BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 10(10)

Molecular docking and analgesic studies of *Erythrina variegata's* derived phytochemicals with COX enzymes

Mir Muhammad Nasir Uddin[1], Talha Bin Emran[2, 3], Muhammad Mamunur Rashid Mahib[2] & Raju Dash[3]*

[1]Department of Pharmacy, University of Chittagong, Chittagong-4331, Bangladesh; [2]Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh; [3]Department of Pharmacy, BGC Trust University Bangladesh, Chittagong-4000, Bangladesh; Raju Dash - Email: rajudash.bgctub@gmail.com; Phone: +8801815913253; *Corresponding author

Received September 21, 2014; Accepted September 22, 2014; Published October 30, 2014

Abstract:
Secondary metabolites from plants are a good source for the NSAID drug development. We studied the analgesic activity of ethanolic extract of *Erythrina variegata* L. (Fabaceae) followed by molecular docking analysis. The analgesic activity of *Erythrina variegata* L. is evaluated by various methods viz., acetic acid-induced writhing test, hot plate and tail immersion test. Subsequently, molecular docking analysis has been performed to identify compounds having activity against COX-1 and COX-2 enzymes by using GOLD docking fitness. The result of preliminary phytochemical screening revealed that the extract contains alkaloids and flavonoids. In analgesic activity tests, the extract at the doses of 50, 100 and 200 mg/kg body weight (b.w.) produced a increase in pain threshold in a dose dependent manner. In acetic acid induced writhing test, the inhibitory effect was similar to the reference drug diclofenac sodium. The extract showed 18.89% writhing inhibitory effect at the dose 200 mg/kg b.w., whereas diclofenac sodium showed 79.42% inhibition of writhing at a dose of 10 mg/kg b.w. The results of tail immersion and hot plate test also showed potential analgesic activity of the extract which is also comparable to the standard drug morphine (5 mg/kg b.w.). Docking studies shows that phaseollin of *Erythrina variegata* L. has the best fitness score against the COX-1 which is 56.64 and 59.63 for COX-2 enzyme. Phaseollin of *Erythrina variegata* L. detected with significant fitness score and hydrogen bonding against COX-1 and COX-2 is reported for further validation.

Keywords: *Erythrina variegata* L., Analgesic, COX-1, COX-2, *in silico* drug discovery, GOLD.

Background:
Natural products are a major source of drugs and about half of the pharmaceuticals in use today are derived from natural products **[1]**. Application of advanced drug screening methods show plant compounds have variety of structures with bioactivities, including anti-tumor, anti-inflammatory, anti-viral and hepatoprotective properties **[2]**. Inflammation and pain are major areas of interest for discovery starting with the identification of morphine form opium **[3, 4]**. The non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs in the control of postoperative pain but often cause a number of side-effects **[5]**. Cyclooxygenase (COX), the key enzyme required for the conversion of arachidonic acid to prostaglandins was first identified over 20 years ago **[6]**. The enzyme exists in at least two isoforms, COX-1 and COX-2. Although both the isoforms catalyze the same biochemical transformation, the two isoforms are subject to a different expression regulation **[7]**. COX-1 is a constitutive enzyme and is

