Pharmacognosy Research [Phcog Res.]
Vol 1, Issue 4, Jul-Aug, 2009 Page 202-207
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 25 April, 2009
Modified: 02 June, 2009
Accepted: 09 June, 2009

PHCOG RES.: Research Article

Modulatory Effect of *Erythrina vareigata* on Experimental Hyperlipidaemia in Male Wistar Rats

Mangathayaru K*, Balakrishna K[a], Kuruvilla Sarah[b], Reddy C Uma Maheshwara*

[*]Sri Ramachandra College of Pharmacy, Sri Ramachandra University, Porur, Chennai – 600 116, India
[a]Captain Srinivasa Murty Drug Research Institute for Ayurveda and Siddha, Chennai – 600 106, India.
[b]Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai – 600 116, India.

**Correspondence: Email, kvmanga@yahoo.com, Phone: 044 24768403*

ABSTRACT

Erythrina vareigata is a medium sized quick growing tree found distributed in deciduous forests throughout India. Its leaves are eaten as a pot herb and different parts of the plant have folkloric reputation as an anti inflammatory in India, China and South east Asia. Bark, wood, root and flowers are richly represented by isoflavones, pterocarpans and biphenyls. Current investigations were aimed at evaluating the hypolipidaemic activity of this popular hypolipidaemic siddha drug. Its nutritive value was estimated by standard reported methods. Ethyl acetate extract of the dried leaves were analyzed for total phenol content by Folin Ciocalteau and Titanium tetrachloride method. Its *in vitro* anti oxidant activity was assessed by DPPH, NO and trichloroacetic acid based reducing power method. Male wistar rats were subjected to high fat diet induced (HFD) hyperlipidaemia concurrent with extract administration for 60 days. While normal control group received standard pellet diet, Positive control group was on HFD only. At the end of experimentation, blood was collected for serum estimation of total cholesterol, high density lipoprotein cholesterol, triglycerides and atherogenic index. The extract had a high total phenol content, beneficially altered lipid parameters and reduced body weight of experimental animals. The leaves are rich in protein, minerals, vitamins and low in carbohydrate and fat. Anti oxidant activity of the polyphenols present in the extract might be mediating the observed hypoplipidaemia. This study validated the traditional medicine claims of its anti obesity effect as well as folkloric tutelage of consumption of leaves as a green vegetable.

Keywords: antioxidant, *Erythrina vareigata,* phenolic content, *hypolipidaemic,* pot herb

INTRODUCTION

Cardiovascular diseases are the leading cause of mortality and morbidity in the developed world and there has been an explosive rise in the incidence of CVD in the Indian subcontinent (1). Its primary cause - atherosclerosis is multifactorial in aetiopathology and elevated plama cholesterol levels is a very important modifiable risk factor. Thus management of dyspilidaemia is considered the prime strategy of halting the progression of atherosclerosis (2). Despite the availability of several hypolipidaemics and the usage of aggressive vascular interventions, cardiovascular diseases continue to account for over 40% of deaths globally (3). Growing global interest in traditional medical practices and awareness of importance of a healthy herb based diet have brought about resurgence of interest in indigenous medicine and drugs.

Erythrina vareigata is a medium sized quick growing tree found distributed in deciduous forests throughout India (4). Locally called 'kalyana murungai', its leaves are eaten as a pot herb in Tamil nadu and is prescribed for its hypolipidaemic, anti obesity effect in Siddha system of medicine (5). Several parts of the plant have folkloric reputation as an anti inflammatory in India, China and

South east Asia. Tetracyclic alkaloids of erythrina type have been isolated from the bark, wood, root and flowers, which are also a prolific source of iso flavones, pterocarpans and biphenyls (6). Anti inflammatory, haemagglutinating, insecticidal, skeletal muscle relaxant effect are the activities reported for the plant (7). Despite usage of the leaves and tender shoots as an edible, the nutritive value has not been estimated so far and the plant has not been scientifically investigated for hypolipidaemic/anti obesity effect.

MATERIALS AND METHODS

Plant material

Fresh green leaves of *Erythrina vareigata* were obtained from Loyola College Campus, Chennai. They were authenticated by Dr Sasikala Ethirajulu, Research officer (Botany), CSMDRIAS, Chennai and a voucher specimen (COP, M/23/07) has been deposited in the Herbarium of Department of Pharmacognosy, SRU. They were dried under shade for four days and milled into a coarse powder.

Preparation of extract

Ethyl acetate extract was prepared to concentrate phenolics from the leaves.Cold maceration of the size reduced, dried leaves in ethyl acetate to complete exhaustion, followed by filtration and evaporation of the filtrate under vacuum yielded a dark greenish pasty residue (Ev) of 1.3%w/w yield. It was dried in a desicator and stored until needed for analysis.

Experimental animals

Male wistar rats weighing 150-170 g were procured from the Department of Laboratory medicine, Tamil Nadu University of Veterinary and Animal Sciences, Chennai. Animals were housed in colony cages and fed water and pellet diet (Hindustan Liver limited , Mumbai) *ad libitum* and acclimatized to animal house conditions one week prior to experimentation. High fat diet constituted oral administration of saturated fat (2:3 ratio of dalda and coconut oil).The experimental protocol received the approval of the Institutional animal ethics committee (IAEC / SRMC & RI / 41 / 2005).

Chemicals and drugs

DPPH, naphthyl ethylene diamine dihydrochloride, trichloroacetic acid were procured from Sigma (St Louis, USA). Sodium nitroprusside, sulphanilamide, potassium ferricyanide, ferric chloride, Sodium chloride, Potassium dihydrogen phosphate, Di potassium hydrogen phosphate, sodium carboxy methyl cellulose, standard vitamins, other solvents and routine chemicals were of analytical grade and purchased from SD Fine Chemicals, Mumbai. Gold winner Dalda -premier quality and Edible coconut oil (Parachute) were purchased from the local market. Atorvastatin calcium was a gift sample from M/S Ordain health care Pvt Ltd., Chennai. Lipid analysis was under taken by commercial enzyme assay kit procured from Randox Laboratories, Antrim, UK.

Experimental studies
Nutritive value determination

Total calorific value, carbohydrate, protein, fat and fibre content of the dried leaf powder were quantitatively estimated (8). Elemental composition was determined by atomic absorption spectroscopy on a Varian Model 10/20 Spectrophotometer. Vitamin estimation was as per Freed, 1966 (9) and total chlorophyll, chlorophyll a & b content of the fresh leaves was evaluated (10) in view of the anti oxidant, DNA protective property of chlorophyll (11). All results are indicated with respect to dried powder.

Total phenolic content

Anti oxidant capacity of polyphenols is suggested to be responsible for their protective effect against cancer, neurodegenerative and cardiovascular diseases (12). In view of the reported presence of isoflavones from other plant parts, the total phenol content of the ethyl acetate extract was estimated in order to ascertain its contribution to the studied activities.

Folin Ciocalteau method (13)

Breifly, To 1 mg/ml solution of the extract in 2:3 methanol, water, 1 ml Folin Ciocalteau reagent, 2 ml 1.5% aqueous sodium carbonate solution were added. After incubation for 90 min in dark, the absorbance of the resulting mixture was measured on a Perkin Elmer lambda 25 UV spectrophotometer against reagent blank. Total phenol content was calculated from the caliberation curve of the absorbance of graded concentrations of gallic acid treated as above.

Titanium tetrachloride method

To 0.1 ml, 1 mg/ml solution of the extract in acetone, 0.5 ml 20% titanium tetrachloride in conc. HCl was added, mixed well in a vortex shaker after adding 9.9 ml of acetone. The absorbance of the resulting solution was measured against reagent blank. Quantitation was based on standard curve of gallic acid.

Determination of in vitro oxidant activity
DPPH assay (15)

To an ethanolic solution of DPPH (200μM), 0.05 ml of the extract dissolved in ethanol were added at different concentrations (5-1000 μg). An equal amount of ethanol was added to the control. Ascorbic acid (20μg) was used as standard for comparision. After 20 min the decrease in absorbance of test mixtures was read at 517 nm using ethanol as blank on a Cistronics Model 117 Spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with that of the control (not treated with the extract) using the formula

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test} \times 100}{\text{Absorbance of Control}}$$

Nitric Oxide Assay (16)

1 ml of 5 mM sodium nitroprusside was mixed with 1 ml of different concentrations of extract (10-1000μ g) / 1 ml of the standard drug ascorbic acid (20 μg) in phosphate buffer (pH 7.4). The mixture was incubated at 25^0 C for 150 min. To 1.5 ml of the incubated solution, 1.5 ml Greiss's reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm using phosphate buffer as blank and percentage inhibition was calculated using the same formula as in DPPH method.

Trichloroacetic acid based reducing power method (17)

1 ml of different concentrations of the extract (100- 800 μg) and of ascorbic acid (20 μg/ml) were mixed with 2.5 ml potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50^0 c for 20 min. 2.5 ml trichloroacetic acid (10%) was added to it and centrifuged at 2000 rpm for 30 min. 2.5 ml of the supernatant was mixed with 2.5 ml of water and 0.5 ml ferric chloride (0.1%) and absorbance was measured at 700 nm using for blank a solution of 2.5 ml potassium ferricyanide, 2.5 ml phosphate buffer, 2.5 ml trichloroacetic acid and 0.5 ml of ferric chloride.

Effect of extracts on rat model hyperlipidaemia (18)

24 animals were divided into 4 groups of 6 animals each. Extract/ atorvastatin calcium suspended in 0.5% Sodium carboxy methyl cellulose was orally administered at the same time between 8.30- 9.00 AM each day. Weight of the animals was taken weekly. The period of experimentation was 60 days. Study design was as follows

Group I - Normal control received standard pellet diet.
Group II - HFD
Group III - HFD plus Ev 100 mg/ kg bw/ day p.o
Group IV - HFD plus atorvastatin calcium 10 mg/kg bw/ day p.o

On day 60 the animals were fasted overnight and blood was drawn from the retro orbital plexus for serum estimation of Total cholesterol (TC), triglycerides (TGL) and high density lipoprotein cholesterol (HDL-C) on a semi auto analyzer (Star Plus). Atherogenic index (A.I) was calculated using the formula

$$A.I = \frac{TC - HDL - C}{HDL - C}$$

Statistical Analysis

The data obtained was analyzed using one way ANOVA followed by Dunnet's test. $P < 0.05$ was considered to be statistically significant.

RESULTS

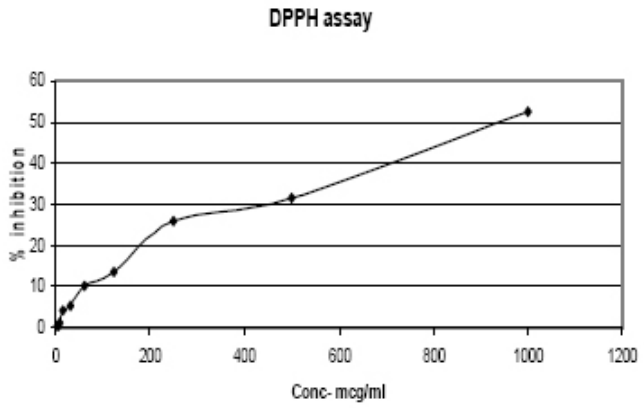
Results of nutritive value determination are summarized in Table 1. Leaves of *Erythrina vareigata* are rich in protein - typical of leguminous leaves, low in carbohydrate, fat and calorific value making it an ideal food for diabetics. Rich in iron, its mineral and vitamin content justify its utility as a green vegetable. Chlorophyll content is presented in Table 2. For comparision, literature values for Spinach, one of the richest sources of chlorophyll among green leafy edibles is given. The leaves have almost half the chlorophyll content of spinach, significant considering the fact that, green vegetables are rich in chlorophyll and have a greater chlorophyll a content relative to b (19). Total phenol content (in gallic acid equivalents) of the ethyl acetate extract as per Folin Ciocalteau method was found to be 17 ±0.06 and by Titanium tetrachloride method, 19±0.09, indicating significant proportion of polyphenolics in the extract.There is no statistically significant difference in phenol content between the two methods. Results of the *in vitro* anti oxidant assay systems are indicated in Figure 1. Graded increase in extract concentration has brought about increase in % inhibition of the nitrogen centered stable free radical, DPPH and nitric oxide. Increasing extract concentration increased absorbance due to complex formation between potassium ferrocyanide and ferric chloride in trichloroacetic based reducing power method. These results amply demonstrate presence of radical scavengers in the extract.

Table 1. Nutritive value of Erythrina vareigata leaf powder.

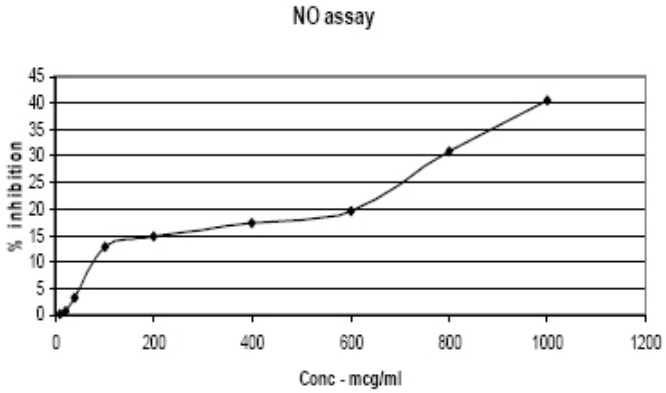
Each 100 g of leaf powder contains					
Colorific value (Kcal)	- 36	Minerals (mg)		Vitamins	
Carbohydrate (g)	- 0.95	Calcium	- 7.2	Total carotene (µg)	- 256.2
Protein (g)	- 7.8	Iron	- 3.26	Thiamine (mg)	- 0.067
Fat (g)	- 0.12	Magnesium	- 2.64	Riboflavin (mg)	- 0.112
Fibre (g)	- 0.2	Potassium	- 2.64	Niacinamide (mg)	- 0.065
		Sodium	- 7.22	Pyridoxine (mg)	- Traces
		Zinc	- 0.18	Folic acid (mg)	- 0.32
		Phosphorous	- 11.77	Vitamin C (mg)	- 2.66
				Choline (mg)	- 1.41

Table 2. Chlorophyll content of Erythrina vareigata leaf powder.

Chlorophyll (mg)	Ev* ± SD	Spinach (Lit. value)
TC	14.21± 0.02	24.3
Ca	8.75 ± 0.01	14.74
Cb	5.47 ± 0.01	9.55



