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Anti-Inflammatory Effect of *Ixora coccinea* Linn On Stem Cells of Human Exfoliated Deciduous Teeth Cells

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Abstract: *Ixora coccinea*, commonly known as a jungle of geranium and red *Ixora* evergreen shrubs found throughout India. The plant is crucial for several medicinal purposes, including inflammation, wound healing, diarrhea, and cancer. This study aimed to determine the anti-inflammatory activity of *Ixora coccinea* extracts and the expression of inflammatory genes in cell lines treated with *Ixora coccinea* extracts. First, the roots of *Ixora coccinea* were extracted using a methanol solvent. MTT assay was carried out to determine the viability of cells after treatment with different concentrations of the extracts. Then, the Hen egg test-chorioallantoic membrane (HET-CAM) assay was used as an in vivo model for chronic inflammation. This method was performed to evaluate anti-inflammatory activity by inhibiting membrane irritation on the chorioallantoic membrane, in which irritation was induced by sodium dodecyl sulfate (SDS). Then, the commercial cell line of stem cells of human exfoliated deciduous teeth cells (SHED) was cultured and total RNA was extracted, followed by reverse transcription cDNA synthesis. Multiplex PCR was performed to detect the expression levels of TNF-α, IL-1β, IL-6, IL-8, and TGF-β in both cell types. The result showed that the highest viability of cells was 78.3 % at a concentration of 1.56 mg/ml. It was found that *Ixora coccinea* root extracts showed good anti-inflammatory at a concentration of 1.56 mg/ml. *Ixora coccinea* root extracts showed good anti-inflammatory activity with 80% inhibition on the chorioallantoic membrane compared to Indomethacin, which showed 85% inhibition. *Ixora coccinea* root extracts showed potent anti-inflammatory effects by enhancing immune response through upregulation of inflammatory cytokines, including IL-1β, IL-6, IL-8, and TGF-β as detected by Multiplex PCR. However, the *Ixora coccinea* root extracts do not affect TNF-α and GM-CSF. In conclusion, the present study has successfully determined the activities of *Ixora coccinea* extracts. The current study shows a novel finding *that Ixora coccinea* root extracts possess anti-inflammatory effects through immunomodulatory mechanisms.

Keywords: *Ixora coccinea* Linn, anti-inflammatory, HET-CAM assay, SHED.

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1. INTRODUCTION

In recent years, there has been a rise in interest in studying medicinal plants as complementary medicine for treating various diseases. The discovery of many clinically useful drugs that play an essential role in treating human diseases by screening natural sources such as microbial fermentations and plant extracts for the new pharmacologically active agents. Herbal medicines are safer than synthetic ones because plant extracts' phytochemicals target the biochemical pathway. With the passage of time and advances in medicine, synthetic drugs gradually began to replace natural treatments, even though the former has some side effects as well[1]. While many synthetic medicines benefit humanity and are used safely, many medications have been linked to severe side effects. The use of herbs for medicine is very popular among the rural people because of its low cost and less adverse effect.[1] Screening of phytochemicals compounds from the plant has led to the development of a new medicinal drug with efficient protection and treatment roles against various diseases.[2] Phytochemicals present in medicinal plants such as alkaloids, tannins, saponin, flavonoids, phenols, steroids and carotenoids may have several disease prevention.[3] Phytochemical with various therapeutic applications has various biological function including anti-inflammatory, anti-allergic, anti-cancer, anti-bacterial, anti-viral and analgesic functions.[4] Plants are a great origin of many anti-inflammatory drugs that inhibit various inflammatory in the immune system.[5] Inflammation is a critical protective response to microbial infection, cell or tissue injury, and irradiation with characteristic features such as swelling, redness, heat, and pain.[6] *Ixora coccinea* Linn is in the Rubiaceae family, also called '*Pokok Jejarum*' in Malay. This plant has been known in India since ancient times and the root has some repute in native medicine. Almost all parts of the *Ixora* coccinea plant including the roots, leaves, bark, and flowers are shown to contain various active phytochemicals and are considered useful for several diseases including antihepatotoxicity, antimicrobial, and anti-inflammatory.[7] Analysis has previously shown that *Ixora coccinea* is commonly considered useful for several diseases, including antihepatotoxicity, antimicrobial, and anti-inflammatory.[8] Roots and flowers are used in dysentery, dysmenorrhea, leucorrhoea, hemoptysis, and catarrhal bronchitis.[2] The roots are also used in hiccups, nausea, loss of appetite and externally for the treatment of sores, eczema and chronic ulcer. The root of *Ixora coccinea* contains phenolic acid, aldehyde, sterols, stearic acid, isoprenoid, palmitic acid and myristic acid.[9] Natural products with anti-inflammatory activity have long been used as a traditional remedy for inflammatory conditions such as fever, pain, migraine and arthritis.[10] Phenolic acids also promote the anti-inflammation capacity of human beings[11]. Previous studies have shown that *Ixora coccinea* root extract has promising anti-inflammatory in rats using carrageenan-induced paw edema pellet and cotton pellet test.[12] The roots of *Ixora coccinea* as natural source of bioactive molecules with potent wound healing, anti-microbial activity and antioxidant.[13,14] To our knowledge, no study has identified the immunologic properties of Stem Cells from Human Exfoliated Deciduous Teeth (SHED) treated with *Ixora coccinea* root extract. Therefore, this research aims to determine the expression of genes in the inflammatory pathway in SHED treated with *Ixora coccinea*. Besides, this research also aimed to determine the anti-inflammatory activity of *Ixora coccinea* extracts using Hen's Egg Test-Chorio Allontoic Membrane assay (HET-CAM Assay). The main advantage compared to the previous methods is identification both in vitro and in vivo research on the anti-inflammatory effect of *Ixora coccinea* root extract.

