

IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS AND METHANOL EXTRACTS OF *ERYTHRINA INDICA* LAM LEAVES

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Summary

Erythrina indica Lam (Family: Fabaceae) is used in Indian folk medicine for rheumatism, itching, burning sensation, fever, asthma, leprosy and inflammation. Traditionally, it is widely used in the treatment of inflammation and related disorders. However there is no scientific report available on its anti-inflammatory activity. Hence, the present study was designed to investigate the anti-inflammatory activity of aqueous and methanol extracts of *E. indica* leaves by *in-vitro* methods viz. albumin denaturation, membrane stabilization assay and proteinase inhibitory activity. Both the extracts were also studied for their phytoconstituents using different phytochemical tests. Linear regression analysis was used to calculate IC₅₀ value. Results showed that, the aqueous and methanol extracts of *E. indica* leaves exhibited significant anti-inflammatory activity by inhibiting the heat induced albumin denaturation with IC₅₀ values 453.54±9.63 and 149.93±10.22 µg/ml respectively. Both the extracts were effective in the stabilization of Red Blood Cells membrane with the IC₅₀ values of 697.27±15.40 and 660.77±2.18 µg/ml respectively. Proteinase activity was also significantly inhibited by aqueous (IC₅₀=251.98±12.45 µg/ml) and methanol extracts of (IC₅₀=53.94±5.55µg/ml) *E. indica*. Results obtained were comparable to that of aspirin, a standard anti-inflammatory drug. From the results, it is concluded that, the aqueous and methanol extracts of *E. indica* Lam leaves exhibited significant *in-vitro* anti-inflammatory activity, which may be due to the presence of flavonoids and related polyphenols.

Keywords: *Erythrina indica*, Albumin denaturation, Membrane stabilization, Proteinase inhibitory.

Introduction

Inflammation is a complex localized response to foreign substances such as bacteria or in some instances to internally produced substances (1, 2) with fever usually presenting as one of its sequel (3). Inflammation underlies almost all disease conditions (2,4,5) and it is fundamentally a protective response, the ultimate goal of which is to get rid of the organism of both the initial cause of cell injury (for example microbes and toxins) and the consequences of such injuries (2,6). Various medicinal plants provide relief from symptoms comparable to that obtained from allopathic medicines (7). The majority of clinically important medicines belong to steroidal or nonsteroidal anti-inflammatory drugs (8). Though these drugs have potent activity, they have a number of severe adverse effects such as gastrointestinal disturbances and body fat redistribution. Hence, there is a need to develop safe and new anti-inflammatory agents with minimum side effects. In this scenario, use of plant derived products to treat inflammation and related condition becomes a viable and valid approach (9).

Erythrina indica Lam is a middle-sized quick growing tree found in Bengal and many parts of India especially in southern India. It is commonly known as ‘Mandara’ in Hindi ‘Indian coral tree’ in English. It grows up to 18 m in height, the leaves are trifoliolate, flowers are borne in dense racemes, coral red and used traditionally for the treatment of liver trouble, joint pain, dysentery, convulsion, as a diuretic, laxative and an anthelmintic (10, 11, 12,). Its powdered bark is used in Indian folk medicine for the treatment of rheumatism, itching, burning sensation, fever, asthma, inflammation and leprosy (13). Aqueous extract of Sri Lankan *Erythrina indica* leaves reported to exhibit sedative but no analgesic activity (14). It is also reported to exhibit anti-diuretic activity (15).

Although *E. indica* is widely used in ethnomedicine for the treatment of inflammatory disorders, its anti-inflammatory properties have not yet been pharmacologically evaluated. Hence, the present study was designed to investigate the *in-vitro* anti-inflammatory activity of aqueous and methanol extracts of *E. indica* leaves.

Materials and Methods

Plant material

The fresh leaves of *E. indica* Lam. were collected from the mature plant in and around the city of Mumbai, Maharashtra, India during month of August 2008 and dried under shade. The plant was authenticated by Dr. Ganesh Iyer, Botanist, Ramnarayan Ruia College, Matunga, Mumbai. A voucher specimen (2007/08/07) has been kept in our laboratory for future reference.

Preparation of plant extracts

The dried powdered leaves of *E. indica* were defatted using petroleum ether (60-80 °C) and successively extracted with methanol in soxhlet extractor. The aqueous extract was prepared by cold maceration method. Both the extracts were filtered through vacuum filter and the filtrates were concentrated in vacuum evaporator. Dried extracts were used for the further studies.

Phytochemical evaluation

The aqueous and methanol extracts of *E. indica* leaves were studied for their phytoconstituents using different phytochemical tests ⁽¹⁶⁾.

Chemicals and reagents

Bovine albumin fraction was procured from Central Drug House Pvt. Ltd, New Delhi. Casein and Trypsin were purchased from Hi media Lab. Ltd, Mumbai. All the other chemicals and reagents were of pure analytical grade and obtained from local supplier.

