Uncovering Green Power: Phytochemical insights and exploration of anticancer, antioxidant and antimicrobial potential of *Ixora coccinea* leaf extract

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ABSTRACT

Ixora coccinea, belonging to the family Rubiacae is an ornamental evergreen shrub widely recognized as Jungle flames or Flame of the woods & is prevalent across various parts of India. Plants have long served as reservoir of therapeutic compound, among them is *Ixora coccinea*, a flowering plant native to tropical Asia and widely used in traditional medicine—has garnered increasing scientific interest due to its rich phytochemical profile and diverse pharmacological activities. It is a medicinal plant with many active constituents that are responsible for wound healing and have anticancer properties. Medicinal plants not only considered free from harmful side effects, they also have potential to reduce subsidiary or negative effects on modern medicine. In traditional Indian medicine, including Ayurveda and various folk practices, different parts of the plant are used to address diverse health conditions. The potential of ***Ixora coccinea*** as a source of bioactive compounds has led to a growing body of research focused on its pharmacological benefits. A key step in unlocking the therapeutic potential of any plant is to understand its phytochemical composition. Phytochemicals are naturally occurring compounds found in plants that possess significant biological activity.

Key words – Phytochemicals, anticancer, antioxidants, antimicrobial, Ixora coccinea leaf extract, cell lines

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INTRODUCTION

Ixora coccinea is a small, ornamental shrub that typically reaches a height between 1 to 4 feet. It is recognized for its lush, green foliage with leaves ranging from 5 to 10 cm in length, arranged in clusters. This plant stands out in gardens and landscapes due to its dense growth and vivid, attractive flower clusters. The flowers, which are tubular and star-shaped, measure about 1 to 2 cm in length and appear in tight, spherical bunches at the tips of its branches. While red is the most common colour, the blooms may also appear in shades of orange, pink, or yellow. The stems start off green and gradually become woody as the plant matures.

Because of its appealing appearance and compact form, *Ixora coccinea* is widely used as a decorative garden plant. Recent research has highlighted the plant's potent antimicrobial potential. Extracts made from its leaves, flowers, and roots—especially those prepared using ethanol or methanol—have demonstrated significant effectiveness in combating various microbial pathogens. These include common and harmful bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This antimicrobial activity is attributed to the presence of several active compounds within the plant.

Ixora coccinea is rich in bioactive molecules such as flavonoids, alkaloids, tannins, and phenolic compounds. Among these, Quercetin and Rutin are commonly identified phytoconstituents, known for their medicinal properties. These compounds have been associated with antioxidant and anticancer effects. The antimicrobial action of the plant is thought to be primarily due to the presence of flavonoids, terpenoids, and phenolic substances that disrupt microbial cell walls and interfere with essential microbial processes. Experimental methods like the Minimum Inhibitory Concentration (MIC) and Agar Well Diffusion technique are commonly used to assess this activity.

Oxidation is a necessary process in living organisms, vital for generating energy that drives cellular functions. However, it also leads to the continuous production of oxygen free radicals and reactive oxygen species (ROS), which can damage cells and tissues. These oxidative processes have been linked to a range of health disorders, including cancer, cardiovascular diseases, diabetes, and aging. Furthermore, lipid peroxidation—the degradation of fats and fatty acids—not only spoils food but also generates harmful radicals like peroxyl and hydroxyl that are associated with carcinogenesis and genetic mutations. To counteract this, antioxidants play a crucial role in neutralizing ROS and preventing oxidative stress-related diseases. Antioxidant activity in plants and their extracts is often evaluated using radical scavenging methods such as ABTS and DPPH assays.

Given the global burden of cancer, there is growing interest in natural compounds as potential therapeutic agents due to their diverse structures and biological functions. Plant-derived bioactive compounds have shown promising anticancer properties through mechanisms such as promoting apoptosis, inhibiting cell growth, and affecting key signalling pathways involved in tumour development. Unlike many synthetic drugs, natural compounds tend to exhibit lower toxicity, making them suitable for both therapeutic and preventive applications. The MTT assay is one of the standard methods used to assess the cytotoxicity and anticancer activity of these compounds.

Fig.1 Ixora coccinea

MATERIALS & METHOD

EXTRACTION OF SAMPLE:

The sample was found in a garden during inspection. It was carefully removed and placed in a zipped bag. The sample was then secured for further analysis.

