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Exploring Erythrina Variegata for respiratory inflammatory therapy

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A B S T R A C T :

This study investigates the therapeutic potential of Erythrina variegata in the treatment of inflammatory respiratory diseases. Various parts of the plant stem bark, leaves, and roots were extracted using ethanol and methanol and subjected to in-vitro assays to evaluate their anti-inflammatory and antioxidant activities. Phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, phenols, and saponins, which are known to modulate inflammatory and oxidative stress responses.The ethanolic stem bark extract exhibited potent COX-2 inhibition, suggesting strong anti-inflammatory activity. The leaf extract showed significant antioxidant potential, evident through DPPH and nitric oxide radical scavenging assays, along with notable membrane stabilizing effects. The root extract demonstrated moderate activity in protein denaturation and membrane stabilization models.The results indicate that Erythrina variegata exhibits multi-targeted actions enzyme inhibition, antioxidant defense, and cellular protection making it a promising candidate for phytotherapeutic intervention in conditions such as asthma, chronic bronchitis, and COPD. Further in-vivo and clinical studies are warranted to validate these findings.

Keywords: Anti-inflammatory, Antioxidant, Erythrina Variegata

1. Introduction

When anything bad enters our body, like germs, an injury, or anything that can cause harm, then the body starts one of its own systems to remove that bad thing. We call this system inflammation. It means the body starts fighting on its own so that we can get well. There are two types of inflammation – one is quick (acute) which gets cured in some time, and the other is long-lasting (chronic) which troubles the body for a long time. If the inflammation persists for a long time, it can lead to bigger problems like asthma, COPD (chronic obstructive pulmonary disease) and cystic fibrosis, which weaken our respiratory system.

When there is any infection in the lungs, or we breathe polluted air, or have an allergy, then initially there is mild inflammation. But if the inflammation does not get cured in time, it becomes chronic. The wall inside the lungs starts becoming flat, the lung passages start getting smaller and the size of the muscles increase. In such a situation, the lungs are not able to function properly. Because of this people have continuous coughs, difficulty in breathing, wheezing like sound comes when they breathe, and get tired quickly after doing some activity.

Now when there is inflammation, some special protectors wake up inside our body which we call immune cells, like neutrophils and macrophages. These protectors send out some chemical signals like cytokines, which call more soldiers to fight. For example, in cystic fibrosis, thick mucus gets formed inside the lungs, in which bacteria get stuck and then the body keeps fighting more. In COPD, cigarette smoke or pollution damages the lungs and the lung cells start breaking down. In asthma, certain special cells of the body react overly because of allergy and breathing becomes difficult.

Doctors give some medicines to control this problem like inhaled steroids (which reduce swelling), bronchodilators (which open up the lung passages), and some special injections like biologics. But these medicines also have some side effects like mouth infection, weak bones (osteoporosis), shaking of hands (tremors), and increased risk of getting infection. Plants contain some powerful things that can reduce inflammation. One such plant is Erythrina variegata, which we call Coral Tree.

Fig 1:- Erythrina Variegata Plant

Erythrina variegata L., synonym Erythrina indica, belongs to the family Fabaceae and is commonly referred to by various vernacular names—Paribhadra (Sanskrit), Coral tree (English), Dadap (Hindi), and Panervo (Gujarati). The genus Erythrina consists of approximately 110 species of trees and shrubs known for their ornamental as well as medicinal value.

TAXONOMY

Kingdom: Plantae-plants
Division: Magnoliophyta -Flowering plants
Class: Magnoliopsida-Dicotyledons
Family: Fabaceae (legume family)
Subfamily: Papilionoideae
Genus: Erythrina L.-Coral Tree
Species: Erythrina variegata L.

Erythrina variegata is a tree that has been used as medicine since ancient times in India, Southeast Asia and the Pacific Islands. The leaves, bark and root of this tree help cure different diseases. The leaves clean the stomach, reduce pain and swelling, are also useful in fever and liver diseases, and help in regulating periods and milk formation in women. The bark is used to treat eye diseases, stomach pain, skin diseases and for killing worms. The root is used for cough, fever, fits, cancer and insect bites, and in some places people also use it to get rid of evil eye and witchcraft. This tree contains some special substances like alkaloids and isoflavonoids, which reduce pain and swelling, kill bacteria, strengthen bones, and calm the brain.These widespread and diverse uses strongly support the pharmacological validation of the plant's potential in treating inflammatory, neurological, respiratory, and microbial conditions, as observed in the present experimental findings.

