

Some Pharmacological Assays Of Loban (Styrax Benzoin Dryand)

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ABSTRACT

The present paper deals with Loban a resinous excudate from the plant *Styrax benzoin*, found in crystals of white and greyish brown colour. Antioxidant, Anti inflammatory and Antidiabetic activity was carried out. Antioxidant assay was estimated by DPPH method as described by Siddartha baliyan et.al., (2022), anti inflammatory assay by protien denaturation adopted by Zephy D and Ahmed (2015) and Maritim et al., (2003) method. While anti diabetic assay measured in vitro alpha amylase inhibition prescribed by method of Bernfeld (1955). white crystals of Loban shows highest antioxidant potential nearly 90% of inhibition at 100µg/ml than greyish brown crystals of loban at all tested concentration, exhibit higher radical scavenging efficiency. The anti inflammatory assay shows white crystals reveals 45% of denaturation/inhibition at 100µg/ml sample and crystal greyish shows 55% of inhibition, reflects samples contain bioactive constituents, capable of stabilizing protiens and preventing denaturation. The antidiabetic assay indicate greyish brown loban crystals exhibit better inhibitory activity than white crystals, suggesting its higher effectiveness in suppressing enzyme activity. Therefore, Loban crystals reveals good amount of antioxidant, anti inflammatory and antidiabetic properties.

Key words : Loban, *Styrax-benzoic*, antioxidant, anti inflammatory, antidiabetic.

1. INTRODUCTION

Styrax benzoin Dryand is an evergreen aromatic tree, native of Sumatra, Indonesia , Malaya Java growing to the height of 30-32 meter tall, occassionally with buttresses, leaves simple, decussate, ovo-elliptic, flowers on axial or terminal racemes, Fruit globose and indehiscent, belong to family *Styraceae*. Now-a-days cultivated in Western countries and South east Asia. Its product resin is very popular and known as Loban or Sambrani which is widely marketed and used across India. Gum resin (Loban) extracted from cuts in the trunk of trees, which is having Vanilla-like scent, with antiseptic property. Hence, used in Ayurveda, Homeopathy , Aromatherapy and in the perfume industry.

Gum resin (Loban) is highly priced for its warm sweet, slightly balsamic aroma, processed and available in the form of white or greyish black crystals or granules or in different colours. *Styrax* also known as *Storax*. Loban is widely used in Hindus for poojas and rituals for creating a sacred atmosphere in the form of poojas, meditation, aartis, prayers to remove negativity as essential part of Indian spiritual practices to

attain divine atmosphere. Thus, plant having several therapeutic properties as emollient, astringent, haemostatic properties, used in the treatment of eczema, pigmentation disorders, cracked skin, healing wounds, etc. Meanwhile in Latin Americans used in the treatment of constipation, bronchitis, heart disease and in Leprosy.

The plant possess several bio active compounds such as Cinnamic acids and their esters, such as benzoic acid, Siarasinolic acid, Sumaresinolic acid, Vanillin different Lignans such as oxyneolignan, benzofuran lignans, neolignane, tetrahydrofuranoid lignans, terpenoids, sesquiterpene, phenyl ethylene, phenylpropyl cinnamate and resinols, etc. Consequently tree posses antipyretic (Kabiruddin, 2007), anti bactericidal (Alruways et.al., 2020). Haemostatic, Styptic (Nadkarni, 1976) and Vermifuge (Najmul Ghani) along with anti bacterial, anti cancer, antiseptic, absorbent, expectorant, nacroctic , sedative properties. Therefore, plant is used in treatment bronchitis, cold sore, constipation, dermatosis, fungal infection, insomnia, jaundice, bronchitis, cold sore, constipation, dermatosis, fungal infection, insomnia, jaundice, toothache, rheumatism, ulcer, whooping cough, wound healing etc. Hence, an attempt has been made to study its antioxidant, anti inflammatory and anti diabetic properties of two different white and greyish coloured loban in our department, in future that it can be used as traditional home remedy.