2. MATERIAL AND METHODS

2.1. Collection of plants material and preparation of extracts

The roots of *Ixora coccinea* were collected from Pulai Chondong, Kelantan, Malaysia in 2018, were identified and verified by local people experienced in herbs. The roots were washed under running water, and dried in the oven at 40°C for 1 and 1.5 hours. The ground root was mixed with 70% methanol in a weight/solvent volume ratio of 1/10, and the mixture was shaken overnight with 130 strokes/min. The extract was centrifuged at 1400 xg for 5 min, filtered using Whatman paper, and then concentrated using an automatic concentrator at 60°C. The concentrated extract was stored at -75°C before being dried by a rotary evaporator at 50°C until the extraction became powder, and the extract was stored at -75°C.

2.2. Cell viability assay (MTT assay)

SHED was obtained from All Cells, USA. SHED was cultured in Alpha Minimum Essential Medium (α-MEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% of penicillin or streptomycin until confluence at 37°C in a humidified atmosphere of 5% CO_2. The cells were monitored closely for 24 hours. The cells were examined carefully for signs of contamination or deterioration. The culture medium was changed every other day until the culture is approximately 50% confluent. Once the culture reaches 50% confluence, changed the medium every day until the culture is approximately 80% confluent. Sterile equipment and procedure were implemented in this experiment. For in vitro cell viability by *3-(4, 5-Dimethylthiazol-2-yl)-2,5-*Diphenyltetrazolium *Bromide* (MTT) assay, the protocol was followed according to the guideline proposed by Mosman[15]. Confluence SHED cells were trypsinized and seeded. 3.26 x 10^6 cells were counted in a 1000 µl sample. MTT test needed 1.0 x 10^4 cells per unit for each well. For the treatment group, various concentrations of *Ixora coccinea* (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml, and 1.56 µg/ml were prepared by serial dilution before adding the cells. Then, the plate was incubated for 24 hours in a humidified incubator at 37°C with 5% CO_2. After that, 10 µl of MTT solution (5mg/ml) was added into all the wells before incubating the plate for 4 hours. Following the incubation period, the formazan crystal that was formed by the viable cells was dissolved by adding 100 µl of DMSO to each well and gentle shaking of the palate was done to aid the process. The absorbance for each well was measured at 540 nm in the microtiter plate. The dose-response curve was plotted to calculate the concentration that kills 50% of SHED cells (IC_{50}).

2.3. Hen's Egg Test-Chorio Allontoic Membrane assay (HET-CAM assay)

2.3.1. Prepare of test sample

Hen Egg Test-Chorio Allontoic Membrane assay (HET-CAM) was performed according to the procedure used by Burgermeister et al.[16] The fertilized egg was sterilized with 70% ethanol and all dirt on the external surface removed

using gauze. The eggs were incubated in an automatic eggs incubator with rotation several times, maintained in 60% relative humidity at 37°C for 75 hours. After 75h, eggs were examined to determine the fertilized eggs as well as to identify the optimal position for the placement of agarose pellets in HET-CAM. This was performed by candling the eggs in a dark room. The fertilized eggs showed a web of blood vessels. However, dead embryos showed blood rings. Both unfertilized eggs and dead embryos were removed. At the pointed end, approximately 3-4 ml of albumin was aspirated using the 18 G needle with 10 ml syringe. At the snub end whereas the air sac is located, a small hole made by using a push pin. At two-thirds of the height of the pointed end, a square about 1 x 1cm was marked on the shell where the chorioallantoic membrane was best developed. In one corner of this square, the shell was punctured by a push pin without piercing the membrane. After that, suction was applied to the hole of the air sac by a rubber teat causing the chorioallantoic membrane to drop from the shell membrane immediately (Figure 1). The shell and shell membrane was then removed. Finally, the opening of the shell was covered with sellotape and eggs were reincubated for a further 75 h.