responsible for the supply of prostaglandins which maintain the integrity of the gastric mucosa and provide adequate vascular homeostasis whereas COX-2 is an inducible enzyme and is expressed only after an inflammatory stimulus **[8]**. Literature studies indicate that direct tissue contact of NSAIDs gives the side effects like gastric upset, irritation, and ulceration **[9]**, and also confirms that gastrointestinal side effects of NSAIDs such as irritation and GI bleeding are due to the presence of a free carboxylic group in the parent drug **[10, 11]**. Thus, developing new agents with minimum or without side effects is an extensive research area in the present scenario. The genus *Erythrina* comprises of about 110 species of trees and shrubs. The name "coral tree" is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originally from India to Malaysia, but is native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas). It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250 m (800ft) in elevation. The coral tree is cultivated particularly as an ornamental tree and as a shade and soil improvement tree (it fixes nitrogen) for other tree crops such as coffee and cacao **[12, 13]**. Leaves are stomachic, anthelmintic, laxative, diuretic, gatactagogue and emmenagogue; applied externally for dispersing venereal buboes, relieve pain of the joints and inflammations; juice is poured in to the ear to relief earache and is used as an anodyne in toothache. The bark is astringent, febrifuge anti-bilious and anthelmintic; useful in dysentery and as a collyrium in ophthalmia. The roots are emmenagogue **[14, 15]**. Phytochemical studies **[16]** suggest that *Erythrina variegata* L. contain compounds like Isoquinoline, Isococcolinine, Erythrinin A, Erythrinin C, Erythrinin B, Osajin, Alpinum Isoflavone, Erythrabyssin II, Erycristagallin, 6-Hydroxygenistein, Lupiwighteone, Hypaphorine, Erysovine, Erysopitine, Erysotrine, Erysonine, Erythratidine, Erythrinine, Erythramine, Erythraline, Nororientaline, Erybidine, L-Reticuline, Coreximine, Euchrenone B10, Eryvarins Q, Abyssinone V, Phaseollin, Campesterol, Scoulerine, Erystagallin A, Orientanol B, Robustone, Stachydrine, Erysovine. Therefore, it is of interest to evaluate the analgesic activity of the ethanolic bark extract of *Erythrina variegata* L. and also the compound for this activity by *in silico* molecular docking analysis **[17].**

Methodology:

Plant material collection and identification

The whole plant was collected from Chittagong University hilly forest, Bangladesh on March 2012. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession No. 36148).

Preparation of plant material

Barks were sundried for 7 days and later dried in drier at 40°C for about an hour. The dried bark were then ground into powder using high capacity grinding machine and stored in airtight plastic container with necessary markings for identification and kept in cool, dark and dry place for the investigation. The bark of the plant material was extracted with ethanol. After completion of the extraction, the liquid was filtered using a sterilized cotton filter **[18]**. It was then evaporated by rotary evaporator for about 1 hr. Further, it was kept for drying till it solidifies from liquid form. Then solvent was completely removed and obtained 6 g (yield 1.5%) dried crude extract which was used for preliminary phytochemical group tests and then subjected to for rest of the experiments.

Preparing animals

For the experiment Adult Swiss albino mice (BALB/c) weighing between (12-300) g of either sex were collected from animal sources department of ICDDRB, Dhaka. The animals were maintained under normal laboratory condition and kept in standard polypropylene cages at room temperature of (30 ± 2)°C and 60% to 65% relative humidity and provided with standard diet and water. The institutional animal ethical committee approved all protocols for animal experiment. Each group consists of five mice and to denote individual animal, they were marked as group I, II, III, for test samples at the doses of 50, 100 and 200 mg/kg body weight and a control and positive control group was also maintained for every tests.

Preliminary phyto-chemical screening

One gram of the ethanol extract of *E. variegata* was dissolved in ethanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents **[19]**.

Acetic acid-induced writhing test

The anti-nociceptive activity of the extract was studied using acetic acid-induced writhing model in mice **[20]**. The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test samples at the doses of 50, 100 and 200 mg/kg body weight. Positive control group received standard drug diclofenac sodium at the dose of 10 mg/kg body weight. Test samples were administered orally 30 min before intra-peritoneal administration of 0.6% acetic acid but diclofenac sodium was administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 30 min **[21]**.

Hot plate method

The paws of mice are very sensitive to temperature at (55 ± 0.5)°C, which are not damaging to the skin. The animals were placed on Eddy's hot plate kept at a temperature of (55 ± 0.5)°C. A cut off period of 30 sec **[22]**, was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped at 0, 30, 60 90 and 120 min after oral administration of the samples **[23]**. The animals of test groups received test samples at the doses of 50, 100 and 200 mg/kg body weight. Positive control group received standard drug diclofenac sodium at the dose of 10 mg/kg b.w. and saline water.

Tail immersion test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice animals of the control, positive control and test groups were treated with diclofenac sodium (10 mg/kg body weight), saline water (10 ml/kg body weight) and test samples at the doses of 50, 100 and 200 mg/kg body weight respectively. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of

the next three readings. A latency period of 30 sec was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was taken as the index of anti-nociception and was determined at 0, 30, 60, 90 and 120 min after the administration of the test drugs and standard **[24]**. Definition of groups and their treatments were as some of the hot plate test. The hot plate latencies and tail flick were sequentially measured at 0, 30, 60, 90 and 120 min with the same cut off time of 30 sec for the safety of animals.

Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM. Data were analyzed by paired sample t test. The results obtained were compared with the control group. The criterion for statistical significance was ***p< 0.01 and *p< 0.05. All the statistical tests were carried out using SPSS statistical software.

In silico molecular docking analysis

For docking analysis Gold 4.12 is used to predict the potent active compound *E. variegata* against the active site of COX-1 and COX-2 enzymes where compounds are collected from the literature review.

Ligands preparation

From the literature review, all compounds-Isoquinoline, Isococcolinine, Erythrinin A, Erythrinin C, Erythrinin B, Osajin, Alpinum Isoflavone, Erythrabyssin II, Erycristagallin, 6-Hydroxygenistein, Epilupeol, Lupiwighteone, Hypaphorine, Erysovine, Erysopitine, Erysotrine, Erysonine, Erythratidine, Erythrinine, Erythramine, Erythraline, Nororientaline, Erybidine, L-Reticuline, Coreximine, Euchrenone B10, Eryvarins Q, Abyssinone V, Phaseollin, Scoulerine, Erystagallin A, Orientanol B, Robustone, Stachydrine, Erysovine are drawn in Symyx Draw 4.0 and then prepared for docking using the Sybyl 7.3 Molecular Modeling Suite of Tripos, Inc. 3D conformations were generated using Concord 4.0 **[25]**, hydrogen atoms were added and charges were loaded using the Gasteiger and Marsili charge calculation method **[26]**. Basic amines were protonated and acidic carboxyl groups were de-protonated prior to charge calculation. The AMPPD ligand was minimized with the Tripos Force Field prior to docking using the Powell method with an initial Simplex **[27]** optimization and 1000 iterations or gradient termination at 0.01 kcal/(mol*A). Input ligand file format was mol2 for all docking programs investigated.

Protein preparation and active site determination

The crystal structure COX-1 and COX-2 enzymes are collected protein data bank **[28]** pdb id: 2OYE (COX-1) and 6 COX (COX-2). Two enzymes are prepared according to the docking protocol of Gold. The active site of these enzyme identified according to the giving information by Harman et al. 2007 **[29]** for COX-1 and Kurumbail *et al*. 1996 **[30]** for COX-2.

Docking using GOLD (Genetic Optimization for Ligand Docking)

GOLD utilizes genetic algorithm to explore the rotational flexibility of receptor hydrogens and ligand conformational flexibility **[31]**. In GOLD docking was carried out using the wizard with default parameters population size (100); selection pressure (1.1); number of operations (10,0 00); number of islands (1); niche size (2); and operator weights for migrate (0), mutate (100) , and crossover (100) were applied. The active site with a 10 Å radius sphere was defined by selecting an active site residue of protein. Default Genetic Algorithm settings were used for all calculation s and a set of 10 solutions were saved for each ligand. GOLD was used by a GoldScore fitness function. GoldScore is a molecular mechanism like function and has been optimized for the calculation of binding positions of ligand. It takes into account four terms:

$$\text{Fitness} = S_{(hb\_ext)} + 1.3750*S_{(vdw\_ext)} + S_{(hb\_int)} + 1.0000*S_{(int)}$$
$$S_{(int)} = S_{(vdw\_int)} + S_{(tors)}$$

Where, S_{hb_ext} is the protein-ligand hydrogen bonding and s_{vdw_ext} are the vanderwaals interactions between protein and ligand. S_{hb_int} are the intramolecular hydrophobic interactions whereas $S_{vdw\_\ int}$ is the contribution due to intra molecular strain in the ligand.

