonoids, alkaloids, tannins, phenols, saponins, terpenoids, and steroids, which were known to exhibit gastroprotective as well as antioxidant properties. Quantitatively, the presence of significant amounts of flavonoids (4.58±1.22 mg/100 g), phenols (2.45±0.54 mg/100 g), and alkaloids (10.24±1.46 % w/w) was observed. All the above-mentioned phytochemicals are known to possess radical scavenging properties along with their ability to inhibit lipid peroxidation, enhance mucosal protection, and decrease inflammatory responses. The TLC spot results, exhibiting three different spots at Rf values of 0.95, 0.7

The oxidative stress is a factor significantly contributing to the development of gastric ulcers due to the production of reactive oxygen species and mucosa damage. In the scavenging capacity experiment for DPPH radicals, the MEMP showed concentration dependent antioxidant property with an inhibition value of 67.67±1.95% at 200 µg/ml level, equivalent to that obtained for a control antioxidant like Ascorbic Acid. In addition, significant antioxidant properties were observed in hydrogen peroxide scavenging experiments.

The antimicrobial assays indicated wide spectrum antibacterial activity of MEMP against both Gram-positive and Gram-negative microbes. Maximum bacterial inhibition was found to be against Pseudomonas aeruginosa and Escherichia coli having zones of inhibition of 25 mm and 24 mm, respectively, at 250 µg/ml concentration. In addition, the MIC assays supported the antimicrobial properties of the extract. Because microbial infection, especially by Helicobacter pylori, is involved in the development of ulcers, the antimicrobial activity of Mimosa pudica is considered an important mechanism in its action as anti-ulcer agent.

The results obtained from the molecular docking analysis with gastric H(+),K(+)-ATPase (PDB code 2XZB) showed good binding ability of phytoconstituents like mimosine (-8.2 kcal/mol), quercetin (-7.6 kcal/mol), and luteolin (-7.6 kcal/mol) compared to ranitidine (-5.4 kcal/mol). It is suggested that these phytoconstituents can possibly exert inhibitory action against gastric proton pump activity resulting in decreased gastric acid secretion.

The in-vivo anti-ulcer study provided more evidence for the gastroprotective property of Mimosa pudica. The extract exhibited substantial gastroprotective effect in the pyloric ligated model, aspirin induced ulcers, and ethanol induced ulcers by decreasing the ulcer index. At 500 mg/kg, the extract exerted gastroprotective properties up to 55.60%, 64.50%, and 65.23% for the pyloric ligated, aspirin induced ulcers, and ethanol induced ulcers, respectively. These properties were similar to those of standard medications like Ranitidine and Sucralfate. Furthermore, histopathological

investigations showed that there was a decrease in gastric congestion, edema, hemorrhage, and necrosis. Overall, results indicate that Mimosa pudica exhibits gastroprotective activity.

CONCLUSION

This study revealed that Mimosa pudica contains good levels of antioxidant, antimicrobial, and anti-ulcer activity. Of the different extracts examined, the methanolic extract contained very potent biological activity, due to the presence of essential phytochemicals such as flavonoids, alkaloids, phenolics, tannins, and saponins. This extract contained high free radical scavenging capacity using the DPPH and hydrogen peroxide methods. In addition, molecular docking results proved high binding energy of mimosine, quercetin, and luteolin against gastric H(+),K(+)-ATPase, possibly inhibiting the process of gastric acid secretion. Moreover, in vivo antiulcer studies performed on pylorus ligation, aspirin induced, and ethanol induced ulcers, showed significant anti-ulcer index activity similar to other antacid drugs. Thus, the results prove the effectiveness of using Mimosa pudica in ulcer treatment.

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