Until now there was not much scientific study about this plant, so in this research scientists have tested different parts of the plant (stem bark, leaves, and roots). They made an extract of the plant in liquids like ethanol and methanol, and then checked it to see how much antioxidant and anti-inflammatory it works. Antioxidant test like DPPH and nitric oxide scavenging tests were done. And in an anti-inflammatory test, its work of stopping COX-2 enzyme, reducing TNF-alpha chemical, preventing protein denaturation (deterioration of protein) and keeping HRBC (blood cell membrane) stable was seen.The result showed that the ethanolic stem bark extract inhibited the COX-2 enzyme well. The leaf extract performed good antioxidant and membrane protecting functions. The root extract also showed slightly less support in inflammation and membrane protection. Meaning, this plant proves its old medicine story true and shows that it can help in diseases like asthma, COPD.

Finally, it is said that Erythrina variegata is a promising plant which can become a good and safe treatment for respiratory diseases in the future. But still more research and testing is needed so that we can completely confirm how safe and effective this plant is.

Material and Methods

1.Collection of plant material

The E. variegata plant was collected from the department of biotechnology, Kakatiya University, Warangal where the plants were grown and maintained in proper condition. The collected plant materials were washed thoroughly under a running tap to remove dirt. After that the parts were separated and shade dried for 10 days and were made into powder form for further use.

2.Preparation of extract

The 3 grams of each plant part powder was taken along with 30 ml of each solvent in separate conical flask the solvents used here were aqueous, butanol ethanol, chloroform and methanol and were incubated in orbital shaker for about 48 and later the extracts were filtered using Whatman filter paper. The final extracts were kept in a rotating shaker for 48 hours at 28 ℃. After 48 hours, the extract was filtered and subsequently subjected for preliminary screening by using standard methods protocol.

3.Phytochemical Screening

A preliminary qualitative phytochemical screening of the aqueous extract of Erythrina variegata was performed to detect alkaloids, phenolic compounds, flavonoids, saponins, tannins, glycosides, steroids, carbohydrates, and various phytochemical constituents using standards.

3.1 Test for alkaloids

For the identification of alkaloids, Mayer's and Hager's tests were employed. In the Mayer's test, a few drops of Mayer's reagent were added to the plant extract, resulting in the formation of a creamy white or yellow precipitate, indicating the presence of alkaloids. Similarly, Hager's reagent gave a creamy white precipitate, further confirming the presence of alkaloids.

3.2 Test for flavonoids

To detect flavonoids, two standard tests were performed. In the alkaline reagent test, the addition of NaOH to the plant extract resulted in an intense yellow coloration, which turned colorless upon acidification, confirming the presence of flavonoids. The ferric chloride test involved mixing the extract with 10% ferric chloride solution, which led to the formation of a dark precipitate, indicating flavonoid content.

3.3 Test for glycosides

The presence of glycosides was determined using Molisch's test and concentrated H_2SO_4test. In the Molisch's test, the addition of Molisch's reagent followed by concentrated sulphuric acid resulted in a violet ring at the interface of the two layers, confirmingglycosides. The H_2SO_4 test similarly produced a brownish-red precipitate, further supporting the presence of glycosides.

3.4 Test for saponins

Saponins were tested using the foam test, where vigorous shaking of the aqueous extractled to the formation of a persistent foam layer, indicating saponins.

3.5 Test for tannins

Tannins were identified by multiple tests. The ferric chloride test resulted in a dark greento bluish-black coloration upon reaction with the extract, indicating the presence of phenolic tannins. The ellagic acid test yielded a muddy precipitate upon reaction withalcohol and $FeCl_3$, further indicating tannins. The gelatin test produced a white precipitate when the extract was treated with gelatin and NaCl solutions, confirming tannin presence.

3.6 Test steroids

Steroids were confirmed by the alkaline reagent test and ferric chloride test. An intense yellow color that turned colorless on acidification confirmed steroid presence. The alkali reagent test also supported this with the same result.

4.In-vitro inflammatory and antioxidants assay

4.1 Cell-Based Assay

Cell cultures preparation: RAW 264.7 murine macrophage cell line was cultured in RPMI1640 Medium (Sigma, St. Louis, MO, USA), supplemented with 10% heated fetal bovine serum (FBS) (Sigma, St. Louis, MO, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were incubated at 37°C with 95% humidity in a 5% CO2 atmosphere. Subculturing was performed every three days.

4.1.1 Determination of nitric oxide (NO) production

The RAW 264.7 cell line was cultured in RPMI 1640 (BIOCHROM AG) supplemented with 10% heated fetal bovine serum, 100 IU/ml penicillin, and 100 µg/ml streptomycin.Cells were grown at 37°C and 5% CO_2 in humidified air. They were seeded in 96-well plates at a density of 1×10^5 cells/well and allowed to adhere for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2. After that, the medium was replaced with fresh medium containing 100 µg/ml of lipopolysaccharide (LPS) along with the test sample at various concentrations and incubated for another 24 hours. NO production was determined by measuring nitrite accumulation in the supernatant using Griess's reagent(100 µl), which was added to the 96-well plates, and absorbance was read using a microplate reader at 570 nm. The inhibition of NO production was calculated, and IC_{50}values were determined using the Prism program.