Extraction is the initial process used to isolate valuable natural compounds from raw plant materials. Depending on the principle involved, different extraction techniques can be applied, such as solvent extraction, distillation, mechanical pressing, and sublimation**.** Among these, solvent extraction is the most commonly used method. The extraction process generally involves a series of steps. First, the solvent penetrates the solid plant material. Once inside, the desired compounds, known as solutes, dissolve into the solvent. These dissolved substances then diffuse out of the solid material and into the surrounding liquid. Finally, the solution containing the extracted compounds is collected for further processing or analysis.

PREPARATION OF SAMPLE

The sample was initially dried at a controlled temperature of 50°C. Once completely dried, it was ground into a fine powder and mixed with methanol in a 1:5 ratio using a 250 ml beaker, which was covered with aluminum foil to prevent evaporation. The mixture was then incubated in a hot water bath at 50°C for a duration of four hours to facilitate extraction. After incubation, the mixture was filtered using Whatman filter paper, and the clear extract was collected in a separate 50 ml beaker. This filtrate was then subjected to further heating at 50°C until all the solvent evaporated and the extract reached a semi-solid state. Finally, the semi-solid extract was weighed, and the yield was documented.

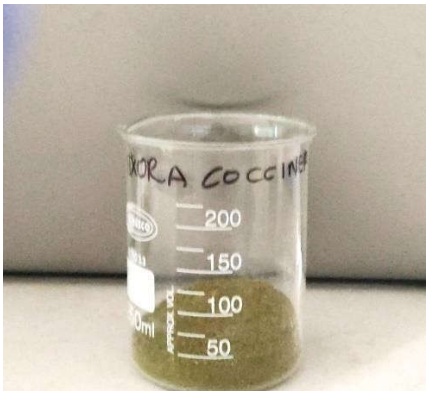


Fig.2 Extracted plant powder (*Ixora coccinea*)

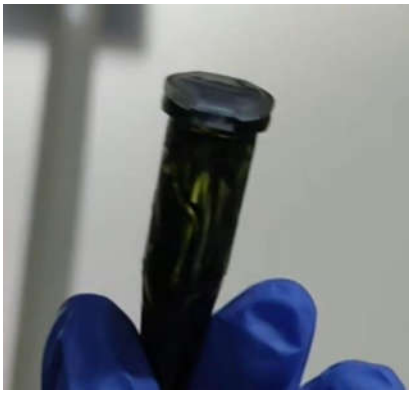


Fig.3 Semi-solid Sample extract

1. PHYTOCHEMICAL ANALYSIS:

Phytochemical analysis is a process of identifying and measuring the chemical compound (phytochemical) produced by plants. It is used to detect the bioactive compounds, understand their health benefit, validate traditional medicine and ensure the quality, safety & effectiveness of plant-based product.

Materials & Methods for Phytochemical Analysis

To qualitatively test for various phytochemicals in a plant extract, several standard assays were performed. For carbohydrates, Molisch's test involved mixing 0.2 ml of sample with Molisch's reagent followed by the addition of concentrated sulfuric acid along the test tube wall, with the formation of a dark purple ring indicating a positive result. Tannins were detected using Braymer's test, where 0.2 ml of extract was heated with water, filtered, and treated with ferric chloride, producing a dark green colour. Terpenoids were tested by Salkowski's method using chloroform and concentrated sulfuric acid, with a reddish-brown interface indicating presence. Saponins were identified via the foam test, in which extract and water were shaken vigorously, and persistent foam indicated a positive result. For flavonoids, the extract was mixed with distilled water and ferric chloride solution; a blue-green coloration confirmed their presence.

2. MINIMUM INHIBITORY CONCENTRATION (MIC):

The Minimum Inhibitory Concentration (MIC) assay was conducted to evaluate the antimicrobial efficacy of *Ixora coccinea* extract against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Ciprofloxacin (0.1 mg/ml) served as the standard, and *Ixora coccinea* extract was prepared at 50 mg/ml in DMSO, with further dilutions made using

Mueller-Hinton broth. A control containing 1% DMSO without any test sample or standard was used. The bacterial inoculum, standardized to 1×10^6 CFU/ml using McFarland standards, was cultured in Mueller-Hinton broth and incubated at 37 ± 2 °C for 24–48 hours.

PROCEDURE: Determination of Minimum Inhibitory Concentration (MIC):

A stock solution of the test substances was initially prepared and subjected to a serial 1:1 dilution in 96-well microtiter plates, with each well receiving 90 µL of the diluted sample. To this, 10 µL of bacterial inoculum was added. For the control, 90 µL of broth containing 1% DMSO was mixed with 10 µL of inoculum. The plates were incubated at 37°C for 24 hours. Following incubation, 20 µL of 0.015% Resazurin solution was added to each well and further incubated for 2 to 4 hours to monitor any color change. Resazurin, a blue dye, is reduced to pink resorufin by metabolically active bacteria, indicating growth. Wells that retained the blue color signified inhibited bacterial activity, and the lowest concentration at which no visible color change occurred was recorded as the Minimum Inhibitory Concentration (MIC).

3. WELL DIFFUSION METHOD:

The well diffusion method was employed to evaluate the antimicrobial activity of a test compound against selected bacterial strains. The organisms used in the assay included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Inocula were prepared by culturing the bacteria in Mueller Hinton broth for 16–18 hours at 37°C. The cell density of each suspension was adjusted to approximately 1×10^6 cells/mL using the 0.5 McFarland standard. The test sample used was *Ixora coccinea* extract dissolved in DMSO at a stock concentration of 100 mg/mL. The standard antimicrobial agent used for comparison was ciprofloxacin (0.1 mg/mL), while DMSO served as the negative control. For the assay, 100 µL of bacterial inoculum was evenly spread onto 90 mm Mueller Hinton agar plates. Wells of 5 mm diameter were punched into the agar, and varying volumes of the test compound (50 µL and 100 µL), standard (20 µL), and control were added to the wells. The plates were then incubated at 37°C for 24 hours, and the zones of inhibition around each well were measured to assess antimicrobial effectiveness.

4. ABTS RADICAL SCAVANGING ASSAY:

Free radicals are generally very reactive molecules possessing an unpaired electron which are produced continuously in cells either as by-products of metabolism or by leakage from mitochondrial respiration. The free radicals produced in-vivo include the active oxygen species such as super-oxide radical O_2^-, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl).

During metabolism, oxygen consumption involves the constant generation of free radicals and reactive oxygen species (ROS). H_2O_2 and O_2 can interact in the presence of certain transition metal ions to yield a highly- reactive oxidizing species, the hydroxyl radical (OH). The hydroxyl radical, one of the ROS, is an extremely reactive free radical formed in biological systems and reacts rapidly with molecules found in living cells, for example, sugars, lipids, DNA bases, amino acids.

Oxygen free radicals have been shown to be responsible for many pathological conditions. Free radicals and Reactive Oxygen Species (ROS) cause DNA damage, lipid per-oxidation, protein damage. They are known to be involved in the pathogenesis of a wide variety of clinical disorders as cancer, cardiovascular diseases, inflammatory diseases, asthma and aging. Free radicals like the hydroxyl radical, hydrogen peroxide, superoxide anion mediate components of the inflammatory response, with production of migratory factors, cyclic nucleotides and eicosanoids. Superoxide radicals amplify the inflammation process, increasing vascular permeability, adhesion of polymorphonuclear leucocytes to the endothelium and stimulation of platelet aggregation.

Some enzymatic and non-enzymatic systems exist in the bodies which are involved in the detoxification process like superoxide dismutase, catalase. Aerobic metabolism entails the production of reactive oxygen species, thus there is a continuous requirement of antioxidants for their inactivation. Thus, the steady state of pro-oxidants and antioxidants may be disrupted in favor of the former, leading to oxidative stress, which may affect all types of biological molecules. Antioxidants are a group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves.

Plants possess antioxidant principles. Various classes of phytochemicals have been shown to have antioxidant property which is due to the presence of substituted groups such as carbonyl, phenolic, phytyl side chain, electron withdrawing group, electron donating group etc. Phenolic antioxidants donate hydrogen to the radical and convert it to stable non radical product.

PRINCIPLE:

ABTS assay is based on scavenging of light by ABTS radical. An antioxidant with an ability to donate hydrogen atom will quench the stable free radical, a process which is associated with change in absorption which can be followed spectroscopically. The relatively stable ABTS radical has a green color and is quantified spectrophotometrically at 734nm.

REQUIREMENTS - Phosphate Buffered Saline (PBS, pH 7.4) was prepared by dissolving 0.14196 g of disodium hydrogen orthophosphate, 0.1560 g of sodium dihydrogen orthophosphate, and 0.7305 g of sodium chloride in 25 mL of distilled water, adjusting the pH to 7.4 with dilute sodium hydroxide, and making up the volume to 100 mL with deionized water. A 7 mM ABTS solution was prepared by dissolving 38.4 mg of ABTS in PBS and adjusting the volume to 10 mL, while a 2.45 mM ammonium persulfate (APS) solution was made by dissolving 5.59 mg of APS in PBS up to 10 mL. To generate the ABTS radical solution, equal volumes (10 mL each) of the ABTS and APS solutions were mixed and kept in the dark at room temperature for 16 hours to form the mother stock. A working solution was then prepared by diluting 1.7 mL of the mother stock to 50 mL with PBS, achieving an absorbance of 1.000. Quercetin standard (1000 µg/mL) was prepared by dissolving 1 mg of quercetin in 1 mL of PBS.

PROCEDURE:

The assay is performed as per Auddy (2003) [8]. ABTS radical cations are produced by reacting ABTS and APS on incubating the mixture at room temperature in dark for 16 hours. The solution thus obtained is further diluted with PBS to give an absorbance of 1.000. Different concentrations of the test sample and the reference standard (highest volume taken was 50µl) are added to 950µl of ABTS working solution to give a final volume of 1ml, made up by adding PBS. The absorbance is recorded immediately at 734nm. The percent inhibition is calculated at different concentrations and the IC_{50} values are calculated using Graph pad prism software.

CALCULATIONS:

Calculating percentage growth inhibition:

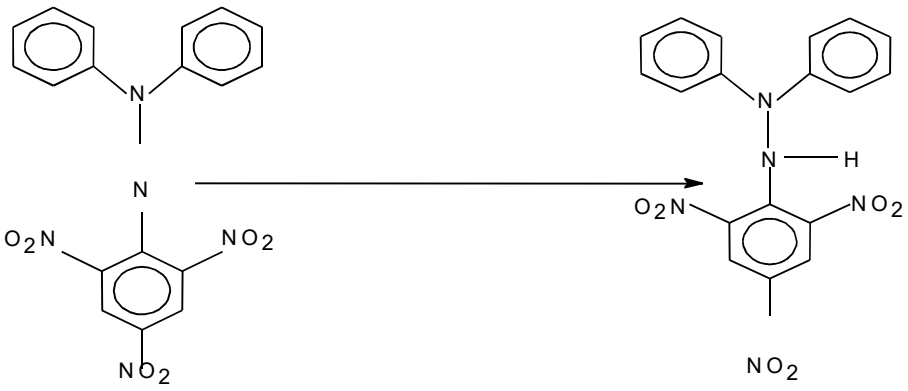
$$\%\ \boldsymbol{Inhibition} = \frac{(\boldsymbol{OD\ of\ Control - OD\ of\ Sample})}{\boldsymbol{OD\ of\ Control}} \times \mathbf{100}$$

5. DPPH Anti-Oxidant Assay

The generation of reactive oxygen species (ROS) beyond the antioxidant capacity of the biological system gives rise to oxidative stress. Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases such as atherosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS. Antioxidants are a group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation (as reducing agents), metal ion chelation (thereby eliminating potential free radicals), sparing of antioxidants (co-antioxidants). Antioxidants lower the burden of free radicals and they have the ability to take up the free radicals and reduce the free radical and make it stable. The DPPH scavenging assay is a simple chemical experiment for the primary evaluation of any compound for its simplicity and low cost for free radical scavenging activity.

PRINCIPLE:

DPPH [1, 1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple color. Antioxidants reduce DPPH to 1, 1-diphenyl-2-picryl hydrazine, colorless compound which is measured at an absorbance of 590nm.



1,1-diphenyl-2-picryl hydrazyl **(Purple colored)**

1,1-diphenyl-2-picryl hydrazine **(Colorless)**

METHODOLOGY:

For the DPPH assay, DPPH (EEC No. 217-591-8, Sigma, USA) was used and stored below 0°C, while HPLC-grade methanol (Ranbaxy Chemicals) served as the solvent. A working DPPH solution was prepared by dissolving 1 mg of DPPH in 6 mL of methanol. The test sample was prepared by dissolving 10 mg in 1 mL methanol to create a stock solution of 10 mg/mL, followed by two-fold serial dilutions starting from 800 µg/mL. According to the method of Rajakumar et al., 80 µL of DPPH solution was mixed with varying concentrations of the test solution, and the final volume was adjusted to 240 µL with methanol. After mixing, the reaction mixture was incubated at 25°C for 15 minutes, and absorbance was measured at 510 nm using a plate reader. A control (without sample) was also included. The IC_{50} value, representing the concentration required to inhibit 50% of DPPH activity, was determined using GraphPad Prism 5.0 via non-linear regression of percentage inhibition data. IC_{50} values were not calculated for compounds showing less than 50% inhibition. The sample's relative antioxidant activity was assessed by comparing its IC_{50} value to that of a standard—where a higher IC_{50} indicates lower activity, and a lower IC_{50} suggests higher antioxidant potential**.**

