



ANTI-OXIDANT AND ANTICANCER PROPERTIES OF ETHANOL LEAVES EXTRACT OF ERYTHRINA INDICA

R. Priya¹, P. Mani² and S. Maneemegalai^{1*}

¹Department of Biochemistry, Bharathidasan University Constituent College for Women, Orathanadu - 614 625, Tamil Nadu, India.

²Department of Biotechnology, Annai College of Arts and Science, Kumbakonam – 612 503, Tamil Nadu, India.

*Corresponding Author: S. Maneemegalai

Department of Biochemistry, Bharathidasan University Constituent College for Women, Orathanadu - 614 625, Tamil Nadu, India.

Article Received on 06/11/2017

Article Revised on 26/11/2017

Article Accepted on 16/12/2017

ABSTRACT

The aim of the study was to explore the pharmacological importance of the ethanol leaves extract of *Erythrina indica*. The dried leaves of this plant were extracted with ethanol; rotary evaporated and lyophilized which was used for antioxidant and anticancer properties. Antioxidant activities based on hydrogen peroxide scavenging assay showed most appreciable results of 83.6%, nitric oxide radical scavenging assay showed a moderate activity of 66.1%, moreover DPPH photometric assay and Superoxide radical scavenging assay showed least values of 21.7% and 19.7%. Further, the extract also revealed promising anticancer activity against the adenocarcinomic human alveolar basal epithelial cancer cells (A549) which showed concentration dependent activities and at the maximum tested concentrations of 1000µg/ml, it showed 78.89% cytotoxicity. All the above predictions proved that the ethanol extract from *Erythrina indica* showed significant results on both anticancer and antioxidant activities which will be useful for its application in near future in the field of biomedicine.

KEYWORDS: *Erythrina indica*; Antioxidant; Anticancer; Leaves extract; Ethanol extraction; A549 cell line.

INTRODUCTION

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of human kind. The search for eternal health and for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, minerals, etc. and the development of a variety of therapeutic agents.^[1] In recent years, ethnomedical studies received much attention as this brings to light the numerous little known and unknown medicinal virtues especially of plant origin. They obviously deserve evaluation on modern scientific lines such as phytochemical analysis, Pharmacological screenings and clinical trials.^[2]

Erythrina is a genus of flowering plants in the pea family, Fabaceae. It contains about 130 species, which are distributed in tropical and sub tropical regions worldwide. They are trees, growing up to 30 m (98 ft) in height. The generic name is derived from the Greek word, "erythros" meaning "red," referring to the flower color of certain species.^[3] It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. The coral tree is cultivated particularly as an ornamental tree and as a shade and soil improvement tree (it fixes nitrogen) for other tree crops such as coffee and cocoa.^[4] *Erythrina*

indica is called as "Kalyana murungai" in Tamil language.

The preliminary phytochemical investigation showed the presence of alkaloids, carbohydrates, amino acids, tannins, steroids, flavonoids.^[5] There were research articles explored the importance of its leaves, the leaves juice used to heal wounds and sores. Leaves paste applied for muscular pain in cattle. Leaves extract possess nematicidal property. The root extract possess antimicrobial activity. Bark is astringent and used as febrifuge and anthelmintic.^[6] It is also used as an antidote to strychnine. The leaves are aperient; they also encourage the start of menstruation and of milk secretion. The bark is helpful in gallstone, liverishness, an expectorant, febrifuge, and vermifuge.^[7] In view of the wide range of medicinal uses of *Erythrina indica* as mentioned in earlier literatures, the present investigation was carried out on the medicinal significances of *E. indica* leaves, yet its therapeutic efficacy has been assessed only in few cases.

