Journal of Applied Pharmaceutical Science Vol. 6 (07), pp. 147-155, July, 2016
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2016.60722
ISSN 2231-3354

Phytochemical analysis, Anti inflammatory activity, *in vitro* antidiabetic activity and GC-MS profile of *Erythrina variegata* L. bark

Nagaraj Santhiya, Suriyamoorthy Priyanga, Subrhamanian Hemmalakshmi, Kanakasabapathi Devaki*

Department of Biochemistry, Karpagam University, Coimbatore-641 021, India.

ARTICLE INFO

Article history:
Received on: 07/03/2016
Revised on: 04/04/2016
Accepted on: 22/04/2016
Available online: 28/07/2016

Key words:
E. variegata, Phytoconstituents, DPPH, Glycosylation of hemoglobin, GC-MS.

ABSTRACT

Many beneficial medicinal products are used to treat various serious diseases and disorders like diabetes, cancer and cardiovascular diseases without side effects. Hence our study focused to investigate the phytochemical analysis, quantification of bioactive compounds (alkaloid, phenols, flavonoids), *in vitro* free radical scavenging activity, anti-inflammatory activity (Membrane stabilization assay), *in vitro* anti-diabetic activity by enzyme inhibition activity, glucose uptake assay in isolated rat hemi-diaphragm and in yeast cells and also record GC-MS profile of *Erythrina variegata* L. bark (methanolic extract) which has boundless medicinal properties. The results of this study showed the evidence that the extracts when tested for their phytochemicals and free radical scavenging activity were found to have considerable antioxidant potential. This plant also exhibit better *in vitro* enzyme inhibitory activity, inhibition of non- enzymatic glycosylation of hemoglobin, *in vitro* anti-diabetic activity proved by glucose uptake assays and anti-inflammatory activity. GC-MS analysis of methanolic extract of *Erythrina variegata* L. bark revealed the presence of 58 compounds which are responsible for pharmacological activities. The results of this study indicate that the methanolic extract of *Erythrina variegata* L. bark has significant pharmacological properties.

INTRODUCTION

Medicinal plants are the bio resources given by natural world used to heal a group of human diseases to evaluate their probable sources for new drugs (Priyanga *et al.,* 2014). Plant parts have been a typical source of medicine from an ancient time and have been the primary source of drugs in Indian systems of medicine and other ancient systems in the world (Devi *et al.,* 2011). Phytochemicals are the natural bioactive compounds present in plants and the most substantial bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates and phenolic compounds (Priyanga *et al.,* 2014; Sowmya *et al.,* 2015). Inflammation is a serious problem and the commonly used drug for managing of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to the formation of gastric ulcers (Sangita *et al.,* 2012).

* *Corresponding Author*
Dr. K. Devaki, Department of Biochemistry, Karpagam University, Coimbatore 641 021, India. Telephone number: 091-0422-6453777, Fax : 091-0422-2980022, E-mail: dr.devaki.bc@gmail.com

Diabetes mellitus is a chronic endocrine disorder that disrupts the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases categorized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not synthesize enough insulin or cells do not respond to the produced insulin (West, 2000; Sangeetha *et al.,* 2015).

According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 (Megha *et al.,* 2013). Nearly 400 traditional plants have been stated for the treatment of diabetes (Upwar *et al.,* 2011).

Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitors of crucial enzymes like alpha amylase and alpha glucosidase associated with type 2 diabetes and lipid peroxidation in tissues (Reddy *et al.,* 2010). Crude extract acquired from the *E. variegata* was evaluated for its radical scavenging properties and assessed that it could be a rich source of natural oxidants with potential applications (Anwar, 2006).

Various parts of this plant is used in alternative system of medicine for wind damp obstruction syndrome, rheumatic joint problems, lower back, knee pain, asthma, nerve depression, epilepsy and insect bites (Devaki *et al.*, 2015).

Different parts of *E. variegata* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic and also it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds (Kumar *et al.*, 2010).

The plant bark is astringent, febrifuge, anti-bilious, anthelmintic, and it is too useful in opthalmia and skin diseases. The leaves are used in fever, inflammation and joint pain and the juice of the leaves are used to relieve earache and toothache. The roots are used in bronchitis, febrifuge and as an insecticide. The roots are also used in the treatment of cancer, convulsions and used to treat pimples. It has the repute to stimulate lactation and menstruation and is used as laxative, diuretic and expectorant (Baskar *et al.*, 2010). The present study was focused to evaluate the phytochemical analysis, anti-inflammatory activity, *in vitro* antidiabetic activity and to record the GC-MS profile of *Erythrina variegata* L. bark (methanolic extract).

MATERIALS AND METHODS

Plant material

The plant material for the proposed study was collected from Kodaikannal, Dindigul district, Tamil Nadu, India. The plant was authenticated by Dr. G.V.S. Moorthy, Botanical Survey of India, TNAU campus Coimbatore, with the voucher number BSI/SRC/5/23/2013-14/Tech/1500.

Preparation of Methanol extract

50 g of powdered plant material (Bark) was weighed and extracted with 250 ml of methanol for 72 hours using occasional shaker. The supernatant was collected and concentrated at 40°C. It was stored at 4°C in an air tight bottle for further studies.

Preparation of animals

Albino Wistar male rats, weighing 100-150g were obtained from the institutional animal house of Karpagam Academy of Higher Education, Coimbatore and were used for the study. The animals were housed at a room temperature of 25±2^{0}C, relative humidity of 75±5% and 12hrs dark-light cycle; animals were fed with standard laboratory diet and water ad libitum. The study was approved by IAEC and the experiments were conducted according to the ethical norms and Institutional Animal Ethics Committee Guidelines.

Phytochemical analysis

The qualitative analysis of secondary metabolites was carried out by following the methods of Trease and Evans (1996) and Harborne (1987).