2. METHODS AND MATERIAL

Two different coloured samples of Loban white and greyish crystals were purchased from local market of Kolhapur (Laxmipuri) in the month of August 2025. One gram of each type of sample was dissolved in 100ml of distilled water and heated few minutes. There after filtered through filter paper, 1ml of different concentration (20,40,60,80,100 µg/ml) were taken in each testubes and 15ml of 0.1% methanolic DPPH (2,2-diphenyl-1-picrylhydrazyl) were added in each sample test tube and incubated for 30 minutes in dark condition. Antioxidant activity of Loban has estimated for their free radical scavenging activity by using DPPH free radicals (Siddartha, 2022). The test sample were observed for discoloration from purple to yellow in colour and absorbance was read at spectrophotometer of 510nm and converted into percentage of antioxidant activity (% scavenging) was carried out by using formula.

$$\text{Scavenging (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

The reduction of DPPH absorption is indicative of the capacity of the sample to scavenge free radicals, lower the absorbance suggests higher free radical scavenging activity (Odumosu et al. 2015). The anti inflammatory activity was carried out by protein denaturation method (In vitro) as prescribed by Zephy .D., Ahmad J.(2015) and Maritim et al.,(2003) method, in which sodium salt of Diclofenac used as reference drug. The reaction mixture 10ml consist of 0.4ml of egg albumin 5.6ml of phosphate buffer saline pH 6.4 and different concentration of Loban samples (20,40,60,80,100) µg/ml. This mixture was incubated at 37°C ± 2 in a incubator for 15min, later heated at 70°C for 5min. After 10 minutes of cooling the absorbance was measured at 660nm using vehicle on blank. The precentage of inhibition was calculated by

$$\% \text{ Inhibition} = \frac{\{C - T\}}{C} \times 100$$

Meanwhile antidiabetic assay was measured assay by alpha amylase method prescribed by Zephy D. (2015) and Maritim AC. (2003). Different concentration of test solution 100 μl was allowed to react with 500 μl 0.1M phosphate buffer pH 6.9, containing α -amylase enzyme incubated at 25°C for 10 min. After incubation 1000 μl of dinitro salicylic acid reagent was added to both control and test sample. After 10 minutes in boiling water, absorbance was recorded at 540nm using spectrophotometer and percentage of inhibition of α -amylase was calculated by using formula,

$$\text{Inhibition \%} = \frac{\{\text{Abs540 control} - \text{Abs540 extract}\}}{\text{Abs540 control}} \times 100$$

3. RESULT AND DISCUSSIONS

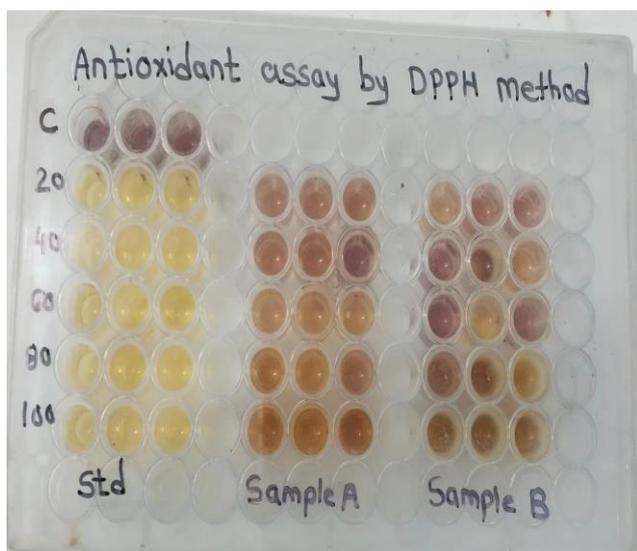
A unanic drug, Loban-resinous exudates from plant *Styrax benzoin* has been used since ancient times having several pharmacological activities (Md. Kausar et al., 2024). The present investigation on two different colours of loban, its anti oxidative property was shown in table 1. Fig 1b & 1c. The activity was evaluated by DPPH free radical scavenging method. Fig. 1b & 1c. Inhibition percentage increased with increasing concentrations for all samples, suggesting that concentration dependant antioxidant effect. The standard ascorbic acid found to be highest antioxidant potential (Table-1, Fig.1b & 1c). Both the samples shows higher potential nearly 90% inhibition at 100 $\mu\text{g/ml}$. Sample B (greyish) reveals better activity as compared to sample A (white) at all tested concentrations, reflecting a higher scavenging efficiency, both the samples have lower than standard have ability to neutralize free radicals and their potential as natural antioxidant agent. A concurrent result was reported by Mandana Jafari et al., (2019) resinous natural product Shilajit. While black Sumatran incense has documented active antioxidant properties against DPPH (Md. Kausar ali ., 2024). Meanwhile two different Shilajit from Pakistan and Afghanistan revealed good scavenging activity (F. Al-Salman et al.,2020), alongwith even age old religious tree *Ficus religiosa* (Siddartha Baliyan et al., 2022).