MATERIALS AND METHODS

Preparation of sample

Leaves of *Erythrina indica* were collected from the available local trees of Thanjavur district, Tamil Nadu, India. The collected leaves were shade dried and grinded

well to a fine powder. The powdered leaves samples were extracted three times repeatedly using 99.9% ethanol and the insoluble contents during the sample extraction were separated using centrifugation at 3000 rpm for 15 min. The resulting supernatant was rotary vacuum evaporated at 40°C and further dried under lyophilization. The dried sample content was stored under dark conditions and further sample preparation procedures were applied with respect to individual analysis carried out below. For antioxidant activities, 100 to 1000µg/mL sample concentration and for anticancer activities, 300 to 1000µg/mL concentrations of distilled water prepared samples with an interval of 100µg/mL were analyzed. All the experimental values were represented from the mean of triplicate experimental procedures.

Antioxidant activities

Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) solution. Different sample volumes were added to a hydrogen peroxide solution (0.6 mL, 40mM).^[8] Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging activity of the positive standard activity was determined using 1mM ascorbic acid was calculated.

$$\% \text{ Scavenged } [H_2O_2] = [(A_C - A_S)/A_C] \times 100.$$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample.

DPPH photometric assay

A total of 1 ml of a 0.3 mM DPPH ethanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature (30°C). After 30 min. the absorbance (Ab) values were measured at 518 nm and converted into the percentage antioxidant activity using the following equation.^[9] Scavenging capacity% = 100 - [(Ab of sample - Ab of blank) × 100/Ab of control]

Ethanol (1.0 ml) plus extract solution (2.5 ml) was used as a blank, while DPPH solution plus ethanol was used as a negative control. The positive controls were DPPH solution plus each 1 mM Morin (flavonol).

Nitric oxide radical scavenging assay

Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different sample concentrations and incubated at 25°C for 180 min. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained ethanol extracts at

the same concentrations with no sodium nitroprusside. A volume of 150 µL of the reaction mixture was transferred to tubes. The absorbance was measured at 546 nm OD spectrum. The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the ethanol extracts and gallic acid were calculated using the following formula.^[10]

$$\% \text{ nitrite radical scavenging activity} = [(A_C - A_S)/A_C] \times 100.$$

Whereas, A_C is the absorbance of control sample and A_S is the absorbance in the presence of the samples from extracts or standards. The standard of positive activity was done using 1mM gallic acid.

Superoxide radical scavenging assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT).^[11] Briefly, each 3.0 ml reaction mixture contained 0.05 M PBS (pH 7.8), 13 mM methionine, 2µM riboflavin, 100µM EDTA, NBT (75µM) and 1.0 ml of different test sample solutions and 1 mM rutin as the positive control. The tubes were kept in front of a fluorescent light (725 lumens, 34 W) and absorbance was read at 560 nm after 20 min. The entire reaction assembly was enclosed in a box lined with aluminium foil. The inhibition of superoxide anion was estimated by the equation.

$$\text{Inhibition \%} = [(A_0 - A_S)/A_0] \times 100$$

Where, A_0 is the absorbance of the control, and A_S is the absorbance of the tested sample.

Anticancer activity

MTT assay

A549 cell line was purchased from the National centre for cell science (NCCS), Pune, India. The purchased monolayer of A549 cells was washed twice with MEM without Foetal calf serum (FCS) to remove the dead cells and excess FCS. Then, 1ml of medium (without FCS) containing defined concentrations of the sample extract was added in respective 24 microtitre plate wells. The control well was prepared with cells containing 1ml MEM without any added test sample. The titer plate was incubated at 37°C in 5% CO₂ environment and observed for cytotoxicity using MTT as earlier described by Masters.^[12]

The percentage growth inhibition was calculated using following formula,

$$\% \text{ cell inhibition} = 100 - \{(A_t - A_b) / (A_c - A_b)\} \times 100$$

Where,

A_t = Absorbance value of test compound

A_b = Absorbance value of blank

A_c = Absorbance value of control

Further, the results were observed by inverted microscopy using Calcein AM, denotes viable cells and

Ethidium homodimer III, denotes dead cells which were used for staining adherent cells on well plates under fluorescent microscope (ZEISS LSM 880, Germany). Fluorescence was recorded using a 490 nm excitation filter and a 520 emission filter for Calcein AM, a 545 nm excitation filter and a 620 emission filter for Ethidium homodimer III and a 495 nm excitation filter and above range of 520 emission filter was used for combined stain (Calcein AM and Ethidium homodimer III).

