

## EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF CRUDE *LAWSONIA INERMIS* LEAF EXTRACT USING EGG ALBUMIN DENATURATION ASSAY

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### ABSTRACT

Hydro alcoholic extract of leaf extract of *Lawsonia inermis* (Family: Lythaceae) was judged for anti-inflammatory activity by *In-vitro* Egg albumin protein denaturation assay. Diclofenac sodium was used as standard drug. Crude extract was partially purified by colum chromatography. Maximum anti-protein denaturation activity and phyto-compounds were noted in fraction E of obtained from Colum. Maximum absorption was noted in the range of 220-240 nm in UV spectrophotometer. FT-IR studies partly suggest presence of phenolics and alkaloids in active fraction. The present findings exhibited a concentration dependent inhibition of protein denaturation in range of 4 mg/ml to 10 mg/mL for leaf extract whereas Diclofenac sodium at the concentration ranges of 2 mg to 10 mg/mL. Present study divulge that *Lawsonia inermis* possessed marked *In- vitro* anti-inflammatory effect against the denaturation of protein. The effect was reasonably due to the presence of alkaloids and other bioactive compound like 'Lawsone'.

**KEY WORDS:** Colum chromatography, *In-vitro* Anti-inflammatory, protein denaturation, *Lawsonia inermis*.

### INTRODUCTION

By the prehistoric times medicinal plants have been identified, exploited, and are used as a natural remedy to cure number disease. Such biological activities are confined to secondary metabolites in plants. Plant makes many of these compounds for performing biological functions like defense against insects, fungi and herbivorous mammals.

Indian medicinal plants are the essence of Ayurveda and Ayurvedic treatments. Ayurvedic Herbs played important role in Ayurvedic treatment, from ancient time to this most modern time. More than 12,000 bio-active molecules from plants are known to us. More or less these chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines proved to be beneficial with minimum side effects just like conventional drugs.

Medicines such as aspirin, digitalis, opium and quinine derive directly from traditional plant medicines, accounting for around a quarter of the modern pharmacopoeia. They are in general far cheaper, and many can be home-grown or picked for free. One such common but deserted plant is *Lawsonia innermis* although it is easily available and cheap.

The common name is Henna and it belongs to the family Lythaceae. It is indigenous to northern Africa, western and southern Asia Henna is a medium sized herb with many branches. Henna consists of white fragrant flowers. The leaves, bark, roots and fruits are the useful parts of the Henna plant. The leaves encompassed of alpha-glucoside which is a coloring substance.

The phyto-chemicals present in the Henna are phenols, anthroquinones and glycosides. Lawsone is the active constituent of the Henna leaves. Lawsone is the main colouring constituent of the Henna. Henna leaves are used as a prophylactic agent against skin diseases by applying the henna paste on the affected areas. Henna leaves show high anti-fungal, Anti-Inflammatory and Anti-analgesic property Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions.

The commonly used drug for inflammation are non-steroidal anti-inflammatory drugs, which pose several adverse effects especially skin irritation and gastric irritation leading to formation of rashes and ulcers respectively. Hence present study was carried out with the evaluation of possible *In-vitro* anti-inflammatory effect of *Lawsonia innermis* extract using protein denaturation assay. This work will be helpful for making topical anti-inflammatory sprays or ointment

## MATERIALS AND METHODS

### Collection of plant material

Dried leaves of *Lawsonia inermis* Linn. (Family: *Lythaceae*) (Common name Hena) were procured in the month of July-August, 2016 from Sangli region (Western Maharashtra), India. Just after procurement, the leaves were ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

### Drugs and chemicals

Diclofenac sodium was procured from College of Pharmacy, Solapur (M.S) India. All other chemicals were of analytical grade obtained commercially and were standardized prior to actual work.