Inflammation is a biological phenomenon in which some biochemicals released from the tissue associated with leukotrienes, bradykinins etc., (Vane and Botting 1987) in response to wounds, allergen or auto immune conditions (Snehal and Savjani., 2015). It may be a protective mechanism of the organisms in response to external and internal stimuli by means of mechanical or by pathogens or any proliferative factors (Erdemoglu and Callis, 2008). In order to overcome inflammation traditional medicines has played a vital role and use of different herbal medicines, with this, we carried out anti inflammatory acitivity of loban by protien denaturation method presented by Chatterjee et al., (1996), where diclofenac Sodium was used as standard, the result shows concentration dependant increase in percentage inhibition in all tested samples Table 2. Fig. 2,3,4 at lower concentration, anti inflammatory activity was weak as gradually increased with increase in concentration at 60-100 $\mu\text{g/ml}$ where a maximum activity was recorded (Table . 2 Fig. 2,3,4). The grey coloured loban reveals about 55% of inhibition, while white colored loban about 45% inhibition. This indicates grey loban may contain some bioactive compounds which capable of stabilizing protiens and prevent denaturation indicates its anti inflammatory properties, a similar result was published by Shenoy and Shirwaikar (2002) in *Hyptis suaveolens* ,Madhu

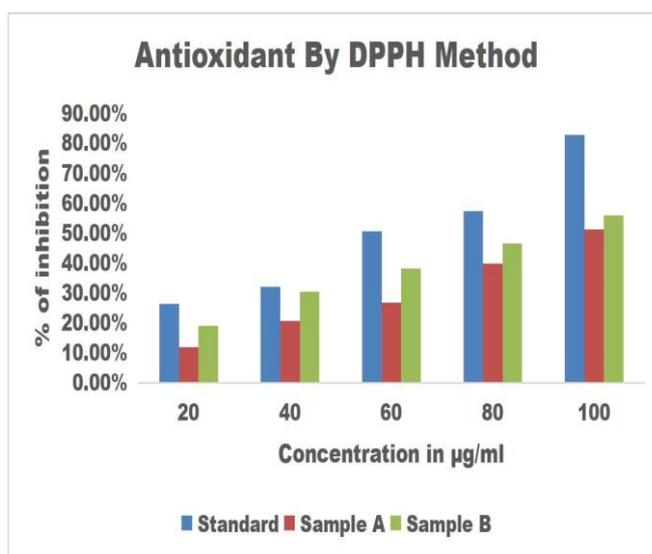
and Narayan (2018) reported a significant anti-inflammatory activity in *Solanum virginianum* in methanolic extract. A parallel document was published by Nishat Fatima et al., (2022) presence of a steroid compound guggulesterone responsible for anti-inflammatory drug.

RESULT:
Observation table: 01.