RESULTS AND DISCUSSION

Antioxidant activities

Medicinal plants are the nature's gift to human society to make disease free healthy life. It plays a vital role to buildup and preserves our health. In our country more

than thousands of medicinal plants are recognized. In recent years, ethnomedicinal studies received much attention as this brings light to many plant based medicines.^[13] They obviously deserve evaluation on modern scientific lines such as phytochemical screenings like antioxidants, anticancer activities etc. An appreciable antioxidant and a concentration dependent hydrogen peroxide scavenging activity was seen with the ethanol leaves extract of *E. indica* which was maximum in the highest observed concentration (1000 µg/ml) of sample extract with 83.6%. Further, negative control was determined using distilled water with 0% activity and positive control was examined with ascorbic acid with 99.3% activity (Fig. 1).

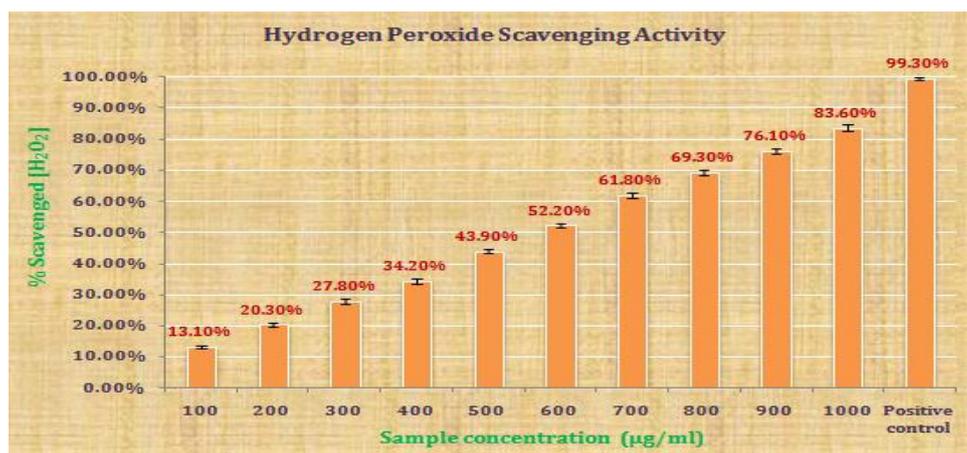


Fig.1: Hydrogen peroxide scavenging assay on ethanol leaves extract of *E. indica*.

Comparatively less appreciable DPPH scavenging activity was observed in ethanol leaves extract of the *E. indica*. This activity is also dependent on concentration and a maximum of 21.7% was achieved at the highest

tested concentration (1000 µg/ml) (Fig. 2). Moreover, the sensitivity of the test was predicted with distilled water as negative control with 0% activity and morin as the positive control with 99% activity.

