Prayogik Rasayan

In vitro erythrocyte membrane stabilization potential in some Sri Lankan medicinal plant extracts

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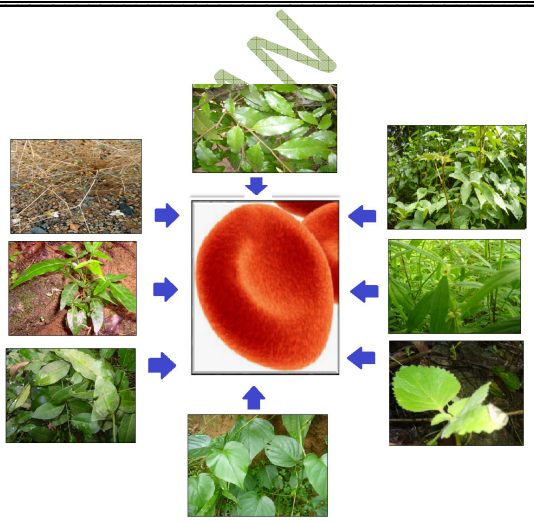
Abstract

The present study was undertaken to evaluate red blood cell (erythrocyte) membrane stabilization potential in hydroalcoholic extracts of eight medicinal plants that have been utilized as anti-inflammatory remedies in Sri Lankan folk medicine.10% suspensions of red blood cells in normal saline were incubated with 1000 µg/mL of each extract. The absorbance of the released hemoglobin was measured spectrophotometrically. The most potent extracts that possess ≥ 50% stabilization potential were subjected to concentration-response studies and EC_{50} values were determined. *Hibiscus furcatus* extract has shown the highest membrane stabilizing potential with an EC_{50} of 51.92 µg/mL which is even better than the reference drug, diclofenac sodium (EC_{50}=131.9 µg/mL). Moreover, a considerable activity was observed in *Leucas zeylanica* and *Plectranthus zeylanicus* (EC_{50}=81.35 and 219.6 µg/mL respectively). Thus, the present study revealed that the plants utilized in folklore medicine in Sri Lanka as anti-inflammatory remedies are capable of stabilizing the cellular membranes.



Keywords: medicinal plants, membrane stabilization, red blood cells

1. Introduction

Human red blood cell membrane consists of a lipid bilayer, which is a common component in all other cell membranes and membrane bound organelles, thus the resemblance of these membranes is widely used in scientific research. Lysosomes are membrane-enclosed organelles that contain an array of hydrolytic enzymes to catalyze the degradation of all kinds of biomolecules in the cells. Lysosomes and their contents play an important role in inflammation and inflammatory disorders[1]. Resemblance of lysosomal membrane to red blood cell membrane could be used to extrapolate the effect of drugs on the stabilization of lysosomal membrane by evaluating their effect on red blood cell membrane stabilization. Thus red blood cell membrane stability test is well established and numerous reports indicate its application to evaluate the effect of plant extracts in stabilization of cellular membranes and thereby to predict the possible anti-inflammatory potential[2,3,4,5].

Plant materials have been used in many aspects of Indian Ayurvedic medicine and indigenous medicine in Sri Lanka over thousands of years. Among the native flora of Sri Lanka, more than 1400 plants are used in indigenous medicine[6] and a large number of plants are extensively used to alleviate the pathological conditions caused by inflammation[7,8,9]. However, the scientific evidences are insufficient to explain the biochemical activities of these plants, thus their therapeutic usage. Therefore, the present study is undertaken to evaluate the red blood cell membrane stabilization potential in plant extracts prepared from eight medicinal plants that have been used in traditional medicine in Sri Lanka as anti-inflammatory remedies.

2. Methodology

2.1. Preparation of plant extracts

Leaves of *Argyreia populifolia* (Convolvulaceae), *Atalantia ceylanica* (Rutaceae), *Hibiscus furcatus* (Malvaceae), *Olax zeylanica* (Olacaceae), as well as, whole plants of *Leucas zeylanica* (Lamiaceae), *Plectranthus zeylanicus* (Lamiaceae) and *Munronia pinnata* (Meliaceae) were collected in Gampaha District - Western Province of Sri Lanka in 2015.

The seeds of *Mollugo cerviana* (Molluginaceae) was purchased from Ayurvedic retail outlet at the Market Place, Nittambuwa, Sri Lanka. The plant materials were authenticated by comparison with the herbarium specimens at the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. A voucher specimen of each plant is deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka.
The plant materials were thoroughly washed and dried in shade (30 ℃) for four-six days. Dried plants were powdered using a domestic grinder. The powdered plant materials (10-15 g) were extracted in 70% methanol-water (300 mL) by heating for 2 hours at 60 ℃. The extracts were evaporated into dryness with the use of rotary evaporator (BÜCHI, R-114, Germany) or a vacuum centrifuge (Thermo, Germany).

2.2 Effect on Human Red Blood Cell Membrane Stability

Initially, the membrane stability of red blood cells incubated with 1000 µg/mL of the extracts were tested according to an *in vitro* model described by Gandhidasan et al.,[10] with slight modifications. Briefly, fresh venous blood (3 mL) from healthy volunteers (n=21) was collected in to a tube containing 3 mL of sterile Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride).

Separation of red blood cells was performed by centrifuging the blood samples using bench centrifuge (Sigma/2-16P) at 3000 rpm for 3 min. The packed cells were suspended in an equal volume of isotonic saline and centrifuged again. The process was repeated two more times until the supernatants were clear. A 10% human red blood cell (HRBC) suspension was prepared then with normal saline and kept at 4℃ undisturbed until used.