Quantification of bioactive compounds

The bioactive compounds alkaloid, phenols and flavonoids were quantified according to the standard procedure. The total alkaloid present in the methanolic extract of *Erythrina variegata* L. bark was determined by the modified method of Harborne (1973). The total phenols present in the sample were estimated by (Singleton and Rossi, 1965) and the total flavonoid content by (Ordon *et al.*, 2006).

In vitro free radical scavenging activity

The free radical scavenging activity of methanolic extract of *Erythrina variegata* L. bark were confirmed by reducing power activity by the method (Oyaizu, 1986) and the DPPH radical scavenging activity by the method (Blois, 1958).

In vitro anti -diabetic assays

α-Amylase inhibitory activity

The α-amylase inhibitory activity was determined according to the method described by (Jyothi *et al.*, 2011). Briefly, the total assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in the concentration range 10-100µg/ml were incubated for 10 min at room temperature followed by the addition of 200 µl of 1% starch in all the test tubes. The reaction was terminated with addition of 400 µl of 3, 5 Dinitro salicylic acid (DNSA) color reagent tubes were placed in boiling water bath for 5 minutes, cooled at room temperature and diluted with 15 ml of distilled water and the absorbance was measured at 540nm. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts prepared with DMSO. The results were expressed as % inhibition calculated using the formula:

$$\text{Inhibition activity (\%)} = \frac{\text{Abs (control)} - \text{Abs (extract)}}{\text{Abs(control)}} \times 100$$

In vitro inhibitory assay for the α-glucosidase activity

The extract (50 μL) and 100 μL of α-glucosidase solution (1.0 U/mL) in 0.1M phosphate buffer (pH 6.9) was incubated at 25^{0}C for 10min.Then, 50 μL of 5mM p-nitrophenyl-α-D-gluco pyranoside solution in 0.1M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25^{0}C for 5min before reading the absorbance at 405 nm in the spectrophotometer (Apostolidis *et al.*, 2007). The α- glucosidase inhibitory activity was expressed as percentage inhibition. The IC_{50} of the extract was calculated.

$$\text{Inhibition activity (\%)} = \frac{\text{Abs (control)} - \text{Abs (extract)}}{\text{Abs(control)}} \times 100$$

In vitro glucose uptake by isolated rat hemi-diaphragm

Glucose uptake by rat hemi-diaphragm was estimated by the modified method described by (Walaas and Walaas, 1952; Chattopadhyay *et al.*, 1992). Albino rats of either sex weighing between 160-180g were selected and maintained as described

above. The animals were sacrificed by decapitation and diaphragm was dissected out quickly with minimal trauma and divided into two halves. The hemi-diaphragm was then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and was placed in small culture tubes containing 2 ml Tyrode solution with 2% glucose was incubated for 30minutes at 37^0C in an atmosphere of 100% O_2 with shaking. Four sets containing five numbers of graduated test tubes were treated as follows:

Group I: Served as control which contain 2 ml Tyrode solution with 2% glucose and 2.0ml of distilled water.

Group II: Contain 2 ml Tyrode solution with 2% glucose, regular insulin (Biocon) 0.62ml of 0.4 units per ml solution and 1.38 ml of distilled water.

Group III: Contain 2 ml Tyrode solution with 2% glucose, 0.6 ml of *E. variegata* (200µg/ml) and 1.4ml of distilled water.

Group IV: Contain 2 ml Tyrode solution with 2% glucose, 0.6 ml of *E. variegata* (200µg/ml), 0.62ml of 0.4 units per ml solution of Insulin and 0.78ml of distilled water.

Following incubation, the hemi-diaphragms were taken out and weighed. The glucose content of the incubated medium was measured by GOD-POD method. Glucose uptake per gram of tissue was the difference between the initial and final glucose content in the incubated medium. The uptake of glucose was expressed as mg/g of moist tissue/30 min.

Glucose uptake in Yeast cells

Yeast cells were prepared according to the method of (Dinesh *et al.*, 2009), briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortexed and further incubated at 37°C for 60 min.

After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metformin was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated. All the tests were performed in triplicates.

Non-enzymatic glycosylation of haemoglobin assay

Antidiabetic activity of *Erythrina variegata* L. bark was investigated by estimating degree of non-enzymatic haemoglobin glycosylation, by colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and sodium azide (0.02%) solutions were prepared in phosphate buffer (0.01 M,) pH 7.4. 1 ml each of above solution was mixed and 1 ml of each concentrations of plant sample were added to the mixture. The mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug and % inhibition was calculated (Megha *et al.*, 2013; Gupta *et al.*, 2013). All the tests were performed in triplicate.

Membrane stabilization assay

Blood was collected freshly and mixed with equal volume of Alsever's solution. It was then centrifuged at 3000g 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 hyposaline and 0.5mL HRBC suspension was added and incubated for 30 minutes at 37^0C and then centrifuged at 2500 rpm for 20 minutes (Patel *et al.*, 2012).

Absorbance was measured at 560 nm. Aspirin was used as the standard and control was taken without the extract.

Gas Chromatography - Mass Spectroscopy (GC-MS) analysis of the methanolic extract of *Erythrina variegata* L.bark

GC-MS analysis of the methanolic extract of *Erythrina variegata* L. bark was performed using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard Non-polar column with dimensions of 30 mts x 0.25 mm ID x 0.25 µm Film.

The carrier gas used is Helium at flow of 1.0ml/minute. The injector was operated at 250^0C and the oven temperature was programmed as follows; 60^0C for 15 minutes, then gradually increased to 280^0C for 3 minutes. The identification of components was based on comparison of their mass spectra with those of Wiley and NBS libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST) attached to the GC-MS instrument and the results obtained have been tabulated.

Statistical analysis

All the experimental results were entered using three parallel measurements of the Mean ± Standard deviation (n=3).

RESULTS AND DISCUSSION

Various herbs and species are used in Indian cooking such as onion, Garlic, Ginger, Turmeric, Cardamom, Coriander etc has many medicinal properties (Vijayameena *et al*., 2013). Among this category one of the significant medicinal plant which show countless medicinal properties is *Erythrina variegata* Linn. The antibacterial and anti-inflammatory properties of *Erythrina variegata* Linn were documented in Chinese herbal medicine for the treatment of pyrexia, scabies and septicaemia (Baskar *et al.*, 2010).