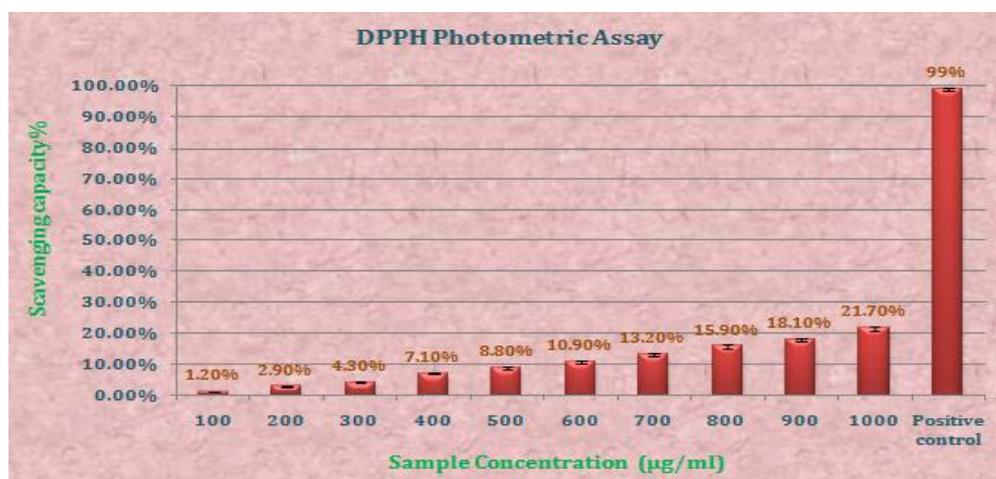


Fig.2: The DPPH photometric assay on ethanol leaves extracts of *E. indica*.

A concentration dependent Nitric Oxide Radical enhanced scavenging activity was observed in the tested samples from *E. indica*. The highest nitric oxide scavenging activity of 66.1% was revealed in the sample extract at the 1000µg/ml tested concentration and lowest

activity of 5.2% was recorded with 100µg/ml. Further, the negative control (distilled water) showed no activity and positive control, gallic acid showed 94.5% scavenging activity.

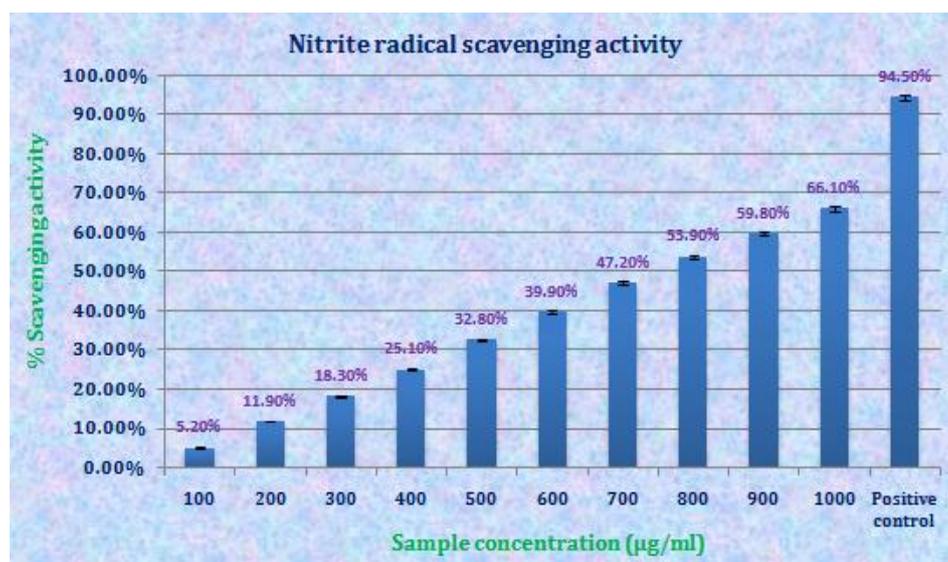


Fig.3: Nitric oxide radical scavenging activity of the tested samples at different concentrations of *E. indica*.