### Preparation of extract

The powder plant material (500 g) was extracted with 1000 mL distilled water by boiling for 45 minutes. After boiling remained extract was kept mixed overnight with 80 % ethanol and mixed with decocted extract. Finally extract was filtered and evaporated to dryness to yield the dry extract (Yield 30%). Extracted material was subjected to Colum chromatography for partial purification. Five fractions namely A, B, C, D, E &F were collected (Figure 1). (Dama and Jadhav., 1998)



Figure 1: Collection Colum fraction (A to F)

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

All five grades were initially tested for anti-protein denaturation activity. Most active E grade was chosen for further study. All reactions were carried out in clean test tubes without interruption of soap solution. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.8) and 0.2 to 2 mL of varying concentrations of active grade E so that final concentrations become 0 to 10 mg/mL. Similar volume of double-distilled water and DMSO served as control. These mixtures were incubated at (37<sup>0</sup> C) in a BOD incubator for 30 min and then heated at 75 for 10 min. After cooling, their absorbance was measured at 660 by using vehicle as blank. Diclofenac sodium at the final concentration of (10 mg/mL) was used as reference drug and treated similarly for determination of absorbance.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

Where,  $V_t$  = absorbance of test sample,  $V_c$  = absorbance of control.

The extract/drug concentration for 50% inhibition (IC<sub>50</sub>) was determined by plotting percentage inhibition with respect to control against treatment concentrations as shown in Figure 2.

### Spectroscopic Evaluation

Among all five fractions 'E fraction' was chosen for spectroscopic analysis. UV Scan range was set in range of 220nm -700nm. Where as FT-IR peak values were noted in the range of 1000 to 4000 cm<sup>-1</sup> wavelength and peak values were evaluated using IR explainer software.

### Phyto-chemical Analysis

Phyto-chemical analysis was done for both crude and fraction E to detect active compound. Evaluation assay was carried out as suggested by Seru *et al.*, 2013.

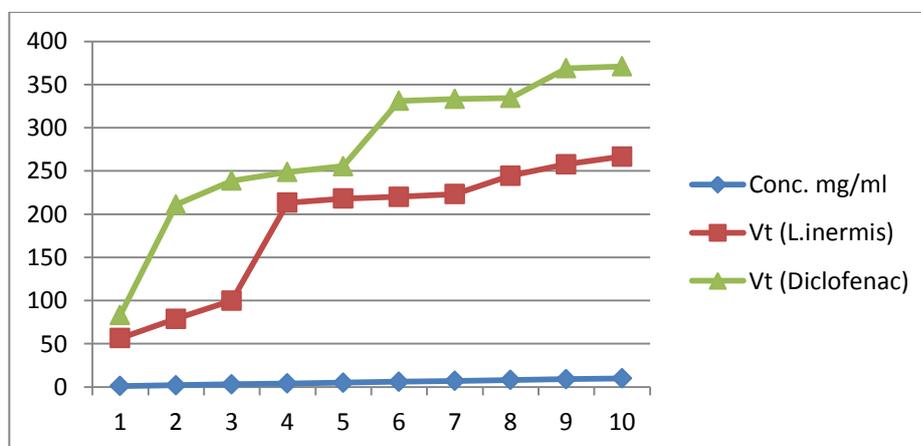
## RESULTS AND DISCUSSION

In the present investigation, the in vitro anti-inflammatory effect of *Lawsonia inermis* leaf extract was evaluated against denaturation of egg albumin. The results are summarized in Table 1. Similar results have been summarized by Sangita *et al.*, (2012). UV spectroscopic analysis revealed maximum absorption in the range of 220-240nm. This points to the presence of some phenolics and alkaloids in active fraction E (Pathade *et al.*, 2009). FT-IR structural analysis revealed broad peaks in the range of 3359<sup>-1</sup> and 1105 cm<sup>-1</sup> shows the presence of OH group and phenolics and alkaloids in fraction as also shown by Dama *et al.*, (2016). Phyto-chemical analysis also confirmed the presence of active compounds like phenolics and alkaloids with carbohydrate moiety shown in Table 2 and figure 2, 4, 5.

The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by leaf extract. The concentration range was 4 mg/ml to 10 mg/mL. