RESEARCH PAPER

"Development And Characterization of Ixora Coccinea L. Flower-Loaded Nanoparticles with *In Silico* and *In Vivo* Gastroprotective Efficacy"

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ABSTRACT

Background: A peptic ulcer refers to an acid-induced injury to the digestive tract, leading to a mucosal break that extends into the submucosa, and leading contributors of death globally.

Objectives: Explore *in-vitro* antioxidant and antiulcer potential of *Ixora coccinea* (IC) flower extract with nano formulation (NEIC), and nano formulation with curcumin (NEICC) against ethanol induce model and pylorus ligation ulcer challenge with histological studies.

Method: NEIC and NEICC were formulated and evaluated for particle size, PDI and SEM. EEIC (IC_{50}: 35.54, 33.9, 35.2), NEIC (IC_{50}:30.18, 30.6, 31.6) and NEICC (IC50: 29.1, 25.8, 27.9) was evaluated with ·NO, ·OH and ·H_2O_2 scavenging assay with ascorbic acid (standard; IC_{50}: 28.7, 19.5, 25.4). Results showed prominent antioxidant potential as compare to ascorbic acid with IC_{50} values. EEIC, NEIC, and NEICC provide significant protection to the gastric mucosa.

Result: The findings revealed a significant antioxidant capacity when compared to ascorbic acid. EEIC, NEIC, and NEICC provide substantial protection to the gastric mucosa in ulcer-induced challenge.

Conclusion: NEIC and NEICC significantly improved the biochemical parameters in the pylorus ligation-induced ulcer model, evidenced by a notable reduction in acidity, UI as well as increased pH of gastric content. Present study suggests that NEIC and NEICC possesses cost effective treatment for antiulcer and antisecretory action.

Keywords:

How to cite this article: Rangum Charitha1 Kalyani Kakad2 Sneha Ramesh Nawale*3. "Development And Characterization of Ixora Coccinea L. Flower-Loaded Nanoparticles with In Silico and In Vivo Gastroprotective Efficacy". Int J Drug Deliv Technol. 2026;16(87s):1084-1096. DOI: 10.25258/ijddt.16.87s.111.

INTRODUCTION

Globally, peptic ulcer disorder has recognized as deadly disease in 20th and 21st centuries, that involves gastric and duodenal ulcers. Ulcers are an open sore on mucus membrane characterized by sloughing of swollen dead tissue usually present in skin of lower extremes and GIT [1]. It is characterized by an imbalance between aggressive and defensive factors [2,3]. Due to a elevated pervasiveness of *H. pylori* infection, fatality consistently increasing and became an important cause for hospitalization globally [4, 5] and appealed nearly 3 lakh lives around the World (2013) [6, 7]. Among PUD patients, endured surgery, almost 86% had colonic ulcers, though 14% with stomach ulcers and its primary complications with 5.7% fatality rate [8]. Globally, *H. pylori* infection and the unsupervised use of aspirin have been identified as major cause of PUD [9, 10]. Anxiety, Zollinger-Ellison condition, and smoking leading to lower the PG level, are major factors [11].

Adequate research has been conducted to identify herbal drugs for prevention of ulcer formation. Previous studies have suggested the potential use of medicinal plant-based formulations as antiulcer agents, involving various genera such as Euphorbia, Allium, Emblica, Curcuma, and Lawsonia [12, 13]. *Ixora coccinea* (Family: Rubiaceae), a bush, popular as hedging plant in subtropic provinces of India with corymb-like cymes with red, orange, pink, or yellow blooms (2–5 inches wide). *Ixora coccinea* flowers (ICF) are recognized as a hepatoprotective, anticancer, and chemotherapy-protective agent [13]. Secondary metabolites found in the flowers include cycloartenol, lupeol, β-sitosterol, ursolic and oleanolic acid [14]. Lupeol is also found in the leaves, used to treat dermatological disorders. *Ixora coccinea* flowers possess carminative, astringent, bitter, and digestive properties. *Ixora coccinea* flowers are beneficial in treatment of dysentery, painful menstruation, haemoptysis, catarrhal bronchitis, abscesses and ulceration [15].

Natural medicines are generally used globally since eras but effectiveness of most of beneficial herbs be dependent on the absorption and delivery of secondary metabolites. Most secondary metabolites cannot cross the cytoplasmic membrane owing to their considerable molecular size or else low solubility in aqueous medium, thereby shows less absorption and deprived bioavailability [16, 17]. Because of this most of secondary metabolites are

least or non-considerable for *in vivo* action in spite of their promising *in vitro* potential. It is recommended to incorporate natural secondary metabolites with nanosystem, as nanostructured systems could be capable of strengthening biological action of secondary metabolites by reducing the dose and complications, enhancing bioactivity [18]. Several nano-oriented advances levegered with purpose of improving the technological aspects of herbal drugs [19]. Nanosuspension has been developed as efficacious formulation for water insoluble constituent release with pronounced manner [20]. Certain limitations of previous particle size reduction technologies, pharmaceutical scientists are seeking a comprehensive approach that can effectively address formulation challenges related to the poor solubility of natural drugs, bringing it to the desired level [21, 22].

Traditionally *Ixora coccinea* was reported to possess antiulcer action so, efforts have made to access antiulcer activity of *Ixora coccinea* with its nano formulation.

MATERIALS AND METHODS

Flowers of *Ixora coccinea* were identified, collected, authenticated by P. Suresh Babu (Plant scientist) from the Government Degree College, Kukatpally, Hyderabad (TS), India. Crude flower material was cleaned, dried under concealer for around ten days, powdered and crude material was stored for further use.

Preparation of Ethanolic extract of *Ixora coccinea*

Crude powdered material of *Ixora coccinea* flowers was extracted with ethanol by maceration and preliminary phytochemical screening was performed to identify various secondary metabolites *viz.* flavonoids, phenolic compounds, steroid, and terpenoids [23].

GC-MS study and quantitative estimation of phenols and flavonoids of *Ixora coccinea*

Crude *Ixora coccinea* flowers extract was analysed for identification of secondary metabolites by GC-MS instrument (Agilent 6890 series), department of technology, Osmania University, Hyderabad.

Total phenolic and flavonoids of *Ixora coccinea* was estimated as per the approach given by Singleton and Rossi, 1965 and Fattahi S *et al*., 2014 respectively [24-27].

Formulation of *Ixora coccinea* nanosuspension

Bottom-up approach was used for formulation of NEIC and NEICC, which is based on principle of solubilizing the drugs in a solvent, convert them into Nano-sized particles/formulation [28].

Formulation of NEIC and NEICC: Polyvinyl alcohol solution was prepared by dissolving PVA in water (Solution I). For preparation of **NEIC and NEICC**, extract solution is prepared by dissolving extract in minimum quantity of ethanol (Solution II) and extract along with curcumin (250 mg) in minimum quantity ethanol (Solution II) respectively. PVP solution and extract solutions were mixed, sonicated and chitosan was added drop by drop into a beaker for formulation of **NEIC and NEICC**.

Characterization of Nanosuspension

Particle size Distribution, poly dispersity index (PDI) and SEM

NEIC and NEICC was studied for particle size, PDI and SEM. Particle size and PDI of **NEIC and NEICC** was determined by Microtrac, Model- W-3275, USA.

Morphological features and particle size of **NEIC and NEICC** were identified with scanning electron microscope field emission EM (Shimadzu), in Osmania University Hyderabad, TS, India at acceleration voltage of 15KV at 25°C to generate high-resolution images [29].

In vitro Drug delivery studies

In vitro drug delivery was executed by accurately weighing nanosuspension equivalent to 10 mg of extract and transferred into empty capsules. Dissolution was performed in accordance with USP/NF guidelines using a USP Type I (Basket) dissolution apparatus and medium (phosphate buffer; pH 6.8, speed-50 rpm) at RT. At pre-set rests, aliquots were collected, samples were analysed for the cumulative per cent drug dissolved/released at their respective wavelengths [30].

Entrapment efficiency of NEIC and NEICC

Centrifugation was done for NEIC and NEICC at 3000 rpm for 10 min, filtered through Whatman No. 1 filtered paper. Aliquots were diluted to 1:10 same solvent and scanned at 200 to 600 nm with Shimadzu UV-1800, Mumbai [31]. Entrapment efficiency (%) was calculated.

FT-IR study

Fourier transform infrared spectroscopy

KBr pellets of Ethanolic extract of *Ixora coccinea* (EEIC), Nanosuspension of *Ixora coccinea* (NEIC), Nanosuspension of *Ixora coccinea* with curcumin (NEICC) were prepared and analysed to identify characteristic bands on IR affinity 1 spectrophotometer (Shimadzu, Japan) [32].

In vitro antioxidant assay:

Antioxidant potential was carried out for *Ixora coccinea* ethanolic extract (EEIC), Nanosuspension of *Ixora coccinea* (NEIC), Nanosuspension of *Ixora coccinea* with curcumin (NEICC) using three methods; NO radical, OH radical and H_2O_2 radicle scavenging assay as per the method reported [33-36].

Animals

Wistar rats (180-200 g) used in the present findings, were obtained from Jeeva Life Sciences, Hyderabad. Study design was permitted with IAEC (Reg.No. GRCP/col/05/2021) of CPCSEA.

Acute Toxicity Studies

Standard rat pellets, tap water, and libitum were given to the female mice. According to OECD-425 guidelines, a study on acute oral toxicity was conducted for *Ixora coccinea* extract (up-and-

down/staircase method) at 2000 mg/kg bd. wt. by oral route [37].

Assessment of Antiulcer activity

EEIC, NEIC and NEICC was evaluated for *in- vivo* anti-ulcer potential with following methods:

Ethanol-induced ulcer model

Thirty-six healthy Wistar Albino rats, 180-200 g, were selected and used in study

Group-I Normal control

Group-II Disease control administered absolute alcohol orally (5 mL/kg, *bd.wt.*) at 10th day.

Group-III EEIC was administered, 200 mg/kg body weight *p.o* for 10 days, and absolute alcohol on the 10th day.

Group-IV NEIC was administered, 100 mg/kg body weight *p.o* for 10 days, and absolute alcohol on the 10th day.

Group-V NEICC was administered, 125 mg/kg body weight *p.o* for 10 days, and absolute alcohol on the 10th day.

Group-VI Omeprazole was administered, 20 mg/kg body weight *p.o* for 10 days, and absolute alcohol on the 10th day.

One hour after ingesting the ulcerogenic substance, the study animals were sacrificed, and their stomachs were removed, operated and ulcer sites were measured as total area (mm²) with millimeter-square paper to calculate the ulcer index (UI) [38].

Pylorus ligation-induced ulcer model

36 healthy Wistar Albino rats (180-200 g) were chosen and grouped:

Group-I Control group

Group-II Disease control

Group-III EEIC administered, 200 mg/kg body weight

Group-IV NEIC administered, 100 mg/kg body weight

Group-V NEICC administered, 125 mg/kg body weight

Group-VI Standard omeprazole administered, 20 mg/kg body weight

Post 1 h of receiving treatment, stomach of animals opened by making a tiny midline opening underneath the xiphoid procedure with local anaesthetic ether. Pyloric section was dragged and ligated to prevent traction on the pylorus as per published method [39]. The abdomen was gently swapped, and the abdominal partition was stitched shut. Four hours post pyloric ligation, the rats were euthanized using CO_2, stomach was dissected and detached to allow the contents to drain. Gastric juice was then assessed and centrifugated (10 min) at 2000 rpm. An aliquot of 1 mL of the supernatant was taken to determine the acidity and pH. On millimetre-square paper, ulcer areas were measured and macroscopically observed. UI was used to express the total area (mm^2), and percentage inhibition was determined. Stomachs were fixed in formalin (10%; 24 h), and given at Varun histopath, Hyderabad [40].

