

## IN VITRO ANTI-DIABETIC AND ANTIBACTERIAL ANALYSIS - "ERYTHRINA VARIEGATA" LEAVES

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### ABSTRACT

Diabetes is one of today's most common metabolic disorder and the number of patients is still increasing by day today life. The aim of the present study is to evaluate the *In vitro* anti-diabetic analysis and antibacterial activities of hydroethanolic extract of *Erythrina variegata* leaves. The *in vitro* analysis against diabetes is determined by inhibiting the action of  $\alpha$ -amylase. The antibacterial activity of hydroethanolic extract of *Erythrina variegata* leaves were tested against Gram positive and Gram negative species by the zone of inhibition. The result of the present study showed that the hydroethanolic extract reveals effective inhibition against  $\alpha$ -amylase enzyme and antibacterial activity against the tested organisms. Thus the active constituents may act as a leading target for multi-target drug resistance and anti-diabetic agent.

**KEYWORDS:** *Erythrina variegata*, hydroethanol, Antibacterial activity Anti diabetic activity.

### INTRODUCTION

The medicinal plants are mainly used for curing the infectious diseases owing to the presence of general bioactive compounds such as tannins, flavonoids, phenolic compounds and terpenoids.<sup>[1]</sup> Many of the infectious diseases were incite by pathogens and microbes, which are treated with herbal remedies or by natural products either in pure compounds or standardized plant extract. The secondary metabolites of medicinal plants with unknown biological activities have been investigated as a source of medicinal agents for antibacterial treatment.<sup>[2]</sup>

Currently, medicinal plants continue to play a chief constituent in the management of diabetes mellitus, especially in the developing countries.<sup>[3][4]</sup> There is revived interest in functional foods and plant-based medicines modulating physiological effects in the prevention and medicant of diabetes. The majority of anti-diabetic medicinal plants comport their blood glucose lowering effect through stimulation of insulin release from pancreatic beta cells or through alteration of some hepatic enzymes involved in glucose metabolism.<sup>[5]</sup>  $\alpha$ -Amylase is one of the main enzymes in human that is responsible for the breakdown of starch into simple sugars and reduces the rate of glucose absorption. Hence the attractive targets like *in vitro* inhibition of  $\alpha$ -

glucosidase and  $\alpha$ -amylase enzymes are currently prevalent. The role of  $\alpha$ -amylase is an important strategy in the management of diabetes in the post parenteral blood glucose level after mixed carbohydrate diet.<sup>[6]</sup>

*Erythrina variegata* is belonging to the family of Fabaceae and commonly known as 'Kalyanmurungai'.<sup>[7]</sup> Every single part of *Erythrina variegata* is used for traditional medicinal purposes - anti-asthmatic, anti-epileptic, febrifuge, sedative, fever, inflammation, bacterial infection.<sup>[8]</sup> An Indian type of *Erythrina variegata* leaves is used as a medicant for roundworm, threadworm and to relieve joint pain.<sup>[9]</sup>

### MATERIALS AND METHODS

#### Collection of sample

The leaves of *Erythrina variegata* was collected from Madurai district and was authenticated by Botanical survey of India, Coimbatore.

#### Preparation of plant extracts

The leaves were collected shade dried at room temperature for 15 days. After shade drying 100g of leaf sample was macerated and it was treated with the solvent hydroethanol, for 72hrs at room temperature. The extracts were filtered by Whatmann filter paper No.1 and these filtrate was evaporated and dried by using hot

air oven. The dried powder was used for the further analysis. The percentage yield was found to be 10%.

**Collection of test Organism:** The strains used for the present study are *Bacillus subtilis*, *Staphylococcus aureus*, *Escherchia coli* and *Pseudomonas aeruginosa*. Fresh cultures were obtained from Sri Ramakrishna Hospital, Coimbatore. Strains were maintained 4<sup>0</sup>c for further use.