Phytochemical screening and Quantification of bioactive compounds

The phytochemical screening of the methanolic extract of *Erythrina variegata* bark were carried and the results are given in the Table 1. The methanolic extract of *Erythrina variegata* shows the presence of major phytoconstituents like alkaloids, flavonoids, steroids, terpenoids, saponins, tannins, phenols and glycosides.

Herbal extracts hold diverse phytochemicals with biological activity that can be of valuable therapeutic index. The protective effect of fruits and vegetables has been qualified by phytochemicals, which are the non-nutrient plant compounds (Devi *et al.*, 2011).

Table 1: Phytochemical constituents of *Erythrina variegata* L.

Phytochemical constituents	Solvent (methanol)
Alkaloids	+
Steroids	+
Flavonoids	+
Tannins/phenols	+
Aminoacids and Proteins	+
Sugars	+
Glycosides	+
Saponins	+
Terpenoids	+

The *Erythrina variegata* L. bark methanolic extract contains alkaloids, phenols, flavonoids, tannins, steroids, terpenoids, glycosides and saponins as their secondary metabolites which is responsible for their pharmacological actions.

The total alkaloid content present in methanolic extract of *Erythrina variegata* L. bark was found to be 220 ± 0.56 mg/g. The total flavonoid content present in *Erythrina variegata* L. bark was found to be 200 mg/ g ± 0.72. The total phenol content present in methanolic extract of *Erythrina variegata* L. bark methanolic extract was found to be 130 mg/g ± 0.62 (T able -2).

Phytochemicals play a vital role in plant defense against prey, microorganisms, stress as well as interspecies protections, these plant components have been used as drugs for eras. Hence, phytochemical screening serves as the first early step in predicting the kinds of potential active compounds from plants (Chew *et al.*, 2011).

Table 2: Quantitative estimation of phytochemicals.

S. No	Parameters	Content (mg/g)
1.	Alkaloid	220 ± 0.56
2.	Flavanoid	200 ± 0.72
3.	Phenol	130 ± 0.62

The values are expressed as Mean ± SD of triplicates.

***In vitro* free radical scavenging activity**

In DPPH assay different concentrations of extracts were used (100 – 500 µg/ml). The percent inhibition of the *Erythrina variegata* L. bark methanolic extract was found to be increased in dose dependent manner, and the graph showing the results were given below. In Figure1, methanolic bark extract of *Erythrina variegata* showed maximum activity of 60.71% at 500µg/ml whereas ascorbic acid at the same concentration exhibited 67.85% inhibition respectively. IC_{50} values were found to be 400 ± 1.05 µg/ml and 300 ±0.76 µg/ml for the extract and the reference standard, ascorbic acid respectively.

DPPH radical scavenging test is worked on the principle, interchange of hydrogen atoms between the antioxidant and the constant DPPH free radical. DPPH is a stable free radical at room temperature which receives an electron or hydrogen radical to form a steady diamagnetic molecule. DPPH radical is condensed to the corresponding hydrazine, a colour change from violet to yellow in solution indicates the scavenging behavior of the crude plant sample due to bioactive compounds such as phenolic compounds, flavonoids, terpenoids and their derivatives (Priyanga *et al.*, 2015).

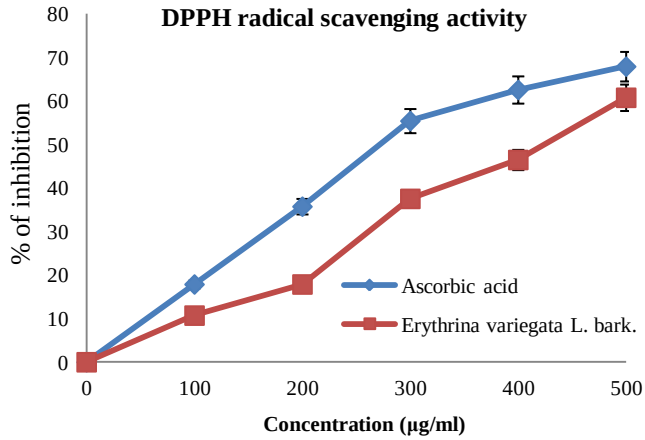

Fig. 1: DPPH radical scavenging activity of *E. variegata* L. bark extract. The values are expressed as Mean ± SD of triplicates

The reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity. Standard curves of ascorbic acid as well as the methanolic extract of *Erythrina variegata* L. bark are shown in Figure 2.

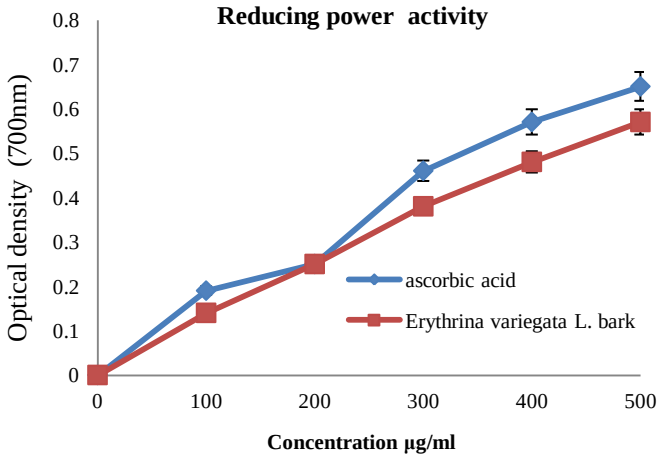

Fig. 2: Reducing power activity of *E. variegata* L. bark extract. The values are expressed as Mean ± SD of triplicates

The *Erythrina variegata* L. bark methanolic extract antioxidant capability increases with the increasing concentration (100–500µg/ml) like the antioxidant activity of standard curve.