UI, acidity and pH

Ulcer index was calculated. 1mL of purified H_2O was used to diluted 1 mL of gastric juice and diluted juice was used to determine the pH (Digital pH Meter, Elico) and was titrated with 0.01N NaOH (phenolphthalein-Indicator) until a persistent pinkish colour. Total acidity was expressed in mEq/L.

1mL of purified H_2O was used to diluted 1 mL of gastric juice and titrated with 0.01N NaOH (Topfer's reagent-Indicator) until a canary yellow colour. Free acidity was then calculated [41].

Total acidity / Free acidity = Vol. of NaOH X N X 1000 mEq/L

***IN-SILICO* DOCKING STUDIES**

Molecular docking was performed on selected secondary metabolites of *Ixora coccinea* against gastric proton pump, to understand the Ligand Target Interactions, generated with mCule.Com Web Server. mCule was used for creating docking simulation experiments. Protein structure PBD ID: 7UT0 (H^+K^+-ATPase inhibitor) was obtained from PDB (www.rcsb.org/pdb) and active sites were generated, extra chains were removed, attributes of the SBD site sphere obtained from the discovery studio visualizer. Chemical Structures of the bioactive phytoconstituents were drawn in mCule docking ligand imported site.7UT0 protein is docked against bioactive phytoconstituents of *Ixora coccinea* which are identified with GC-MS studies. The docking score was displayed on the mCule website by downloading the pose and resultant poses were visualized through the Discovery studio visualizer. More negative score, more favourable binding [42].

Statistical analysis

The results are presented as mean ± SEM, with statistical analysis by one-way ANOVA followed by Dunnett's test, with significance levels ($p < 0.0001$ and 0.001).

RESULTS

Ixora coccinea flowers crude material was extracted and preliminary phytochemical investigation was performed, results revealed the occurrence of flavonoids, triterpenoids, steroids, and phenols, proved through GC-MS. GC-MS confirmed the presence of rutin, kaemferol-3-rutinoside, Lupeol acetate, quercetin-7-oleate, oleanolic acid, stigmasterol, beta tocopherol, geranyl acetate and triterpenol acetate in crude *Ixora coccinea* flower extract (Fig.1 Table: 1). Total flavonoid and phenolic of the *Ixora coccinea* extract was estimated, yielding 74.25 mg Quercetin/g and 202 mg GAE/g, respectively (Fig.2- Fig.3).

Characterization of Nanosuspension of *Ixora coccinea*

Ethanolic *Ixora coccinea* flower extract was encapsulated in the form of nanoformulation. Nanosuspensions of *Ixora coccinea* and Nanosuspensions of *Ixora coccinea* with curcumin

were formulated to rise biological potential. Due to the challenges of slow absorption, insufficient and inconsistent bioavailability, and particle size impacting the in vivo bioactivity of key secondary metabolites, NEIC and NEICC were analyzed for their particle size and morphological characteristics using SEM (Fig. 4 & Fig. 5) results showed particles are within the accepted range (100-200 nm).

Mean particle size of NEIC and NEICC was found to be 169.8nm (Fig. 6) and 96.5 nm (Fig. 7) respectively and had distribution of 60% which showed the good dispersibility of the particles and stability of formulations (Fig. 8 and Fig. 9). Entrapment efficiency of NEIC and NEICC was found to be 54.5% and 74.40 % respectively (Fig. 10). Percentage drug release was performed for EEIC, NEIC and NEICC (at 37 ± 0.5°C) was reported in (Table 2). EEIC, NEIC and NEICC showed dissolution of 49.11, 57.20 and 73.16 % in 120 mins respectively. Dissolution of NEICC is 73.16 % in 120 mins indicating that the NEICC has improved solubility and bioavailability of *Ixora coccinea* extract (Fig. 11).

Nanonisation of NEIC and NEICC exhibited increased dissolution, improved wetting, and greater particle surface area, leading to enhanced solubility due to improve *in vivo* release [43]. NEIC and NEICC attributable to improve biological action of secondary metabolites with reduced dose and unwanted effect [44]. SEM images of NEIC and NEICC nanosuspension revealed their smooth texture and utmost particles were having sphere-shaped with even topology.

FTIR Spectra of Ethanolic extract of *Ixora coccinea*

Fourier transform infrared spectroscopy of EEIC showed peak at 3399.68 cm^{-1} Phenolic OH stretching, 2703.35 cm^{-1} H-C=O: C-H (Aldehyde), 1490.07 cm^{-1} C=O & C=C, 1429.31 cm^{-1} C-H bending, 1245.10 cm^{-1} C-O (Carboxylic acid),1148.66 cm^{-1} C-OH stretching functional groups (Figure 12). NEIC showed the sharp characteristic peak at 3299.38 cm^{-1} Phenolic OH stretching, 2702.39 cm^{-1} H-C=O: C-H (Aldehyde), 1313.58 cm^{-1} C-H bending, 1244.14 cm^{-1} C=O & C=C, 1141.91 cm^{-1} C-N aliphatic amines, 1032.93 cm^{-1} C-O stretchings, 1021.35 cm^{-1} C-OH stretching functional groups (Figure 13). NEICC showed the sharp characteristic peak at 3031.26 cm^{-1} Phenolic OH stretching, 2708.17 cm^{-1} H-C=O: C-H (Aldehyde), 2187.37 cm^{-1} CH (Alkane), 1590.38 cm^{-1} benzene,1378.20 cm^{-1} C-H bending, 1373.38 cm^{-1} C=O & C=C, 1225.82 cm^{-1} Aromatic C-O stretching, 1066.72 cm^{-1} C-O stretchings, 1021.35 cm^{-1} C-OH stretching functional groups (Fig. 14). The prominent peaks representing NEIC, NEICC, and EEIC appear in the spectra; not shown any shifting of absorption peak. Overlay of EEIC, NEIC & NEICC was performed, indicates that all the functional groups present in the EEIC also present in the NEIC & NEICC (Fig. 15).