Std. Ascorbic acid (20 µg), % inh.- 93.58



Std. ascorbic acid (20 µg), % inh. - 7.51

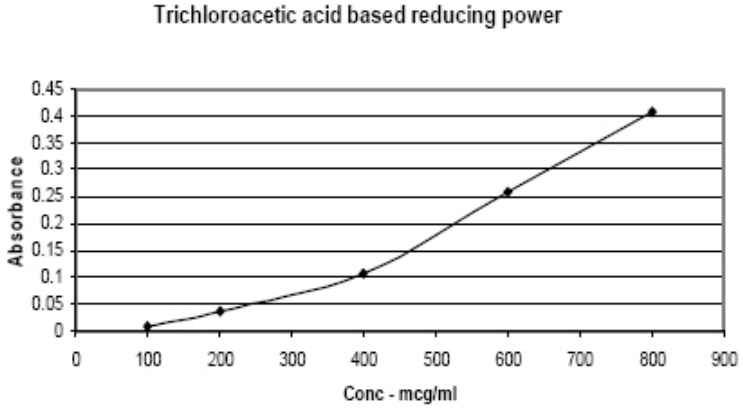


Figure 1. in vitro anti oxidant activity of alcohol extract of Ev

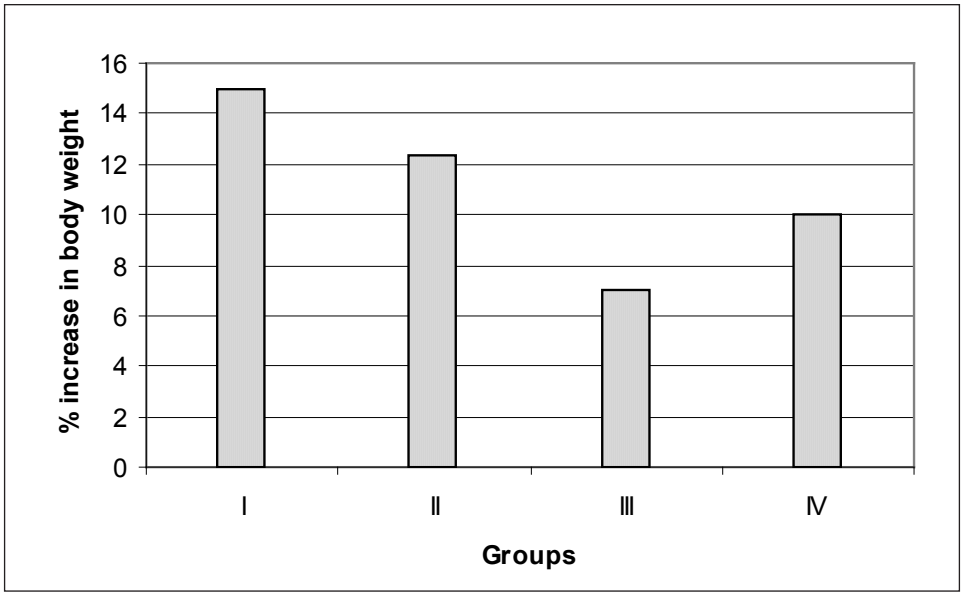


Figure 2. Body weight increase of experimental animals

Table 3. Serum lipid profile of experimental animals.

Group	Treatment	TC (mg/dl)	% change	TGL (mg/dl)	% change	HDL (mg/dl)	% change	A.I	% change
I	Normal diet	51.67 ± 2.71		71.7 ± 2.89		36.99±2.79		0.463±0.041	
II	HFD	73.3 ± 4	+41.86	157.52 ± 4.76	+119.7	26.69± 1.54	-27.84	1.817 ± 0.35	+292.44
III	HFD+Ev (100mg/kg)	46.13±0.91	-37	83.96 ± 4.05	-46.7	35.6± 2.69	+33.38	0.3 ± 0.084	-83.5
IV	HFD+At ca (100mg/kg)	48.02 ± 3.6	-34.5	87.96 ± 3.21	-44.2	30.42 ± 1.13*	+14	0.467 ± 0.083	-74.3
One way ANOVA	FDfP	9.773,20< 0.001		70.123,20<0.001		4.493,20<0.01		15.73,20<0.001	

Results of the influence of the extract on rat model hyperlipidaemia is presented in Table 3. High fat administration has increased levels of total cholesterol, triglycerides and decreased HDL-C in group II animals relative to normal control. A.I - a parameter indicating relative risk of atherogenicity was elevated by 292%. Ev administration to group III has decreased levels of TC (-37%) and TGL (-47%) and increased. HDL-C (+33%) in a statistically significant manner. A.I was brought down by 84% compared to positive control, while Atorvastatin calcium decreased it by 74%. From Dunnet's Ev is found to be the more effective hypolipidaemic.

All groups in comparison to group II, Values are mean ± SEM; n=6 animals in each group, * ns

Percentage increase in body weight of experimental animals due to dietary lipemia is summarized in Figure 2. The least increase in body weight is noted with respect to Ev administered group III compared to other groups, signifying anti obesity effect.

DISCUSSION

Nutritive value parameters of *Erythrina vareigata* quantified in this study justify utility of the leaves as a pot herb. Its phenolic and chlorophyll content presumably contribute to the demonstrated anti oxidant activity considering the reported DNA protective property of chlorophyll (11) and anti oxidant potential of phenolics. Presence of isoflavones in other plant parts, explain the phenolic content of the leaves, which may also be represented for phenolics. Also some isoflavones from this plant are attributed with Phospholipase A2 inhibition (20). Thus anti inflammatory, anti oxidant effect of the constituent phytomolecules, are possibly mediating the observed hypolipidaemic influence considering the pro inflammatory nature of oxidative changes associated with hyperlipidaemia. Current investigations have validated traditional medicine claims for the plant as an anti obesity, hypolipidaemic as well as folkloric tutelage of consumption

of leaves as a green vegetable. Also modulation of HFD induced hyperlipidaemia in rats by the ethyl acetate extract of Ev *during* cholesterol feeding is relevant to conditions likely to be present in man. The future work will focus on the isolation of major phytomolecules from the extract for biopotency assessment.

