EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY PROPERTIES OF LEAVES OF *JEFFREYCIA ZEYLANICA* (PUPULA)

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Abstract:

Background: The medications known as non-steroidal anti-inflammatory drugs are frequently used to treat inflammation and relieve pain. But non-steroidal anti-inflammatory drugs have adverse side effects, as an alternative we can use plant extract**.** *Jefferycia zeylanica* (Pupula) is an endemic plant which is used to treat inflammatory conditions.

Objectives: To investigate the *in vitro* anti-inflammatory activity of aqueous, methanol, dichloromethane, and hexane extracts of *Jeffreycia zeylanica* leaves using the egg albumin denaturation method and Human Red Blood Cell membrane stabilization method.

Methods: Matured leaves of *J. zeylanica* were collected, washed, and air dried. Then the leaves were ground into a fine powder using a grinder. The extractions were obtained using the maceration method and concentrated using a rotary vacuum evaporator. The egg albumin denaturation and Human Red Blood Cell membrane stabilization methods were used to evaluate the anti-inflammatory activity of the plant. Diclofenac sodium was used as the positive control.

Results: In egg albumin denaturation method, hexane leaves extract (IC50 154.9 µg/mL) showed the highest inhibition of protein denaturation compared to Diclofenac Sodium (IC50 179.2 µg/mL). **P values and R^2** of the plant extracts suggested a statistically significant correlation (P <0.05) between concentration and % inhibition of egg albumin denaturation. In the Human Red Blood Cell method, Diclofenac sodium indicated an **IC_{50} value of 77.05 µg/mL. Dichloromethane extract of *J. zeylanica* leaves indicated an IC_{50} value of 188.6 µg/mL. In this method also, all the plant extracts indicated a** statistically significant correlation (P<0.05) between concentration and % protection of red blood cell membrane. **Dichloromethane** extract showed the highest efficacy and potency similar to the positive control diclofenac sodium.

Conclusion: *Jeffreycia zeylanica* leaves extracts showed considerable anti-inflammatory activity. Hexane leaves extract of *J. zeylanica* indicates the highest potential activity using the protein denaturation method. The **dichloromethane** leaf extract of *J. zeylanica* indicated the highest potential activity using the Human Red Blood Cell membrane stabilization method. Further studies are necessary to determine the mechanisms and to evaluate active compounds that affect the anti-inflammatory activity of the plant.

Key words: anti-inflammatory activity, egg albumin denaturation method, HRBC method, *Jeffreycia zeylanica*, endemic.

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INTRODUCTION: Inflammation is a defense mechanism that enables the body to protect itself against infection, burns, toxic chemical allergens, or any other harmful stimuli. Inflammation is a substantial reaction to damage, disease, or destruction. Classic hallmarks that portray inflammation are redness, pain, swelling, and disturbed physiological functions. In synthetic medicine, Non- Steroidal Anti- Inflammatory Drugs (NSAIDs) are commonly used to manage pain and inflammation. But these NSAIDs can cause severe adverse effects such as acute renal failure, upper and lower GI complications, increased risk of cardiovascular harm and some of which may be life-threatening. Other than that, these NSAIDs may interact with other medications and cause unwanted side effects. As an alternative to these NSAIDs, the use of plant materials has been popular nowadays because natural plant compounds contain fewer side effects. *Jeffreycia zeylanica* is an herbaceous plant that is native to Sri Lanka. It exhibits a variety of ethnomedical traits. It is a member of the family ASTERACEAE. Extracts of *J. zeylanica* have been utilized in a variety of folk medicines as therapies for cancer, microbial infections, and inflammation and for treating wounds, bone fractures, and snake venom.

METHODOLOGY: Leaves of *Jeffreycia zeylanica* were collected, washed, and air-dried. When the dry weight of the leaves reached a constant level, obtained a fine powder by grinding the crude plant leaves. Leaf extracts were prepared using cold maceration, and aqueous, methanol, DCM (dichloromethane), and hexane were used as the solvents. The standard plant extraction concentrations were 2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 15.625 µg/mL. Diclofenac sodium was used as the reference drug and the same standard concentrations were used. Data were analyzed using the Graph pad Prism 8 software (version 8.2.1).

