



# EVALUATION OF ANTI-INFLAMMATORY AND NITRIC OXIDE SCAVENGING ACTIVITY OF *BAUHINIA VARIEGATA* L. LEAVES BY *IN VITRO* METHOD

Natasha N. Aggarwal<sup>1</sup>, Santanu Saha<sup>2\*</sup>, D.G. Banylla Felicity<sup>1</sup>, B.C. Revanasiddappa<sup>1</sup> and M. Vijay Kumar<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Paneer, Deralakatte, Mangalore-575018 (Karnataka) India.

<sup>2\*</sup>Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Paneer, Deralakatte, Mangalore-575018 (Karnataka) India.

## Abstract

Antioxidant plays a major role in treatment of most of the ailments including inflammatory diseases. Inhibition of free radical generation is the foremost step to stop inflammatory reaction in body and antioxidant is successfully carrying out that activity. Hence, the present study was carried out to evaluate preliminary anti-inflammatory and antioxidant properties of ethanolic extract of leaves of *Bauhinia variegata* L. by *in vitro* models. The anti-inflammatory activity of the plant extract (BVE) was evaluated by the assay of inhibition of bovine serum albumin and egg albumin denaturation and antioxidant property by nitric oxide scavenging assay. BVE showed the protein denaturation inhibitory effect with IC<sub>50</sub> value 35.84 µg/ml and at 50 µg/ml concentration showed 63.82% inhibition of protein denaturation in bovine serum albumin denaturation assay. The standard drug diclofenac sodium at the same concentration showed 77.52% inhibition. In egg albumin denaturation assay IC<sub>50</sub> value for standard drug and BVE was calculated 33.08 and 40.32 µg/ml respectively. Standard drug diclofenac sodium showed 65.92% inhibition of protein denaturation at concentration of 50 µg/ml in egg albumin denaturation assay and at the same concentration test drug BVE showed 46.77% of inhibition. Whereas, BVE showed 36.62% of nitric oxide scavenging activity at 50 µg/ml concentration and IC<sub>50</sub> value was calculated 64.81 µg/ml in the study. The results of this preliminary study suggested the anti-inflammatory and antioxidant property of the plant.

**Key words:** Anti-inflammatory, Antioxidant, *Bauhinia variegata*, Bovine serum albumin, Fabaceae, Nitric oxide scavenging assay.

## Introduction

Inflammation, a pathological condition is associated with most of the diseases, like, arthritis, gastritis, spondylitis, diabetes, and asthma. As a defensive mechanism of body, inflammation is caused by tissue damage due to external and internal stimuli; and characterized by pain, redness, heat, swelling and loss of function of the area (Simmons, 2006; Fangkrathok, 2013; Vane and Botting, 1995; Kumar *et al.*, 2011). These external and internal stimuli produce free radicals in form of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in mammalian body (Cheeseman, 1993). Previous studies have helped to establish the etiology of development of inflammation which attributed to the over

production of nitric oxide (NO<sup>•</sup>) from macrophages and neutrophils present at the site of tissue injury/infection (Billiar, 1995; Billiar and Harbrecht, 1997; Boscá *et al.*, 2005; Diaz *et al.*, 2012; Sharma *et al.*, 2007; Tang *et al.*, 2004; Tripathi *et al.*, 2007). Hence, the present study was carried out to search for a safe and potent anti-inflammatory drug from natural sources with nitric oxide scavenging potential.

India has vast and diversified botanical resources. These resources have helped to develop traditional system of medicine. Though the traditional system of medicine is well versed and commonly practiced throughout India, it has its own limitation that is lack of scientific evidence. *Bauhinia variegata* L. (Fabaceae), a common Indian medicinal plant which is being traditionally used as

\*Author for correspondence : E-mail: santanusaha@nitte.edu.in

anthelmintic, astringent, anti-leprotic, liver tonic, antibacterial etc (Kirtikar and Basu, 2006; Bhatnagar *et al.*, 1973; Mali *et al.*, 2007; Nadkarni, 2009). As per the traditional systems the plant is also useful in the treatment of dysmenorrhoea, skin diseases, wounds, edema, dysentery, ulcers, eye disease, piles, hemorrhoids and snake bite (Azevedo *et al.*, 2006; Mohamed *et al.*, 2009; Yadava *et al.*, 2003; Raj Kapoor *et al.*, 2009; Bodakhe *et al.*, 2007). Hence, in this study the ethanolic extract of leaves of *B. variegata* L. was evaluated for anti-inflammatory and antioxidant activity by *in vitro* models assay of inhibition of bovine serum albumin and egg albumin denaturation and nitric oxide scavenging assay respectively.

