GC-MS Analysis, Gastroprotective and In Silico Docking Studies of Phytoconstituents from Ixora Javanica Flowers

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ABSTRACT: Ixora javanica belongs to the family Rubiaceae having phytoconstituents that are useful in the treatment of various diseases. In our study, the methanolic extract of Ixora javanica flowers was screened for its antiulcer activity. The extract was analyzed using GC-MS to identify significant active constituents, and these constituents were subjected to molecular docking to study their affinity towards the H+/K+ ATPase enzyme. The dry powder of the Ixora javanica flowers was extracted by methanol as the solvent for the soxhlation technique. The extract was then subjected to the GC-MS examination. The in-vivo antiulcer activity was screened using ethanol, indomethacin in pylorus ligation induced gastric ulcer models. The pharmacological evaluation of the extract was carried out using 200 and 400 mg/kg bd. wt. Omeprazole (20 mg/kg, bd.wt, p.o) was used as the standard. Ulcer scores were calculated. Glide 5.6 (Schrodinger Inc.) was used for generating docking simulation studies. The various phytochemical constituents identified from GC-MS study were formononetin, ferulic acid, quinic acid, palmitic acid, oleanolic acid and maslinic acid in higher amounts. The extract exhibited a substantial reduction in the ulcer scores in ethanol, indomethacin, and pylorus ligation prompted gastric ulcer prototypes. A decrease in the ulcers might be due to the presence of phytochemical constituents like terpenoids, flavonoids, phenolic acids, glycosides, alkaloids, saponins, and tannins present in Ixora javanica flowers, which clearly showed that the extract possesses gastroprotective activity. Molecular docking studies confirmed the H+/K+ ATPase inhibitory effect. Among the identified constituents, formononetin, ferulic acid, and rutin have shown the highest docking scores when compared to other compounds.

Keywords: Anti-ulcer, Docking, H+/K+ ATPase, GC-MS, Ixora javanica, Omeprazole.
1. INTRODUCTION

Gastric ulcers are an important disease of the digestive system and affect 5–10% of the adult population, becoming a global problem due to their higher morbidity and mortality, as well as their medical, social and economic impact. Population upsurge, the scanty supply of drugs, the expensive cost of treatments, side effects of several synthetic drugs, and development of resistance to presently used drugs for transmittable diseases have led to the augmented emphasis on the use of plant materials as a source of remedies for a varied variety of human illness. Helicobacter pylori, genetic factors, alcoholic beverages and the use of non-steroidal anti-inflammatory drugs (NSAIDs) are the main contributing factors for gastric ulceration. Antacids, anticholinergics, proton pump inhibitors and histamine H$_2$-receptor antagonists are drugs currently used for the treatment however, they cannot be tolerated in the long-term because of their safety profile. Thus, medicinal plants have become attractive options for the development of newer agents due to their lower side effects. Ixora javanica, belonging to the family Rubiaceae, is traditionally beneficial for numerous infirmities like hepatic disorder, cancer, microbial infection, antioxidiant, pain, edema etc and has been predictable for several therapeutic properties. The flowers and leaves of Ixora javanica are used as a sedative stomachic tonic, intestinal antiseptic and as astringent. The flower parts of this plant are used to heal sores and chronic ulcers. Peptic ulcer disease had been a significant cause of morbidity and mortality for almost more than a century. The term peptic ulcer refers to an acid peptic injury of the digestive tract, resulting in mucosal break reaching the submucosa. Peptic ulcers are usually located in the stomach or proximal duodenum, but they can also be found in the esophagus or Meckel’s diverticulum. Traditionally, a hypersecretory acidic environment, together with dietary factors or stress was thought to cause most peptic ulcer diseases. However, the discovery of Helicobacter pylori infection and the ubiquitous use of Non- Steroidal Anti-Inflammatory Drugs (NSAIDs) in the mid-20th century have changed this perception. Recently, herbal compounds have played an important role in the discovery and development of modern drugs against ulcers and other diseases due to their potentially improved safety and efficacy over conventional treatments. Its flowers are traditionally used for chronic ulcers. There have been no reports on anticiultr activity of Ixora javanica in the current literature. Therefore, the present study assessed the anticiultr activity of the methanolic extract of Ixora javanica and their isolated compounds affinity with H$^+$/K$^+$ ATPase inhibitory effect so that their binding interactions can be studied, with the aim of developing a safe and effective drug for treating gastric ulcers.