The reaction mixture consisted of hypotonic saline (0.25% w: v NaCl) (1 mL), 0.15 M sodium phosphate buffer (pH 7.4) (0.5 mL), and the extract in 0.5 mL of normal saline was added with 0.25 mL of 10% HRBC in normal saline. The reaction mixture was maintained at pH 7.4 ± 0.2 and was incubated at 56℃ for 30 min. The tubes were cooled under running water for 20 min, then the mixture was centrifuged at 3000 rpm for 3 minutes, and the absorbance of the supernatants (released hemoglobin) was measured at 540 nm using a spectrophotometer (UV-1800, SHIMADZU). Two controls were used where control$_1$ with 0.5 mL of isotonic saline instead of the extract and the control$_2$ with 0.5 mL of extract solution without red-blood cells. Diclofenac sodium was used as the reference drug.

The percentage membrane stabilizing activity was determined using the following equation.

$$\% \text{ Membrane stability} = \frac{100 - (\text{Abs with the extract} - \text{Abs of control}_2)}{\text{Abs of control}_1} \times 100$$

The extracts that have displayed membrane stabilization of ≥ 50% at an initial concentration of 1000 µg/mL were further subjected to concentration-response study and their EC_{50} values were determined.

2.3 Statistical analysis

All experiments were conducted in duplicates and statistical analysis of the data was performed by Analysis of Variance (ANOVA). P value <0.05 was considered to denote a statistically significance. All data were presented as mean values ± standard deviation (SD). EC_{50} values were determined by Graph Pad Prism 6.01 software.

2.4 Ethical consideration

Ethical approval was obtained from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna.

3. Results and Discussion

The preliminary screening revealed that out of the eight extracts, only three extracts i.e. *H. furcatus, L. zeylanica* and *P. zeylanicus* were capable of stabilizing red blood cell membrane above 50% at an initial concentration of 1000 µg/mL (Table 1). Therefore, concentration response studies were conducted for the above three extracts and EC_{50} values were determined.

Extract	Membrane stabilization (%) at 1000 µg/mL
A. populifolia	2.5 ± 1.2
A. ceylanica	4.3 ± 2.8
H. furcatus	71.7 ± 1.3
L. zeylanica	72.4 ± 1.5
M. cerviana	11.7 ± 1.9
M. pinnata	34.3 ± 5.3
O. zeylanica	16.1 ± 3.1
P. zeylanicus	56.6 ± 2.5
Diclofenac sodium	62.9 ± 1.8

Table1: Membrane stabilization potency in plant extracts and in the reference drug at 1000 µg/mL

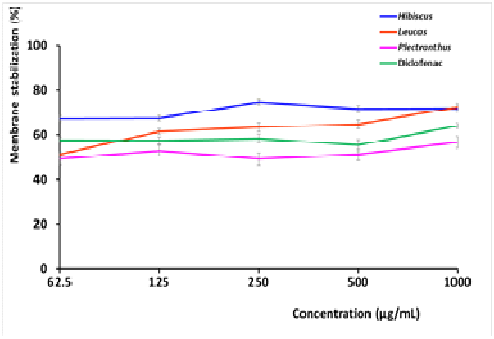


Fig 1: Concentration-response graphs for the most potent extracts and the reference drug

As depicted in Fig.1, the effect of the extracts on membrane stabilization was highest at 1000 µg/mL, however, none of the extracts have displayed a typical sigmoidal curve normally observed in dose-response studies, suggesting that increased stabilization of red blood cell membrane was not directly proportional to the increase in concentration of the extract. Interestingly, the EC_{50} values for *H. furcatus* and *L. zeylanica* was lower than that of the reference drug,

diclofenac sodium (Table 2) indicating their high potency as membrane stabilizing agents.

Extract	EC_{50} (µg/mL)
H. furcatus	51.92
L. zeylanica	81.35
P. zeylanicus	219.6
Diclofenac sodium	131.9

Table 2: EC_{50} values of the most potent extracts and the reference drug

Membrane stabilization is a process of maintaining the integrity of biological membranes such as erythrocyte and lysosomal membranes against osmotic and heat induced lysis. During inflammation, lysosomal hydrolytic enzymes are released into the sites causing damages to the surrounding organelles and tissues. Therefore stabilization of lysosomal membrane is important in limiting the inflammatory response as it could lead to reduced release of lysosomal constituents. As the lysosomal membrane cannot be used in isolation, the resemblance of red blood cell membrane to lysosomal membrane is normally used to predict the anti-inflammatory activity of various compounds.

Even though the previous reports have revealed the presence α-tocopherol[11], a secondary metabolite with anti-oxidant and membrane stabilization potential[12], in *M. pinnata,* a prominent membrane stabilization activity was not observed for this extract in our study. Nevertheless, *L. zeylanica* extract has proven strong membrane stabilizing potential and the recent findings have indicated that the extract contains α-tocopherol[13]. Therefore, the membrane stabilization in *L. zeylanica* could be correlated to the presence of α-tocopherol. Furthermore, our previous studies have revealed a strong anti-inflammatory activity in *P. zeylanicus*[14] and the present observations further corroborate those findings. However, the phytochemical constituents in *H. furcatus*, should be investigated in depth as it has shown the highest membrane stabilizing potency in comparison to the other extracts employed in this study and even the reference drug. Thus, experiments are in progress to identify the bioactive secondary metabolites in *H. furcatus*. We believe that these findings would shed a new light to the field of anti-inflammatory activity studies of Sri Lankan medicinal plants.

4. Conclusion

Our investigations clearly indicated that popular Sri Lankan medicinal plants, *H. furcatus, L. zeylanica* and *P. zeylanicus* could effectively stabilize the red blood cell membrane and specially the activity of *H. furcatus* and *L. zeylanica* were found to be remarkable. However, further studies are necessary to characterize the compounds responsible for this activity and to evaluate possible cytotoxic effects of these extracts.

5. Acknowledgements

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6. References

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