Calculating percentage growth inhibition:

$$\%\ \boldsymbol{Inhibition} = \frac{(\boldsymbol{OD\ of\ Control - OD\ of\ Sample})}{\boldsymbol{OD\ of\ Control}} \times \mathbf{100}$$

6. EVALUATION OF CYTOTOXICITY OF TEST SAMPLES IN BREAST CANCER CELLS (MDA-MB-231)

MATERIALS, REAGENTS AND METHOD:

CELL LINE: Breast cancer Cell (ATCC- HTB-26)

CELL CULTURE: MDA-MB-231 cell line was procured from ATCC, stock cells were cultured in DMEM with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100µg/mL) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cells were dissociated using 0.05% trypsin and centrifuged at 1000 rpm for 5 mins. The culture media was discarded and the cell pellet was gently re-suspended using 2 ml DMEM complete media. The viability of the cells was checked and a single cell suspension of 5.0 x 10^5 cells/ml was prepared.

SOURCE OF REAGENTS: DMEM, FBS, Penstrep, Trypsin-procured from Invitrogen.

PREPARATION OF TEST SOLUTIONS:

For cytotoxicity testing, the standard drug used was Doxorubicin. A 3.7 mM stock solution was prepared, and from this, serial two-fold dilutions were made in plain culture media (specific to each cell line as per the procedure) to obtain concentrations ranging from 100 µM to 3.125 µM. The dilutions were prepared stepwise: 100 µM was obtained by mixing 8.1 µL of 1 mM stock with 291.8 µL of media, followed by sequential dilutions—each made by mixing equal volumes (150 µL) of the previous concentration with media—to reach final concentrations of 50, 25, 12.5, 6.25, and 3.125 µM.

For the test samples, a 32 mg/mL stock solution was prepared using plain media. From this, serial two-fold dilutions were prepared to obtain a range of concentrations from 640 µg/mL down to 10 µg/mL. This was achieved by initially diluting 20 µL of the 32 mg/mL stock with 180 µL of media to get 3.2 mg/mL, which was further diluted (e.g., 60 µL of 3.2 mg/mL with 240 µL media) to reach 320 µg/mL, followed by serial two-fold dilutions to generate 160, 80, 40, 20, and finally 10 µg/mL concentrations, all using plain media.

TEST PROCEDURE:

To each well of the pre-labelled 96-well microtiter plate, 100 µL of the prepared cell suspension (50,000 cells/well) was added and incubated at 37ºC with 5% CO_2. After 24h of incubation, the supernatant was removed and the monolayer was rinsed with DMEM. To each pre-designated well, 100µL of test drugs at various concentrations were added and incubated for 24hrs. After incubation, the test solutions in the wells were discarded and 100 µL of MTT reagent (4 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37º C in 5% CO_2. The supernatant was removed and 100 µL of DMSO was added and the plates were gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at 590 nm wavelength using a multimode plate reader, Spectra max i3X, Molecular devices. The percentage growth inhibition was calculated using the following formula and the concentration of test drug to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line using GraphPad Prism 5.0 software.

CALCULATING INHIBITION:

% Inhibition = ((OD of Control – OD of sample)/OD of Control) x 100

STATISTICAL EVALUATION: IC_{50} Value

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process i.e. an enzyme, cell, cell receptor, or microorganism) by half. The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonists on reversing agonist activity. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist.

IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on a sigmoid dose-response curve (variable) and computed using Graph Pad Prism 5 (Graph pad, San Diego, CA, USA).

NONLINEAR REGRESSION:

In statistics, nonlinear regression is a form of regression analysis in which observational data are modelled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximation.

RESULTS & DISCUSSION

IXORA COCCINEA EXTRACT:

The dried powder of *Ixora coccinea* (19g) were subjected to Solvent extraction using Methanol. A total of 862 mg of crude extract was obtained from the plant material subjected to the extraction process.

Sample	Dry weight of the sample	Solubility	Yield
Ixora coccinea	19g	Methanol	862mg

Table 1: Yield summary after crude extraction

1. RESULTS OF PHYTOCHEMICAL ANALYSIS:

Preliminary phytochemical screening of the crude plant extract was conducted using standard qualitative methods to identify the presence of various bioactive compound classes, including alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, and steroids. The Molisch's test confirmed the presence of carbohydrates by the formation of a purple ring at the interface. A dark green coloration indicated the presence of tannins, while a reddish-brown layer in the Salkowski test confirmed terpenoids. The absence of stable foam upon shaking suggested that saponins were not present. Flavonoids were detected through the appearance of a yellow solution that became colorless, indicating their reaction with dilute alkali or specific reagents. Finally, phenols were identified by the development of a blue or green color upon the addition of iron (III) chloride, signifying the formation of a colored complex with phenolic compounds.