2. MATERIALS AND METHODS

2.1 Plant material

The flowers of Ixora javanica were collected and air-dried under shade, powdered mechanically and stored in an airtight container. The powder was extracted using a soxhlet apparatus and ethanol as solvent, dried, and stored in a refrigerator for further use. The plant part was authenticated (Ij 27022018) by a botanist at the New Government Degree College, Kukatpally, Hyderabad, India.

2.2 Phytochemical screening

The preliminary phytochemical screening was performed with the methanolic extract of Ixora javanica flowers (MEIJ) for the detection of various phytochemicals.

2.3 Identification of phytochemical constituents using GC-MS

The GC-MS analysis was carried out by Shimadzu, GCMSQP2010 model instrument coupled with mass spectroscopy as the detector. The ZB-5MS Column with dimensions 30m×0.32mm×0.25µm was used for analysis. The oven temperature was adjusted to 50°C and the solvent cut time 5 min. The column flow is 1.5 mL. The inlet temperature was kept at 250°C, and the source temperature of 210°C and an interface temperature of 260°C.

2.4 Animals

Wistar albino rats of both sexes weighing up to 200-250 g were used. The animals were accommodated in enclosures under standard conditions. All the experimental protocols were duly approved by the Institutional Animal Ethics Committee (Protocol Apvl No: I175/PO/Re/S/08/ CPCSEA).

2.5 Acute toxicity Studies

Acute toxicity testing was conceded out on Wistar albino mice by the oral route at a dose of 2000 mg/kg of the methanolic extract of Ixora javanica flowers as per the OECD-guidelines 425.

2.6 Experimental models

2.6.1 Ethanol-induced gastric ulcer model

After 48 h fasting, rats were divided into 4 groups of 6 animals (each group) and treated orally with distilled water (10 ml/kg), extract (200 and 400 mg/kg), and omeprazole (20 mg/kg). Sixty minutes after this procedure, every animal received ethanol (1 ml/200 g). One hour later, the rats were euthanized, stomachs were removed, opened along the greater curvature, and the ulcer score was determined.

2.6.2 Indomethacin induced gastric ulcer model

Rats were fasted for 48 h and treated orally with vehicle (distilled water, 10 ml/kg), extract (200 and 400 mg/kg), and omeprazole (20 mg/kg). One hour after the treatment, 60 mg/kg of indomethacin was administered orally to all the groups. Four hours later, the animals were euthanized, stomachs were removed, opened along the greater curvature, and the ulcer score was determined.

2.6.3 Pylorus ligation induced gastric ulcer model

Rats were fasted for 36 h and divided into 4 groups of 6 animals in each and treated orally with distilled water (10 mL/kg), extract (200 and 400 mg/kg), and omeprazole (20 mg/kg). The pylorus was ligated under light thiopental sodium anaesthesia with care taken not to cause bleeding or to occlude blood vessels. Six hours after ligation, the animals were sacrificed by an overdose of thiopental sodium, and the stomach part was isolated, contents were collected, measured for volume, and subjected to analyse the acidity against 0.1 N NaOH to pH 8 using a pH meter. The total
Acidity was calculated. Each stomach was examined for lesions.\textsuperscript{12,13}

2.6.4 Determination of pH

A fraction of 1mL gastric juice was diluted with 1mL of distilled water, and the pH of the solution was measured using pH meter.\textsuperscript{12,13}

\[
\text{Acidity} = \left( \frac{\text{Vol. of NaOH} \times N \times 100}{0.1} \right) / \text{mEq/L}
\]

2.6.5 Determination of total acidity

A fraction of 1mL gastric juice diluted with 1mL of distilled water was taken into a 50 mL conical flask, and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted.\textsuperscript{12,13} The total acidity is expressed as mEq/L by the following formula:

\[
\% \text{ protection} = \left( \frac{([\text{Ulcer Score of control} - \text{Ulcer Score of the test}])}{\text{Ulcer Score of control}} \right) \times 100
\]

2.6.6 Determination of Free acidity

The Topfer’s reagent was used. A fraction of gastric juice was titrated with 0.01N NaOH until the canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