In similar to the above predictions, the superoxide radical scavenging test also revealed a concentration dependent activity observed from the samples of *E. indica* with a highest activity of 19.7% was recorded with 1000 µg/ml and lowest activity of 0.7% was observed at 100 µg/ml. Moreover, the negative control (distilled water), showed no activity and positive control, rutin observed 96.3 ± 2.4

% activity. Similar to this present study, an earlier study investigated the ethanol extract of the stem bark of *E. indica* was screened for its *in vitro* antioxidant activity using Ferric thiocyanate and thiobarbituric acid methods and it was found that it possess significant antioxidant activity.^[14]

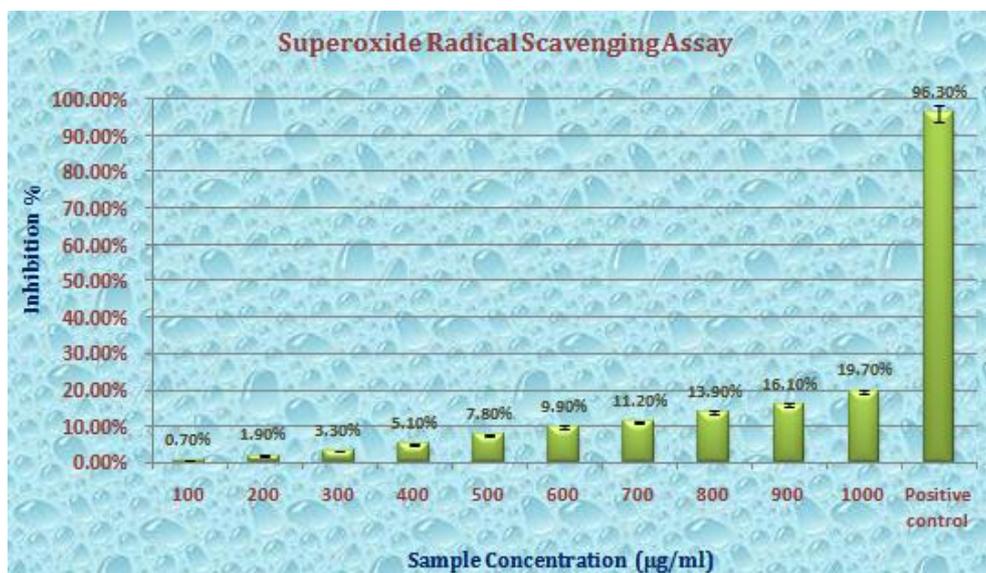


Fig.4: The superoxide radical scavenging activity of the samples at different concentrations of *E. indica*.

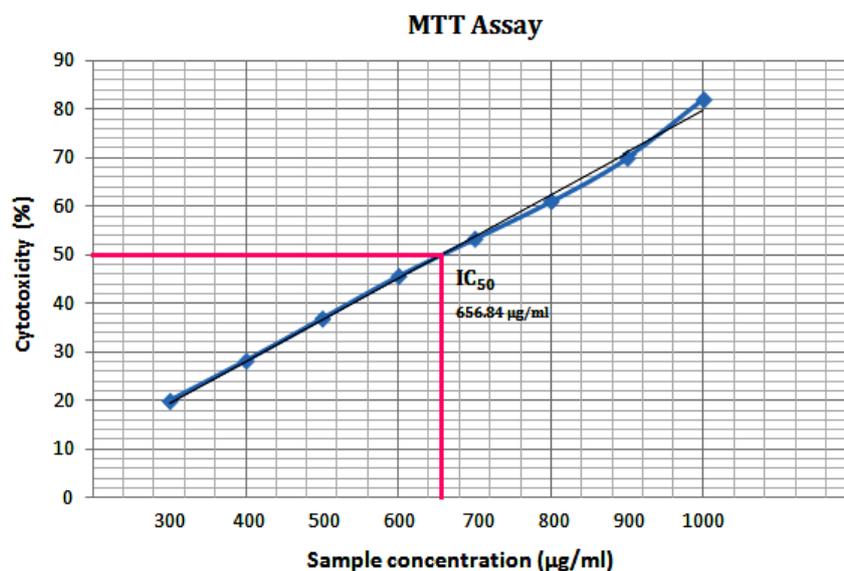
Anticancer activity

The anticancer effect of the ethanol leaves extract was studied against A549 cancer cell culture. These cells were treated with this extract at different concentrations, ranging from 300 to 1000 µg/ml, respectively (Table 1). The data of IC₅₀ (Half inhibitory concentration) are shown in graphical representations (Fig 5). The leaves extract exhibited significant inhibition of cell proliferation of A549 cancer cell line which is directly proportion to its tested concentrations.