In vitro antidiabetic assays

In vitro glucose uptake assay by isolated rat hemi-diaphragm (Erythrina variegata L. bark methanolic extract)

The estimation of glucose content in rat hemi-diaphragm is a usually employed and reliable method for *in vitro* study of peripheral uptake of glucose. Antihyperglycemic behaviors of most efficient plants were in part explicated by the ability of the phytoconstituents to increase glucose transport and metabolism in muscle and to stimulate insulin secretion (McCue *et al.*, 2004). Glucose uptake assay by isolated rat hemi-diaphragm **(***Erythrina variegata* L. bark methanolic extract) were done using the extract concentration of 200µg/ml and the activity were shown below.

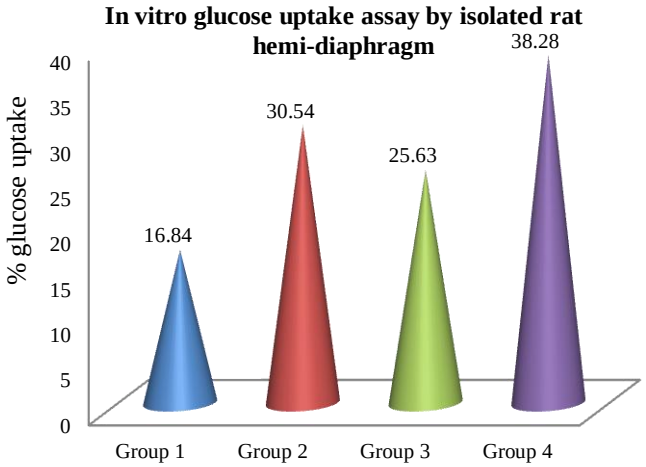

Fig. 3: *In vitro* glucose uptake assay by isolated rat hemi-diaphragm of *E. variegata* L. bark extract
Values are expressed as Mean ± SD of triplicates

Among the 4 groups, Glucose uptake were increased when treated with insulin alone compared to that of bark extract, and the bark extract were somewhat nearer to that of insulin, but when combine both the extract and insulin, glucose uptake by diaphragm were increased more and this indicates that insulin has some synergistic effect with the extract in uptake of glucose.

Glucose uptake by Yeast cells

The rate of glucose transport across cell membrane in yeast cells system was identified and the results are given below. The amount of glucose lingering in the medium later a specific time serves as a marker of the glucose uptake by the yeast cells (Wagner and Bladt, 2004).

In the diabetic patients, regulation of glucose level in the blood can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a selection of dietary conditions is one of the most significant and closely regulated processes observed in the mammalian species (Megha *et al.*, 2013)

E. variegata L. bark methanolic extract increased the glucose uptake in yeast cells. It increases with increasing concentration and the results are comparable with that of the standard which was shown in figure 4.

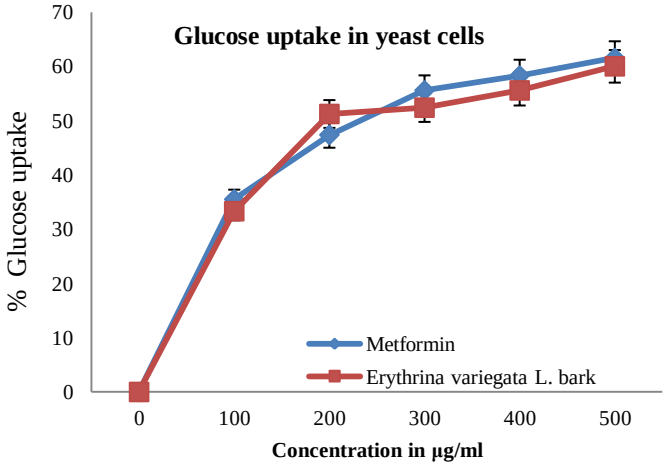

Fig. 4: Glucose uptake in Yeast cells assay of *E. variegata* L. bark extract. Values are expressed as Mean ± SD of triplicates

Inhibitory assay of α-amylase & α-glucosidase activity of methanolic bark extract of *Erythrina variegata* L.

Among the different anti-diabetic therapeutic approaches one major strategy is dropping gastrointestinal absorption of glucose by inhibition of carbohydrate metabolizing enzymes alpha-amylase and alpha- glucosidase. Pancreatic and intestinal glucosidases are the key enzymes of dietary carbohydrate digestion and inhibitors of these enzymes may be effective in retarding glucose absorption. This is because only monosaccharides are readily taken up from the intestine and all other carbohydrates have to be broken-down enzymatically before they can be absorbed. In previous reports *M. uniflorum* leaves showed α-amylase and α-glucosidase inhibitory potential when compared with standard drug indicated that extreme inhibition of pancreatic - amylase could result in the irregular bacterial fermentation of undigested carbohydrates in the colon and therefore gentle –amylase inhibition action is desirable (Priyanga *et al.*, 2015). The inhibitory action of methanolic bark extract of *Erythrina variegata* L. were increased as the concentration increases in both the extract and the standard acarbose and both the graphs were shown in Figure 5 and 6.

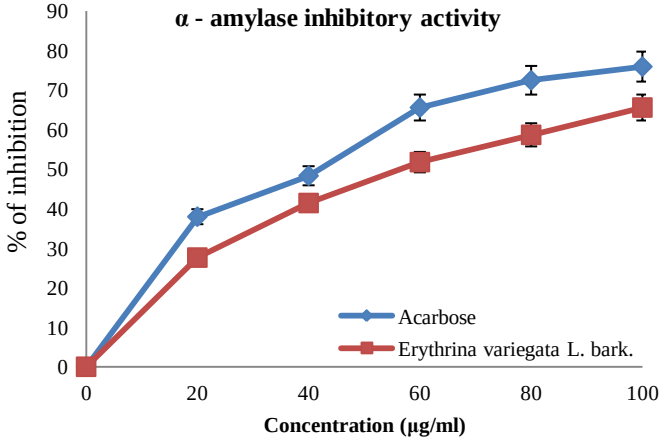

Fig. 5: α - amylase inhibitory activity of *E. variegata* L. bark extract. Values are expressed as Mean ± SD of triplicate.