2.6.7 Determination of Gastric volume

After sacrificing the animal, the stomach is dissected out, gastric juice is collected, drained into tubes & was centrifuged at 1000 rpm for 10 min, and volume is noted.\textsuperscript{14}

2.6.8 Percentage protection

Percentage protection was calculated by using the formula:

2.6.9 Histopathology

For histopathology assessment, the dissected stomach tissues were fixed in a 10% buffered formalin solution. Sections were deparaffinised and were stained with haematoxylin and eosin.\textsuperscript{12}

2.7 Docking studies

Docking simulations predicted the binding orientation of drug candidates to their protein targets. Glide 5.6 (Schrodinger Inc.) was used for generating docking simulation studies. Docking simulations were performed in Dell precision T-1500 workstation Intel (R) Core (TM) i7 CPU 860 @GHz; 12.0 GB Ram, 1 TB Hard disk. Protein-ligand interactions were visualized using Maestro 9.1. Proton pump inhibitors (PPIs) block the gastric H+/K+ ATPase, inhibiting gastric acid secretion. The crystallized x-ray structure of H+/K+ ATPase (PDB ID: 5A5N) was retrieved from the RCSB protein bank. Protein-ligand interactions were stimulated though flexible glide-ligand docking with XP extra precision mode. The best-docked structures were chosen using the glide score function. The more negative the glide score, the more favourable the binding. Additionally, the docked ligand poses were visualized, and the different ligand-receptor interactions were studied.\textsuperscript{15} In the present study, six compounds, namely formononetin, ferulic acid, rutin, oleanolic acid, ursolic acid, and maslinic acid, are docked against H+/K+ ATPase (PDB ID: 5A5N).

3. STATISTICAL ANALYSIS

Data were expressed as mean ± S.E.M. Comparisons between means of different groups were analyzed by ANOVA followed by the Dunnett’s test. The Graph Pad Prism software package, version 8 (Graph Pad Software, Inc., San Diego, CA, USA), was used to perform all statistical investigations.

4. RESULTS AND DISCUSSION

2.1 Phytochemical screening

The preliminary phytochemical investigation for the methanolic extract of \textit{Ixora javanica} flowers showed the presence of flavonoids, tannins, steroids, glycosides, carbohydrates, alkaloids, saponins, and terpenoids.

4.2 Identification of phytochemical constituents using GC-MS

The crude methanolic extract of the \textit{Ixora javanica} flower was subjected to GC-MS analyzer. Nearly 40 bio compounds were identified from extract, namely formononetin, ferulic acid, quinic acid, palmitic acid, oleanolic acid, maslinic acid, rutin, serine, supraene, malonic acid, 2-4-butyl-4-methyl-5-oxo (1,3) dioxdole carboxylic acid, erythro 4- hydroxy arginine lactone in high amounts. Fig 1 shows the GC-MS chromatogram of the extract.
4.3 Acute toxicity studies

Methanolic extract of *I. javanica* flowers was tested on female Wistar Albino mice up to a dose of 2000 mg/kg bd.wt., p.o. Methanolic extract of *I. javanica* flowers did not exhibit any signs of toxicity and mortality up to 2000 mg/kg, bd.wt. All animals were safe even after 14 days of observation. The pharmacological evaluation was done using 200 and 400 mg/kg bd. wt.

4.4 Ethanol-induced gastric ulcer model

Methanolic extract of *I. javanica* flower extracts has shown a significant reduction in ulcer scores at 200 mg/kg, bd.wt (p<0.001), 400 mg/kg, bd.wt (p<0.001) and standard omeprazole 20 mg/kg, bd.wt. showed significant inhibition of ulcers by 44%, 65.05%, and 87.78%, respectively.

4.4.1 Macroscopic appearance of the gastric mucosa in Ethanol-induced ulcer models

In figure 2, disease control 4-5 bands of thick and dark red erosions with 0.5-1 mm in width were observed. In MEIJ, 200 mg/kg bd.wt 1-2 bands of thick and dark red erosions and erythema were observed. In MEIJ, 400 mg/kg bd.wt 3-4 bands of light red erosions and erythema were observed. In Omeprazole, 20 mg/kg bd.wt no lesions, but slight erythema was observed. A significant reduction in ulcers was observed in the test extract and the standard groups when compared to the disease control group.