REFERENCES

1. Salim Y., Stephanie O., Sonia A. Global burden of cardiovascular diseases Part I. General considerations, the epidemiologic transition, Risk factors, and impact of urbanization *Circulation* **104**: 2746–2753 (2001)
2. Gould A., Roussouw J.E., Santanello N.C., Heyse J.F, Furberg C.D.. Cholesterol reduction yields clinical benefits: impact of statin trial. *Circulation*. **97**: 946 (1998)
3. Murray C.J.L. (ed) *The Global Burden of Disease: A Comprehensive Assessment of Mortality and Disability from Diseases, Injuries, and Risk Factors in 1990 and Projected to 2020*. (Harvard School of Public Health, Boston Mass , 1996)
4. Anonymous *Wealth of India-Raw Materials, vol. 3*. (CSIR-NISCAIR, New Delhi, 1952) 197
5. Yoganarasimhan S.N., *Medicinal Plants of India*. Vedam eBooks, New Delhi, 2000) 149
6. Deshpande V.H., Pendse A.D., Pendse R.. Erythrinins A, B and C, Three new isoflavones from the bark of Erythrina vareigata. *Ind J Chem* **15B**: 205–207 (1977).
7. Telikepalli H., Gollapudi S.R., Shokri A., Velaquez L., Sandmann R.A., Veliz E.A., Rao K.V.J., Madhavi A.S., Mitscher L.A.. Isoflavonoids and a cinnamylphenol from the root extracts of *Erythrina vareigata*. *Phytochemistry* **29**: 2005–2007 (1990).
8. *British Pharmacoepia*. Vol. **III** (British Pharmacoepia Commission, London, 2007).
9. Freed M. *Methods of Vitamin assay*. (Interscience, New York, 1966)
10. Harborne J.B.. *Modern methods of Phytochemical analysis*. (Chapman & Hall, Madras, 1998)227
11. Park K.K., Surh Y.J., Stewart B.C., Miller J.A. Chemoprotective activities of chlorophyllin: Inhibition of mutagenicity and covalent bonding of various ultimate carcinogens. *Proc. Am. Assoc. Cancer Res* **35**: 139 (1994)
12. Neuhouser M.L.. Dietary flavonoids and cancer risk: evidence for human population studies. *Nutr Cancer* **50**: 1–7 (2004).
13. Wrolstad B. *Handbook of Food Analytical Chemistry* . New Jersey, 2005), pp 459–470.
14. N.A. Michael Eskin, Hoehn E., Frenkel C.. A simple and rapid quantitative method for total phenols. *J Agric Food Chem* **26**: 973–975 (1978)
15. Shirwaikar A., Rajendran K., Dinesh Kumar C. *in vitro* antioxidant studies of Annona squamosa Linn. leaves. *Ind J Exp Biol*, **42**: 804 (2004).
16. Schulz J.B., Lindnau J., Seyfriend J., Dichgans J.. Glutathione oxidative stress and neuro degeneration. *Eur J Biochem* **267**: 4904 (2000).
17. Jay Prakash G.K., Singh R.P., Sakariah K.K.. Antioxidant activity of grape seed extracts on peroxidation models *in vitro*. *J Agri Food Chem* **55**: 1018 (2001)
18. Pranab S., Mengi S.A. Evaluation of Hypolipidaemic activity of Acorus calamus Linn. in rats. *Indian drugs*. **40**:25–29 (2003).
19. Baxter J.H. Absorption of chlorophyll phytol in normal men and in patients wtih Refsum's disease. *J Lipid Res* **9**:636–641 (1968).
20. Hegde V.R., Dai P., Patel M.P., Paur M.S., Das P., Dai J., Bryant R., Cox P.A. Phospholipase A_2 inhibitors from from an Erythrina species from Samoa. *J Nat Prod* **60**: 537–539 (1977).