S R. N O	Sample Code	Concentration (µg/ml)	Absorbance at 510nm				% Inhibition	IC50 (µg/ml)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.93	1.93	1.93	1.93	-	
2	Standard	20	1.45	1.42	1.40	1.42	26.42%	72.49
	(Ascorbic Acid)	40	1.31	1.29	1.33	1.31	32.12%	
		60	0.95	0.97	0.93	0.95	50.77%	
		80	0.82	0.85	0.79	0.82	57.51%	
		100	0.35	0.32	0.32	0.33	82.90%	
3	Sample A	20	1.70	1.74	1.68	1.70	11.91%	98.71
		40	1.58	1.52	1.50	1.53	20.72%	
		60	1.39	1.42	1.42	1.41	26.94%	
		80	1.18	1.12	1.20	1.16	39.89%	
		100	0.91	0.98	0.94	0.94	51.29%	
4	Sample B	20	1.56	1.53	1.61	1.56	19.17%	94.05
		40	1.34	1.30	1.38	1.34	30.56%	
		60	1.20	1.18	1.19	1.19	38.34%	
		80	1.01	1.04	1.06	1.03	46.63%	
		100	0.85	0.88	0.82	0.85	55.95%	



Images of the activity:1b



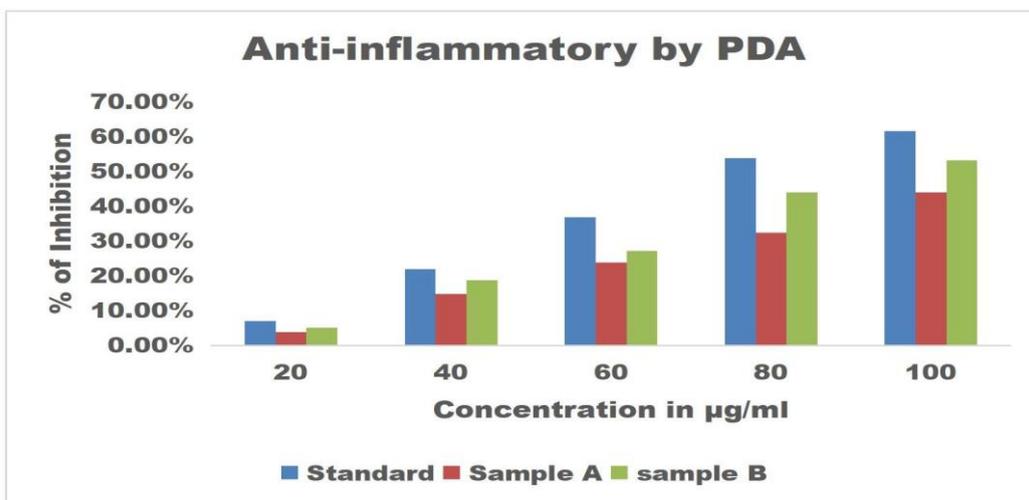
Graphical Data: 1c

Inflammation is a biological phenomenon in which some biochemicals released from the tissue associated with leukotrienes, bradykinins etc., (Vane and Botting, 1987) in response to wounds, allergen or auto immune conditions (Snehal and Savjani., 2015). It may be a protective mechanism of the organisms in response to external and internal stimuli by means of mechanical or by pathogens or any proliferative factors (Erdemoglu and Callis, 2008). In order to overcome inflammation traditional medicines has played a vital role and use of different herbal medicines, with this, we carried out anti inflammatory activity of loban by protien denaturation method presented by Chatterjee et al. (1996), where diclofenac Sodium was used as standard, the result shows concentration dependant increase in percentage inhibition in all tested samples Table 2. Fig. 2a & 2b, at lower concentration, anti inflammatory activity was weak as gradually increased with increase in concentration at 60-100µg/ml where a maximum activity was recorded (Table . 2 Fig. 2a & 2b). The grey coloured loban reveals about 55% of inhibition, while white colored loban about 45% inhibition. This indicates grey loban may contain some bioactive compounds which capable of stabilizing protiens and prevent denaturation indicates its anti inflammatory properties, a similar result was published by Shenoy and Shirwaikar (2002) in Hyptis suaveolens, Madhu and Narayan (2018) reported a significant anti inflammatory activity in Solanum virginianum in methanolic extract. A parallel document was published by Nishat Fatima et al., (2022) presence of a steroid compound gugglesterone responsible for anti inflammatory drug.