4.4.2 Histopathology studies of ethanol-induced gastric ulcerated rat stomach

Rat’s stomach in the disease control group, gastric mucosal hyperplasia, gastric pits, and inflammation were observed. In MEIJ 200 mg/kg, bd. wt. group, gastric mucosa scant inflammatory cells appeared. No gastric pits but slight hyperplasia was observed. In MEIJ 400 mg/kg., bd. wt. group, scant inflammatory cells, and gastric mucosal thickness appeared to be normal, no pits were observed. In the standard omeprazole (20 mg/kg bd.wt.) group, mucosal thickness appeared to be normal, no inflammation and no pits were observed (Figure 3).
Ethanol is an ulcerogenic agent that induces gastric mucosa by promoting disturbances of mucosal microcirculation, ischemia, and appearance of free radicals, endothelin release, degranulation of mast cell and inhibition of prostaglandins and decrease of gastric mucus production. Oleanolic acid (terpenoid) treatment caused a significant increase in PGE2 content in the gastric epithelial cells, which resulted in a decrease in ulcers. Ursolic acid (terpenoid) decreases in the LPO level while the increase in SOD, CAT, and GSH levels. Phenols exhibit antioxidant properties by the virtue of scavenging free radicals by breaking radical chain reactions, attenuating peroxides level, and triggering antioxidant defense enzyme systems contributing to the antiulcer effect. Tannins have astringent effects which stimulate protein precipitating and vasconstriction resulting in information of impenetrable protective barriers preventing gastric ulcer by reducing the number of ulcer score. Formononetin (flavonoid) decreases the abruptly increased MDA concentration due to exposure to ethanol. Glycosides possess potent antioxidant properties by decreasing lipid peroxidation and increasing antioxidant level. Alkaloids have gastro protective effects by stimulating nitric oxide manufacture, acting by modifying the levels of pro-inflammatory cytokines (IL-8), and reducing the action of myeloperoxidase (MPO) and lipid peroxidation, signifying a potential antioxidant activity. MEIJ significantly reduced the ulcer index and afforded significant protection against ethanol-induced ulcers. Terpenoids, flavonoids, tannins, glycosides, and alkaloids present in MEIJ could be the reason for its antiulcer activity in ethanol-induced ulcers.

4.5 Indomethacin induced gastric ulcers

Methanolic extract of I. javanica flowers has shown a significant reduction in ulcer scores at 200 mg/kg., bd.wt. (p<0.001), 400 mg/kg., bd.wt. (p<0.001) and standard omeprazole 20 mg/kg bd. wt. (p<0.001) when compared to the disease control group. Results were shown in Table 1. The extract at a dose of 200 mg/kg bd. wt., 400 mg/kg bd. wt. and standard drug omeprazole at 20 mg/kg bd. wt. showed significant inhibition of ulcers by 47.78%, 63.05%, and 82.63%, respectively.

4.5.1 Macroscopic appearance of the gastric mucosa in indomethacin-induced ulcer model

Figure 4 depicts the macroscopic appearance of the gastric mucosa in the indomethacin-induced ulcer model. In disease control (group I), grade 5 lesions, along with haemorrhage, were observed. In group II (MEIJ, 200 mg/kg bd. wt), 2-3 erosions with <5 mm in length and 0.5–1 width erythema were observed. In group III (MEIJ, 400 mg/kg bd. wt) pits in the gastric mucosa were observed. Group IV (Omeprazole, 20 mg/kg bd. wt) no lesions, but small petechiae were observed. A significant reduction in ulcers was observed in the test extract and the standard groups when compared to the disease control group.

![Fig 4: Macroscopic appearance of the gastric mucosa in indomethacin induced ulcer model](image-url)
were observed. In MEIJ (400 mg/kg bd.wt.) group, petechiae were observed and omeprazole, 20 mg/kg bd.wt., group, erythema was observed, as shown in figure 5. A significant reduction in ulcers was observed in the test extract and the standard groups when compared to the disease control group.