Phytochemical screening

Preliminary phytochemical screening of the crude ethanolic extracts of the barks of *E. variegata* revealed the presence of alkaloid and flavonoids **Table 1 (see supplementary material).**

Acetic acid-induced abdominal writhing test

The analgesic effect ethanolic extracts of *E. variegata* at dose level of 50, 100 and 200 mg/kg body weight on acetic acid induced writhing in mice was exhibited. Doses of the extract inhibited writhing response induced by acetic acid in a dose dependent manner in which group III (200 mg/kg b.w.) exhibits the highest 18.89% of inhibition and is comparable to the reference drug diclofenac sodium (79.42%) **Table 2 (see supplementary material).**

Hot plate test

The tail withdrawal reflex time following administration of the ethanolic extracts of *E. variegata* at dose level of 50, 100 and 200 mg/kg b.w. was found almost remain the same consistency with increasing dose of the sample which is comparable to the reference drug **Table 3 (see supplementary material).**

Tail immersion test

The tail withdrawal reflex time following administration of the ethanolic extracts of *E. variegata* at dose level of 50, 100 and 200 mg/kg b.w. was found almost remain the same consistency with increasing dose of the sample which is comparable to the reference drug **Table 4 (see supplementary material).**

Docking analysis

The compounds of *E. variegata* mentioned above were subjected to dock in the active site of COX-1 and COX-2 enzyme by gold docking method. The results of docking analysis of COX-1 and COX-2 enzyme are listed in **Table 5 (see supplementary material).** After docking the ligand protein complex was saved in pdb format then subjected for analysis in the Accelrys Discovery Studio Visualizer. Docking studies showed that phaseollin has the best gold fitness score against the COX-1 which is 56.64 and 59.63 for COX-2 enzyme. Molecular analysis showed that phaseollin form two hydrogen bonds with residues of the active site of COX-1 enzyme. It is that phaseollin made bonds with TYR354 with a distance 2.98478 Å between 4

no oxygen of phaseollin with hydrogen of Tyr354. Another hydrogen bond was formed between 1 no hydrogen of phaseollin and O of SER499 where bonding distance is 1.86273 Å. Similarly, In COX-2 enzyme two hydrogen bonds are formed between the oxygen of SER499 and hydrogen of TYR354 with the hydrogen at 1st and oxygen at 4th position of phaseollin where bonding distance is 1.48447 Å and 1.54784 Å respectively. Interaction between the phaseollin with the COX-1 and COX-2 are represented in **Figure 1**.

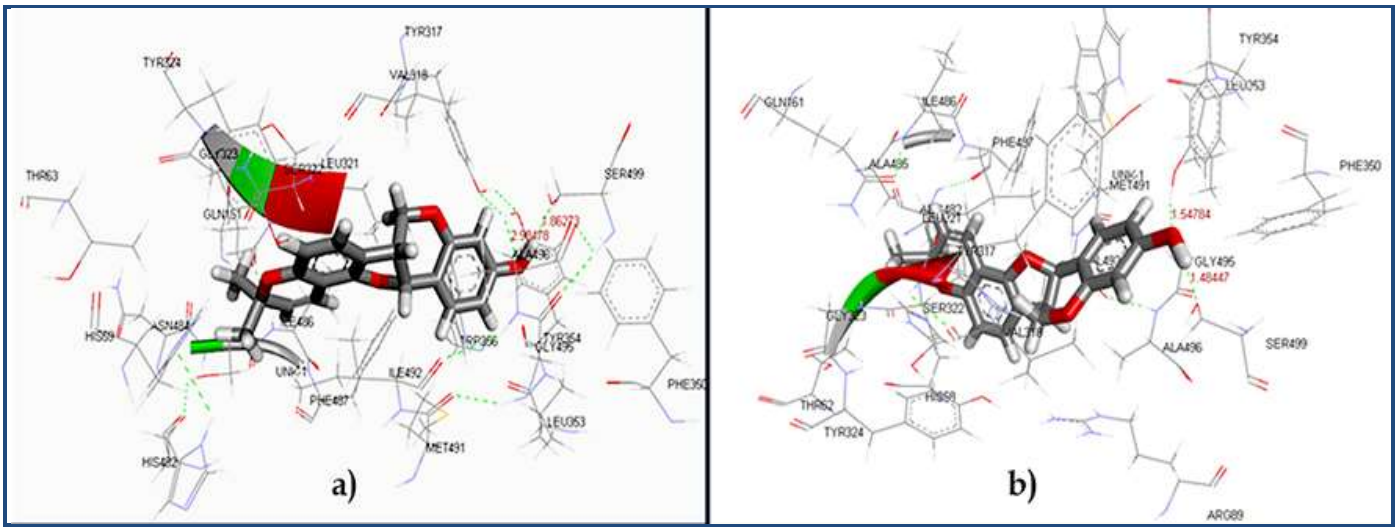

Figure 1: Interaction of phaseollin with a) COX-1 and b) COX-2.