Fig 1: Windowing the eggs

2.3.2. In vivo HET CAM Assay

The test substances, including the control, were dissolved in 2.5% agarose solution at 60°C and reached 5 mg/ml final concentration. 5 mg of sodium dodecyl sulfate was dissolved in 1 ml of 2.5% agarose solution to induce irritation. Indomethacin was dissolved in a 2.5% agarose solution with sodium dodecyl sulfate (SDS) for positive control. *Ixora coccinea* root extract was dissolved in a 2.5% agarose solution without sodium dodecyl sulfate. CAMs were treated with 2.5% agarose solution as a blank control. 10 µl of these gelling solutions were prepared with a final concentration was 50 µg/pellet. Five eggs were utilized for every test. At day six, commonly chorioallantoic membrane had formed approximately a diameter of 2 cm. Thus, one pellet of test compound, including control (50 µg/pellet), was placed on it. The eggs then return to the incubator for 24 hours and then evaluated under the stereo microscope. The inhibition of the membrane irritation was evaluated according to a scoring system which was followed by conversion of the score index in the proportional inhibition of inflammation by Burgermeister et al.[16]: inhibition < 40%, no anti-inflammatory effect; inhibition 40-55%: uncertain anti-inflammatory effect; inhibition 55-70%: weak anti-inflammatory effect; inhibition 70-85%: good anti-inflammatory effect; inhibition > 85%: strong anti-inflammatory effect.

2.4. RNA extraction & cDNA synthesis

The total RNA was extracted from 1 x 10^7 SHED cells using the RNeasy Mini Kit (QIAGEN, USA) as instructed by the manufacturer. First, the cell was lysed in the RLT buffer, then added to via shredder and followed with centrifuging. Next, the lysate was homogenized with 70% ethanol and transferred into the RNeasy mini spin column and centrifuge. Then, RW1 and RPE buffer were added to dissolve the complete RNA. Then, the cDNA synthesis was performed using RevertAid™ H Minus First Strand cDNA synthesis (Fermentas, USA). Incubation times were 60 minutes at 42°C. The reaction was terminated by heating at 70°C for 5 min.

2.5. Multiplex PCR

The treated SHED cells were performed multiplex PCR using MPCR Kit for Human Inflammatory Cytokines Set-1 (Maxim Biotech., USA) to detect the relative expression levels of the human GM-CSF, TNF-α, IL-1β, IL-6, IL-8, and TGF-β genes in SHED cells. The final reaction volume comprising 2.5 U of Taq DNA polymerase, 1X MPCR buffer mixture, and 1X MPCR primers solution was added to 200 mg of cDNA from the MPCR kit. cDNA samples were amplified according to the following parameters: denaturation and amplification steps at 96°C (1 min) and 66°C (4 min) for two cycles followed by 94°C (1 min), 66°C (2 min) for 35 cycles and elongation step at 70°C for 10 min. Electrophoresis was conducted to analyze the PCR products on 2 % agarose gel for 120 minutes at 60 V, and the bands were observed. Molecular Imager Gel Doc XR System (Bio-Rad, USA) was used to calculate the intensity of each band. In addition, GAPDH, house-keeper genes, was used to normalize against positive control.

2.6. Phytochemical analysis

Phytochemical analysis of the ethanol extract of *Ixora coccinea* was accomplished using a Hewlett Packard 5890 series Gas Chromatograph with 5973N Mass Selective Detector

equipped with an Elite-5MS (5%Diphenyl / 95% Dimethyl Polysiloxane) Fused capillary column (30 x 025µm ID x0.25µm Df). 70 Ev electron was used for ionization in GC-MS analysis. Helium was used as the carrier gas at a flow rate of 1.0ml/min. The samples were analyzed with an initial oven temperature of 50°C for 2 min, rising at 20°C/min to 280°C, and then held for 10 minutes. The injection was performed in splitless mode, and the injection port temperature was 250°C. Data were acquired in the MS scan mode (range 40-650m/z). The mass spectrum of GC-MS was interpreted using the database of the National Institute Standard and Technology and Wiley Registry of Mass Spectral Data, New York (Wiley). The chemical compounds were identified by comparing the mass spectrum stored in the NIST and WILEY. In addition, information on the component of the test materials, such as retention time name, molecular weight, and structure, was obtained in this analysis.