***In-vitro* Antioxidant assay**

In NO radical, OH radical and H_2O_2 radical scavenging assay, IC_{50} value for EEIC, NEIC, NEICC and standard drug (ascorbic acid) was found to be 35.54, 30.18, 29.11 and 28.7; 33.90, 30.61, 25.80 and 19.51; 35.29, 31.31, 27.90 and 25.41 µg/mL respectively, which showed prominent anti-oxidant potential of EEIC, NEIC and NEICC (Table 3). NEICC has showed enhanced anti-oxidant potential; might be due to curcumin which acts as permeability enhancer. Phenolic compounds present in medicinal plants have various categories; chief category amongst are the flavonoids, have powerful antioxidant capabilities [45]. Flavonoids have been proved as high scavengers of most reacting species, inclusive of singlet oxygen, and numerous free radicals [46] implicated in pathophysiology of various chronic diseases. Thus, antioxidant potential of *Ixora coccinea* extract and its formulations might be due to phenolic and flavonoids [47]. Prominent amount of phenolic and flavonoid content is also present in *Ixora coccinea viz*. 202 mg GAE/g and 74.25 mg Quercetin/g respectively, which can be correlated to the antioxidant potential.

Acute toxicity study was carried out for *Ixora coccinea* extract, The animals showed no indications of toxicity. Therefore, the extract was considered safe at this dosage level. EEIC, NEIC and NEICC showed significant antiulcer potential against the alcohol-induced ulcer (35.30, 42.32. and 58.04 % protection, $p < 0.0001$, Table 4; Fig. 16). Abdominal damage due to alcohol induction includes generation of ROS [48]. Ethanol is identified to decrease concentration of endogenous NO [49] and is an important defence mediator for gastric mucosa [50]. The antiulcer activity of EEIC, NEIC, and NEICC was assessed using the pyloric ligation ulcer model in rats (33.12, 48, 53.28 % protection, $p < 0.0001$, $p<0.001$). Pyloric ligation ulcer method, ulcers are developed due to the self-assimilation of gastric mucosa by gastrointestinal acid. (Table 5 & Fig.17) [51]. Histopathology study of NEIC showed normal mucosal layer and inflammatory cells in submucosal layer and NEICC and standard omeprazole showed normal morphology mucosa, submucosa, and muscular layer (Fig.18) [52].

In silico docking studies was carried out for selected secondary metabolites of *Ixora coccinea* against DDAH1 (PDB ID: 7UT0). Glide score obtained was tabulated in Table 6, which represents approximates the ligand binding free energy (figures 19). The predicted G-score for antiulcer activity with selected bioactive (-8.0 to -5.2) was found in the following order; Kaemperol-3-rutinoside (-8.0) > Quercetin-7-oleate (-7.5) > rutin (-7.5) > stigmasterol (-7.1) > campesterol (-5.3) > β-tocopherol (-5.2) > geranyl acetate (-5.2) whereas standard omeprazole has -6.0. All the seven bioactive compounds of *Ixora*

coccinea are predicted to possess antiulcer action (Table 6 & Fig.19).

Therefore, the NEIC and NEICC improved efficacy due to the nanosize formulation. This may be because these formulations rendered the secondary metabolites to more water soluble and enhanced diffusion in the stomach wall. Thus, the significant antiulcerogenic activity found in present work could presently be ascribe to total phenolic and flavonoid of *Ixora coccinea*.

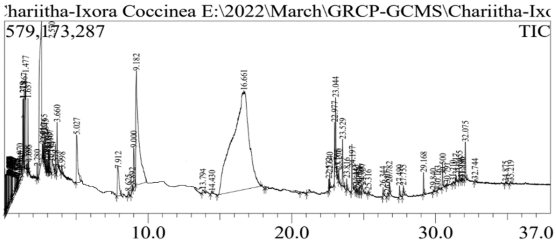


Fig 1: GC-MS spectra of Ixora coccinea flower extract

Table 1: GC-MS results of Ixora coccinea flower extract

S. No.	RT	Name of the compound	Molecular weight	Peak Area %
1	16.661	Rutin	286.24	49.21
2	2.570	Kaempferol-3-rutinoside	610.517	9.00
3	32.744	Lupeol acetate	426.72	0.05
4	3.660	Quercetin-7-olate	302.23	0.72
5	9.182	Oleanolic acid	456.7	10.07
6	31.655	Stigmasterol	412.69	0.19
7	30.163	Beta tocopherol	416.68	0.07
8	1.477	Geranyl acetate	196.29	1.98
9	5.027	Triterpenol acetate	196.29	1.81
10	31.417	Campesterol	400.68	0.11