Discussion:

The present study has established analgesic potential of *E. variegata* using acetic acid induced writhing test for visceral pain and tail immersion and hot plate tests for pain mediated by central activity. Acetic acid induced writhing in mice is a model of visceral pain, which is highly sensitive and useful for screening peripherally acting analgesic drugs. *E. variegata* plant extracts caused dose dependent anti-nociception against chemical induced pain in mice. Ethanolic extracts of the bark of *E. variegata* were treated in test animals at a dose of 50, 100 and 200 mg/kg b.w. The ethanolic extracts of *E. variegata at* the dose of 200 mg/kg b.w. were found to exhibit the highest 18.89% writhing inhibitory response, where the reference drug diclofenac sodium showed about 79.42% writhing inhibitory response at a dose of 10 mg/kg b.w.

The tail immersion test is considered to be selective to examine compounds acting through non-opoid receptor; the extract increased mean basal latency, which indicates that it may act via centrally, mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non-steroidal anti-inflammatory drugs inhibit only peripheral pain **[32]**. The extract inhibited both mechanisms of pain, suggesting that the plant extract may act as a narcotic analgesic.

The hot plate and tail immersion tests are widely used for assessing central anti-nociceptive activities. Opioid agents exhibit their analgesic effects both via supra-spinal and spinal receptors **[33]**. The present experiments, ethanolic extracts of *E. variegata* exhibited a statistically significant. It seems quite possible that the lower doses of the extract have more potent central anti-nociceptive effect. It has been suggested that the opioid mechanisms mediate anti-nociceptive effect of ethanolic extracts of *E. variegata*.

Ethanolic extract of *E. variegata* produced a dose-dependent anti-nociceptive effect on the glutamate-induced paw licking response. Recently, found that the nociceptive response induced by glutamate appears to involve peripheral, spinal and supraspinal sites of action and is greatly mediated by both NMDA and non- NMDA receptors as well as by the release of nitric oxide or by some nitric oxide-related substance. Hence, an effect of the plant extract directly on the receptors or second messengers related to these transmitters could avoid the nociceptive response. The effect of *E. variegata* against nociception induced by glutamate is of great interest since glutamate plays a significant role in nociceptive processing in both central and peripheral nervous systems **[34]**.

Advances in computational techniques have enabled virtual screening to have a positive impact on the discovery process. Virtual screening utilizes docking and scoring of each compound from a dataset and the technique used is based on predicting the binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure **[35]**. Some recent studies have focused on certain factors such as the size and diversity of the ligand dataset, wide range of targets and the evaluation of docking programs **[36]**. In our present studies, by means of gold docking, we docked 33 compounds of *E. variegata* into the active site of the COX-1 and COX-2 enzymes. In view of the above, fitness score values were measured using GOLD 4.12 showed that phaseollin has the highest fitness score of 56.64 was noticed with COX-1 and fitness score of 59.63 was observed for COX-2, suggesting that more interaction of phaseollin has more in COX-1 and COX-2 than the other compounds.

Conclusion:

Results show that plant extract of *E. variegata* possesses moderate analgesic potential. Though the involvement of

opioid receptor has been determined using naloxone, further studies are needed using different agonists (such as adrenergic, serotonergic etc.) to completely understand the exact mechanisms of its anti-nociceptive activity. It seems possible that *E. variegata* contains chemical constituents with analgesic property for consideration in drug development. In the present study the analgesic activity of the ethanolic bark extract of *E. variegata* was done and its compound was successfully docked onto the both COX-1 and COX-2. Thus, phaseollin could be considered as a potent analgesic molecule against COX-1 and COX-2 for further validation.

Competing interests:
All authors declare that they have no competing interests.