2. RESULTS FOR MINIMUM INHIBITORY CONCENTRATION (MIC):

Test Organism	***MIC for Ixora coccinea* (mg/ ml)**					
	1	*2*	*3*	*4*	*5*	*6*
Staphylococcus aureus	*0.312*	*0.625*	*1.25*	*2.5*	*5*	*10*
Psedomonas aeurogenosa	*0.312*	*0.625*	*1.25*	*2.5*	*5*	*10*
E.coli	*0.312*	*0.625*	*1.25*	2.5	*5*	*10*

Table 2: Plate format for MIC of standards against M. *tuberculosis*

Fig 4: Row A – S. aureus, **Row B –** pseudomonas,**Row C –** E. coli
Lane 1-6 -> lower to higher concentration **(0.312 -10 mg/mL)**

MIC results of *Ixora coccinea* is tabulated in table 2 and fig 6 Test samples shown minimal inhibitory activity against Pseudomonas, E. coli and S. aureus at the concentration of 5mg/ml.

3. RESULTS FOR WELL DIFFUSION METHOD:

Test sample concentration		Zone of inhibition (mm)		
		Pseudomonas	*S. aureus*	*E. coli*
Standard (2ug)		35	30	11
control		-	-	-
Ixora coccinea	1mg	-	-	-
	2mg	-	-	-
	5mg	-	-	10
	10mg	-	-	18

Table 3: Inhibitory activity of test compounds against test organism

The tested sample of methanol extraction of ***Ixora coccinea*** showed inhibitory activity in 5mg, and10mg concentration against *E. Coli.*

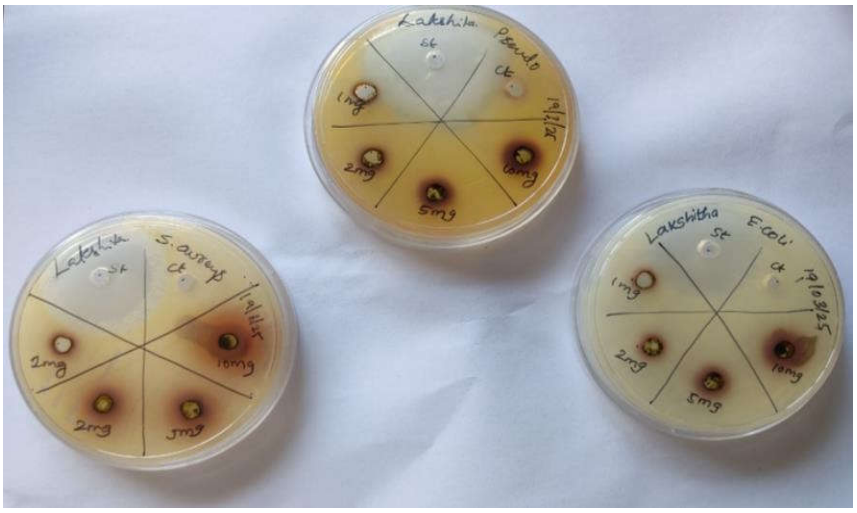


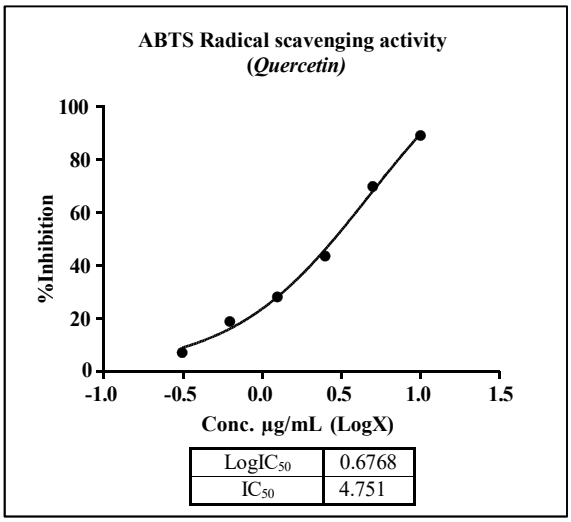
Fig 5: Test organism culture plate with zone of Inhibition