Table 1: Anticancer potentials of ethanol extract of *E. indica* against A549 cancer cells.

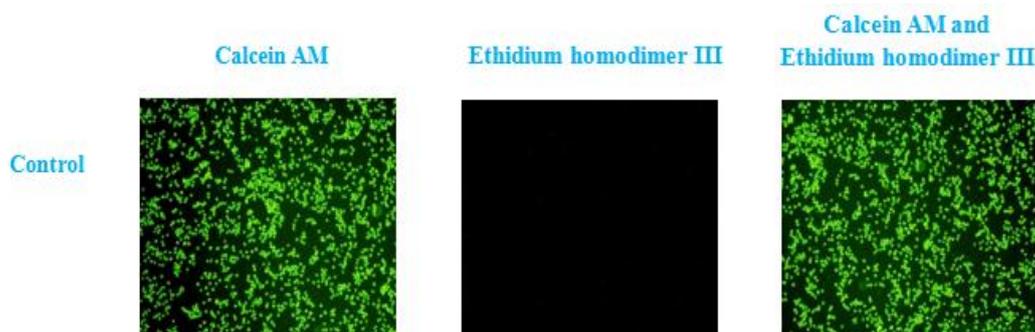
S. No.	Test sample concentration ($\mu\text{g/ml}$)	Cell inhibition/ cytotoxicity (%) of ethanol extract against A549 cell culture, Mean values	Cell inhibition/ cytotoxicity (%) of ethanol extract against A549 cell culture, S. D. values
1	300	19.99	0.14
2	400	28.23	0.23
3	500	36.91	0.36
4	600	45.64	0.39
5	700	53.31	0.45
6	800	61.01	0.57
7	900	69.91	0.71
8	1000	78.89	0.89
9	Control	0.01	0.00

Indications: Green color indicates the nearest IC_{50} value on the tested concentration and red color indicates the less predicted value on different concentration.

**Fig. 5: IC_{50} concentration of the ethanol extract from *E. indica* against A549 cancer cells.**

In the highest concentration (1000 $\mu\text{g/ml}$) the cell inhibition was in the mean value of 78.89%. IC_{50} value of A549 cell lines was 656.84 $\mu\text{g/ml}$ of extract concentration and recorded lowest cell inhibition of 19.99 % in the lowest tested concentration of 300 $\mu\text{g/ml}$. Microscopic examinations were also done at the important concentrations (Fig 6). These results showed

the impact of ethanol leaves extract sample from *E. indica* for its potential anticancer effect against this A549 cancer cells. The research on anticancer activities of the genus *Erythrina* was very less studied till date, so, the observed result may let many research to work for the pharmacological importance of this plant.