## Materials and Methods

### Chemicals and drugs

All the chemicals and reagents used in this study were of analytical grade. The chemicals were obtained in high purity either from Himedia, Mumbai, India or Loba Chemicals, India. Diclofenac sodium was obtained as a gift sample from Cipla pharmaceuticals, Ahmedabad, Gujrat, India.

### Plant material

Leaves of *Bauhinia variegata* L. were collected from Mangaluru, Karnataka. It was authenticated by Dr. Jyothi Miranda, Head, Dept. of Botany, St. Aloysius College, Mangaluru. A voucher specimen was deposited in the herbarium of NGSIM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangaluru.

### Preparation of plant material

Shade dried and coarsely powdered leaves of *Bauhinia variegata* L. was extracted by maceration method with ethanol (95%). The extracts was concentrated by rotary flash evaporator at 40°C temperature under reduced pressure and stored in deep freezer at -20°C (Prashant *et al.*, 2011). The concentrated ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) was subjected to evaluation of anti-inflammatory and antioxidant activity by *in vitro* models.

### *In-vitro* anti-inflammatory activity

- Inhibition of bovine serum albumin denaturation method:

Test extracts BVE and standard drug Diclofenac sodium at different concentration 10-50 µg/ml were mixed separately with 1% aqueous solution of bovine serum albumin. The pH was adjusted to 6.3±0.5 with 1N HCl and then incubated at 37°C for 20 minutes. Each mixture containing test extract and standard drug were then

heated separately at 57°C for 3 minutes and then cooled. The absorbance of those reaction mixtures were measured at 416nm after the addition of 2.5ml of phosphate buffer and using the vehicle as blank (Mizushima and Kobayashi, 1968). The investigation was carried out in triplicates. Percentage of protein denaturation inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100$$

### Inhibition of egg albumin denaturation method

2 mL of test extracts BVE and standard drug Diclofenac sodium at different concentration 10-50 µg/ml were mixed separately with 0.2 mL of egg albumin from hen's egg and 2.8 mL of pH 6.4 phosphate buffered saline. These contents were mixed thoroughly and incubated at 37°C for 15 min. After incubation each mixture was kept in a water bath at 70°C for 5 min. The absorbance was recorded at 660nm, using the vehicle as blank (Chandra *et al.*, 2012). Each of the experiments was performed in triplicates. Percentage inhibition of protein denaturation was evaluated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100$$

### *In-vitro* antioxidant activity

- Nitric oxide radical scavenging method:

Sodium nitroprusside solution is used in the test as it is generating Nitric oxide (NO) radical at a physiological pH. 1ml of each test compound BVE and standard drug Ascorbic acid at different concentration (10-50 µg/ml) in phosphate buffer (pH 7.4) were mixed separately with Sodium nitroprusside (1ml of 10mM). Each mixture was incubated at 25°C for 150 min. To 1 ml of the each incubated solution, 1ml of Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl

**Table 1:** Study of anti-inflammatory activity of ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) by inhibition of bovine serum albumin denaturation method.

Concentration (µg/ml)	Standard Drug	BVE
10	25.58±0.07	5.03±0.05
20	33.67±0.02	17.09±0.08
30	50.13±0.05	25.4±0.03
40	61.65±0.04	37.44±0.01
50	77.52±0.04	63.82±0.06
IC <sub>50</sub>	30.71	35.84
Each value is expressed as mean ± SD of three replicates for three triplicates (n=3)		

**Table 2:** Study of anti-inflammatory activity of ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) by inhibition of egg albumin denaturation method.