2.7. Statistical analysis

Statistical analysis was done using the Mann-Whitney U Test in SPSS software for Windows 18.0. The p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1. Cell viability and assessment

The study showed that the SHED cells have become more fibroblast-shaped (Figure 2). MTT decrease was related to cell-associated viability by active metabolism in instruction to change MTT into a purple-colored formazan product with a maximum absorbance of 570 nm. A phase contrast microscope was reported to readily observe the presence of MTT formazan crystal after extended incubation for 4 hours.[17] The results showed a sizable needle-shaped crystal with some prolonged cell surface in the present study (Figure 3). The dose-response curve showed the percentage of cell viability and proliferation of SHED cells after treatment with different concentrations of *Ixora coccinea* root extracts, as determined by the MTT assay (Figure 4). The results suggested that the viability rate of SHED cells reduced in descending form where the half-maximum inhibitory concentration (IC_{50}) of *Ixora coccinea* root extracts was 3.12 mg/ml with 53 % cell viability. It showed that cell viability was decreased in higher concentrations. Meanwhile, at the lowest concentration of 1.56 mg/ml, the percentage of cell viability had increased to 78.3%. The eggs were opened and tested under a stereo-microscope for vascularization in CAM on the sixth day of incubation (Figure 5). After being induced by SDS, CAM showed positive irritation. Granuloma developed around the pellet, and blood capillaries were found to be highly vascularized (Figure 5a). Nevertheless, the capillary network showed normal when inhibited with *Ixora coccinea* root extracts. Also, no granuloma was generated around the pellet (Figure 5b). This finding was compared to Indomethacin, which also demonstrated inhibition of membrane irritation by which granuloma was hardly recognized and standard capillary network (Figure 5c). Table 1 shows the anti-inflammatory effects (HET-CAM assay) of *Ixora coccinea* extract. The percentage of inhibition membrane irritation of *Ixora coccinea* root extracts and Indomethacin were presented in Figure 6. The results demonstrated no anti-inflammatory reactions, with only 25 % inhibition effects by SDS; *Ixora coccinea* root extracts exhibited good anti-inflammatory effects with 80% inhibition. Indomethacin presented strong anti-inflammatory effects with 85% inhibition. The expression of inflammatory cytokines such as TNF-α, IL-Iβ, GM-CSF, IL-6, IL-8, and TGF-β have been identified by Multiplex PCR. The size of nucleotide for each cytokine was presented as 921 bp (GAPDH), 680 bp (TNF-α), 555 (IL-1β), 424 BP (GM-CSF), 360 BP (IL-8) and 161 bp (TGF-β). The expression level of inflammatory cytokines was presented in Figure 7, which found that IL-1β was expressed in both treated and untreated SHED cells. Meanwhile, the different level of IL-1β was not significant between treated and untreated SHED cells. The gene expression of IL-6, IL-8, and TGF-β was significantly higher in treated SHED cells compared with untreated SHED. However, TNF-α and GM-CSF were not observed in treated and untreated SHED cells. The major chemical constituents have been identified, including Hexadecanoic acid (7.38 %), 9-Octadecenoic acid, Methyl-ester (1.97), 2,6-methoxyphenyl (1.35 %), 13-Docosenamide (1.31%), 3,4,5-tri methoxyphenyl (1.24 %), 4,8,12,16-Tetramethylheptadecan-4-olide (1.13%). Figure 8 shows the GC-MS chromatogram extract of the *Ixora coccinea* root. Table 2 shows Phyto-component identified in the *Ixora coccinea* root extract.

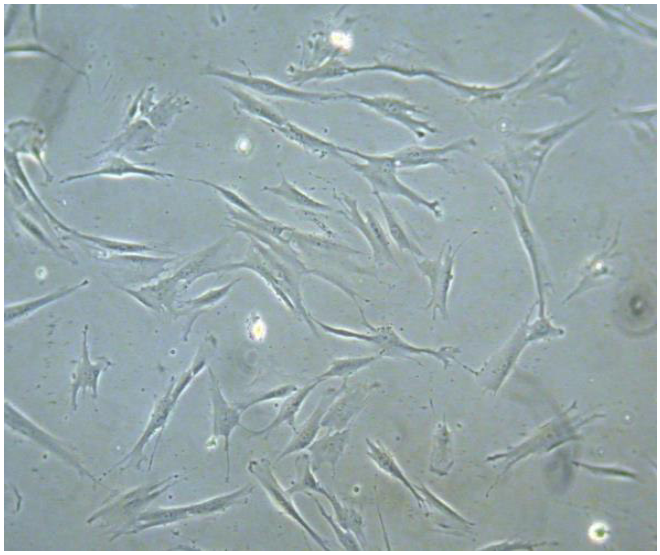


Fig 2: The SHED cells exhibited fibroblast-like morphology.