Fig 5: Macroscopic appearance of the gastric mucosa in pylorus induced ulcer model

**Pylorus ligation** causes accumulation of acid and pepsin, which leads to the auto digestion of gastric mucosa and ulceration. Ferulic acid (phenol) administration of ferulic acid to pylorus ligated animals resulted in a decrease in ulcer index and volume of gastric contents. Total acidity and free acidity was reduced upon administration of ferulic acid along with decrement of lipid peroxidation. Ursolic acid (Terpenoid) treated Wistar Albino rats showed a significant increase in gastric pH and gastric juice volume and total acid- pepsin output. Gallic acid (Phenolic acid) caused a decrease in gastric juice volume, total acidity, free acidity, and pepsin concentration. Pepsin concentration significantly decreased after treatment with gallic acid. Gallic acid decreased the plasma protein leakage from gastric mucosa by strengthening the mucosal defense. The strengthening of mucosal defense is further exemplified by a decrease in cell exfoliation, as seen from the reduction in DNA content of the gastric juice. Tannins possess as an antiulcer agent by its astrin gency property and vasoconstriction effects. Due to the precipitation of micro proteins on the ulcer site, a protective layer was formed, which hinders gut secretions and protects the mucosa from toxins and other irritants. Saponins have also been stated to retain the antiulcer property, possibly due to its surfactant-like properties. MEIJ has shown a significant reduction in the gastric volume, free and total acidity, and an increase in the pH, thus, proving its antiulcer activity. The phenolic acids, terpenoids, tannins, and saponins present in the MEIJ could be responsible for the reduction of ulcers in the pylorus ligated rats. The compounds interacted with the active site of H+/K+ ATPase was found to inhibit H+/K+ ATPase activity. Formononetin, ferulic acid, and rutin have shown the highest docking scores when compared to other compounds. More negative, the glide score is better than the binding affinity. Hydrogen bonding is an exchange reaction where the hydrogen bond donors and acceptors of the free protein and ligand break their hydrogen bonds with water and form new ones in the protein-ligand complex. More negative the score, stronger is the hydrogen bonding. Rutin and oleanolic acid have the highest hydrogen bonding score when compared to other compounds.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ethanol induced</th>
<th>Indomethacin induced</th>
<th>Pylorus ligation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcer Score</td>
<td>% Protection</td>
<td>Ulcer Score</td>
</tr>
<tr>
<td>Disease control</td>
<td>4.75±0.11</td>
<td>-</td>
<td>7.66±0.21</td>
</tr>
<tr>
<td>MEIJ (200 mg/kg)</td>
<td>2.66±0.1</td>
<td>44%</td>
<td>4.00±0.3</td>
</tr>
<tr>
<td>MEIJ (400 mg/kg)</td>
<td>1.66±0.1</td>
<td>65.05%</td>
<td>2.83±0.21</td>
</tr>
<tr>
<td>Omeprazole (20 mg/kg)</td>
<td>0.58±0.08</td>
<td>87.78%</td>
<td>1.33±0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were expressed as (*= p <0.001) vs disease control group and (**= p <0.001, ***= p <0.05) vs standard group.

### 4.7 Ulcer healing study

Methanolic extract of *I. javanica* flowers has shown a significant reduction in the gastric volume, free and total acidity at 200 mg/kg bd. wt. (p <0.001), 400 mg/kg bd. wt. (p<0.001) and standard omeprazole (20 mg/kg bd. wt.) (p<0.001). The MEIJ has shown a significant increase in the pH at 200 mg/kg bd. wt. (p <0.05), 400 mg/kg bd. wt. (p<0.05) and standard omeprazole (20 mg/kg bd. wt.) (p <0.001), as shown in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gastric volume</th>
<th>pH</th>
<th>Free acidity</th>
<th>Total acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.366±0.09</td>
<td>1.86±0.08</td>
<td>58±0.7</td>
<td>46±0.5</td>
</tr>
<tr>
<td>Disease control</td>
<td>2±0.1</td>
<td>1.21±0.05</td>
<td>85.5±0.4</td>
<td>76.166±0.7</td>
</tr>
<tr>
<td>MEIJ (200 mg/kg)</td>
<td>1.466±0.06</td>
<td>3.1±0.04</td>
<td>77.167±0.4</td>
<td>67±0.5</td>
</tr>
<tr>
<td>MEIJ (400 mg/kg)</td>
<td>1.01±0.06</td>
<td>3.6±0.08</td>
<td>68.667±0.8</td>
<td>58.666±0.8</td>
</tr>
</tbody>
</table>