Concentration ( $\mu\text{g/ml}$ )	Standard Drug	BVE
10	29.16 $\pm$ 0.02	6.32 $\pm$ 0.06
20	36.3 $\pm$ 0.07	25.73 $\pm$ 0.03
30	48.87 $\pm$ 0.03	27.6 $\pm$ 0.04
40	57.57 $\pm$ 0.01	44.43 $\pm$ 0.05
50	65.92 $\pm$ 0.03	46.77 $\pm$ 0.05
IC <sub>50</sub>	33.08	40.32

Each value is expressed as mean  $\pm$  SD of three replicates for three triplicates (n=3)

ethylene diamine dihydrochloride) was added (Mondal *et al.*, 2004). Absorbance was measured at 546 nm and percentage of nitric oxide scavenging activity was calculated by using following formula:

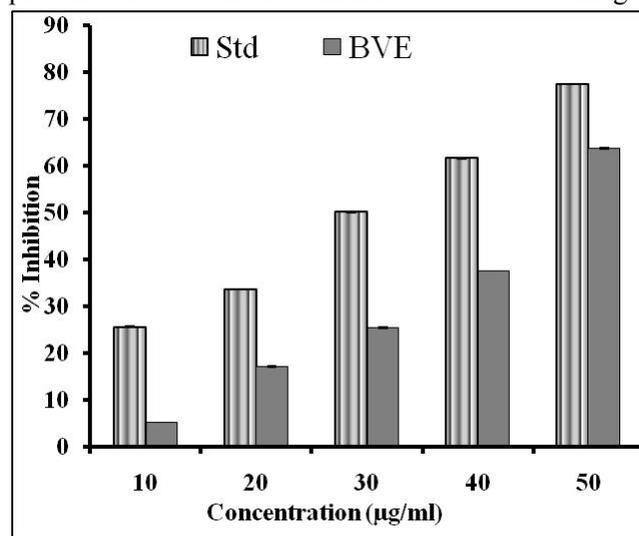
$$\% \text{ Nitric oxide (NO) Scavenging} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100$$

### Statistical analysis

Results are expressed as Mean  $\pm$  SD for three triplicates (n=3). Linear regression analysis was used to calculate IC<sub>50</sub> value.

## Results and Discussion

Denaturation of protein is a common phenomenon of inflammation. Various external influences like heat, stress, injuries, chemicals, microbial infection, etc., induce protein denaturation and which leads to tissue damage,



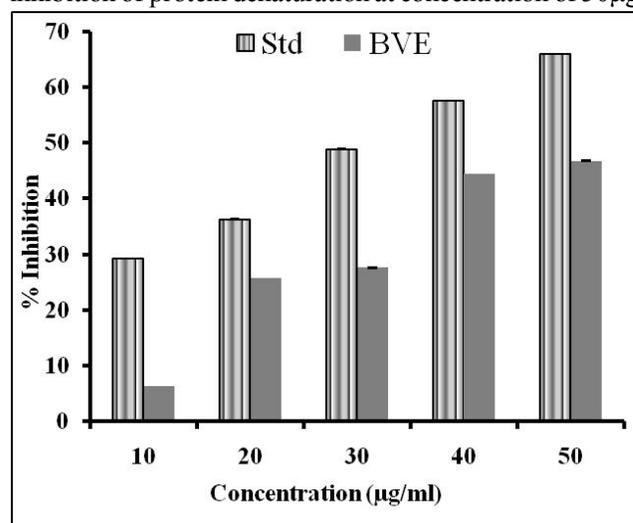
**Fig. 1:** Effect of ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) on bovine serum albumin denaturation. Anti-inflammatory effect of standard drug (Diclofenac sodium) and plant material BVE at different concentration (10-50  $\mu\text{g/ml}$ ) were evaluated by inhibition of bovine serum albumin denaturation method. Each value represents as mean  $\pm$  SD for three triplicates (n=3).

**Table 3:** Study of antioxidant activity of ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) by nitric oxide radical scavenging method.