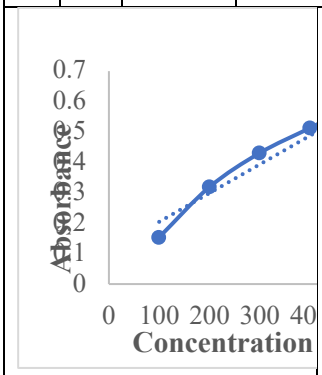


Fig. 2: Total flavonoid content of *Ixora coccinea*

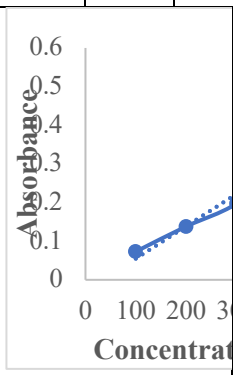


Fig. 3: Total phenolic content of *Ixora coccinea*

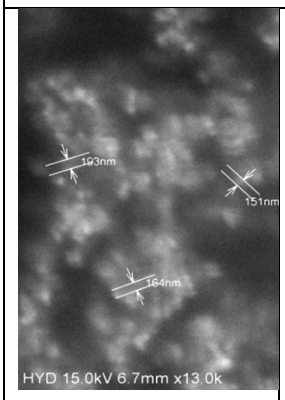


Fig. 4: SEM images-Nanosuspension of *Ixora coccinea* extract

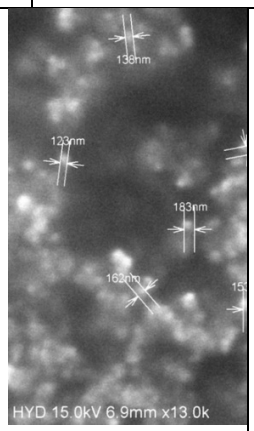


Fig. 5: SEM images-Nanosuspension of *Ixora coccinea* with curcumin extract

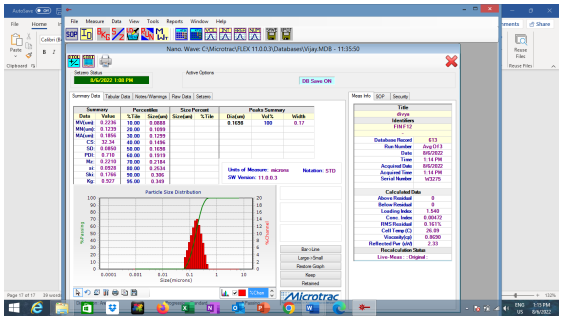


Fig. 6: Particle size analysis of formulation of Nanosuspension of Ixora coccinea using Microtrac

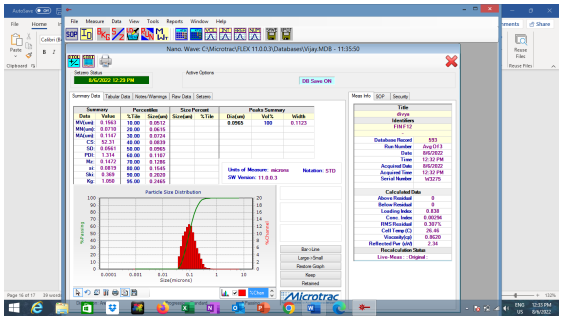


Fig. 7: Particle size analysis of formulation of Nanosuspension of Ixora coccinea with curcumin using Microtrac

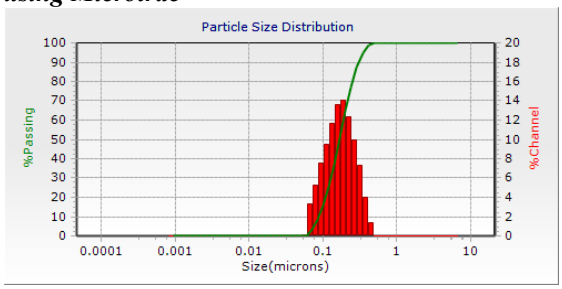


Fig. 8: PDI of Ixora coccinea Nanosuspension using Microtrac

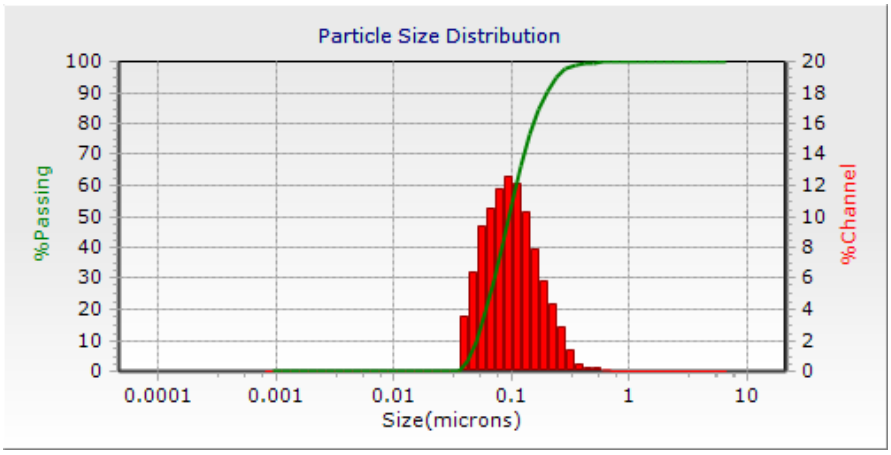
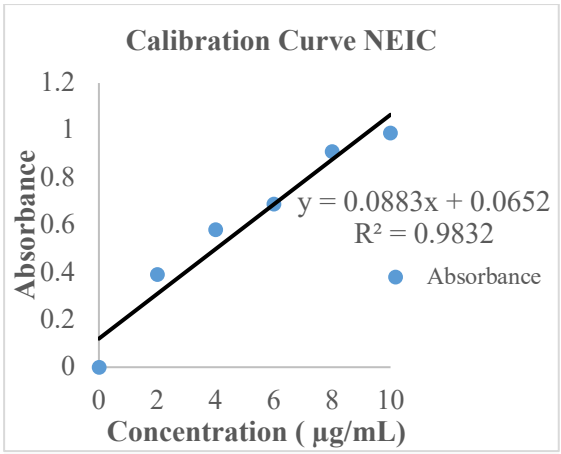


Fig. 9: PDI of Ixora coccinea Nanosuspension with curcumin using Microtrac



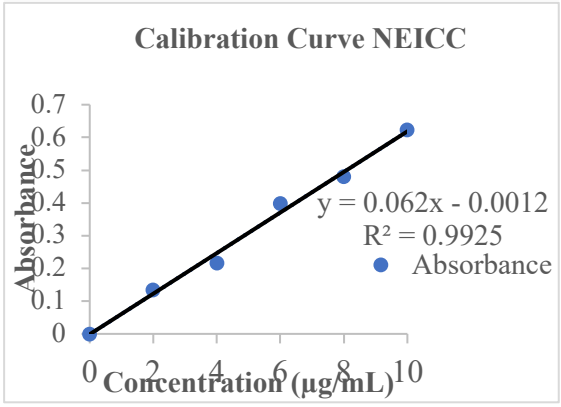


Fig. 10: UV-VIS Spectral Analysis of NEIC & NEICC

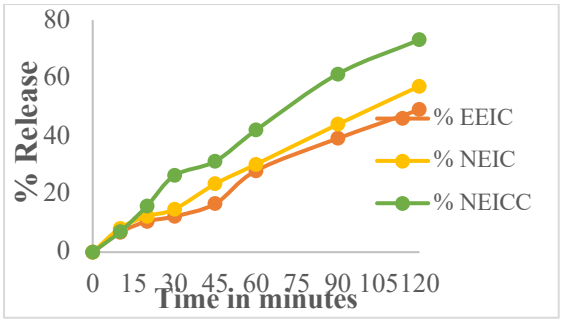


Fig. 11: *In-vitro* drug release of EEIC, NEIC & NEICC

Table 2: *In-vitro* Drug release studies of EEIC, NEIC & NEICC

Time (mins)	% Release of EEIC	% Release of NEIC	% Release of NEICC
0	0	0	0
10	6.98	8.23	6.98
20	10.6	12.42	15.95
30	12.32	14.77	26.39
45	15.40	23.52	31.32
60	28.08	30.29	42.05
90	39.19	44.04	61.39
120	49.11	57.20	73.16