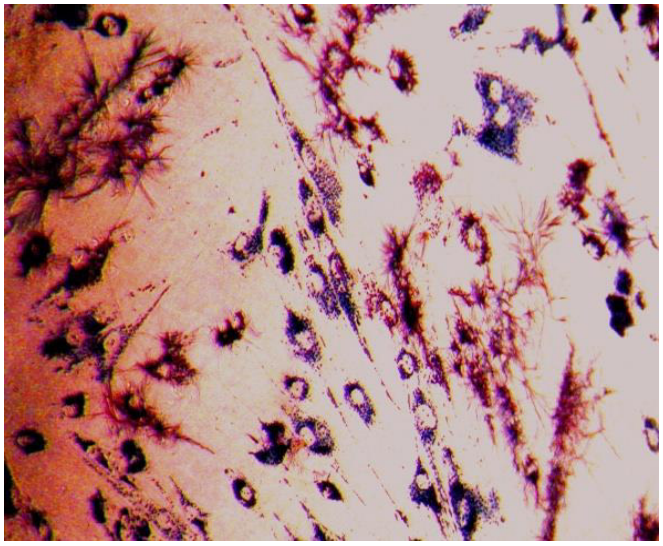


Fig 3: The cells morphology was changed, and formazan crystals appeared after 4 hours incubated with MTT solution

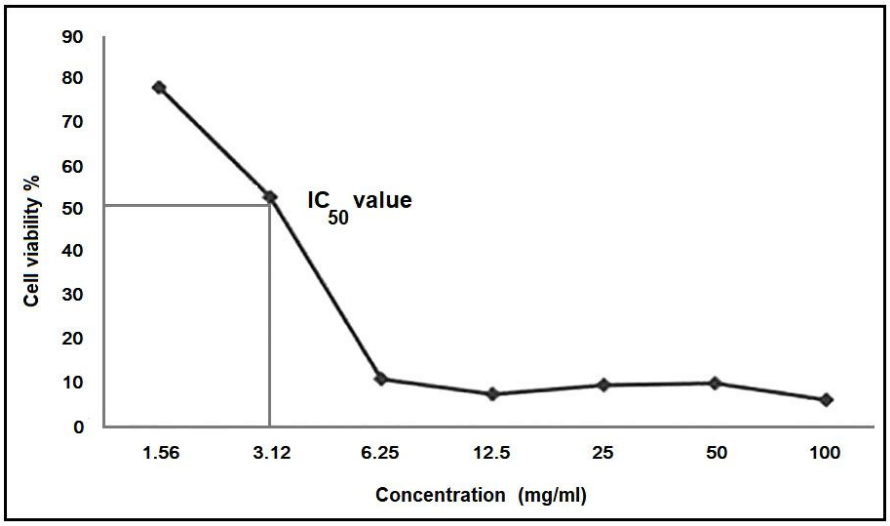
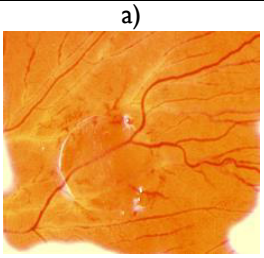
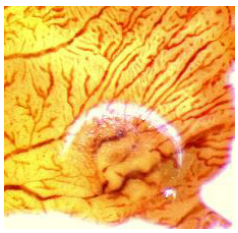
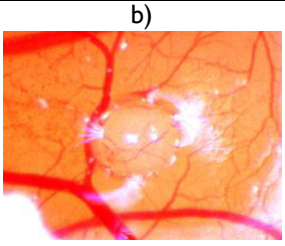
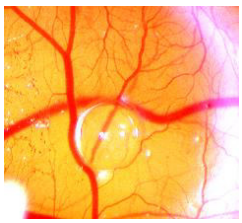


Fig 4: Dose-response curve for cell viability of SHED cells treated with different Concentrations of *Ixora coccinea* root extracts.

Before treatment	After treatment
a) 	
b) 	

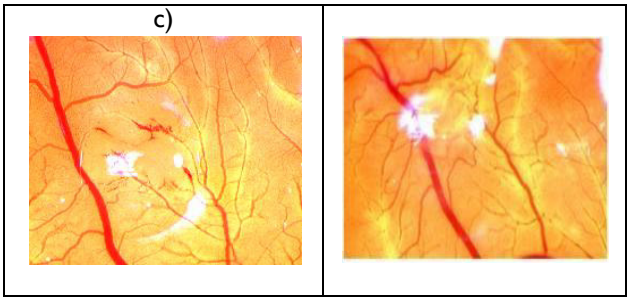


Fig 5: HET-CAM assay (a) Irritation caused by SDS; (b) Inhibition of membrane Irritation by *Ixora coccinea* extracts; (c) Inhibition of membrane irritation Indomethacin