4.1.2 MTT assay

Briefly, after 24 hours incubation with test samples, MTT solution (10 µl, 5 mg/ml inPBS) was added to the wells and then incubated at 37°C at 5% CO_2 atmosphere with 95%humidity for 2 hours. After that, the medium was removed and isopropanol containing0.04 M HCl was added to dissolve the formazan solution, and the absorbance was read using a microplate reader at 570 nm. The test compounds were considered to be cytotoxicwhen the optical density of the sample-treated group was less than 70%.

4.1.3 Inhibitory effect on LPS-induced TNF-α release from RAW 264.7 cells line

The inhibitory effects on the release of TNF-α from RAW 264.7 cells were evaluated using the Quantikine mouse TNF-α ELISA test kit. Cells were seeded in 96-well plates at a density of 1×10^5 cells/well and allowed to adhere for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2. After that, the medium was replaced with a fresh medium containing 100 µg/ml of LPS along with the test sample at various concentrations and incubated for another 24 hours. Subsequently, 50 µl of the supernatant was transferred into a 96-well ELISA plate, and TNF-α concentrations were determined. The inhibition ofTNF-α production was calculated, and IC_{50} values were determined using the Prism program.

4.1.4 Inhibitory effect on LPS-induced PGE2 release from RAW 264.7 cells line(COX-2)

The RAW 264.7 cells were seeded in 96-well plates at a density of 1×10^5 cells/well, and 5µg/ml of LPS was added to stimulate macrophages. The cells were then allowed to adhere for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2. After incubation, the supernatant was collected, and the amount of PGE_2 was determined using a PGE_2 EnzymeImmuno-Assay Kit (Cayman Chemical Company). The amount of PGE_2 was measured relative to that of the positive control.

Statistical analysis

Results were expressed as mean ± SEM of four determinations at each concentration for each sample. The IC_{50} values were calculated using the Prism program.

4.2 Non-Cell-Based Assay

4.2.1 HRBC Membrane stabilization Assay

The HRBC membrane stabilization method was used to study anti-inflammatory activity.Blood collected from a healthy volunteer was mixed with an equal volume of sterilizedAlsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride in water), centrifuged at 3000 rpm, and the packed cells were washed with isosaline (0.90%, pH 7.2) to prepare a 10% (v/v) suspension. The assay mixture contained the test drug, 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%), and0.5 ml of HRBC suspension, with diclofenac sodium as the reference drug. In the controlgroup, 2 ml of distilled water was used instead of hyposaline. All assay mixtures were incubated at 37°C for 30 minutes and centrifuged, and the hemoglobin content in the supernatant was estimated using a spectrophotometer at 560 nm. The percentage of hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100%, and the percentage of protection was determined using the formula:

% Protection = (1 - [Absorbance of sample / Absorbance of control]) × 100

4.2.2 DPPH Radical Scavenging Assay

The free radical scavenging activity of antioxidants was evaluated using the DPPH assay following the procedure of Nuutila et al. (2003). A 0.1 mM DPPH solution was prepared in methanol, and to 3 mL of this solution, 1 mL of the extract at various concentrations(20, 40, 60, and 80 µg/mL) was added. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Methanol with DPPH served as the control, and ascorbic acid was used as the standard antioxidant. The percentage inhibition of DPPH radicals was calculated using the following formula:

% Inhibition = [(A Control – A sample) / A control] × 100

4.2.3 NO scavenging Assay

The nitric oxide (NO) radical scavenging activity of Erythrina variegata leaf extract was evaluated based on the method developed by Garrat (1964) [18]. In this assay, sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide, which reacts with oxygen to form nitrite ions. These nitrite ions were quantified using Griess reagent.In the present study, 0.5 mL of 10 mM sodium nitroprusside in phosphate buffer saline(pH 7.4) was mixed with 0.5 mL of the leaf extract and incubated at 25°C for 150 minutes under light. After incubation, 1 mL of the reaction mixture was treated with 1 mL of freshly prepared Griess reagent, consisting of a 1:1 mixture of 1% sulfanilamide in 5%phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride. The mixture was then kept at room temperature for 30 minutes, and the absorbance was measured at 546 nm using a UV-Visible spectrophotometer. A control was maintained without plant extract for baseline comparison. The percentage inhibition of nitric oxide was calculated to assess the antioxidant potential of the extract. The reduction in absorbance indicated the scavenging ability of the leaf extract against nitric oxide radicals.

Statistical analysis Tests were carried out in triplicate for 3–5 separate experiments. Results were expressed as mean ± SEM of four determinations at each concentration for each sample. The IC_{50} values were calculated using the Prism program.