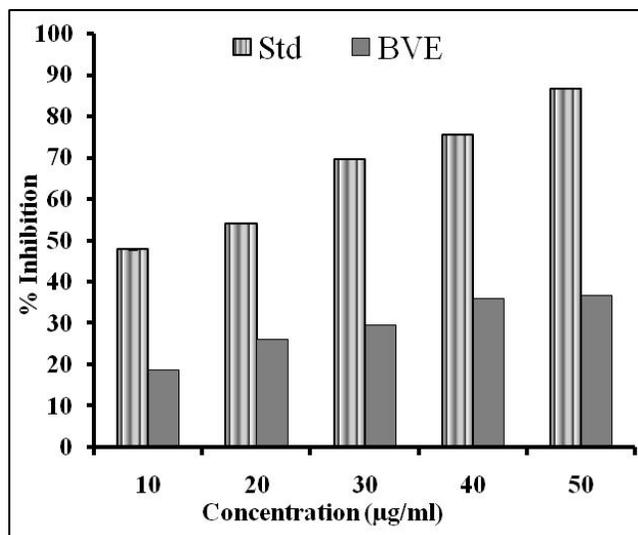
Concentration ( $\mu\text{g/ml}$ )	Standard Drug	BVE
10	47.83 $\pm$ 0.08	18.60 $\pm$ 0.04
20	54.02 $\pm$ 0.03	26.16 $\pm$ 0.02
30	69.70 $\pm$ 0.02	29.65 $\pm$ 0.06
40	75.53 $\pm$ 0.05	36.04 $\pm$ 0.04
50	86.72 $\pm$ 0.01	36.62 $\pm$ 0.03
IC <sub>50</sub>	22.16	64.81

Each value is expressed as mean  $\pm$  SD of three replicates for three triplicates (n=3)

the cause of inflammation (Opie, 1962; Mizushima, 1966; Kishore *et al.*, 2011; Osman, *et al.*, 2016). The function of the anti-inflammatory drug is to inhibit the protein denaturation. As per the table 1, the test drug of this experiment ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) showed the protein denaturation inhibitory effect with IC<sub>50</sub> value 35.84 $\mu\text{g/ml}$  in bovine serum albumin denaturation assay. The result showed that BVE at different concentration (10-50 $\mu\text{g/ml}$ ) inhibited protein denaturation 5.03%, 17.09%, 25.40%, 37.44% and 63.82% respectively. The standard drug diclofenac sodium showed the highest effect 77.52% inhibition of protein denaturation at the concentration of drug 50 $\mu\text{g/ml}$  with the IC<sub>50</sub> value of 30.71 $\mu\text{g/ml}$ . Standard drug also showed 61.65% of inhibition of protein denaturation at the concentration of 40 $\mu\text{g/ml}$  (Fig. 1). As show in table 2, standard drug diclofenac sodium showed 65.92% inhibition of protein denaturation at concentration of 50 $\mu\text{g/ml}$



**Fig. 2:** Effect of ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) on egg albumin denaturation. Anti-inflammatory effect of standard drug (Diclofenac sodium) and plant material BVE at different concentration (10-50  $\mu\text{g/ml}$ ) were evaluated by inhibition of egg albumin denaturation method. Each value represents as mean  $\pm$  SD for three triplicates (n=3).



**Fig. 3:** Effect of ethanolic extract of leaves of *Bauhinia variegata* L. (BVE) on nitric oxide radical scavenging method. Antioxidant activity of standard drug (Diclofenac sodium) and plant material BVE at different concentration (10-50 µg/ml) were evaluated by nitric oxide radical scavenging method. Each value represents as mean  $\pm$  SD for three triplicates (n=3).

ml in egg albumin denaturation assay. At the same concentration test drug BVE showed 46.77% of inhibition. BVE at the concentration of 10, 20, 30 and 40 µg/ml showed 6.32%, 25.73%, 27.60% and 44.43% of inhibition of protein denaturation in egg albumin denaturation assay (Fig. 2). The  $IC_{50}$  value for standard drug and BVE was calculated 33.08 and 40.32 µg/ml respectively.