Determination of in-vitro anti-inflammatory activity

Inhibition of albumin denaturation

Method of *Mizushima et al* (17) was followed with minor modifications. The reaction mixture was consisting of test extracts at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. Aspirin was taken as standard drug. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percentage Inhibition} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Abs}_{\text{Control}} \times 100$$

Membrane stabilization test

Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline (18).

Heat induced haemolysis

The reaction mixture 2 ml consisted of 1 ml of test drug solution and 1 ml of 10% RBCs, instead of drug only saline was added to the control test tube. Aspirin was taken as standard drug. All the centrifuge tubes containing reaction mixture was incubated at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 10 min and the absorbance of the supernatant was read at 560 nm. The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by the formula mentioned above (19).

Proteinase inhibitory action

The test was performed according to the modified method of *Oyedepo et al* (20). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The mixture was incubated at 37°C for 5 min. The 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. Then 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of proteinase inhibitory activity was calculated by the formula mentioned above.

Statistical analysis

The results are expressed as the mean±SD for three replicates. Linear regression analysis was used to calculate IC₅₀ value.

Results and Discussion

Phytochemical analysis

The phytochemical evaluation and extractive yield of the aqueous and methanol extracts of *E. indica* leaves are shown in Table 1. The aqueous extract showed the presence of carbohydrates, proteins, glycosides, saponins, alkaloids, flavonoids, tannins and phenolic compounds, while methanol extract showed the presence of carbohydrates, proteins, steroids, saponins, alkaloids, flavonoids, tannins and phenolic compounds. Extractive yield of the aqueous and methanol extracts were found to be 14.26 and 7.89% w/w respectively.

Table 1: Phytochemical evaluation of aqueous and methanol extracts of *E. indica* leaves

Phytoconstituents	Aqueous Extract	Methanol Extract
Carbohydrates	+	+
Proteins	+	+
Amino acids	-	-
Fats and oils	-	-
Steroids	-	+
Polysaccharides and Glycosides	+	-
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Tannins and Phenolic compounds	+	+
Extractive yield	14.26% (w/w)	7.89% (w/w)

+: Present; -: Absent

In-vitro anti-inflammatory activity***Inhibition of albumin denaturation***

Denaturation of proteins is a well-documented cause of inflammation. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs) etc, have shown dose dependent ability to thermally induced protein denaturation (17). As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extracts to inhibit protein denaturation was studied. Both the extracts were effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Figure 1. The aqueous and methanol extracts of *E. indica* leaves showed maximum inhibition, 65.21 ± 1.77 and $96.84 \pm 0.79\%$ at $800 \mu\text{g/ml}$. IC_{50} values were found to be 453.54 ± 9.63 and $149.93 \pm 10.22 \mu\text{g/ml}$ at correlation coefficient value (r) of 0.933 and 0.955

respectively. Aspirin, standard anti-inflammatory drug showed the maximum inhibition, $75.89 \pm 0.56\%$ at the concentration of $200 \mu\text{g/ml}$.

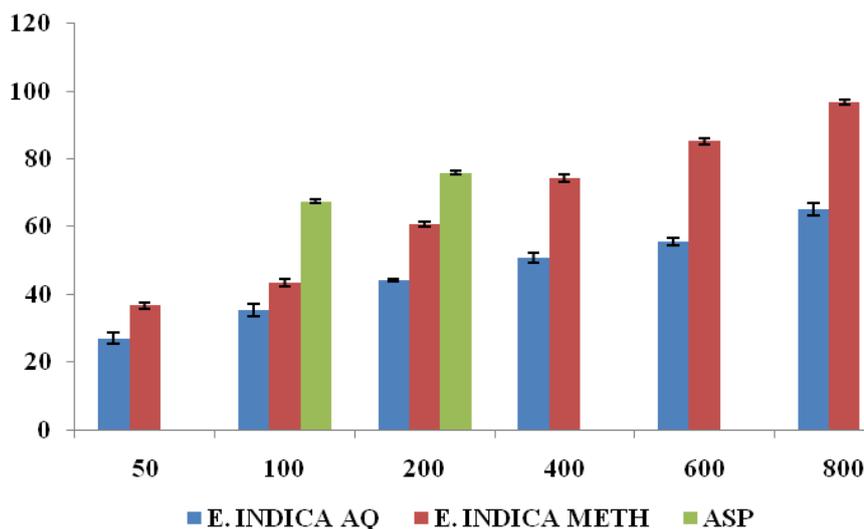


Figure 1: Effect of aqueous and methanol extracts of *E. indica* leaves on albumin denaturation

Membrane stabilization test

Stabilization of the RBCs membrane was studied to further establish the mechanism of anti-inflammatory action of *E. indica* leaves extracts. Both the extracts were effective in inhibiting the heat induced hemolysis at different concentrations. They provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. These extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophil lysosomal constituents include bactericidal enzymes and protease, which upon extracellular release cause further tissue inflammation and damage (21). The plants extracts ($50\text{--}800 \mu\text{g/ml}$) inhibited the heat induced hemolysis of RBCs to varying degree as shown in Figure 2. The aqueous and methanol extracts of *E. indica* showed maximum inhibition 62.98 ± 1.24 and $58.63 \pm 0.27\%$ at $800 \mu\text{g/ml}$ respectively. IC_{50} was observed at 697.27 ± 15.40 and $660.77 \pm 2.18 \mu\text{g/ml}$ at correlation coefficient value (r) of 0.974 and 0.998 respectively. Aspirin, standard anti-inflammatory drug showed the maximum inhibition, $85.92 \pm 0.75\%$ at the $200 \mu\text{g/ml}$. Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the *E. indica* produced this effect surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of the cells and an interaction with membrane proteins (19).