Authors' contributions:
MMNU has designed the study, performed data analysis and interpretation and wrote the manuscript. TBE has wrote the manuscript and also modify the *in silico* parts. MMRM has provided assistance in taxonomical identification and collections of voucher specimen's numbers for the plants and modify the *in silico* parts. RD has performed data analysis and wrote the *in silico* parts.

References:

[1] Clark AM, *Pharm Res*. 1996 **13**: 1133 [PMID: 8865302]
[2] Zhang GL *et al. Zhongguo Zhong Yao Za Zhi*. 2007 **32**: 2089 [PMID: 18306734]
[3] Appendino G *et al. Nat Prod Rep*. 2014 **31:** 880 [PMID: 24823967]
[4] Wang J *et al. Chem Biol Drug Des*. 2014 **11**: 12316 [PMID: 24618048]
[5] Sandri A, *Minerva Med*. 2014 **105:** 313 [PMID: 25078485]
[6] Dubois RN *et al. Faseb J*. 1998 **12**: 1063 [PMID: 9737710]
[7] Chen YF *et al. Health Technol Assess*. 2008 **12**: 1 [PMID: 18405470]
[8] Ishikawa TO *et al. Biochem Biophys Res Commun*. 2009 **378:** 534 [PMID: 19061862]
[9] Lanza FL, *Am J Gastroenterol*. 1998 **93:** 2037 [PMID: 9820370]
[10] Metwally KA *et al. Eur J Med Chem*. 2007 **42**: 152 [PMID: 17052805]
[11] Geronikaki AA & Gavalas AM, *Comb Chem High Throughput Screen*. 2006 **9**: 425 [PMID: 16842224]
[12] Pompeng P *et al. Phytomedicine*. 2013 **20**: 918 [PMID: 23639188]
[13] Kalachaveedu M *et al. J Pharmacol Pharmacother*. 2011 **2**: 285 [PMID: 22025860]
[14] Kongmanila D *et al. Trop Anim Health Prod*. 2012 **44**: 1659 [PMID: 22461199]
[15] Balamurugan G & Shantha A, *J Pharm Bioallied Sci*. 2010 **2**: 350 [PMID: 21180471]
[16] Kumar A *et al. Pharmacogn Rev*. 2010 **4:** 147 [PMID: 22228954]
[17] Zhao H & Caflisch A, *Eur J Med Chem*. 2014 **4:** 731 [PMID: 25108504]
[18] Mazzolin LP *et al. J Ethnopharmacol*. 2013 **150**: 1016 [PMID: 24157378]
[19] Pradeep BV *et al. J Environ Biol*. 2013 **34:** 645 [PMID: 24617153]
[20] Harasstani OA *et al. Inflamm Res*. 2010 **59:** 711 [PMID: 20221843]
[21] Momin MA *et al. Asian Pac J Trop Biomed*. 2014 **4:** 18 [PMID: 24144125]
[22] Franzotti EM *et al. J Ethnopharmacol*. 2000 **72:** 273 [PMID: 10967481]
[23] Suresha RN *et al. J Adv Pharm Technol Res*. 2014 **5:** 129 [PMID: 25126534]
[24] Umukoro S *et al. J Basic Clin Physiol Pharmaco*. 2014 **6:** 2013 [PMID: 24598834]
[25] Hevener KE *et al. J Chem Inf Model*. 2009 **49**: 444 [PMID: 19434845]
[26] Hristozov DP *et al. J Comput Aided Mol Des*. 2007 **21:** 617 [PMID: 18008169]
[27] Osolodkin DI *et al. Chem Biol Drug Des*. 2011 **78:** 378 [PMID: 21679374]
[28] Berman HM *et al. Nucleic Acids Res*. 2000 **28**: 235 [PMID: 17142228]
[29] Harman CA *et al. J Biol Chem*. 2007 **282**: 28096 [PMID: 17656360]
[30] Kurumbail RG *et al. Nature* 1996 **384**: 644 [PMID: 8967954]
[31] Jones G *et al. J Mol Biol*. 1997 **267**: 727 [PMID: 9126849]
[32] de Souza ET *et al. Mar Drugs*. 2009 **7:** 689 [PMID: 20098607]
[33] Hajhashemi V & Klooshani V, *Avicenna J Phytomed*. 2013 **3:** 193 [PMID: 25050274]
[34] Ferreira J *et al. Br J Pharmacol*. 2004 **141:** 787 [PMID: 14967737]
[35] Franca TC *et al. Curr Comput Aided Drug Des*. 2013 **9**: 507 [PMID: 24341424]
[36] Song Y *et al. Curr Med Chem*. 2013 **21**: 329 [PMID: 24164196]