Heat-induced Egg albumin denaturation method- Tubes were prepared with 2.8 mL of PBS, 2 mL of plant extract standard concentrations, and 0.2 mL of egg albumin were added to prepare the test samples, and a control was prepared using 2.8 mL of PBS, 2 mL of distilled water, and 0.2 mL of egg-albumin. Reference drug concentrations were also made using the same procedure. Tubes were incubated at 37 ^{0}C in the water bath for 15-20 minutes using the laboratory shaking water bath, followed by heating at 70 ^{0}C, for five minutes absorbances were taken at 660 nm using a UV spectrophotometer. Using the absorbance values the percentage of egg albumin denaturation was calculated.

Human Red Blood Cell membrane stabilization method- Test samples were prepared using 1 mL phosphate buffer, 2 mL hypotonic saline, 0.5 mL of the plant extract, and 0.5 mL of washed red blood solution were added. A test control was prepared with 1 mL phosphate buffer, 2 mL distilled water, and 0.5 mL of 10% RBC suspension. In order to obtain 10% RBC

suspension, the blood samples were mixed with the same amount of Alsevers solution, The blood solution was centrifuged at 3000 rpm for 10 minutes. Then the supernatant was discarded and again washed two times with isotonic saline. 10% w/v red blood cell solution was made afterward. Diclofenac standard control samples were made using 1 mL phosphate buffer, 2 mL hypotonic saline, 0.5 mL of plant extract, and 0.5 mL of 10% RBC suspension. Assay mixtures were incubated at 37^0 C for 30 minutes. After incubation, the assay mixtures were centrifuged at 3000 rpm for 10 minutes, The absorbances of the supernatant were measured at 560 nm wavelength using a UV spectrophotometer. Using the absorbance values percentage stabilization of the Human RBC membrane was calculated.

Results And Discussion: The results were presented in mean, ± SEM (Standard Error of Mean), and ± SD (Standard deviation). The evaluation of EC_{50} (Half maximal effective concentration) and IC_{50} (half maximal inhibitory concentration) was done using the software.

Heat-induced Egg Albumin Denaturation Method

Table 1

Dose-response curve details of normalized % inhibition of egg albumin denaturation of diclofenac, aqueous, methanol, DCM, and hexane leaves extracts of *J. zeylanica*.

	Diclofenac	Aqueous	Methanol	DCM	Hexane
IC_{50} (µg/mL)	179.2	1297	26.14	568.1	154.9
P value	0.0098	0.0382	0.0054	0.0351	0.0075
R squared	0.9979	0.9962	0.3812	0.9898	0.9114

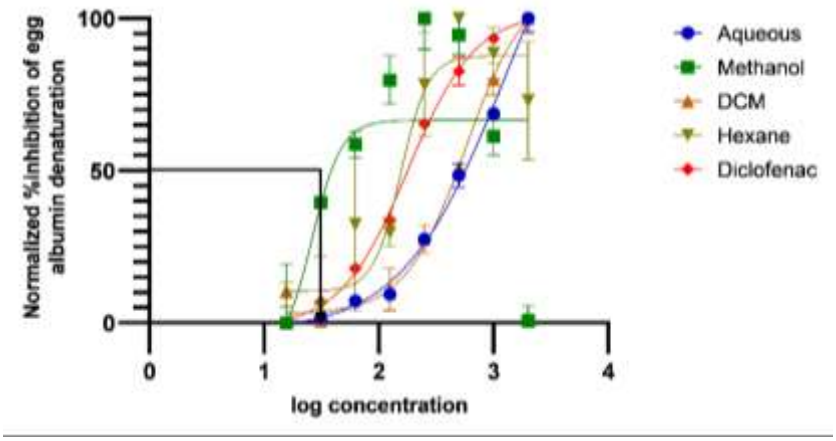

Figure 1: Normalized % inhibition of egg albumin denaturation of the reference drug diclofenac, aqueous extract, methanol extract, dichloromethane extract, and hexane extract of *J. zeylanica* leaves.