Table 1: Anti-inflammatory effect (HEM-CAM assay) of *Ixora coccinea* extract

Test sample	Percentage of inhibitions (%)
Indomethacin	85
Ixora coccinea	80
SDS	25

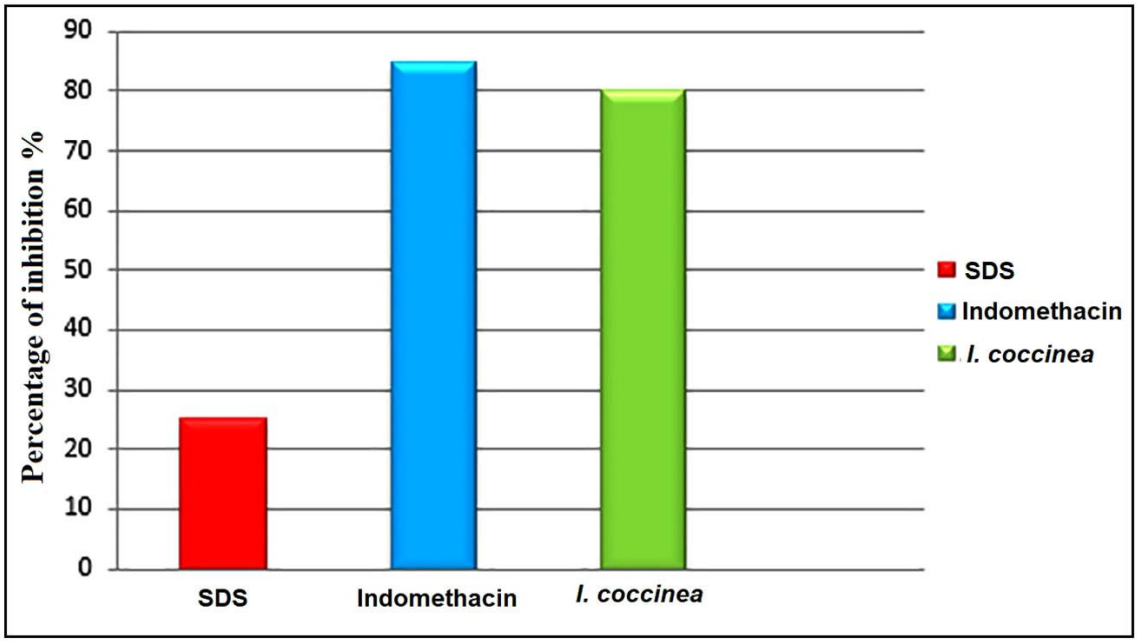


Fig 6: Percentages of inhibition of membrane irritation.

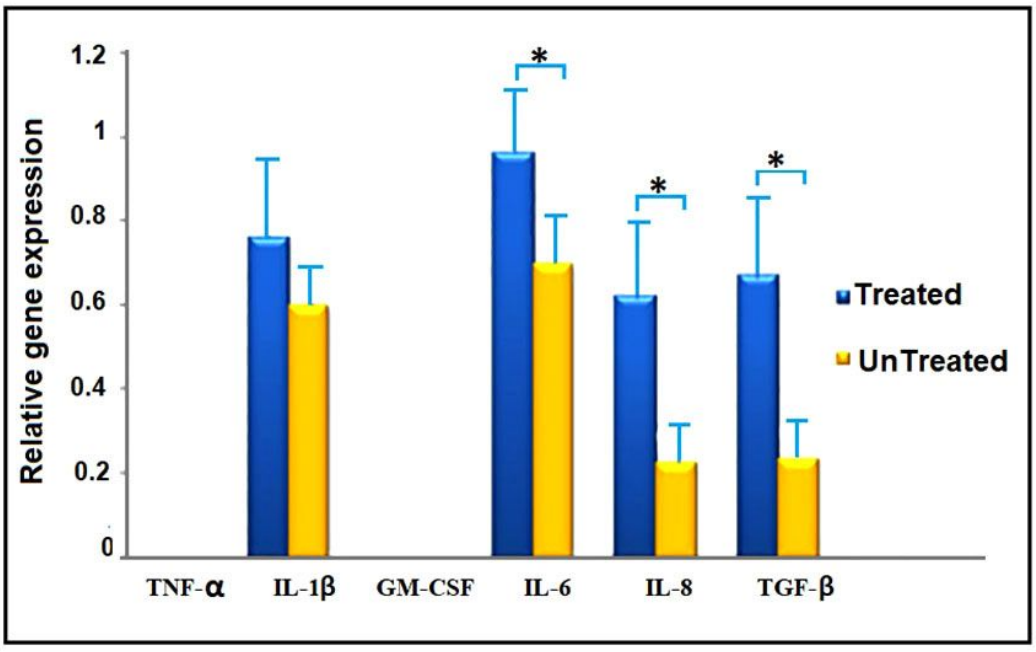


Fig 7: Level of inflammatory cytokine genes expression. (*showed significantly)