Edited by P Kangueane
Citation: **Uddin *et al.*** Bioinformation 10(10): 630-636 (2014)

BIOINFORMATION open access

Supplementary material:

Table 1: Result of chemical group test of the ethanolic extracts of the bark of *Erythrina variegata* L.

Plant Extract	Alkaloid	Carbohydrate	Flavonoid	Glycoside	Glucoside	Saponin	Tannin
Ethanolic extract of *E. variegata*	++	--	++	--	--	--	--

(++): Present; (--): Absent

Table 2: Effect of bark extract of *Erythrina variegata* L. extract on acetic acid-induced abdominal writhing test.

Treatment	Dose (mg/kg)	% of Inhibition
Control	10	-
Diclofenac sodium (Std.)	10	79.42**
Group I	50	0.24
Group II	100	8.47
Group III	200	18.89

Values are mean ± SEM (n=5), * (p< 0.05),** (p< 0.01),*** (p< 0.001) significantly different when compared with the corresponding value of standard group, done by independent sample t-test.

Table 3: Effect of bark extract of *Erythrina variegata* L. extract on hot plate test.

Treatment	Dose (mg/kg)	Response Times (in seconds) 0 min	30 min	60 min	90 min	120 min
Control	10	7.80 ± 1.45	13.76 ± 0.55	14.87 ± 0.86	12.98 ± 0.17	15.02 ± 1.13
Diclofenac sodium (Std.)	10	11.2 ± 1.17	13.95 ± 1.48	21.01 ± 2.34	18.39 ± 2.86	24.26 ± 2.28*
Ethanolic extract of *E. variegate*	50	8.13 ± 0.72	11.01 ± 1.14	13.79 ± 1.24	15.4 ± 1.42	13.88 ± 0.73
	100	9.45 ± 0.08	12.81 ± 1.12	14.23 ± 0.99	14.72 ± 2.73	17.05 ± 1.67
	200	12.66 ± 1.67	15.51 ± 2.96	21.42 ± 2.52*	20.35 ± 3.24	14.5 ± 2.41

Values are mean ± SEM (n=5), * (p< 0.05),** (p< 0.01),*** (p< 0.001) significantly different when compared with the corresponding value of standard group, done by independent sample t-test

Table 4: Effect of ethanolic extract of *Erythrina variegata* L. on tail immersion test.

Treatment	Dose (mg/kg)	Response Times (in seconds) 0 min	30 min	60 min	90 min	120 min
Control	10	4.33 ± 0.42	6.01 ± 1.44	5.45 ± .89	4.41 ± 0.46	3.31 ± 0.38
Diclofenac sodium (Std.)	10	4.71 ± 0.67	11.65 ± 0.58**	13.72 ± 0.79**	14.79 ± 1.20**	10.79 ± 1.26**
Ethanolic extract of *E. variegate*	50	2.79 ± 0.24	4.23 ± 0.26	5.05 ± 0.73	4.68 ± 0.38	3.55 ± 0.49
	100	2.96 ± 0.10	3.46 ± 0.16	4.22 ± 0.34	4.38 ± 0.24	3.94 ± 0.5
	200	2.65 ± 0.15	3.38 ± 0.16	3.85 ± 0.29	3.94 ± 0.30	3.62 ± 0.44

Values are mean ± SEM (n=5), * (p< 0.05),** (p< 0.01),*** (p< 0.001) significantly different when compared with the corresponding value of standard group, done by independent sample t-test.

Table 5: Gold docking result of *Erythrina variegata* L. compounds against COX-1 and COX-2.