When comparing the IC_{50} values of the plant extracts with the reference drug diclofenac sodium belove information can be concluded. The aqueous plant extract has the highest IC_{50} value. Hexane extract has the best-fit results for the regression model indicating an IC_{50} value lower than the reference drug diclofenac sodium. Even though the methanolic extract has the lowest IC_{50} value among all, the coefficient determination of the methanolic extract suggests that the data doesn't fit well with the regression model. All the plant extracts had a significant relationship with the positive control diclofenac sodium ($P \leq 0.05$).

Human Red Blood Cell Membrane Stabilization Method

Table 2: Dose-response curve details of % stabilization of HRBC membrane with reference drug diclofenac sodium, aqueous, methanol, and dichloromethane leaves extract of *J. zeylanica*.

	Diclofenac sodium	Aqueous	Dichloromethane	Methanol
IC_{50} (µg/mL)	77.05	199.5	154.0	371.9
P value	0.0037	0.0063	0.0104	0.0056
R squared	0.9929	0.9873	0.9787	0.9431

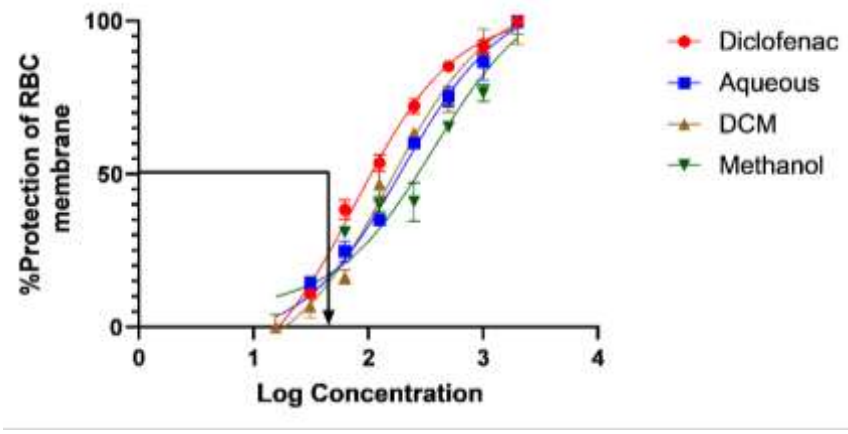

Figure 2: Normalized % stabilization of the Human RBC membrane with reference drug diclofenac sodium and aqueous, methanol, Dichloromethane extracts of *J. zeylanica* leaves.

When comparing the obtained IC_{50} values with the reference drug diclofenac sodium, the highest potential activity was reported by the DCM extract of *J. zeylanica*. And the lowest potential activity was indicated by methanolic plant extract compared to the reference drug. In conclusion, all the plant extracts had a significant anti-inflammatory activity ($P \leq 0.05$).

Furthermore, both methods indicated that *J. zeylanica* has an effective anti-inflammatory activity. The hexane plant extract expresses the highest potential activity in the egg albumin denaturation method. The DCM extract presented the highest potency in the HRBC membrane

stabilization method. When considering the reported anti-inflammatory activity of *J. zeylanica* through the study it is possible to reason out that the leaves of the plant are more soluble in low polarity solvents, evidencing that the plant's anti-inflammatory activity could be supported by nonpolar substances than polar substances.

CONCLUSIONS/RECOMMENDATIONS: *Jeffreycia zeylanica* leaves extracts showed considerable significant anti-inflammatory activity. Hexane leaves extract of *J. zeylanica* indicates the highest potential anti-inflammatory ability using the protein denaturation method. The DCM leaf extract of *J. zeylanica* indicated the highest potential activity using the HRBC membrane stabilization method. The egg albumin denaturation assay has to be repeated to get a better understanding of the effect of organic solvents on protein denaturation activity. Further studies are necessary to evaluate the mechanisms of anti-inflammatory activity.

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