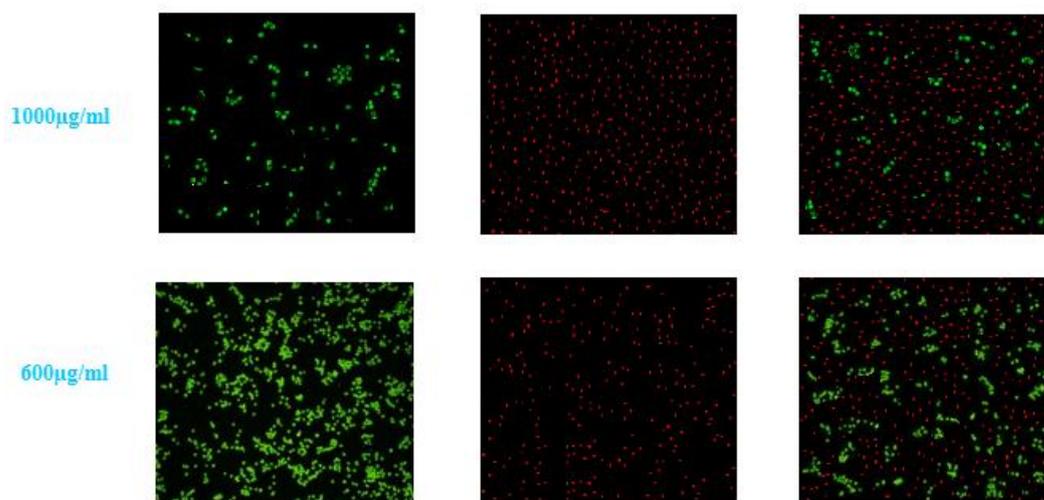


Fig. 6: Effect of different concentrations of ethanol extract from *E. indica* against A549 cancer cell culture.

Moreover, the cytotoxicity of the methanolic extract of *E. variegata* was studied on n-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions was evaluated on *Artemia salina*. The LC50 were found to be 36.68, 4.67, 7.733, and 14.289µg/mL, respectively. They concluded these results clearly indicated the presence of potent bioactive compounds, which might be very useful as antiproliferative, antitumor, pesticidal, and other bioactive agents.^[15] These results showed the impact of the ethanol leaves extract from *E. indica* and its possibilities of uses in the biomedical applications.

ACKNOWLEDGEMENT: None.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES

- Sharma R. Medicinal Plants of India an Encyclopedia. Delhi; Daya Publishing House, 2003.
- Chopra RN, Nayar SL. Glossary of Indian Medicinal Plants. Bangalore; National institute of science communication, 1996.
- Hegde N. Cultivation and uses of *Erythrina variegata* in western India. In: Westley SB, Powell MH (eds.). *Erythrina* in the New Old Worlds Palahi, USA; McGraw-Hill, 1993; 77-84.
- Chawla AS, Krishnan TR, Jackson AH, Scalabrin DA. Alkaloidal constituents of *Erythrina variegata* bark. *Planta Med*, 1988; 54: 526–528.
- Rahman MZ, Sultana SZ, Faruquee CF, Ferdous F, Rahman MS, Islam MS. Phytochemical and Biological investigation of *Erythrina variegata*. *Saudi Pharm J*, 2007; 15: 140–145.
- Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd ed., Bangladesh; Asiatic Society, 2003; 222-223.
- James HR, Christopher D. Synthetic studies on the Erythrina alkaloids Preparation of 2-epi-erythrinitol. *Tetra Hedron Letters*, 1999; 63: 6887-6890.
- Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 1989; 10: 1003-1008.
- Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, Leitao SG. Screening of brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, *Phytother Res*, 2001; 15: 127-130.
- Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. *Biochem Biophys Res Commun*, 1994; 15: 748–755.
- Awah FM, Uzoegwu PN, Oyugi JO, Rutherford J, Ifeonu P, Yao X, Fowke KR, Eze MO. Free radical scavenging activity and immunomodulatory effect of *Stachytarpheta angustifolia* leaves extract. *Food Chem*, 2010; 119: 1409–1416.
- Masters RW. Animal cell culture, Cytotoxicity and viability assays. 3rd ed., New York; Oxford University Press, 2000; 202-203.
- Khan IA, Khanum A. Herbal Medicines for Diseases, Vol I, 1st ed., UAE; Ukaaz Publications, 2005; 126-139.
- Zanoli P, Avallone R, Baraldi M. Behavioral characterisation of the flavonoids apigenin and chrysin. *Fitoterapia*, 2007; 71: S117–123.
- Bhattacharya SK, DeBnath PK, Sanyal Ak, Ghoshal S. Pharmacological studies of the alkaloids of *Erythrina variegata* (mandar). *J Res Indian Med*, 1971; 6: 235–41.