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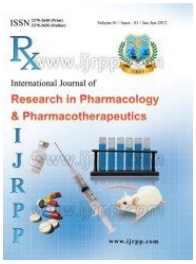

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In vitro screening for cytotoxic activity of ethanolic extract of *Erythrina variegate* lam. (leaves)

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ABSTRACT

Erythrina variegate Lam commonly known as Indian Coral tree, belonging to the family Fabaceae. It contains about 130 species, which are distributed in tropical and sub tropical regions worldwide. Various parts of the plants such as bark, root, stem, leaves, fruits and flowers possess many medicinal values, particularly for treating fever, liver ailments, rheumatism, relieve joint pain, kills the worms etc. The preliminary phytochemical screening of Ethanolic extract of *Erythrina variegate* (EEEV) was carried out and revealed the presence of flavonoids, alkaloids, glycosides, carbohydrates, steroids, saponins and triterpenoids. The aim of the work is to assessed the cytotoxic activity of the EEEV by the hemolytic test and the Isolated Chicken Eye (ICE) test. The cytotoxic effect was found to be negative towards the corneal cells and the human blood cells.

Keywords: Cytotoxicity, *Erythrina variegate*, EEEV, Corneal cells and Human blood cells

INTRODUCTION

Herbal medicine is a scientifically recognized complementary and alternative treatment method with proven efficacy [1]. Recently, World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care. Interest in herbal medicines has increased dramatically in recent years throughout the world, as globally people are seeking herbal remedies [2]. *Erythrina variegate* also known as Indian coral tree, belonging to the family Fabaceae [3]. It is a medium sized quick growing tree found in deciduous forests throughout India [4]. Leaves extract possess nematicidal activity and encourage the start of menstruation and milk secretion. Root extract possess

anti microbial activity. Bark is used as an anthelmintic and liver ailment [5, 6]. Based on the literature survey, we studied the *in vitro* screening for cytotoxic activity of Ethanolic extract of *Erythrina variegate* by using the corneal cells and human blood cells.

MATERIALS AND METHODS

Plant Material

The plant material was collected from Karanthai, Thanjavur district, Tamil Nadu and was identified by the botanist.

Preparation of Plant extract

The dried coarsely powdered sample of *Erythrina variegate* leaves was first extracted with Petroleum ether (60-80°C) in Soxhlet apparatus and then using ethanol as a solvent at 60 - 70ºC.Then the extract was concentrated with the help of hot plate. The solvent was removed by distillation under reduced pressure [7].

Phytochemical screening

The preliminary phytochemical screening was carried to identify the phytoconstituents. The result showed that the presence of carbohydrates, flavonoids, alkaloids, steroids, saponins and triterpenoids [8].

Chemicals

Phosphate buffer, DMSO, Triton X-100, Tryphan blue, Methanol and 1% acetic acid

ASSESSMENT OF CELL VIABILITY

Hemolytic assay

Anticoagulant treated whole blood from Human was taken and diluted ten folds with phosphate buffer. Different concentrations (25, 50, 75 and 100 µg/mL) of compound was prepared by two-fold dilutions with three replicates. All the tubes filled with 100µL of diluted blood was incubated for 45 min at 37° C. Vehicle control 0.5% DMSO was used as negative and Triton X-100 as positive control. Degree of lysis at each concentration was recorded Spectrophotometry at 540 nm. Percentage of Hemolysis for each concentration was calculated by dividing sample's absorbance on positive control absorbance (complete hemolysis) multiplied by 100 [9].

Isolated Chicken Eye test (ICE)

The Isolated Chicken Eye (ICE) test method is an in vitro test method that can be used to classify substances as “ocular corrosives and severe irritants”. The ICE method uses eyes collected from chickens obtained from slaughterhouses where they are killed for human consumption, thus eliminating the need for laboratory animals. The eye was enucleated and mounted in an eye holder with the cornea positioned horizontally. The test substance and negative/positive controls were applied to the cornea. Toxic effects to the cornea was measured by a qualitative assessment of damage to epithelium based on staining with 0.4% tryphan blue and uptake of dye by dead cells determined by microscopic observation [10].