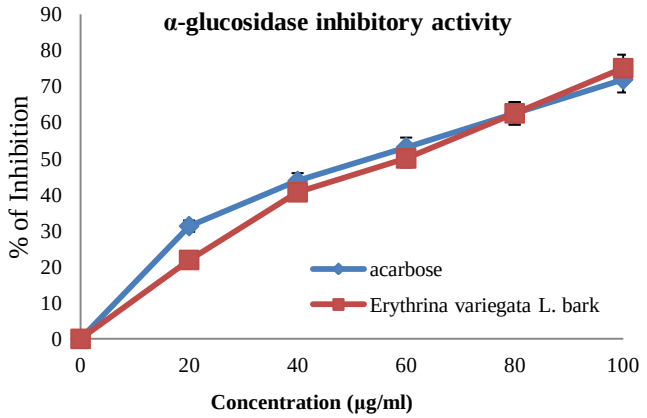

Fig. 6: α-glucosidase inhibitory activity of *E. variegata* L. bark extract. Values are expressed as Mean ± SD of triplicates

Non-enzymatic glycosylation of hemoglobin

The methanolic bark extract of *Erythrina variegata* L. shows an excellent inhibition of glycosylated hemoglobin. The percentage inhibition of glycosylation is dose dependent (Figure 7). The hemoglobin present in the red blood corpuscles has a tendency to get bound to glucose and form an HbA1c. The greater the blood-glucose concentration, the greater is the amount of glucose-bound (glycosylated) hemoglobin. Such glucose hemoglobin linkage is rather constant and lasts for 60 to 120 days (the life-span of red blood corpuscles). Thus the amount of glycosylated hemoglobin is a convinced steer to the concentration of glucose in the blood. Amount of glycated hemoglobin should not be more than 12% (Radhika *et al.*, 2013). In case of standard drug the concentration of drug increases, the formation of glucose-hemoglobin complex decreases and free hemoglobin increases and shows the inhibition of glycosylated hemoglobin.

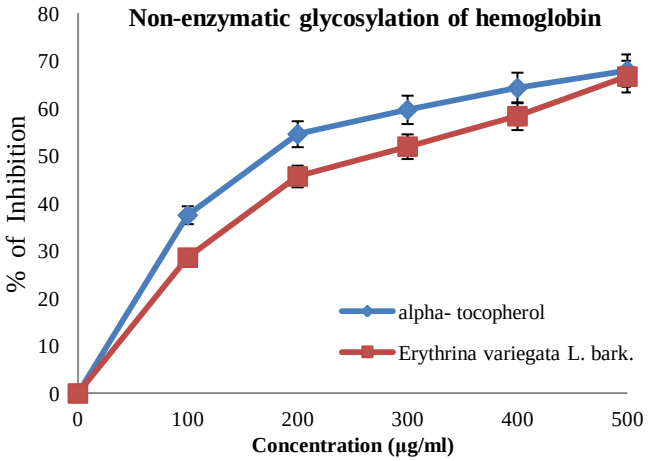

Fig. 7: Non-enzymatic glycosylation of hemoglobin of *E. variegata* L. bark extract.
Values are expressed as Mean ± SD of triplicates

Anti-inflammatory effect of methanolic extract of *Erythrina variegata* L. bark

Chloroform and water extract of *E.variegata* Linn showed significant stabilization towards HRBC (Human RBC) membrane. Inflammation is a common phenomenon and it is a reaction of living tissue. This can be done by using HRBC method. It clearly indicates that the inflammation shortened compared with the control. The anti-inflammatory property of *Erythrina variegata* was due to the presence of alkaloid or steroid in the crude extract (Reddy *et al.*, 2015).

The membrane stabilization was increased as the concentration of the plant extract increases the IC_{50} value of plant extract and the standard was found to be 364.26 ± 0.74 µg/ml and 340.66 ± 0.95 µg/ml respectively.

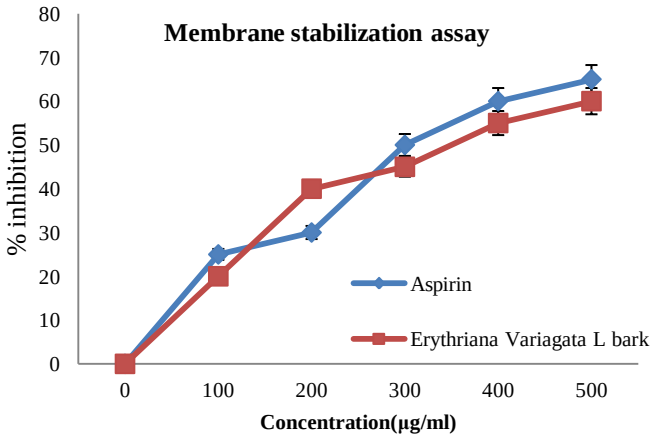

Figure 8: Membrane stabilization assay of *E. variegata* L. bark extract
Values are expressed as Mean ± SD of triplicates

GC-MS spectral analysis of methanolic extract of *Erythrina variegata* L. bark

Gas chromatography-mass spectrometry (GC-MS) is a technique that combines the features of gas-liquid chromatography and mass spectrometry to recognize different substances within a test sample (Joseph *et al.*, 2014).

The results pertaining to GC-MS analysis of the methanolic extract of *E. variegata* lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC. The various compounds present in the entire herb of *E. variegata* detected by the GC-MS are shown in Table 3.

In the GC-MS analysis, 58 compounds were identified in the methanolic extract of *Erythrina variegata* L. bark and were mentioned in table below. The identification of compounds is based on the peak area, molecular weight and molecular formula.

These compounds are responsible for pharmacological activities. Out of 58 compounds, 3 compounds has highest peak area, they are Palmitic acid (17.44), Octadecadienoic acid (13.3) and Diacetyllycorine lactam (12.7).

