



EVALUATION OF *IN-VITRO* ANTI-DENATURATION ACTIVITY OF ISOLATED COMPOUND OF *BUTEA MONOSPERMA* BARK.

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ABSTRACT

The aim of the present study was to evaluate the *in vitro* anti-inflammatory activity of isolated compound (BM-01) of *Butea monosperma* bark by means of anti-denaturation of bovine serum albumin. The stem bark of *Butea monosperma* was extracted with methanol in a static extractor and the obtained extract was column chromatographed using silica gel (100-120 mesh) pooled and processed further. Accordingly the compound was isolated and characterised. In the study for anti-denaturation activity, the isolated compound (BM-01) showed greater percentage of inhibition of bovine serum albumin i.e 85.68% at lowest concentration. Thus the result indicates that the compound (BM-01) exhibited significantly good anti-denaturation activity. The phenolic nature of the isolated compound (BM-01) may be the region for its possible anti-denaturation activity.

Keywords: *Butea monosperma*, Diclofenac sodium, Anti-denaturation, Bovine serum albumin.

INTRODUCTION

Inflammation is the reaction of vascularized living tissue to local injury. The role of inflammation is to contain and isolate injury, to destroy invading microorganisms, inactivate toxins and to achieve healing and repair. However it may be potentially harmful, causing life threatening hypersensitivity reactions and progressive organ damage^[1]. In order to overcome mainly the NSAIDs (Non- Steroidal Anti-inflammatory Drugs) are prescribed. The activity of NSAIDs in rheumatoid arthritis and other inflammatory diseases does not seem to be only due to the inhibition of the production of endogenous prostaglandins (which could be affected at much lower doses than those required in these conditions), but also by preventing the denaturation of proteins (which act as antigens and leads to auto-immune diseases)^[2]. These anti-inflammatory agents inspite of their potency in relieving pain and other consequences of inflammatory responses are also associated with some serious side effects, especially in elderly. NSAIDs on prolonged duration of usage may cause gastric bleeding, ulceration, bone marrow disturbance, kidney and liver dysfunction^[3]. However plant derived drugs is used to treat most of the

inflammatory diseases which are difficult to treat with allopathic medicines. Even today 80% of the world population depends on plant derived medicines for the first line of primary health care because of least/no side effects^[4,5].

When BSA is heated it undergoes denaturation and expresses antigens associated to Type III hypersensitive reaction and which are related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus^[6]. There are emerging ethical issues with regards to the use of animals in the early stages of drug discovery for inflammatory diseases. Thus, the *in vitro* anti-denaturation (stabilization) effects of heat treated (immunogenic) bovine serum albumin (BSA) assay is being used for detecting a wide range of anti-inflammatory compounds.

Butea monosperma (Family: Fabaceae) is a moderate sized deciduous tree, widely distributed throughout India, Burma and Ceylon. The methanolic leaves extract of *Butea monosperma* had been reported to possess anti-inflammatory activity^[7], however isolation and characterisation of active constituents responsible for such activity will play a major role in the development of new anti-inflammatory agent. Hence in the present study an effort had been made to evaluate the *in vitro* anti-denaturation activity of isolated compound from the dried stem bark of *Butea monosperma*.

MATERIALS AND METHODS

The methanolic extract of *Butea monosperma* bark was collected from Natural Remedies Pvt. Ltd, Bangalore and the further work of isolation was carried out under the supervision of Dr. Deepak M, Senior manager, Dept. of photochemistry, Natural Remedies Pvt. Ltd, R & D centre. Bovine serum albumin, triss buffer and diclofenac sodium. All other chemicals and reagents including solvents used were of analytical grade.

Air-dried and powdered stem bark of *Butea monosperma* was extracted with methanol as solvent at 60°-70°C in a static extractor three times each for 2 hrs. The crude extract was then concentrated under vacuum at 45°-50°C, transferred to a watch glass and kept in a dessicator containing fused calcium chloride.

Methanolic extract of *Butea monosperma* bark was passed through a column packed with silica gel (100 – 120 mesh) and developed according to the following lines. The column was built up by passing two column volumes of pet ether before the extract was loaded.

The solvent was kept 5 cm above the bed and the extract was carefully loaded in the form methanolic slurry. Column was then developed with a series of solvent starting with pet ether, ethyl acetate: pet ether, ethyl acetate, ethyl acetate: methanol and methanol as eluants depending on the polarity of compounds. The different fractions thus obtained were taken for TLC study, followed by purification by passing through different column and preparative HPLC. Finally purity of the compound obtained was checked by analytical HPLC and characterized by standardized methods such as NMR, Mass and IR. The bioactive compound obtained was also screened for its anti-denaturation activity.