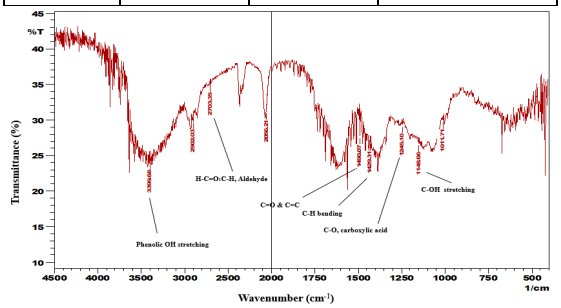


Fig. 12: IR Spectra of Ethanolic extract of Ixora coccinea (EEIC)

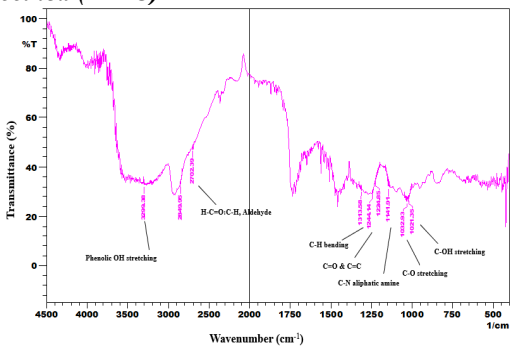


Fig. 13: IR Spectra of Nanosuspension of Ixora coccinea (NEIC)

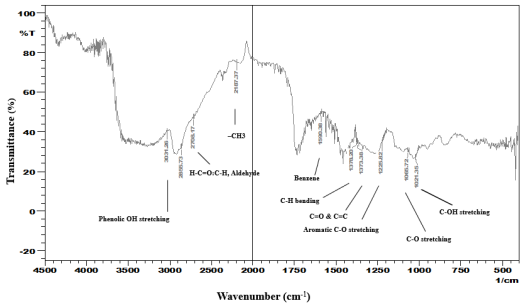


Fig. 14: IR Spectra of Nanosuspension of Ixora coccinea with curcumin (NEICC)

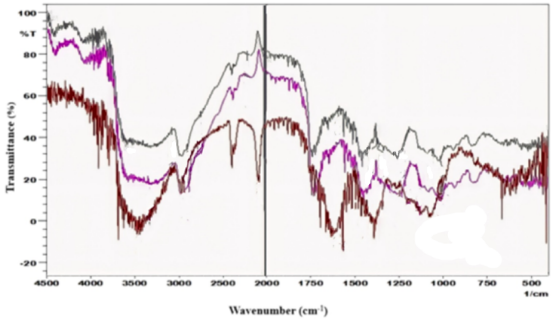


Fig. 15: Overlay of spectrum of EEIC, NEIC & NEICC

Table 3: Antioxidant assay of Ixora coccinea

S. No.	Antioxidant test	Compound	IC_{50} (µg/mL)
1	$NO^{\cdot}$ scavenging assay	EEIC NEIC NEICC Standard	35.54 30.18 29.11 28.7
2	Hydroxyl radical scavenging assay	EEIC NEIC NEICC Standard	33.90 30.61 25.80 19.51
3	Hydrogen peroxide radical scavenging assay	EEIC NEIC NEICC Standard	35.29 31.31 27.90 25.41

Table 4: The effect of Ixora coccinea on ulcers induced by Ethanol

The values are expressed as mean ± SEM (n=6). Statistical analysis was carried out by Dunnett's test. Results were related to the disease group (*P < 0.0001) and the standard group (A = P < 0.0001), with 'ns' indicating non-significant.

Fig. 16: Action of Ixora coccinea on ulcers induced by Ethanol

Table 5: Effect of Ixora coccinea on ulcers induced by the pylorus ligation model

Groups	**Treatment**	**Ulcer Index**	**% Protection**
Group-I	Normal control	-	-
Group-II	Disease control (5 mL/kg, bd.wt., *p.o*)	5.41 ± 0.06	-
Group-III	EEIC (200 mg/kg, bd. wt, *p.o*)	3.50 ± 0.02*A	35.30
Group-IV	NEIC (100 mg/kg, bd. wt, *p.o*)	3.12 ± 0.01*A	42.32
Group-V	NEICC (125 mg/kg, bd. wt, *p.o*)	2.27 ± 0.01*A	58.04
Group-VI	Omeprazole (20 mg/kg, bd. wt, *p.o*)	2.03± 0.03*	62.47

Groups	**Treatment**	**Ulcer index**	**% Ulcer protection**	**pH**	**Gastric volume**	**Total acidity**	**Free acidity**
Group-I	Normal control	-	-	2.16 ± 0.04	0.48 ± 0.1	40.6 ± 1.5	40.8 ± 0.94
Group-II	Disease control	6.25 ± 0.1	-	1.11 ±0.06	2.51 ± 0.1	71 ±1.52	81.16 ±0.9
Group-III	EEIC (200 mg/kg, bd. wt)	4.18 ± 0.10 *#A	33.12	2.72 ±0.03 *A	1.71 ± 0.06 *A	61 ±1.5 *A	66.3±0.88 *A
Group-IV	NEIC (100 mg/kg, bd. wt)	3.25 ± 0.08 *#A	48	3.32 ±0.06 *A	1.48 ± 0.08 **A	51 ±1.5 *A	60.8 ±0.98 *A
Group-V	NEICC (125 mg/kg, bd. wt)	2.92 ± 0.08 *#A	53.28	3.78 ±0.03 *ns	1.01 ±0.08 **B	46 ±1.16 *A	50.6 ±0.91 *A
Group-VI	Omeprazole (20 mg/kg, bd. wt)	2.48 ± 0.1 *#	60.32	3.92 ± 0.06 *	0.53 ± 0.12 *	41 ±1.5 *	42.1 ±0.79 *

The values are presented as mean ± SEM (n=6). Statistical evaluation was carried by Dunnett's test. Results were compared to the disease group (*P < 0.0001, **P < 0.001) and the standard group (A = P < 0.0001, B = P < 0.001), with 'ns' indicating non-significant differences.