Palmitic acid has the property of antioxidant and antimicrobial activities. It has the property of larvicidal effect. Octadecadienoic acid has the property of anti-inflammatory and anti-arthritic activity (Lalitharani *et al.*, 2010). Diacetyl lycorine lactam is the most abundant alkaloid exhibits promising anticancer activity (Wang *et al.*, 2014).

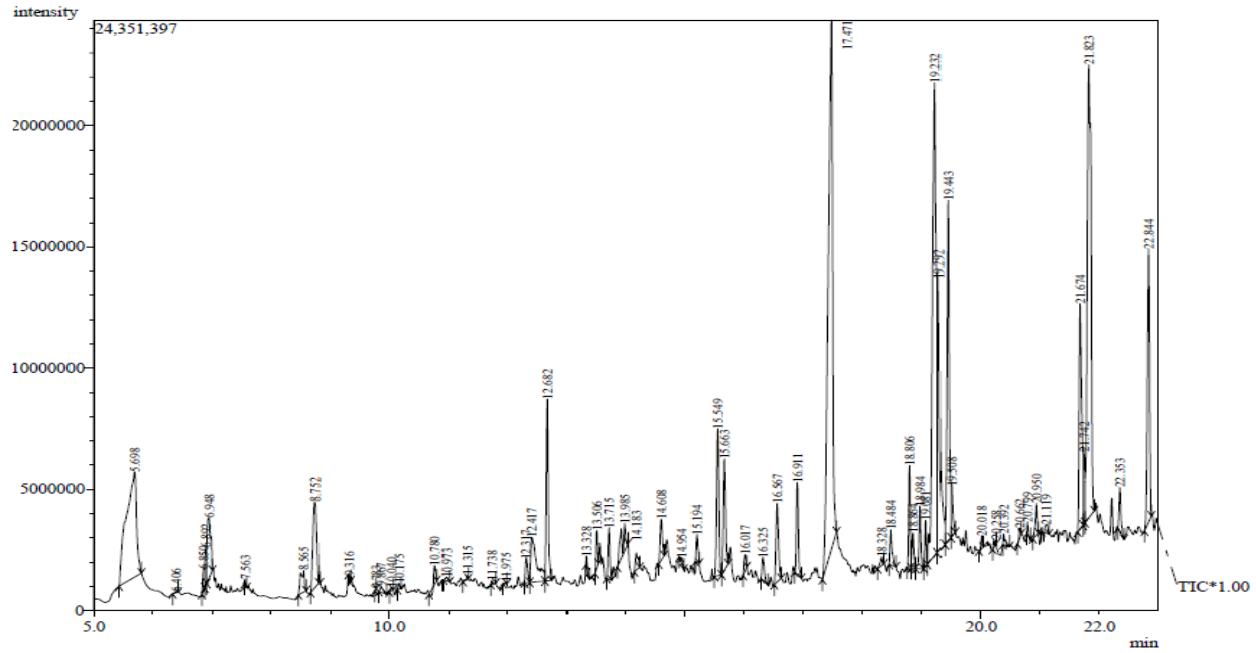

Fig. 9: GC-MS Chromatogram of methanolic extract of *E. variegata* L. bark extract.

Table 3: GC-MS spectral analysis of *E. variegata* L. bark extract.

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1.	5.698	1-Butanol	$C_6H_{12}O_2$	116	8.13
2	6.406	Acetamide	$C_5H_8N_2O_3$	144	0.02
3	6.850	7h-Pyrrolo[1,2-A]Azepine	$C_{10}H_9N$	143	0.33
4	6.892	1H-Indole-3-Ethanol	$C_{12}H_{13}NO_2$	203	0.33
5	6.948	4-Ethynylbenzaldehyde	C_9H_6O	130	1.61
6	7.563	1h-Indene	$C_{25}H_{42}$	342	0.05
7	8.565	1h-Indole	C_8H_7N	117	0.62
8	8.752	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150	2.38
9	9.316	O-Ethyl O-N-Octyl Ethyl Phosphonate	$C_{12}H_{27}O_3P$	250	-0.05
10	9.783	Dimethyl(Divinyl)Stannane	$C_6H_{12}Sn$	204	0.00
11	9.867	2H-Quinoline-1-Carboxylic Acid,	$C_{11}H_{13}NO_2$	191	0.11
12	10.040	1,5-Dihydroxy-1,2,3,4-Tetrahydronaphthalene	$C_{10}H_{12}O_2$	164	0.05
13	10.175	Benzaldehyde	$C_8H_8O_3$	152	0.08
14	10.780	2-Allyl-6-Methoxyphenol	$C_{10}H_{12}O_2$	164	0.19
15	10.97	Malonic Acid	$C_{16}H_{30}O_4$	286	0.07
16	11.315	Thiourea	$C_{19}H_{21}N_3OS$	339	0.04
17	11.738	1,2-Benzenediol	$C_{10}H_{14}O_2$	166	0.02
18	11.975	1,2,4-Cyclopentanetrione	$C_{10}H_{12}O_3$	180	0.01
19	12.317	Phenol	$C_{11}H_{16}O_2$	180	0.44
20	12.417	Quinoline	$C_{10}H_9N$	143	2.02
21	12.682	Caryophyllene Oxide	$C_{15}H_{24}O$	220	2.71
22	13.328	1H-3a,7-Methanoazulene-6-Methanol	$C_{15}H_{24}O$	220	0.03
23	13.506	Rosifoliol	$C_{15}H_{24}O$	220	0.44
24	13.715	"Neoclovenoxid-Alkohol"	$C_{15}H_{24}O$	220	0.70
25	13.985	Phenol,	$C_{11}H_{14}O_3$	194	0.93
26	14.183	Benzoic Acid	$C_{10}H_{12}O_4$	196	0.34
27	14.60	Tetradecanoic Acid	$C_{14}H_{28}O_2$	228	0.70
28	14.954	1-Cyclohexene-1-Methanol,	$C_{11}H_{20}O$	168	0.04
29	15.194	Pluchidiol	$C_{13}H_{20}O_2$	208	0.37
30	15.549	Epiglobulol	$C_{15}H_{26}O$	222	2.48
31	15.663	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	$C_{20}H_{40}O$	296	1.87
32	16.017	Pentadecanoic Acid	$C_{15}H_{30}O_2$	242	0.24
33	16.325	2-Methyl-7-Octadecyne	$C_{19}H_{36}$	264	0.37
34	16.567	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]Dodecane-2,9-Diol	$C_{15}H_{26}O_2$	238	1.47
35	16.911	Hexadecanoic Acid	$C_{17}H_{34}O_2$	270	1.32
36	17.471	Palmitic Acid	$C_{16}H_{32}O_2$	256	17.44
37	18.328	Methyl 2-Hydroxy-Hexadecanoate	$C_{17}H_{34}O_3$	286	0.14
38	18.484	Heptadecanoic Acid	$C_{17}H_{34}O_2$	270	0.54
39	18.806	Methyl 10-Trans,12-Cis-Octadecadienoate	$C_{19}H_{34}O_2$	294	1.15
40	18.864	Linolenic Acid	$C_{19}H_{32}O_2$	292	0.51