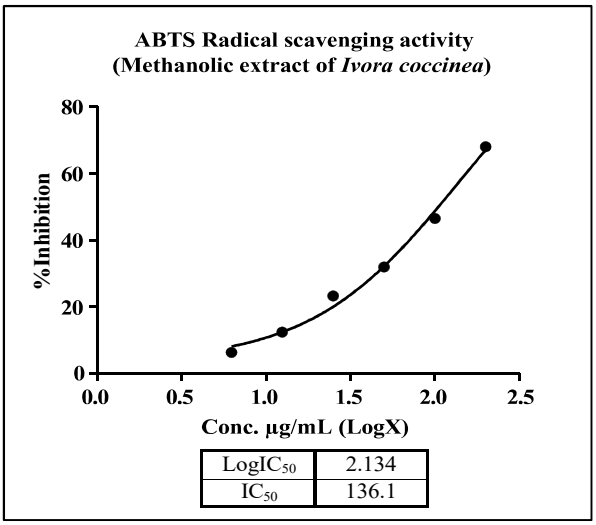
4. ABST RADICAL SCAVEGING ASSAY RESULT:

Sample	Conc. (µg/ml)	Abs at 734nm	% inhibition	IC_{50} (µg/ml)
Blank	0	0.881	0.00	
Quercetin	0.3125	0.819	7.07	
	0.625	0.716	18.81	
	1.25	0.634	28.06	
	2.5	0.498	43.49	
	5	0.267	69.70	**4.751**
	10	0.097	88.95	

Ixora coccinea	6.25	0.825	6.38	**68.03**
	12.5	0.772	12.41	
	25	0.676	23.31	
	50	0.600	31.94	
	100	0.471	46.51	
	200	0.282	67.98	

Table 4: ABST radical scavenging assay against test organism



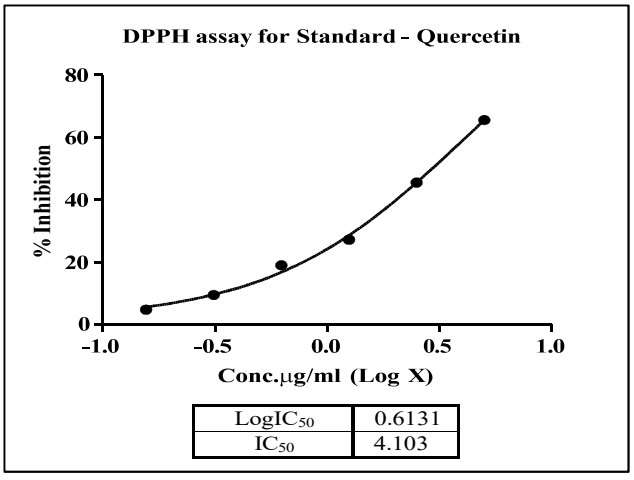


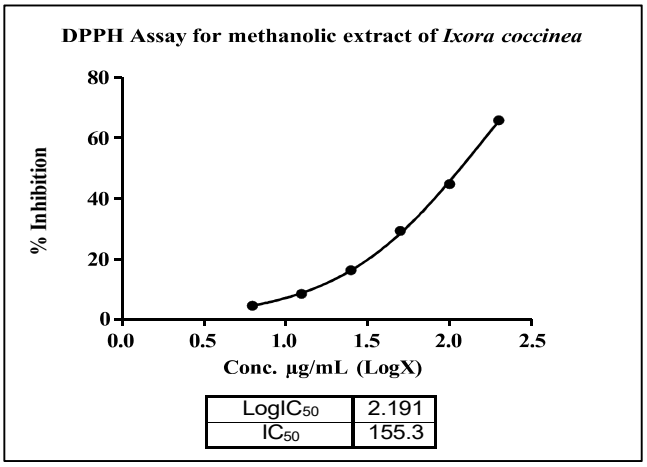
In ABTS assay, sample showed dose-dependent ABTS radical scavenging activity with IC_{50} value of 68.03µg/ml when compared to standard Quercetin with IC_{50} value of 4.750µg/ml.