Table 1: Effect of MEIJ on various groups in different models of rats

Table 2: Effect of MEIJ on gastric contents
4.8 Molecular Docking

Docking studies of MEIJ and standard omeprazole were observed against H+/K+ ATPase protein (PDB ID: 5A5N). The parameters analyzed in the study include glide score, hydrogen bonding, and lipophilicity (Table 3). The results show that formononetin is having highest glide score (-8.37) amongst the test compounds followed by ferulic acid (-8.30), rutin (-7.34), oleanolic acid (-5.53), ursolic acid (-4.30), maslinic acid (-3.60). Standard compound omeprazole has a glide score of -8.63. The hydrogen bonding interactions of these compounds were shown in Figure 6.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the compound</th>
<th>Glide score</th>
<th>Hydrogen bond</th>
<th>Lipophilicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Omeprazole</td>
<td>-8.63</td>
<td>-1.61</td>
<td>-0.13</td>
</tr>
<tr>
<td>2</td>
<td>Formononetin</td>
<td>-8.37</td>
<td>-0.70</td>
<td>-3.32</td>
</tr>
<tr>
<td>3</td>
<td>Ferulic acid</td>
<td>-8.30</td>
<td>-0.56</td>
<td>-3.31</td>
</tr>
<tr>
<td>4</td>
<td>Rutin</td>
<td>-7.34</td>
<td>-3.54</td>
<td>-1.50</td>
</tr>
<tr>
<td>5</td>
<td>Oleanolic acid</td>
<td>-5.53</td>
<td>-1.66</td>
<td>-0.71</td>
</tr>
<tr>
<td>6</td>
<td>Ursolic acid</td>
<td>-4.30</td>
<td>0</td>
<td>-2.31</td>
</tr>
<tr>
<td>7</td>
<td>Maslinic acid</td>
<td>-3.60</td>
<td>-0.96</td>
<td>-1.17</td>
</tr>
</tbody>
</table>

In the figure 6, docking interaction studies were represented having the following interactions. A: Hydrogen bonding interaction of omeprazole with PDB- 5A5N. Omeprazole (total score- 8.63) demonstrated hydrogen bonding interactions with Asn 1064 and lipophilic bond interaction with Val 1008, Phe 1009, Val 1013, Glu 1017, Val 1018, Tyr 1021, Ala 1060, Ile 1074. B: Hydrogen bonding interaction of ferulic acid with PDB- 5A5N. Ferulic acid (total score -8.30) demonstrated hydrogen bonding interaction with Met 1029 and lipophilic bond interaction with Phe 1009, Val 1013, Tyr 1021, Ala 1060, Tyr 1063, Ile 1074. C: Hydrogen bonding interaction of formononetin with PDB- 5A5N. Formononetin (total score – 8.37) demonstrated hydrogen bonding interaction with Tyr 1063 and lipophilic bond interaction with Val 1008, Phe 1009, Val 1013, Glu 1017, Val 1018, Tyr 1021, Ala 1060, Ile 1074. D: Hydrogen bonding interaction of rutin with PDB- 5A5N. Rutin (total score -7.34) demonstrated hydrogen bonding interaction with Arg 1077, Asn 1064 and lipophilic

5. CONCLUSION

The plant extract was prepared using methanol, and the phytochemical screening revealed the presence of phytoconstituents such as terpenoids, flavonoids, tannins, steroids, alkaloids, saponins, glycosides, and carbohydrates. The GC-MS analysis of extract showed the presence of 40 bio compounds. Acute toxicity studies have revealed that the methanolic extract of *Ixora javanica* flowers was found to be safe up to 2000 mg/kg bd.wt. The methanolic extract of *Ixora javanica* flowers has shown a significant reduction in ulcers;

9. REFERENCES


5. CONCLUSION

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9. REFERENCES


thus, proving its antiulcer activity. Molecular docking studies confirmed the H+/K+ ATPase inhibitory effect of the compounds obtained from GC-MS study. Further studies are required to focus on the isolation of specific phytochemicals and elucidating the mechanism of action.

6. AUTHORS CONTRIBUTION STATEMENT

The authors Dr M. Ganga Raju, Anusha, Gayathri and Nikitha conceived the present idea. Anusha, Gayathri, and Nikitha designed and performed the experiments. Dr M. Ganga Raju and Dr. NVL Suvarchala analyzed the data and wrote the paper with input from all authors.

7. ACKNOWLEDGEMENTS

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8. CONFLICT OF INTEREST

Conflict of interest declared none.