OBSERVATION TABLE:02

			Protein denaturation assay					
SR. NO	Sample code	Concentration (µg/ml)	Absorbance at 660nm				% Inhibition	IC50 (µg/ml)
			Test 1	Test 2	Test 3	Mean		

1	Control		1.54	1.54	1.54	1.54	-	
2	Standard(Diclofenac Sodium)	20	1.43	1.44	1.43	1.43	7.14%	76.11
		40	1.20	1.20	1.22	1.20	22.07%	
		60	0.96	0.97	0.98	0.97	37.01%	
		80	0.72	0.72	0.71	0.71	53.89%	
		100	0.59	0.61	0.59	0.59	61.68%	
3	Sample A	20	1.46	1.48	1.50	1.48	3.89%	NE
		40	1.31	1.28	1.35	1.31	14.93%	
		60	1.18	1.20	1.15	1.17	24.02%	
		80	1.02	1.05	1.07	1.04	32.46%	
		100	0.87	0.83	0.90	0.86	44.15%	
4	Sample B	20	1.50	1.45	1.45	1.46	5.19%	96.76
		40	1.25	1.28	1.22	1.25	18.83%	
		60	1.10	1.13	1.15	1.12	27.27%	
		80	0.88	0.85	0.87	0.86	44.15%	
		100	0.71	0.75	0.72	0.72	53.24%	



Graphical data : 2a



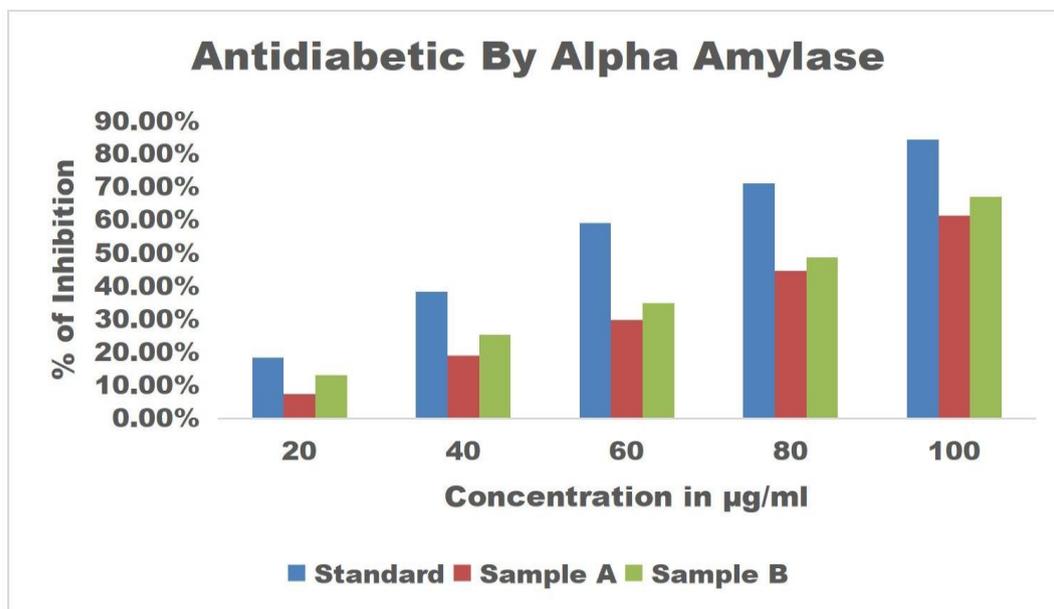
Images of the activity:2b

Diabetic mellitus a physiological disorder, result into hyper glycemia and insufficiency of secretion of insulin with exogenous insulin and other used cations can control many aspects of diabetes, increased oxidative stress enhanced progression of diabetic and its complications or impaired anti oxidant defenses was shown by (Ceriello A. 2000, Baynes and Thorpe J.W. 1999, Baynes 1991) and Saxena .et al., (1993).