41	18.984	Phytol Isomer	$C_{20}H_{40}O$	296	0.86
42	19.081	Methyl 16-Methyl-Heptadecanoate	$C_{19}H_{38}O_2$	298	0.48
43	19.232	9,12-Octadecadienoic Acid (Z,Z)-	$C_{18}H_{32}O_2$	280	13.33
44	19.292	1,Z-5,E-7-Dodecatriene	$C_{12}H_{20}$	164	4.39
45	19.443	Stearic Acid	$C_{18}H_{36}O_2$	284	5.72
46	19.508	9h-Fluorene-4,5-Dicarbaldehyde	$C_{15}H_{10}O_2$	222	0.65
47	20.018	1,7-Dimethyl-9,10-Phenanthrenedione	$C_{16}H_{12}O_2$	236	0.11
48	20.258	5-Bromovaleric Acid	$C_{13}H_{23}BrO_2$	290	0.08
49	20.392	Carbonic Acid	$C_{18}H_{33}C_{l3}O_3$	402	0.12
50	20.662	Methyl Icosanoate	$C_{21}H_{42}O_2$	326	0.12
51	20.799	Octadecanoic Acid	$C_{19}H_{38}O_4$	330	0.08
52	20.950	Eicosanoic Acid	$C_{20}H_{40}O_2$	312	0.46
53	21.119	1-Acetyl-2,2,4-Trimethyl-4-Phenyl-1,2,3,4-Tetrahydroquinoline	$C_{20}H_{23}NO$	293	0.13
54	21.674	6,7,8-Trimethoxy-3,4-Dimethyl-1-Methylsulfanyl-3,4-Dihydroisoquinoline	$C_{15}H_{21}NO_3S$	295	4.37
55	21.742	Isoquinoline	$C_{18}H_{18}N_2O_4$	326	0.76
56	21.823	DiacetylLycorine Lactam	$C_{20}H_{19}NO_7$	385	12.74
57	22.353	Erythrinan-16-Ol	$C_{18}H_{21}NO_3$	299	6.63
58	22.844	2-Amino-5-Isopropyl-6,7-Dimethoxy-1,3-Azulenedicarbonitrile	$C_{17}H_{17}N_3O_2$	295	4.58

CONCLUSION

By considering the above data it can be concluded that the bark of *Erythrina variegata* L has medicinal values since it contains more secondary metabolites and its free radical scavenging activity were found to have considerable antioxidant potential. This plant also reveal better *in vitro* enzyme inhibitory activity (alpha amylase and alpha glucosidase) which are involved in regulation and absorption of carbohydrate, inhibition of non-enzymatic glycosylation of hemoglobin, anti-diabetic activity proved by glucose uptake assay and also exhibits good anti-inflammatory activity. The present data, illustrate that the methanolic extract of *Erythrina variegata* L. bark has good medicinal properties and it will be useful in treating various diseases including diabetes.

ACKNOWLEDGEMENT

We, the authors are thankful to Chancellor, Chief Executive Officer, Vice Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

Apostolidis E, Kwon YI, Shetty K. Inhibitory potential of herb, fruit, and fungal - enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov Food Sci Emerg Technol, 2007; 8:46–54.

Baskar N, Devi PB, Kumar MR. Anti cancer activity of methanol extract of root bark of *Erythrina variegata* Linn. Int J Toxicol Pharm Res, 2010; 2:74-76.

Blois MS. Antioxidant determinations by the use of stable free radical. Nature, 1958; 81:1199-2000.

Chandra S, Chatterjee P, Dey P, Bhattacharya S. Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Med, 2012; 178-180.

Chew YL, Chan EWL, Tan PL, Lim YY, Stanslas J and Goh JK. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminase medicinal plants in Peninsular. BMC complementary Altern Med, 2011; 11-12.

Daksha G, Chandrashekher KS, Pal G. *In vitro* antidiabetic activity of *Pentacyclic tritrprnoida* and fatty acid ester from *Bauhinia purpurea.* Int J Pharm Pharm Tech, 2013; 2:25-28.

Devaki K, Hemmalakshmi S, Priyanga S. HPTLC analysis and *in vitro* antioxidant activity of aqueous bark extract of *Erythrina variegata* L. Isr J Plant Sci, 2015; doi.org/10.1080/07929978.2015.1096608.

Devi RM, Manoharan A. Characteristics of pharmacognostical significance of *Erythrina variegata* Var. and *Ficus racemosa* Linn. bark. J Chem Pharm Res, 2011; 3:707-714.

Dinesh Kumar A, Mitra M, Manjunatha. *In vitro* and *in vivo* studies of antidiabetic Indian medicinal plants: A Review. J Herb Med Toxicol, 2009; 3:9-14.

Harborne JB. Phytochemical Methods 1st edn, London, Chapman and Hall, 1973, 288-504.