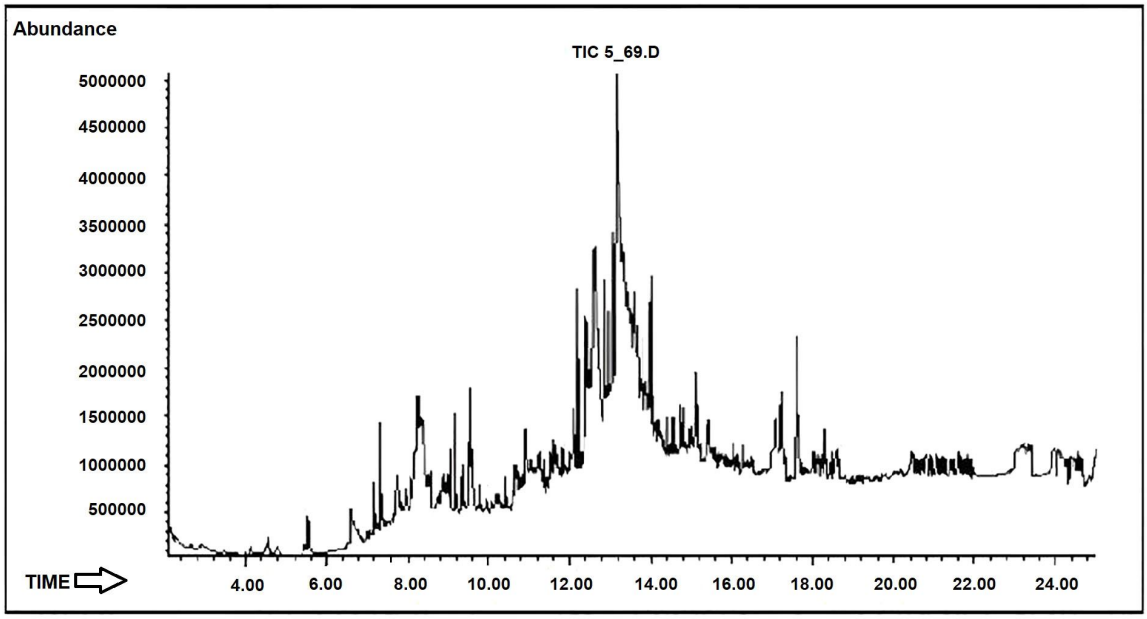


Fig 8: GC-MS chromatogram of methanolic extract of the *Ixora coccinea* Linn root

Table 2: Phyto-component identified in the *Ixora coccinea* root extract

No	RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
1	4.117	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.36
2	12.877	9-Octadecenoic acid, Methyl-ester	$C_{19}H_{34}O_2$	296	1.97
3	9.551	2,6-dimethoxy phenol	$C_8H_{10}0_3$	154	1.35
4	15.118	13-Docosenamide	$C_{22}H_{43}NO$	337	1.31
5	10.895	3,4,5-tri methoxyphenyl	$C_9H_{12}O_4$	184	1.24
6	13.801	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	1.13

Table 3: Activity of phyto-components identified in the *Ixora coccinea* root extract

No	Name of component	Compound nature	Activity
1	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
2	9-Octadecenoic acid, Methyl-ester	Oleic acid	Anti-inflammatory Hypocholesterolemic Cancer preventive, Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic Antiarthritic Anticoronary, Insectifuge
3	2,6-dimethoxy phenol	Phenolic compound	No activity report
4	13-Docosenamide	Amide compound	Antimicrobial
5	3,4,5-tri methoxyphenyl	Phenolic compound	No activity report
6	4,8,12,16-Tetramethylheptadecan-4-olide	Isoprenoid	No activity report

******Source: Dr. Duke's phytochemical and ethnobotanical database (Online database)***

4. DISCUSSION

The viability of SHED cells decreased when the half-maximum inhibitory concentration (IC_{50}) of *Ixora coccinea* extracts was 12.5 mg/ml with 50% cell viability. It exhibited that cell viability was reduced in greater concentrations. The MTT assay is a quantitative and precise colorimetric assay that evaluates cell viability, proliferation, and activation. The theory of MTT assay is based on reducing yellow water-soluble 3-(4,5-dimethylthiazol-2-yl)-2,2-diphenyl tetzolium bromide (MTT) sites of living cells by viable cell ra cytochrome b and cytochrome c transform the soluble form of formazan salt to an insoluble, intracellular purple formazan metabolite. The American National Cancer Institute (NCI) guideline set the crude extract activity limit at 50 percent inhibition (IC_{50}) of proliferation below 30 µg/ml after 72 hours of exposure.[18] However, a crude extract with IC_{50} below 20µg/ml is highly cytotoxic.[19] Sodium dodecyl sulfate (SDS) induced membrane irritation formed a powerfully vascularized and star-like capillary network. Chronic inflammatory cells proliferate, migrate, and recruit to the side of injury, thereby damaging normal tissue. Chronic