5. DPPH ASSAY RESULTS:

Test sample	Conc. µg/ml	OD at 510nm	% Inhibition	IC_{50} (µg/ml)
Control	0	0.932	0.00	NA
Quercetin	0.15625	0.888	4.72	4.103
	0.3125	0.844	9.44	
	0.625	0.755	18.99	
	1.25	0.679	27.15	
	2.5	0.508	45.49	
	5	0.321	65.56	
Ixora coccinea	6.25	0.890	4.56	155.3
	12.5	0.853	8.50	
	25	0.780	16.28	
	50	0.659	29.29	
	100	0.515	44.75	
	200	0.421	54.84	

Table 5: DPPH ASSAY



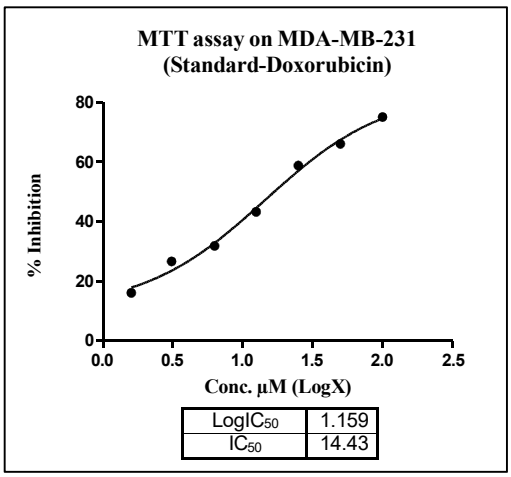


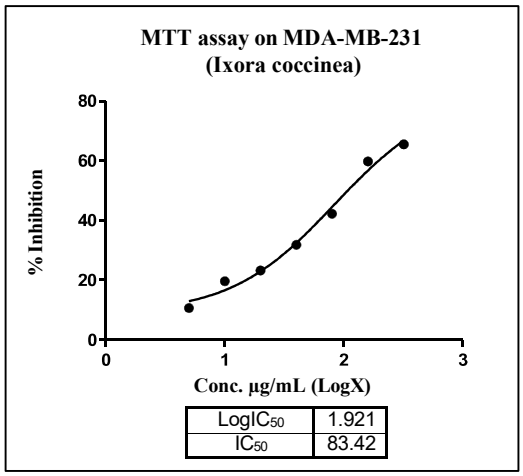
The results suggest that the test sample ***Ixora coccinea***, have shown inhibition more than 50% with IC_{50} value of 155.3µg/ml.

6. RESULTS OF EVALUATION OF CYTOTOXICITY OF TEST SAMPLES IN BREAST CANCER CELLS (MDA-MB-231):

MDA-MB-231		n=1		IC_{50}
SAMPLE	Conc.	OD at 590nm	% Inhibition	
	Control	1.150	0.00	
Ixora coccinea in µg/ml	5.0	1.028	10.63	83.42
	10.0	0.924	19.66	
	20.0	0.883	23.22	
	40.0	0.784	31.83	
	80.0	0.665	42.22	
	160.0	0.463	59.76	
	320.0	0.397	65.48	
Standard-Doxorubicin in µM	1.6	0.965	16.09	14.43
	3.1	0.844	26.62	
	6.3	0.784	31.83	
	12.5	0.653	43.22	
	25.0	0.474	58.79	
	50.0	0.391	66.00	
	100.0	0.287	75.03	

Table 6: Cytotoxicity of test compounds in MDA-MB-231 cells.





The MDA-MB-231 cells were incubated with samples for 24hrs and the samples ***Ixora coccinea*** showed cytotoxicity at IC_{50} value of 83.42µl/mg. The reference standard doxorubicin has shown the IC_{50} value of 14.43 µM against MDA-MB-231 cells.

CONCLUSION-

The present study explored the phytochemical composition and evaluated the in vitro biological potentials of methanolic leaf extract of Ixora coccinea. The phytochemical screening revealed the presence of flavonoids, tannins, terpenoids, carbohydrates, and phenols, which are known to exhibit a wide range of pharmacological effects. The extract showed notable antimicrobial activity, particularly against *Escherichia coli*, as evident from both the MIC and well diffusion assays. The antioxidant potential, assessed by ABTS and DPPH assays, demonstrated moderate free radical scavenging capacity with IC_{50} values of 68.03 µg/ml and 155.3 µg/ml respectively, compared to standard quercetin. Furthermore, cytotoxicity evaluation using MTT assay on MDA-MB-231 breast cancer cells indicated promising anticancer potential with an IC_{50} value of 83.42 µg/ml, although it was less potent than the standard drug doxorubicin.

Collectively, these findings suggest that *Ixora coccinea* possesses valuable phytoconstituents with significant antimicrobial, antioxidant, and anticancer activities. This underscores its potential for further investigation and development as a natural therapeutic agent. Future studies are recommended to isolate individual bioactive compounds, elucidate their mechanisms of action, and evaluate their efficacy in in vivo models to validate the therapeutic applications of this plant.

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