**Antibacterial assay:** Antibacterial activity of the plant extract were assayed against two set of different strains of microbes (two Gram positive and two Gram negative) by well diffusion pursuant to the method Lyudmila et al.,(2005).<sup>[10]</sup> The Muller Hinton Agar was weighed and dissolved in 150 ml of distilled water in a sterile conical flask. The medium was sterilized using autoclave and it was allow to cool at room temperature. Then the cooled medium was poured into sterile petriplates and allow it to solidify for 20 mins. A lawn of bacterial cultures was done by streaking respectively with a sterile loop. Wells were prepared in the plates by using a sterile borer. The wells were filled with 10 $\mu$ l of each increasing concentration extract (25mg/ml, 50 mg/ml, 75 mg/ml respectively) with hydroethanolic solvent along with standard (Amoxicillin). By using micropipette the samples were poured respectively. The plates were incubated at 37<sup>0</sup>c for overnight. The diameters of the inhibitory zones were measured in millilitres.

### In vitro $\alpha$ -amylase inhibitory activity

Amylase inhibitor activity assay was based upon the method of Bernfeld's (2001)for amylase assay.<sup>[11]</sup> Amylase inhibitor extracts were mixed up with amylase and incubated for 30 min at 37°C.The reaction was evoked by adding extract-enzyme mixture to test tubes containing buffered starch solution(2mg starch in 20mM phosphate buffer of pH 6.9 containing 0.4mM NaCL) and was incubated for 20 min. This reaction was abort by adding 3, 5 dinitrosalicylic acid (DNS) reagent to the assay mixture. The assay tubes were kept in a boiling water bath for 5 min, cooled under tap water and the color formed by maltose oxidation was measured at 530 nm. Controls without inhibitor were run simultaneously. One amylase unit is defined as the amount of enzyme that will liberate 1  $\mu$ mol of maltose from starch under the assay conditions (pH 6.9, 37°C, 5 min). Inhibitory activity is expressed as the percentage of inhibited enzyme activity out of the total enzyme activity used in the assay.

## RESULT AND DISCUSSION

### Antibacterial Activity

The antibacterial activity have been screened because of its great medicinal relevance with the recent years, infections have heightened to a great extent and resistance against antibiotics, became an ever increasing therapeutic problem. Plant based antimicrobials have huge therapeutic potential, as they can serve the purpose without any side effects that are often associated with synthetic antibacterial compounds.<sup>[12]</sup>

**Table 1: Represents the antibacterial activity of hydroethanolic extract of *E.variegata*.**

Concentration (mg/ml)	Gram Positive		Gram Negative	
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherchia coli</i>	<i>Pseudomonas aeruginosa</i>
25	12mm	12 mm	8 mm	6 mm
50	10 mm	10 mm	8 mm	10 mm
75	15 mm	15 mm	10 mm	12 mm
Standard-Amoxycillin-1000	20 mm	20 mm	12 mm	15 mm

Various concentrations (25,50,75 mg/ml) of *Erythrina variegata* were taken for the analysis of antibacterial activity along with the standard anti-bacterial agent (Amoxycillin). *E.variegata* showed the inhibitory zone depends upon the concentration. The present study indicates that hydroethanolic extract of *Erythrina variegata* has maximum effective action against organism *Staphylococcus* compared with that of other three organisms *Bacillus subtilis*, *Escherchia coli*, *Pseudomonas aeruginosa*. Among Gram positive organisms *Staphylococcus aureus* showed Maximum zone of inhibition at 15mm for 25mg/ml, Gram negative organisms *E.Coli* showed minimum zone of inhibition at 10mm for 25mg/ml.

### Anti-Diabetic Activity

An attempt has been made to know the *in vitro* anti-diabetic activity of the hydroethanolic extract by using  $\alpha$ -amylase inhibition assay model. Hence, if the extract possess the inhibitory effect on this enzyme it may lead to reduction in post prandial hyperglycaemia in diabetic condition.