RESULTS

Table: No: 1- Percentage of Hemolytic activity among *Erythrina variegate*

S.No	Concentration	OD value	Percentage of Hemolysis
1.	100	0.06	6.6
2.	50	0.04	3.3
3.	25	0.038	3
4.	12.5	0.02	0
5.	6.25	0	0
6.	3.1	0	0
7.	NC DMSO	0.02	
8.	PC SDS	0.6	

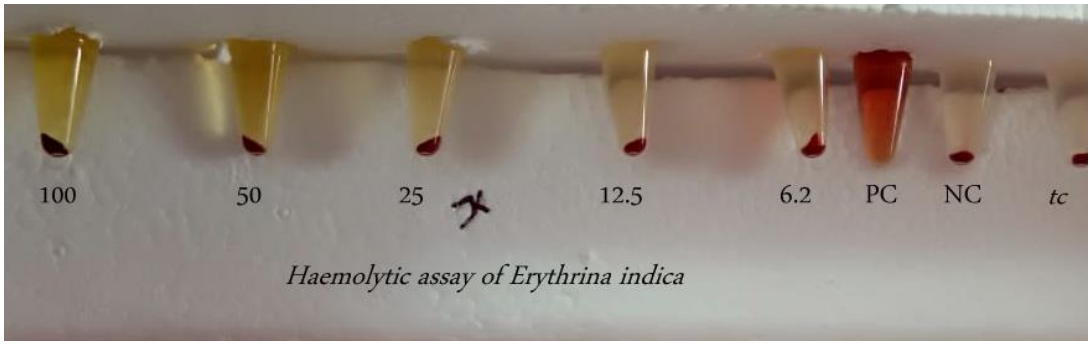

Fig.No:1 – Hemolysis of *Erythrina variegate* at various concentrations

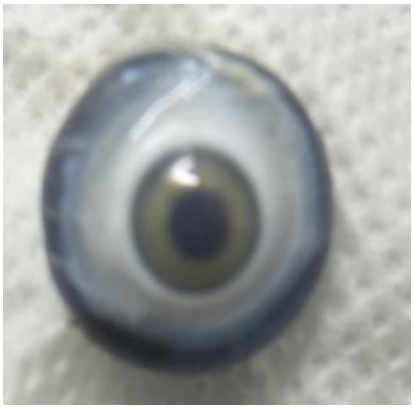

Fig. No: 2- Chicken eyeball

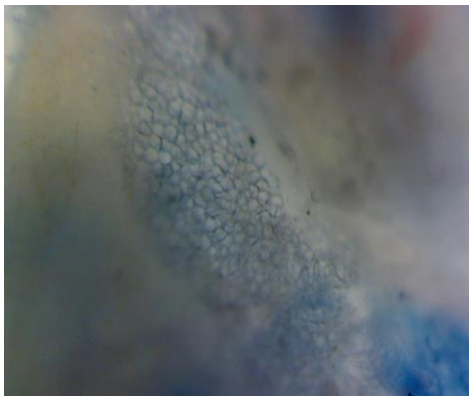

Fig. No: 3- *Erythrina variegate*

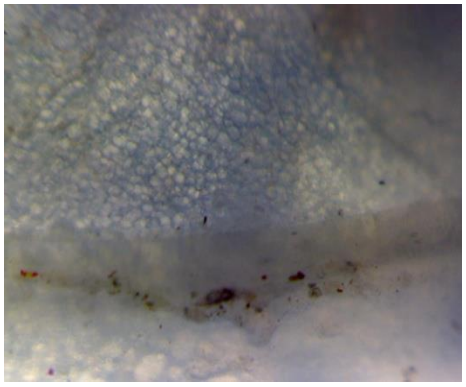

Fig. No: 4- Negative control

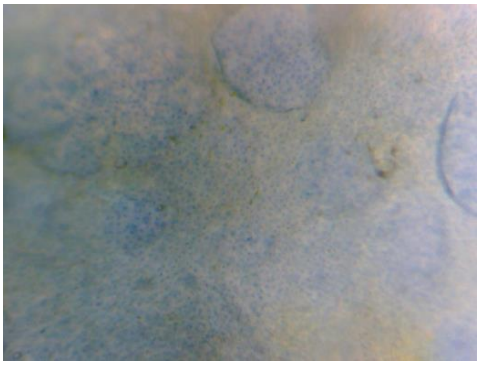

Fig. No: 5- Positive control

The result showed that the Ethanolic extract of *Erythrina variegate* exhibit the negative hemolytic and tryphan blue cytotoxic effect. In hemolytic assay, various concentration of the plant extract does not produce any toxic effect in the human blood samples. In Isolated Chicken eye test (ICE), dead cells absorbed the tryphan blue and it is stained. The viable cells does not absorb the blue colour.

DISCUSSION

In hemolytic assay, the plant extract is biocompatible [11]. Triton-X destabilizes the erythrocyte membrane, which causes hemolysis. The plant extract of *Erythrina variegate* does not interfere with the erythrocyte membrane, so it does not cause hemolysis [12]. In ICE method, viable cells have a blue cytoplasm and number of dead cells per unit volume is determined by light microscopy. Trypan

blue is a negatively charged dye which only stains cells with a compromised cell membrane due to bind with intracellular proteins hence indicating cell death. In contrast, viable cells are absence of trypan blue due to both the cell membrane and dye being negatively charged. Only cells with intact membranes can effectively exclude the dye, so dead cells with compromised membrane becomes stained [13].

Acknowledgement

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