Compounds Name	COX-1 Gold Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	COX-2 Gold Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)
Nororientaline	46.67	5.12	40.14	0.00	-13.65	50.18	3.11	43.88	0.00	-13.27
Erythraline	45.04	0.00	35.92	0.00	-4.35	28.88	0.01	25.77	0.00	-6.56
Erythramine	42.08	1.99	35.25	0.00	-8.38	40.66	7.39	25.85	0.00	-2.27
Erythrinine	39.93	0.53	34.05	0.00	-7.42	31.52	0.00	28.18	0.00	-7.23
Erythratidine	44.39	0.00	38.16	0.00	-8.08	45.40	3.84	38.18	0.00	-10.94
Erysonine	41.54	0.15	34.19	0.00	-5.62	33.52	0.07	29.81	0.00	-7.55
Erysotrine	35.64	0.00	33.51	0.00	-10.44	36.32	0.00	36.25	0.00	-13.53
Robustone	47.42	3.72	36.16	0.00	-6.01	37.73	0.00	32.97	0.00	-7.60
Erycricstagallin	53.50	0.59	49.92	0.00	-15.73	34.54	8.15	31.61	0.00	-17.07
Orientanol B	30.41	0.76	29.43	0.00	-10.81	25.13	4.43	27.30	0.00	-16.83

Erystagallin A	34.21	5.64	39.40	0.00	-25.60	36.50	5.13	45.40	0.00	-31.05
Erysopitine	38.41	0.00	32.58	0.00	-6.38	38.55	0.23	31.99	0.00	-5.66
Scoulerine	50.22	0.41	41.32	0.00	-7.00	48.43	0.01	41.33	0.00	-8.41
Erysovine	34.41	0.47	32.77	0.00	-11.12	37.25	4.32	32.13	0.00	-11.24
Campesterol	-10.60	0.00	34.33	0.00	-57.80	-79.83	0.00	-10.84	0.00	-64.92
Phaseollin	56.64	0.29	53.02	0.00	-16.55	59.63	**3.53**	**42.26**	**0.00**	**-2.00**
Abyssinone V	45.82	2.08	50.14	0.00	-25.20	34.21	5.64	39.40	0.00	-25.60
Eryvarins Q	44.27	5.74	44.94	0.00	-23.27	40.50	2.91	43.75	0.00	-22.56
Euchrenone B10	43.61	0.00	39.47	0.00	-10.66	18.96	1.29	22.20	0.00	-12.86
Coreximine	54.64	0.96	44.08	0.00	-6.92	44.29	2.72	36.35	0.00	-8.41
L-Reticuline	48.78	0.15	49.43	0.00	-19.33	44.55	2.91	45.12	0.00	-20.41
Erybidine	54.45	2.00	44.72	0.00	-9.04	54.43	0.15	47.04	0.00	-10.40
Stachydrine	20.55	8.07	16.18	0.00	-9.76	29.66	9.34	21.79	0.00	-9.65
Hypaphorine	42.97	0.79	38.45	0.00	-10.69	45.03	7.34	37.61	0.00	-14.03
Lupiwighteone	51.89	0.30	47.54	0.00	-13.78	52.95	0.07	48.18	0.00	-13.36
Hydroxygeniste in	47.52	1.43	40.43	0.00	-9.51	44.77	3.39	37.14	0.00	-9.69
Erycristagallin	53.50	0.59	49.92	0.00	-15.73	34.54	8.15	31.61	0.00	-17.07
Erythrabyssin II	54.34	2.00	43.19	0.00	-7.04	33.35	4.77	35.12	0.00	-19.72
Alpinum Isoflavone	49.35	2.95	38.94	0.00	-7.15	35.42	0.00	32.06	0.00	-8.67
Osajin	43.93	3.18	38.14	0.00	-11.69	42.60	0.00	42.84	0.00	-16.30
Erythrinin B	43.68	2.36	40.80	0.00	-14.78	38.52	0.08	41.49	0.00	-18.61
Erythrinin C	50.89	1.27	41.59	0.00	-7.57	38.43	0.11	33.93	0.00	-8.34
Erythrinin A	39.77	0.10	32.65	0.00	-5.22	35.78	0.00	31.13	0.00	-7.02
Isococcolinine	35.88	0.00	32.37	0.00	-8.63	37.18	0.00	34.69	0.00	-10.52
Isoquinoline	31.79	0.52	22.74	0.00	-0.00	32.69	0.00	23.78	0.00	-0.00