Anti-denaturation Activity

The method of Williams *et al*^[8], was employed for the anti-denaturation assay. A solution of 0.2%w/v of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solution of 1000µg/ml of compound (BM-01) was prepared by using methanol as a solvent. From these stock solutions 4 different concentrations of 1, 10, 20, and 30µg/ml were prepared by using methanol as a solvent. 50 µl of these different concentrations was transferred to Eppendorf tubes using 1ml micro pipette. 5ml of 0.2 % W/V BSA was added to all the above Eppendorf tubes. The standard consist 10µg/ml of Diclofenac Sodium in methanol with 5ml 0.2% W/A BSA solution. The test tubes were heated at 72⁰ C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using UV/Vis Double beam spectrophotometer (Elico SL -196) at a wave length of 660 nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula.

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

RESULTS

The introduction of new analytical methods (Thin layer chromatography, high-performance liquid chromatography, infra-red, mass and nuclear magnetic resonance spectroscopy) has led to the isolation of compound (BM-01), which was also identified by TLC and standardized by spectral analysis. The compound (BM-01) might be

assumed as hypogallic acid since its spectral value was found to be same as that of hypogallic acid. The Spectral data's of isolated compound are given in Table 01.

The compound (BM-01) has protected Bovine serum albumin (BSA) against heat denaturation. The BM-01 has shown 32.92% and 21.55% anti-denaturation activity on BSA at concentrations of 20 μ g/ml and 30 μ g/ml respectively. However the compound (BM-01) had shown 85.68% of anti-denaturation effect at 1 μ g/ml concentration. This result was coinciding with the statement of Williams *et al.*, that the anti-denaturation action of compound (BM-01) is more when the concentration is less. The standard Diclofenac Sodium had shown 93.44% of anti-denaturation effect at 10 μ g/ml. The results are as shown in Table-02 and Figure-01.

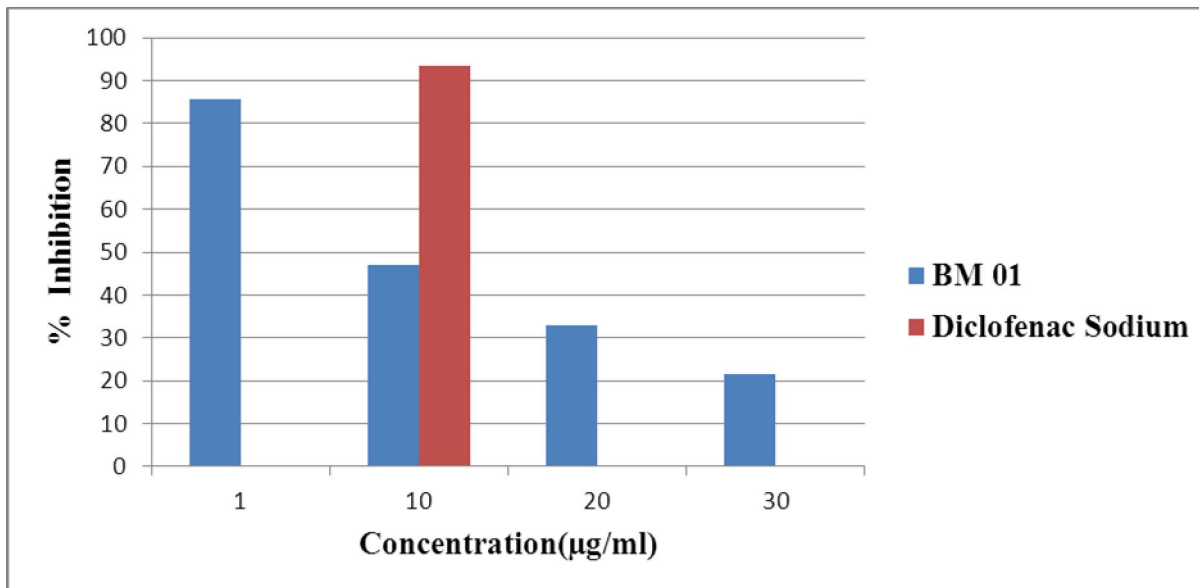
TABLE 1: SPECTRAL DATA'S OF ISOLATED COMPOUND OF BUTEA MONOSPERMA BARK.

Compound	ppm	, cm^{-1}
BM-01	C-1 resonated at 121.6, C-2 at 116.5, C-3 at 149.9 C-4 at 144.8, C-5 at 121.8, C-6 at 115.1 and C-7 resonated at 167.3.	3338.6 and 3186.6 (hydroxyl groups), 1879.4 (carbonyl group), 1532.9 and 1593.7 (aromatic carbon-carbon double bond), 1095 (C-O bending), 1295.9 (O-H bending).

TABLE 2: EFFECT OF ISOLATED COMPOUND OF BUTEA MONOSPERMA BARK ON INHIBITION OF BOVINE SERUM ALBUMIN DENTURATION.

