Research Article

IN-VITRO ANTIOXIDANT AND ANTIINFLAMMATORY ACTIVITIES OF ERYTHRINA INDICA BARK

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ABSTRACT

Erythrina indica Lam (Family: Fabaceae) has good ethno pharmacological value in Indian folk medicine. The present study was undertaken to evaluate two different extracts of Erythrina indica bark for antioxidant and anti-inflammatory activity by simple, reliable, and less time consuming methods. Standard Methanolic and ethyl acetate extracts of Erythrina indica was tested in vitro for its anti-inflammatory activity and antioxidant activity using anti denaturation and reducing power methods respectively. Methanolic extract possess significant anti-inflammatory and antioxidant effect where as ethyl acetate extract possess moderate anti-inflammatory and antioxidant activity.

Keywords: Erythrina indica, Anti denaturation, reducing power.

INTRODUCTION

The ultimate objective of the present study was to predict the antioxidant activity and anti inflammatory activity of different extracts of *Erythrina indica* by using appropriate assay procedures.

In recent years much attention has been devoted to natural antioxidant and their association with health benefits[1]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immuno suppression, neurodegenerative diseases and others[2]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive[3]. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders[4]. Currently, there is a growing interest toward natural antioxidants of herbal resources. Epidemiological and in vitro studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems[5].

Inflammation is a complex localized response to foreign substances such as bacteria or in some instances to internally produced substances with fever usually presenting as one of its sequel[6]. Inflammation underlies almost all disease conditions 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. Various medicinal plants provide relief from symptoms comparable to that obtained from allopathic medicines[7]. It has been suggested that many anti-inflammatory drugs may exert some of their effects by scavenging oxidants, and decreasing formation of reactive oxygen species (ROS) by activated phagocytes[8].

Most of the anti-inflammatory drugs are steroidal or nonsteroidal anti-inflammatory drugs. Though they are very useful, 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.

MATERIALS AND METHODS

(i) Collection and Authentication

Erythrina indica belonging to family Fabaceae has been selected for the study. The plant material was collected from Thirumala forest, Chittor district, Andhra Pradesh, India and authenticated by Dr. Madhava Chetty, botanist, Sri Venkateshwara University, Tirupathi. The bark was shade dried and ground into coarse powder.

(ii) Preparation of Extract

500g of powdered bark of Erythrina indica was extracted continuously using soxhlet apparatus with ethyl acetate and methanol successively for about 48 hours at 30°C. The extracts were concentrated under reduced pressure

using rotary vacuum flash evaporator to get a constant volume.

(iii) Preliminary Phytochemical Screening

The plant is a biosynthetic laboratory, not only for chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, gums, tannins, saponins etc. that exert physiological and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systematic study of a crude drug embraces, thorough consideration of primary and secondary metabolites derived as a result of plant metabolism[5]. The plant material is subjected to preliminary phytochemical screening for the detection of various plant constituents (Tab1).

Table 1: Preliminary Phytochemical Screening for various phytoconstituents

S. No	Test	Ethyl acetate Extract	Methanolic Extract
1	**Carbohydrates** (Benedict's test)	-	+
2	**Proteins** (Biuret test)	-	+
3	**Amino acids** (Ninhydrin test)	-	+
4	**Alkaloids** (Mayer's test)	+	+
5	**Steroids** (Salkowaski's Test)	+	+
6	**Phenolic compounds** (CH_3COOPb)($FeCl_3$)	+	+
7	**Tannnins**	+	+
8	**Cardiac Glycosides** (Kellar killani Test)	+	+
9	**Saponins** (Foam Test)	-	+

(iv) Anti-denaturation Activity

The method of Williams et al, was employed for antidenaturation assay. A solution of 0.2% W/V of BSA was prepared in Tris buffer saline and P^H was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10,000µg/ml of all extracts were prepared by using methanol as a solvent. From these stock solutions 4 different concentrations of 1, 100, 200 and 500µg/ml were prepared by using methanol as a solvent. 50µl of each extract was transferred to Eppendorf tubes using 1ml micro pipette. 5ml of 0.2% W/V BSA was added to all the above Eppendorf tubes. The control consists of 5ml 0.2% W/V BSA solution with 50 µl methanol. The standard consist 100 µg/ml of Diclofenac Sodium in methanol with 5ml 0.2% W/V BSA solution. The test tubes were heated at 72° C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using UV/Vis Double beam spectrophotometer (Elico SL-196) at a wavelength of 660nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula[9].

$$\% \text{ inhibition of denaturation} = \frac{(\text{Abs of control} - \text{Abs of extract})}{\text{Abs of control}} \times 100$$

(v) Reducing Antioxidant Activity

Different concentrations of plant extracts (10 – 500µg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, P^H 6.6) and potassium ferri-cyanide [$K_3Fe(CN)_6$] (2.5 ml,1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer (Elico –SL 196). Increased absorbance of the reaction mixture indicates increase in reducing power[10]

RESUTS

Anti-denaturation Activity:

Table2: Anti denaturation of BSA in presence of *Erythrina indica* extracts

S. No	Type of Extract	Concentration (µg/ml)	% Inhibition
1	Methanolic	1	78.2±0.60
2	Methanolic	100	50.3±0.34
3	Methanolic	200	38.7±0.53
4	Methanolic	500	24.1±0.25
5	Ethyl acetate	1	61.9±0.20
6	Ethyl acetate	100	39.7±0.32
7	Ethyl acetate	200	24.6±0.12
8	Ethyl acetate	500	10.3±0.65
9	Diclofenac sodium	100	88.9±0.46

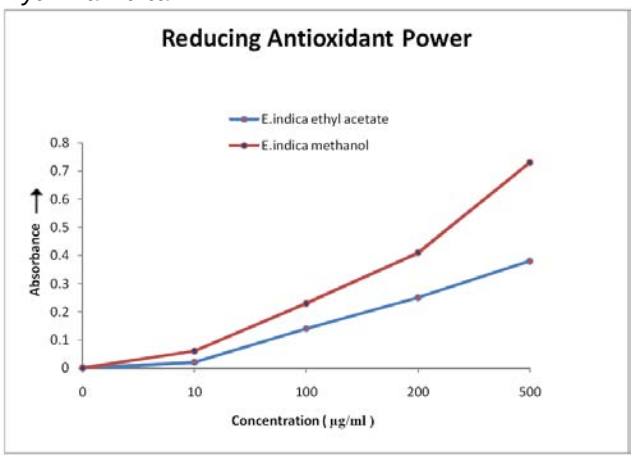
Both methanolic and ethyl acetate extracts of *Erythrina indica* inhibited the denaturation of Bovine serum albumin (BSA). The degree of inhibition of BSA denaturation increased with the decrease in the concentration of both the extracts as stated by Williams et al., that the antidenaturation of the drug will be more at lower concentration. As shown in Table (2), the methanoloc extract has significant antidenaturation activity (78.2 ±0.60%, 1µg/ml), where as ethyl acetate extract has moderate antidenaturation activity (61.9±0.20%, 1µg/ml).

Reducing Antioxidant Power:

Table 3: Anti oxidant activity of *Erythrina indica* extracts

Concentration (µg/ml)	Absorbance	
	Ethyl acetate extract	Methanol extract
10	0.02	0.06
100	0.14	0.23
200	0.25	0.41
500	0.38	0.73

Figure 1: Antioxidant activity of different extracts of *Erythrina indica*



Both methanolic and ethyl acetate extracts of erythrina indica were tested for antioxidant activity. As shown in table (3) Methanolic extract possess significant antioxidant activity, where as ethyl acetate extract has moderate antioxidant activity.

DISCUSSION

In Indian system of medicine, certain herbs are claimed to provide relief of pain and inflammation. The claimed therapeutic reputation has to be verified in a scientific manner. In the present study one such drug Erythrina indica bark was taken. In this study Erythrina indica, traditionally used for various disorders was studied for their in-vitro antioxidant activity and anti-inflammatory activity. The anti-inflammatory activity of this plant material has not been reported before.

Reactive Oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process[11] (Guyton et al., 1997, Halliwell and Gutteridge, 1999). Inflammation is a complex process and ROS play an important role in the pathogenesis of inflammatory diseases[12] (Conner and Grisham, 1996).Thus antioxidants which can scavenge ROS are expected to improve these disorders.

Many literatures have correlated the protein denaturation activity and autoimmune diseases. Many studies on antioxidants have proved that oxidative stress has great importance in generation of autoimmune bodies responsible for autoimmune diseases.

Since most of plants have polyphenolic compounds which has a good reducing, singlet oxygen quenching effect on free radicals. Thus in this study *in-vitro* anti-denaturation of Bovine serum albumin and reducing antioxidant activity was evaluated. The results have clearly demonstrated that the plant have moderate to significant antioxidant and anti-denaturation activity.

Literatures suggest that, the anti-denaturation property of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine rich and aliphatic threonine and lysine residue regions of the BSA (Williams et al). They have also reported that therapeutic molecules could be activating the tyrosine motif rich receptor dually with Many studies on antioxidants have proved that oxidative stress has great importance in generation of autoimmune bodies responsible for autoimmune diseases. Since most of plants have polyphenolic compounds which has a good reducing, singlet oxygen quenching effect on free radicals. Thus in this study *in-vitro* anti-denaturation of Bovine serum albumin and reducing antioxidant activity was evaluated. The results have clearly demonstrated that all the plant species have moderate to significant antioxidant and anti-denaturation activity.

It is well known that, phenolics constitute one of the major groups of compounds antioxidants [13] (Cakir et al., 2003).

The abundance of our plant extracts in polyphenol content should also explain the antioxidant activity results. Therefore, this may be due to phenolic compounds present in the plant extracts may be the reason for the possible antidenaturation and reducing antioxidant activity.

CONCLUSION

The results of the present study suggest that tested plant materials have moderate to potent antioxidant activity and antidenaturation activity. However, we do not know what components in the plant extracts show these activities. More detailed studies on chemical composition of the plant extracts, as well as other in vivo assays are essential to characterize them as biological antioxidants and anti-inflammatory agents which are beyond the scope of this study.

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