Joseph AP, Hemmalakshmi S, Priyanga S, Devaki K. Phytochemical screening, GC-MS analysis and enzyme inhibitory activity of *Passiflora foetida* L. Indo Am J of Pharm Res, 2014; 4:3526-3534.

Jyothi KSN, Hemalatha P, Calla S. Evaluation of alpha amylase inhibitory potential of three medicinally important traditional wild food plants of India. Int J Green Pharm, 2011; 95-99.

Kumar A, Lingadurai S, Jain A, Barman NR. *Erythrina variegata* Linn: A Review on morphology, phytochemistry, and pharmacological aspects. Pharmacogn Rev, 2010; 4:147–152.

Lalitharani S, Ramasamy V, Mohan and Regini GS. GC-MS analysis of ethanolic extract of *Zanthoxy lumrhetsa* (roxb.) DC spines. J Herb Med Toxicol, 2010; 4:191-192.

Mc Cue P, Vattern D, Shetty K. Inhibitory effect of *Clonal oregano* extracts against porcine pancreatic amylase *in vitro*. Asia Pac J Clin Nutr, 2004; 13:401-408.

Megha G, Chaudhari ,Bhoomi B, Joshi, Kinnari N. Mistry, Brijesh Dabhi and Lal S. *In vitro* antimicrobial activity of stem bark of *Bauhinia purpurea*. Int Sci Press, 2013; (India),4:29-35.

Megha G, Chaudhari, Bhoomi B, Joshi, Kinnari N, Mistry. *In vitro* anti diabetic and anti-inflammatory activity of stem bark of *Bauhinia purpurea.* Bull Pharm Med Sci, 2013; 1:139-150.

Ordon LE, Gomez JD, Vattuone MA and Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) swart extracts. Food Chem, 2006; 97:452-458.

Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japan J Nut, 1986; 7:307-315.

Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Med, 2012; 320-33.

Priyanga S, Hemmalakshmi S, Devaki K. Comparative chromatographic fingerprint profiles of ethanolic extract of *Macrotyloma uniflorum* L. leaves and stem. Int J Pharm Cli Res, 2014; 6:288-299.

Priyanga S, Hemmalakshmi S, Devaki K. Comparative phytochemical investigation of leaf, stem, flower and seed

extracts of *Macrotyloma uniflorum* L. Indo Am J Pharm Res, 2014; 4:5415-5420.

Priyanga S, Hemmalakshmi S, Sowmya S, Vidya B, Chella Perumal P, Gopalakrishnan VK, Devaki K. *In vitro* enzyme inhibitory evaluation and free radical scavenging potential of ethanolic leaf extract of *Macrotyloma uniflorum* (L.). Int J of Cur Pharm Rev and Res, 2015; 6:169-177.

Priyanga S., Hemmalakshmi S., Sowmya S., Vidya B., ChellaPerumal P., Gopalakrishnan V. K. And Devaki K. Quantitative evaluation and *in vitro* free radical scavenging ability of ethanolic stem extract of *Macrotyloma uniflorum* L. Der Pharmacia Lettre, 2016; 7:225-233.

Radhika S, Senthil Kumar R, Sindhu S, Sagadevan E, Arumugam P. Phytochemical investigation and evaluation of antihyperglycemic potential of *Premna corymbosa*. Int J Pharm PharmSci, 2013; 5:352-356.

Reddy NVLS, Anarthe SJ, Raghavendra NM. Effect of thermal treatment an phenolic content. J Res Biomed Sci, 2010; 1:72-75.

Reddy SK, Krishna Naik K, Karthikeyan R. Anti-inflammatory activity of the leaf extracts of *Erythrina variegata* L. Inventi Rapid: Ethnopharmacology, 2015; 1:1-2.

Sangeetha MS, Priyanga S, Hemmalakshmi S, Devaki K. *In vivo* antidiabetic potential of *Cyclea peltata* in streptozotocin‐induced‐diabetic rats. Asian J Pharm Cli Res, 2015; 8:103-108.

Sangita C, Priyanka C, Protapaditya D, Sanjib B.Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed, 2012; S178-S180.

Scharff T. Evidence for hexose transport in acetone-dried yeast. Arch Biochem Biophys, 1961; 95:329-335.

Sindhu S, Kavrekar V, Mishra A. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Euro J Exp Bio, 2013; 3:128-132.

Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phospho tugnstic acid reagents. Am J Enol Vitic, 1965; 16:144-158.

Sowmya S, Chella Perumal P, Anusooriya P, Vidya B, Pratibha P, Gopalakrishnan, V. K. *In vitro* antioxidant activity, *in vivo* skin irritation studies and HPTLC analysis of *Cayratia trifolia* (L.) Domin. Int J Toxicol Pharm Res, 2015; 7:1-9.

Trease GE, Evans WC (1996). Pharmacognosy 4th Edition, W B Saunders, USA: 243-283.Hh.

Upwar N, Patel R, Waseem N, Mahobia NK. Hypoglycemic effect of methanolic extract of *Berberis aristata* DC stem on normal and streptozotocin induced diabetic rats. Int J Pharm Pharm Sci, 2011; 3:222-224.

Vijayameena C, Subhashini G, Loganayagi M , Ramesh B. Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*. Int J Curr Microbiol App Sci, 2013; 2:1-8.

Wagner H, Bladt S. 2004. Plant Drug Analysis-A Thin Layer Chromatography Atlas. 2nd Ed, New Delhi, Thompson Press Ltd.

Wang P, Yuan H, Zhang X, Li Y, Shang L, Yin Z. Novel lycorine derivatives as anticancer agents: synthesis and *in vitro* biological evaluation. Molecules, 2014; 19:2469-2480.

West IC. Radicals and oxidative stress in diabetes. Diabet Med, 2000; 17:171-180.

How to cite this article:

Santhiya N, Priyanga S, Hemmalakshmi S, Devaki K. Phytochemical analysis, Anti inflammatory activity, In vitro antidiabetic activity and GC-MS profile of *Erythrina variegata* L. bark. J App Pharm Sci, 2016; 6 (07): 147-155.