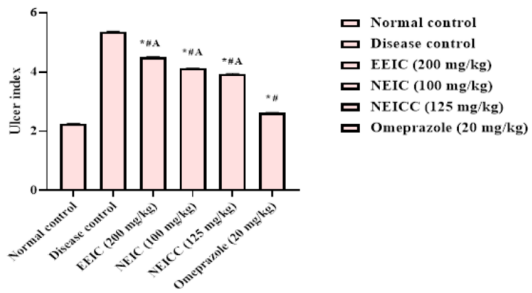


Fig. 17: Effect of Ixora coccinea on Pylorus ligation ulcer model

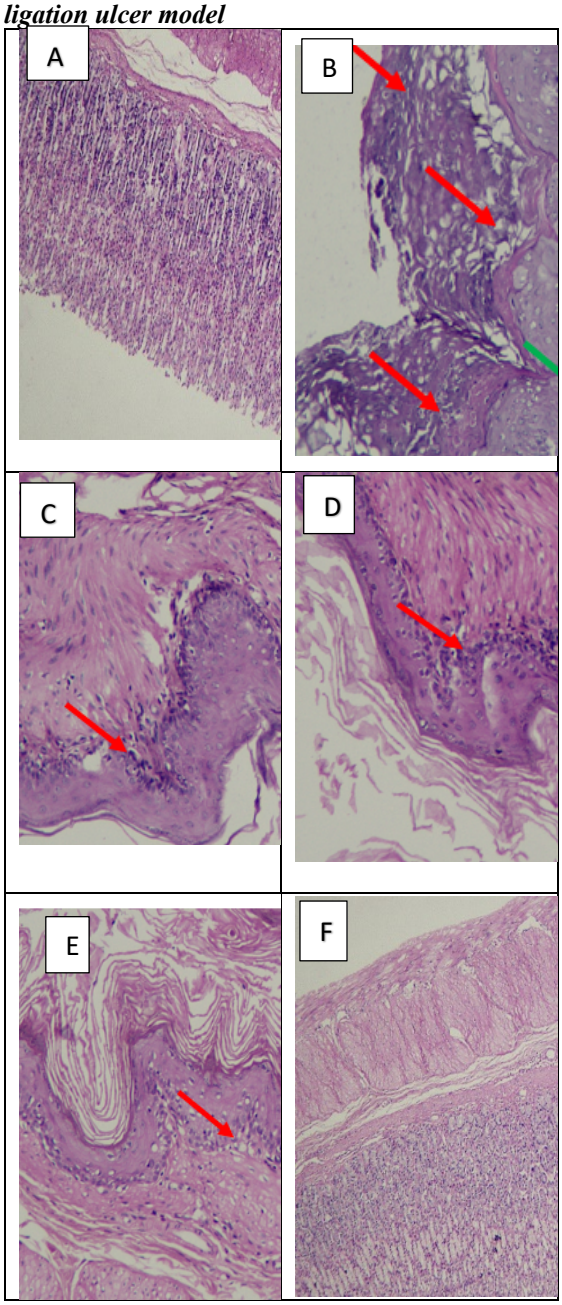


Fig. 18: Histopathological examination of the abdominal mucosa in a pylorus-ligation ulcer model in rats. A. Normal control: Mucosal layer appeared normal in the Normal control group. B. Disease control group, Mucosal ulceration, degeneration, and, infiltration of inflammatory cells was noticed in the mucosal layer of the glandular stomach. Moderate hyperplasia of mucosal epithelial cells along with degenerative changes was noticed in the mucosal layer of the non-glandular stomach. C. Administration of EEIC shows mucosal epithelial cells in the mucosal layer appeared normal, but submucosal inflammation with infiltration of inflammatory cells was observed. D. Administration of NEIC 100 mg/kg shows mucosal layer appeared normal and submucosal infiltration of inflammatory cells was observed. E. Administration of NEICC 125 mg/kg shows Normal morphology mucosal, submucosal, and muscular layer observed. F. Administration of Omeprazole 20 mg/kg shows Normal morphology mucosal, submucosal, and muscular layer observed.