Sl.NO	Compound	Concentration(μ g/ml)	% Inhibition
1	BM-01	1	85.68 \pm 0.086
		10	47.04 \pm 0.150
		20	32.92 \pm 0.410
		30	21.55 \pm 3.040
2	Diclofenac Sodium	10	93.44 \pm 0.173

The values are expressed as mean \pm SD. $p < 0.05$.

**Figure 1**

Effect of isolated compound of *Butea monosperma* bark on inhibition of bovine serum albumin denaturation.

DISCUSSION

The different fractions were obtained from the column and subjected to TLC. However the fraction of 33% ethyl acetate: pet ether shown prominent bands which were further purified by passing through different column and finally by preparative HPLC. The purity of the compound (BM-01) thus obtained was checked by analytical HPLC and further characterised by standardized methods such as NMR, Mass and IR. Spectral value of compound (BM-01) was found to be same as that of hypogallic acid as described by Buniyamin *et al*^[9], hence it was assumed as hypogallic acid.

The various concentrations of compound (BM-01) ranging from 1-30µg/ml were tested for its anti-denaturation activity. The results have clearly demonstrated that the compound (BM-01) at different concentrations have good anti-denaturation activity, however it exhibited highest anti-denaturation activity at its lowest concentration. Literatures suggest that, the anti-denaturation property of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine rich and aliphatic threonine and lysine residue regions of the BSA^[10]. They have also reported that therapeutic molecules could be activating the tyrosine motif rich receptor dually with threonine that regulate signal transduction biological pathways for their overall biological action^[8, 11].

Compounds interacting with the aliphatic regions around the lysine residue on the BSA could be interesting as anti-oxidant with anticancer activity such as the polyphenols, phenyl propanoids and the disulphides^[8, 11, 12, 13]. However the isolated compound BM-01 is phenolic in nature, hence this may be the region for its possible anti-denaturation activity.

CONCLUSION

Our investigation had clearly demonstrated that the isolated compound (BM-01) possess significant anti-denaturation property. The phenolic nature of the compound (BM-01) may be the region for its possible anti-denaturation activity. However further *in vivo* studies have to be performed to authenticate such biological activity.

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REFERENCES:

1. Robbins, Cotron, Vinay k, Abdul KA, Nelson F: Pathologic Basis of Disease. Elsevier publication ,seventh edition, 2008: 47-53.
2. Insel, PA: Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Hardman JG Limbird LE, Molinoff PB, Ruddon RW and Goodman Gilman A, The pharmacological Basics of Therapeutics. McGraw Hill, New York, ninth edition,1996, 617-657.
3. Rang HP, Dale MM, Ritter JM, Moore PK: Pharmacology. Elsevier publication, Churchill Livingstone , sixth edition , 2007; 226-232.
4. Soeken KL, Miller SA, Ernst E: Herbal Medicines for the treatment of rheumatoid arthritis: a systematic review. *Rheumatology* 2003; 42(5): 652-9.
5. Sheetal V, Singh SP: Current and future status of herbal medicines. *Veterinary World* 2008; 1(11): 347-50.
6. Fayyaz A, rafeeq A. Khan, Shahid R: Study of analgesic and anti-inflammatory activity from plant extracts of lactuca scariola and Artemisia absinthium. *J. Islam. Acad. Sci* 1992; 5: 111-114.

7. Ashish M, Saket V, Abhinav PM: A plant review: *Butea monosperma* (Lam.) Kuntze. *Research Journal of Pharmaceutical, Biological and Chemical Science* 2012; 3(1) 700-714.
8. Williams LAD, Connar AO, Latore I, Dennis O, Ringer S, Whittaker JA, Conard J, Vogler B, Rosner H, Kraus W: The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J*, 2008; 57: 327-331.
9. Buniyamin A, Aynide, Doris N, Onwukaeme, Eric K, Omogbai: isolation and characterization of two phenolic compounds from the stem bark of *Musanga cecropioides* r. brown (moraceae). *acta poloniae pharmaceutica-drug research* 2007; 64:183-5.
10. Williams LAD, Rosner H, Conard J, Moller W, Beifuss U, Chiba K: Selected secondary metabolites from *Phytolaccaceae* and their biological/pharmaceutical significance, *Research Signpost*. In : *Recent Res Devel In Phytochem* 2002; 6: 13-68.
11. Rosner H, Williams LAD, Jung A, Kraus W: Disassembly of microtubules and inhibition of neurite outgrowth, neuroblastoma cell proliferation, and MAP kinase tyrosine dephosphorylation by dibenzyl trisulphide. *Biochim Biophys Acta*. 2001; 1540: 166-77.
12. Kannan S: Free radical theory of autoimmunity. *Theror Biol Med Model* 2006; 3: 22.
13. Kawabata T, Packer L: -lipoate can protect against glycation of serum albumins but not low density lipoproteins. *Biochem Biophys Res Commun* 1994; 203: 99-104.

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