### $\alpha$ -Amylase inhibitory activity

The  $\alpha$ -amylase ( $\alpha$  -1,4-glucan-4- glucanohydrolases; E.C. 3.2.1.1) is one of the major substance released by the pancreas (about 5–6%)<sup>[13]</sup> and salivary glands.  $\alpha$ -amylase plays a vital role in digestion of starch, glycogen which is commonly found in microorganisms, plants and higher organisms.<sup>[14]</sup> The human  $\alpha$ -amylase is one of the calcium- containing enzyme which constitute a mixture of 512 amino acids present in a single

oligosaccharide chain, it has molecular weight of about 57.6 kDa.<sup>[13]</sup>

The final elements of  $\alpha$ -amylase is oligosaccharides which has alternating length enriched with  $\alpha$ -

configuration and  $\alpha$ -limit dextrins<sup>[15]</sup>, which is composed of maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units that contain both  $\alpha$ -1,4 and  $\alpha$ -1,6 linkages.<sup>[13]</sup>

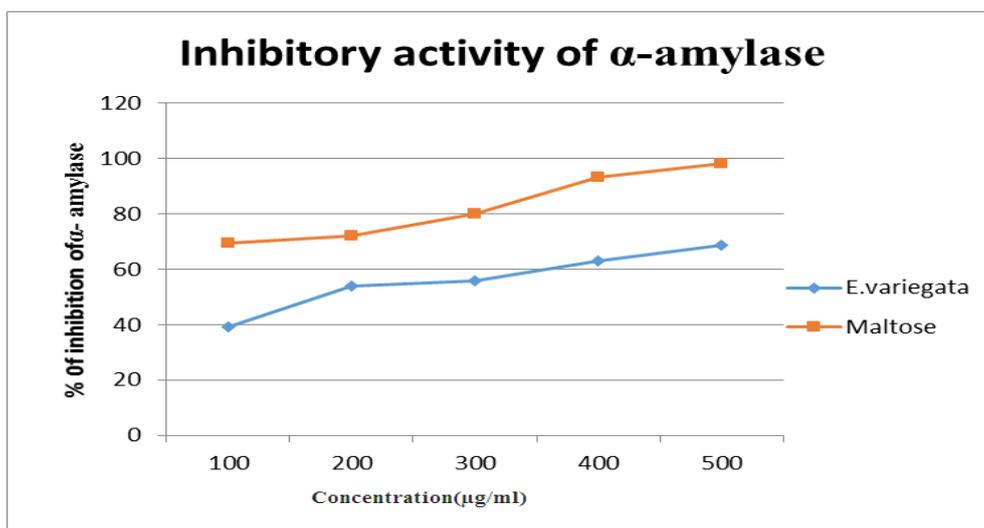


Fig 1: Inhibitory activity of hydroethanolic extract of *E. variegata* on  $\alpha$ -amylase enzyme

The present study indicates that the hydroethanol extract of *E. Variegata* exhibited inhibitory activity of  $\alpha$ -amylase. As the concentration was increased the maximum inhibitory activity was also increased, at the concentration of 500  $\mu$ g/ml respectively.  $\alpha$ -Amylase is one of the therapeutic approach for treating early stage diabetes which reduces the post prandial hyperglycemic by slowing down the absorption of glucose. Inhibitors present in the plant extracts can hinder the carbohydrate digestion thereby it reduces the postprandial rise in blood glucose. Active constituents present in the *E. variegata* is the discovery of natural products with the inhibitory potential on key enzymes related to Diabetes.<sup>[4][16]</sup> *E. variegata* leaves showed  $\alpha$ -amylase inhibitory potential when compared with standard drug indicated that maximum inhibition of pancreatic amylase<sup>[17]</sup>. It results in the unbalanced bacterial fermentation of undigested carbohydrates in the colon and shows gentle amylase desirable inhibitory action.

## CONCLUSION

Thus the present study concludes that the leaves of *Erythrina variegata* have considerable antibacterial activity and a potent inhibitor of  $\alpha$ -amylase activity. Future studies is to isolate the active constituents which is responsible for the antidiabetic actions and infectious diseases.

## CONFLICT OF INTEREST

Have no desire for conflict of interest.

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