Observation Table:03

SR.NO	Sample code	Concentration (µg/ml)	α-amylase enzyme inhibition assay				IC50 (µg/ml)	
			Absorbance at 540nm					% Inhibition
			Test 1	Test 2	Test 3	Mean		
1	Control		1.74	1.74	1.74	1.74	-	
2	Standard (Acarbose)	20	1.42	1.45	1.40	1.42	18.39%	65.81
		40	1.07	1.09	1.05	1.07	38.50%	
		60	0.71	0.74	0.69	0.71	59.19%	
		80	0.50	0.53	0.48	0.50	71.26%	
		100	0.29	0.28	0.25	0.27	84.48%	
3	Sample A	20	1.61	1.65	1.58	1.61	7.47%	84.65
		40	1.42	1.44	1.38	1.41	18.96%	
		60	1.28	1.20	1.20	1.22	29.88%	
		80	0.98	0.95	0.97	1.96	44.82%	
		100	0.71	0.68	0.62	0.67	61.49%	
4	Sample B	20	1.49	1.51	1.55	1.51	13.21%	82.41
		40	1.30	1.34	1.28	1.30	25.28%	
		60	1.11	1.13	1.16	1.13	35.05%	
		80	0.89	0.86	0.92	0.89	48.85%	

		100	0.58	0.55	0.60	0.57	67.24%	



Graphical data : 3a



Many herbal preparations used for treating diabetic and becomes a persistant aspect of present day health care in developing countries Mahabir and Gulliford (1997). Existing several medicinal plants possess active biochemical constituents to treat diabetic (Sofowora, 1984) and other allied ailments. (Bailey and Day 1989). We performed anti diabetic assay of loban both white and grey coloured by in vitro amylase inhibition, prescribed by the method of Benfeld (1955) Zephy D et al.(2015) and Maritim (2003). The different concentration of loban both samples were react with 0.1M phosphate buffer pH 6.9 containing α -amylase enzyme (fungal diastage 0.5%). After incubation 1% starch solution 0.1M phosphate buffer were added. In case of control enzyme was replaced by buffer, soon after 1000ul of dinitro salicylic acid

reagent added to both control of all test samples. Absorbance was recorded at 540nm in spectrophotometer. percentage of α amylase inhibition was calculated. The results were depicted in Table 3. Fig 3a & 3b, suggests a clear concentration dependant inhibitory effect. The standard Acarbose shows highest inhibition in all tested concentration indicating its strong antidiabetic potential. The grey coloured loban exhibit better inhibitory activity than white coloured loban (Table. 3. Fig 3a & 3b), indicating its higher effectiveness in suppressing enzyme activity. Consequently both samples of white and grey coloured loban illustrated notable inhibitory amylase activity showing its potential to reduce carbohydrate breakdown and glucose absorption, contributing to effective anti diabetic properties. A similar condition was published by Yasser Hussein and Shakukath Ara Khenum (2016) in *Dracaena cinnabari* extracts. Gomathi et al., (2017) published anti diabetic activity in vitro condition of *Plumaria acuminata* leaves A huge number of substances obtained from plants that possesses anti diabetic potential (Rajendran et al., 2018; Saminathan, K and S. Kavimanim 2015 and Maritin et al., 2003).

Accordingly, Loban it acts as potential anti oxidant, anti inflammatory, and anti diabetic drug with some formulation it can be used as best medicine , home drug, even then it needs for some clinical trials with less side effects.

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