inflammation facilitates the spread of inflammatory cells, migrating and recruiting to injury, destroying the normal. In this analysis, SDS induced chronic inflammation, showing that grain is highly vascularized and that the pellets containing SDS are enclosed in a star-like network of capillaries. It was consistent with several previous HET-CAM studies, which showed that the inflammation was most frequently illustrated by granuloma development with high blood vessels[20,21]. In addition, the blood vessel is usually star-like around granuloma, including activated chronic inflammatory disorders, such as tuberculosis.[22] In this study, positive regulation of Indomethacin showed good anti-inflammatory effects with an inhibition of 85%. Similar to the previous study, it was found that Indomethacin induced significant anti-inflammatory activity in the HET-CAM assay.[23] No granuloma was created and a regular blood vessel network emerged. *The Ixora coccinea* extract was a strong anti-inflammatory operation with an inhibition of 80%. Previous research shows the anti-inflammatory activity of *Ixora. coccinea* root extracts showed inhibition of carrageenan-induced paw edema (acute inflammatory model) and pellet granuloma (chronic inflammatory model).[12] Excitingly, this was the first study demonstrating the inhibitory effects of *Ixora coccinea* on the sixth-day chicken embryo's chorioallantoic membrane. The key benefit of using a chick embryo chorioallantoic membrane was that it was easy to use, fast and cost-effective. Inflammatory cytokines gene expression, including TNF-α, IL-1β, GM-CSF, IL-6, IL-8, and TGF-β, were identified using multiplex PCR. This study did not express TNF-α and GM-CSF in treated and untreated SHED cells with *Ixora coccinea* root extract. This finding was consistent with the previous study by Nurul et al.[24] that did not identify TNF-α and GM-CSF in SHED cells and human fetal osteoblastic (hFOB) as control. It might be because bioactive present *Ixora coccinea* root extracts do not affect these cytokine expressions. In this analysis, in *Ixora coccinea* Linn root-treated SHED cells, IL-1β was expressed significantly higher than in untreated SHED cells. Also, gene expression upregulation may be linked to the fact that *Ixora coccinea* root extracts showed an immunomodulatory impact. On the other hand, a previous study revealed that *Tanacetum pseudoacillae* displayed lipopolysaccharide inhibition of IL-1β in RAW cells (mouse leukemia monocyte-macrophage line).[25] The expression of IL-6 in SHED cells was higher than in untreated cells. Similar findings in the previous immunostimulatory herbal research showed that IL-6 was strongly induced in peripheral mononuclear blood cells (PBMC) after the treatment.[26] It may indicate that the upregulation of the medicinal plant's inflammatory cytokines, such as IL-6, may promote immune response, prevent pathogen-associated symptoms and inhibit chronic disease.[27] *Ixora coccinea* root extracts substantially encouraged the expression of IL-8 and TGF-β in treated SHED cells. It may be because the momentary expression of these cytokines increases the early inflammatory process of wound healing. Previous studies also showed that *TGF-β* was enriched by Aloe Vera extracts in cultured human keratinocytes and Macrotyloa uniflorum extract in wound healing, thereby reducing hypertrophic scarring.[28]

5. CONCLUSION

In conclusion, the present study has determined that *Ixora coccinea* root extracts showed a strong anti-inflammatory operation and immunomodulatory impact by enhancing the immune response via stimulated expression of inflammatory cytokines in SHED cells. The MTT assay showed the IC_{50} of *Ixora coccinea* extracts was 12.5 mg/ml with 50% cell viability while indicating it is not toxic. The HET-CAM analysis showed the *Ixora coccinea* extract was a strong anti-inflammatory operation with an inhibition of 80%. In addition, the inflammatory cytokines expression of IL-6 IL-1β, IL-6, IL-8, and TGF-β in intreated SHED cells was higher than in untreated cells.

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7. AUTHORS CONTRIBUTION STATEMENT

Professor. Dr Normastura Abd Rahman conceptualized and analysed the data of the study. Dr. Norliana Ghazali designed the methodology, and wrote the original draft, formal analysis, writing-reviewing, and editing the manuscripts. Ms. Noor Hafizah Mohd Shah performed data analysis and discussed the methodology.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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