4.2.4 Inhibition of albumin denaturation

The assay was performed following the method of Mizushima et al. with minor modifications. The reaction mixture consisted of extracts at different concentrations (10–50 µg/ml) and a 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted to 6.5 using 1N HCl and incubated at 37°C for 20 minutes, followed by heating at 57°C for 30 minutes. The denaturation process was stopped by cooling the samples, and the turbidity was measured using a spectrophotometer at 660 nm. Aspirin was used as the reference standard, and the control was prepared without the extract. The inhibition of protein denaturation was determined by centrifuging at 2500 g for 10 minutes, and the absorbance of the supernatant was read at 560 nm. The experiment was performed in triplicate, and membrane stabilization activity was calculated and expressed as a percentage.

4.2.5 Membrane stabilization assay

Blood was collected freshly and mixed with equal volume of Elsevier's solution. It was then centrifuged at 3000 g for 15 minutes. The cells were washed with isosaline and a 10 % suspension was made with isosaline. Different concentrations of methanolic extract (100-500µg/ml) were prepared in isosaline. To 0.5 mL of the extract, 1 mL phosphate buffer, 2 mL hypersaline and 0.5mL HRBC suspension was added and incubated for 30 minutes at 370C and then centrifuged at for 20 minutes. Absorbance was measured at 560 nm[18,19]. Aspirin was used as the standard and control was taken without the extract served as negative control.

Result

Table 9. Comparative Analysis of Erythrina variegata Parts Based on Anti-inflammatory and Antioxidant Assays Relevant to Respiratory Disease

Plant part used	Assay performed	Erythrina variegata IC50 Value (µg/ml)	Interpretation
Bark	**NO inhibition**	**47.1+0.21**	**Moderate**
Bark	**COX inhibition**	**9.27+0.72**	**Strong**
Bark	**TNF-α inhibition**	**>100**	**Weak**
Leaf	**HRBC stabilization**	**13.86**	**Moderate**
Leaf	**DPPH Radical Scavenging**	**48.20**	**Moderate**
Leaf	**NO inhibition**	**49.93**	**Moderate**
Roots	**HRBC stabilization**	**6.24**	**Strong**
Roots	**Denaturation inhibition**	**277.65**	**Very weak**

Discussion

Erythrina variegata is one such plant which contains many special chemicals that benefit our body. The leaves, bark, and roots of this plant contain different chemicals that reduce inflammation and oxidative stress (damage to cells) in our body. The leaves contain some special things like flavonoids, alkaloids, and phenolic compounds that protect the body from inflammation and strengthen its immunity. Chaals (bark) contains alkaloids, tannins, and glycosides that boost the body's immunity and reduce inflammation. The roots also contain flavonoids and alkaloids that control inflammation and protect the body.When scientists did anti-inflammatory tests, it was found that the extract of the bark of the tree blocked the COX-2 enzyme very well. The COX-2 enzyme is the thing that increases inflammation. If this enzyme is blocked, inflammation reduces, which is very important in diseases like asthma and COPD. The leaf extract was also very good but it blocked another chemical, nitric oxide, at a moderate level. Excess of nitric oxide also increases inflammation, so it is important to prevent it. Root extract was slightly less effective, but it still helped in reducing inflammation.Then another test was done to see which part can protect the HRBC membrane (outer layer of blood cells) when there is swelling in the body. In this test, both leaves and bark worked quite well, but the extract of bark was slightly more effective. This means that these parts help in protecting the cell wall during swelling, so that the cells do not break and get damaged.In antioxidant tests, leaf extract was the best. It showed good capacity to kill DPPH and nitric oxide which are important for our body as they neutralize free radicals (which cause damage to the body). Root extract also provides antioxidant protection but to a lesser extent than leaves.Finally, when protein denaturation was tested, root extract was most effective. This extract protects body proteins from damage during inflammation. Leaves and bark also help but roots had a slightly greater effect.

So, if we look at everything, the bark inhibits more COX-2 enzyme, the leaves provide more antioxidant protection and keep the membranes stable, and protect the root proteins. All these parts together make Erythrina variegata a plant that can help in diseases like asthma, COPD, and chronic bronchitis.In this way, this plant can become a natural and safe way to protect our lungs and body from inflammation and damage. It absolutely proves our old medicinal methods to be true.

Conclusion

This study has shown that the Erythrina variegata plant may be helpful in treating respiratory diseases such as asthma, COPD, and bronchitis. The ethanolic extract of its stem bark is the most anti-inflammatory, which inhibits the COX-2 enzyme. Leaf extract contains antioxidants and protects cell membranes, and the root extract is slightly less effective but protects proteins and keeps the cell membrane stable. All these results support the traditional uses of the plant and point to the need for future research so that the plant can be validated for clinical use.

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