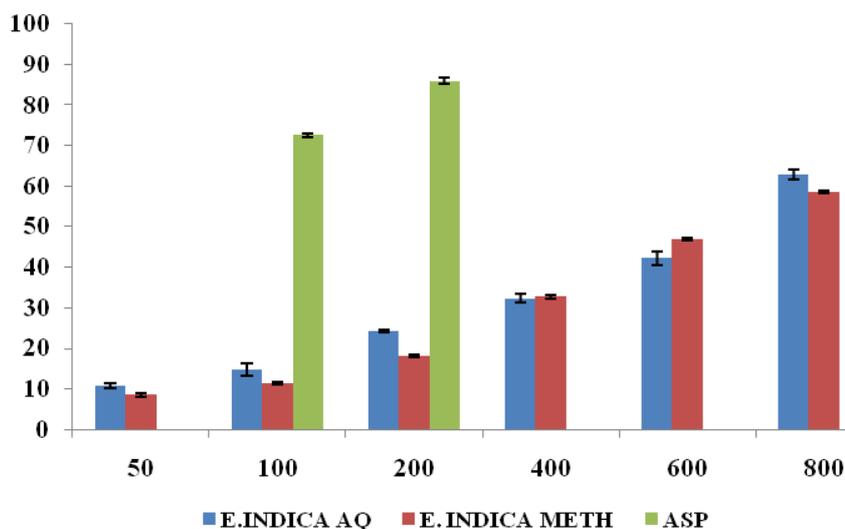


Figure 2: Effect of aqueous and methanol extracts of *E. indica* leaves on membrane stabilization

Proteinase inhibitory action

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors (22). The plants extracts of *E. indica* exhibited significant antiproteinase activity at different concentrations as shown in Figure 3. The aqueous and methanol extracts of *E. indica* showed maximum inhibition 79.67 ± 2.13 and 96.44 ± 1.17 % was at $800 \mu\text{g/ml}$ respectively. IC_{50} value was found to be 251.98 ± 12.45 and $53.94 \pm 5.55 \mu\text{g/ml}$ at correlation coefficient value (r) of 0.990 and 0.847. Aspirin showed the maximum inhibition $92.87 \pm 0.76\%$ at the $200 \mu\text{g/ml}$.

Conclusion

In conclusion, present study revealed the *in-vitro* anti-inflammatory activity of aqueous and methanol extracts of *Erythrina indica* Lam leaves. The presence of flavonoids and related polyphenols may be responsible for the activity. Further investigations are required to find active component of the extract and to confirm the mechanism of action

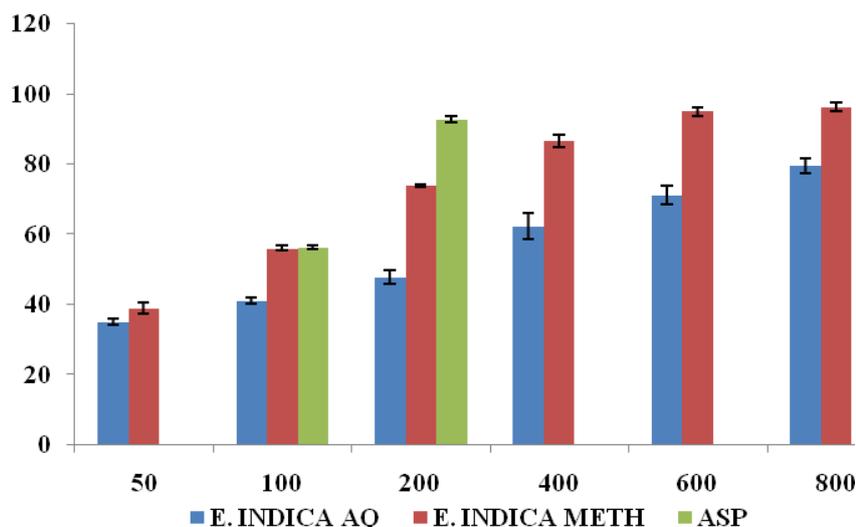


Figure 3: Effect of aqueous and methanol extracts of *E. indica* leaves on proteinase inhibitory activity

The phytochemical evaluation of aqueous and methanol extracts of *E. indica* leaves showed the presence of flavonoids. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the anti-inflammatory activity of many plants (23-24). Hence, the presence of flavonoids in aqueous and methanol extracts of *E. indica* leaves may be contributed to its anti-inflammatory activity.

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