Free radical like  $NO^{\bullet}$  converts to peroxynitrite ( $ONOO^{\bullet}$ ) by reacting with oxygen. The peroxynitrite causes cell death by damaging cell membrane which leads to the tissue damage, a major cause of inflammation (Diaz *et al.*, 2012; Darley-Usmar *et al.*, 1995, Ilhami *et al.*, 2005). The antioxidants can inhibit the production of free radicals and it can alter the process of inflammation. Thus, the antioxidant property is also a possible mechanism of action of a drug for treating inflammation. In this study, BVE showed 36.62% of nitric oxide scavenging activity at 50 µg/ml concentration (Table 3, Fig. 3) whereas, the standard drug showed 86.72% inhibition at the same concentration. The  $IC_{50}$  value for standard drug and BVE was calculated 22.16 and 64.81 µg/ml respectively in nitric oxide scavenging assay.

### Conclusion

The present study was designed to ascertain the anti-inflammatory and antioxidant property of *Bauhinia variegata* L. The preliminary *in vitro* pharmacological studies for anti-inflammatory activity by denaturation of bovine serum albumin and egg serum albumin and

antioxidant activity by nitric oxide scavenging assay was carried out with ethanolic extract of leaves of *Bauhinia variegata*. The results showed the prominent anti-inflammatory and antioxidant activity of the extract, which also justify the traditional claim for the drug. However, further studies need to be carried out to understand the mechanism for anti-inflammatory and antioxidant property of the drug.

### Acknowledgement

Authors are thankful to Nitte (Deemed to be University) and NGSM Institute of Pharmaceutical Sciences for providing all the necessary facilities and infrastructure for the studies.

### References

- Azevedo, C.R., F.M. Maciel, L.B. Silva, A.T.S. Ferreira, M. da Cunha, O.L. Machado, K.V.S. Fernandes, A.E. Oliveira and J. Xavier-Filho (2006). Isolation and intracellular localization of insulin-like proteins from leaves of *Bauhinia variegata*. *Braz. J. Med. Bio. Res*, **39**: 1435-1444.
- Bhatnagar, L.S., V.K. Singh and G. Pande (1973). Medicobotanical studies on the flora of Ghatigaon forests, Gwalior, Madhya Pradesh. *J. Res. Ind. Med.*, **8**: 67-100.
- Billiar, T.R. (1995). Nitric oxide. Novel biology with clinical relevance. *Ann. Surg.*, **221**: 339-349.
- Billiar, T.R. and B.G. Harbrecht (1997) Resolving the nitric oxide paradox in acute tissue damage. *Gastroenterology*, **113**: 1405-1407.
- Bodakhe, S.H. and A. Ram (2007). Hepatoprotective properties of *Bauhinia variegata* bark extract. *Yakugaku Zasshi*, **127**: 1503-1507.
- Boscá, L., M. Zeini, P.G. Través and S. Hortelano (2005). Nitric oxide and cell viability in inflammatory cells: A role for NO in macrophage function and fate. *Toxicology*, **208**: 249-258.
- Chandra, S., P. Chatterjee, P. Dey and S. Bhattacharya (2012) Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. *Asian Pac. J. Tropic. Biomed.*, **2**: 178-180.
- Cheeseman, K.H. and T.F. Slater (1993). An introduction to free radical biochemistry. *Br. Med. Bull.*, **49**: 481-493.
- Darley-Usmar, V., H. Wiseman and B. Halliwell (1995). Nitric oxide and oxygen radicals: a question of balance. *FEBS Letters*, **369**: 131-135.
- Diaz, P., S.C. Jeong, S. Lee1, C. Khoo and S.R. Koyyalamudi (2012). Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. *Chin. Med.*, **7**: 26.
- Fangkrathok, N., J. Junlatat and B. Sripanidkulchai (2013). *In vivo* and *in vitro* anti-inflammatory activity of *Lentinus polychrous* extract. *J. Ethnopharmacol.*, **147**: 631-637.