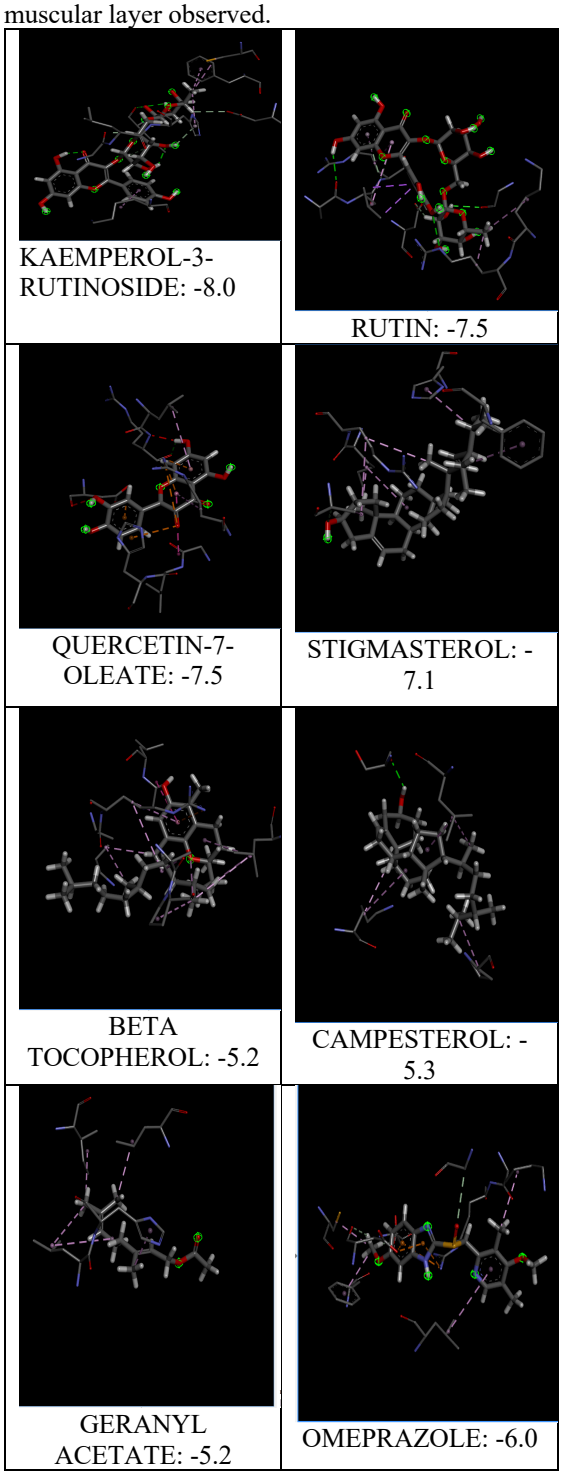


Fig. 19: Docking with 7UT0: (H^+K^+ -ATPase inhibitor)

Table 6: mCule docking results against 7UT0: (H^+K^+-ATPase inhibitor)

Ligands	G-Score
Kaemperol-3-rutinoside	-8.0
Quercetin-7- oleate	-7.5
Rutin	-7.5

Stigmasterol	-7.1
Beta tocopherol	-5.2
Campesterol	-5.3
Geranyl acetate	-5.2
Omeprazole (Standard)	-6.0

DISCUSSION

Impaired mucosal resistance, stomach motility and decreased gastric mucosal blood flow causes most frequent GIT condition (peptic ulcer). OH, NO and H2O2 radical generated in biological systems are highly reactive free radical having capacity to harm living cells with various mechanism. NO radical reacts with ROS where numerous cellular components' structural and functional behaviours of cells are changed. EEIC, NEIC and NEICC demonstrated prominent ROS radical scavenging potential, might be because of phenolic and flavonoids compounds which are already proved as radical scavengers.

Ixora coccinea flower extract was encapsulated to increase bioactivities of secondary metabolites as limited bioavailability and particle size distribution are main factor influencing the in vivo fate of major secondary metabolites. NEIC and NEICC these nanosuspensions were showed 60% particle distribution indicates good dispersibility and stability. Dissolution of NEIC and NEICC has showed improved solubility and bioavailability. NEICC showed more dissolution as curcumin acts as a penetration enhancer for bioactive compounds of *Ixora coccinea*. Metabolism of alcohol results initiation of free radicals plays a critical role in both acute and chronic ulceration of stomach. Increased acid-pepsin cumulation of pylorus obstruction results in pylorus ligation-induced ulcers. Free radicals generated also plays important role in ulcer formation. In alcohol induced ulcer and pylorus-ligated ulcer method, EEIC, NEIC and NEICC have showed a significant ($p<0.0001$, $p<0.001$) reduction in ulcer formation comparable to standard Omeprazole. Pylorus-ligated rats, EEIC, NEIC and NEICC confirmed a significant decline in UI, acidity, gastric volume and raised pH. *Ixora coccinea*'s phytochemical screening disclose the presence of flavonoids, phenolic and steroids, had proved to have antiulcer properties; their presence was further confirmed with GC-MS. Flavonoids have ability to interact with key enzymes such as Cox, Lipo-oxygenase, Phospholipase A, NADH-Oxidase or glutathione reductase which are involved pathophysiology of gastric ulcers. Phenolics are also having capacity to counteract deteriorating effects of ROS and having capability to stimulate mucus, biHCO3, and PG's secretions in the gastric lumen. Hence flavonoids, phenolic, terpenoids, and steroids of *Ixora coccinea* extract can be accountable for the antiulcer capability. Phenolic OH group of curcumin is a key component to enhance mucus secretion and have capability to reduce lipid peroxides (LPs), to increase superoxide dismutase, catalase, and glutathione peroxidase levels, promotes ulcer healing. Molecular Docking Studies was performed for selected seven bioactive compounds to see the binding affinity of compounds to 7UT0 (H+-K+ ATPase inhibitors). These selected compounds showed good G-score viz. Kaemperol-3-rutinoside (-8.0), Quercetin-7-oleate (-7.5), rutin (-7.5), stigmasterol (-7.1), campesterol (-5.3), β-tocopherol (-5.2), geranyl acetate (-5.2) and standard omeprazole (-6.0). All the seven bioactive compounds are predicted to possess antiulcer action.

CONCLUSION

The study report proven that pre-treatment with *Ixora coccinea* flower extract and formulations caused a beneficial effect on alcohol and pylorus ligation-induced ulcer model.

ACKNOWLEDGEMENT

The authors wish to oblige gratitude to Principal and GRES society for ongoing support and encouragement throughout this research.

Funding:

The authors have no funding sources to declare.

Data Availability Statement:

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Disclosure of AI Use:

The authors used ChatGPT and Perplexity for language editing, grammar correction, and improving manuscript clarity. All AI-generated suggestions were carefully reviewed and edited by the authors, who take full responsibility for the final manuscript.

Conflicts of interest

There are no conflicts be of interest to report.

Author contribution statement

SN, RC, KK contributed to this article. SN contributed to conceptualisation, supervision. SN, RC contributed to the literature search, data acquisition and calculations and writing of the preliminary draft of the manuscript. RC and KK edited the manuscript and made critical revisions. All authors re viewed and approved the final manuscript.

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