- Ilhami, G., A.A. Haci and C. Mehmet (2005). Determination of *in vitro* antioxidant and radical scavenging activities of propofol. *Chem. Pharm. Bull*, **53**: 281-285.
- Kirtikar, K.R. and B.D. Basu (2006). Indian Medicinal Plants, Vol. II, 2<sup>nd</sup> ed, International Book Distributors, Dehradun, India, 898-900.
- Kishore, G., G. Siva and E.S. Sindhu (2011). *In vitro* Anti-Inflammatory and Anti-Arthritic Activity of Leaves of *Physalis Angulata* L. *Int. J. Pharmaceut. Ind. Res.*, **1**: 211-213.
- Kumar, V., Z.A. Bhat, D. Kumar, P. Bohra and S. Sheela (2011). *In-vitro* anti-inflammatory activity of leaf extracts of *Basella alba* Linn. var. *alba*. *Int. J. Drug Dev. Res.*, **3(2)**: 176-179.
- Mali, R.G., S.G. Mahajan and A.A. Mehta (2007). Rakta Kanchan (*Bauhinia variegata*): Chemistry, Traditional and Medicinal uses- a review. *Pharmacogn. Rev.*, **1**: 314-319.
- Mizushima, Y. (1966). Screening test for antirheumatic drugs. *Lancet*, **288**: 443.
- Mizushima, Y. and M. Kobayashi (1968). Interaction of anti inflammatory drugs with serum proteins, especially with some biologically active proteins. *J. Pharm Pharmacol.*, **20**: 169-173.
- Mohamed, M.A., M.R. Mammoud and H. Hayen (2009). Evaluation of antinociceptive and anti-inflammatory activities of a new triterpene saponin from *Bauhinia variegata* leaves. *Zeit. Natur. C*, **64**: 798-808.
- Mondal, S.K., G. Chatraborty, M. Gupta and U.K. Mazumder (2004). *In vitro* antioxidant activity of *Dispyros malabarica* Kostel bark. *Ind. J. Exp. Biol.*, **44**: 39-44.
- Nadkarni, A.K (2009). Dr. K.M. Nadkarni's Indian Materia Medica, Vol. I, 2<sup>nd</sup> ed, Popular Prakashan Pvt. Ltd, Bombay, India, 184-185.
- Opie, E.L (1962). On the relation of necrosis and inflammation to denaturation of proteins. *J. Exp. Med.*, **115**: 597-608.
- Osman, N.I., N.J. Sidik, A. Awal, N.A. Adam and N.I. Rezali (2016). *In-vitro* xanthine oxidase and albumin denaturation inhibition assay of *Barringtonia racemosa* L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis. *J. Intercult. Ethnopharmacol.*, **5**: 343-349.
- Prashant, T., K. Bimlesh, K. Mandeep, K. Gurpreet and K. Harleen (2011). Phytochemical screening and extraction: A review. *Int. Pharmaceutica. Scientia*, **1(1)**: 98-106.
- Raj Kapoor, B., N. Muruges and D. Rama Krishna (2009). Cytotoxic activity of a flavanone from the stem of *Bauhinia variegata* Linn. *Nat. Prod. Res.*, **23**: 1384-1389.
- Sharma, J.N., A. Al-Omran and S.S. Parvathy (2007). Role of nitric oxide in inflammatory diseases. *Immunopharmacol*, **15**: 252-259.
- Simmons, D.L. (2006). What makes a good anti-inflammatory drug target? *Drug Discov. Today*, **11**: 210-219.
- Tang, S.Y., M. Whiteman, Z.F. Peng, A. Jenner, E.L. Yong and B. Halliwell (2004). Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. *Free Radic. Biol. Med.*, **36**: 1575-1587.
- Tripathi, P., P. Tripathi, L. Kashyap and V. Singh (2007). The role of nitric oxide in inflammatory reactions. *FEMS Immunol. Med. Microbiol.*, **51(3)**: 443-452.
- Vane, J.R. and R.M. Botting (1995). New insights into the mode of action of anti-inflammatory drugs. *Inflammation Research*, **44(1)**: 1-10.
- Yadava, R.N. and V.M. Reddy (2003). Anti-inflammatory activity of a novel flavonol glycoside from the *Bauhinia variegata* Linn. *Nat. Prod. Res.*, **17**: 165-169.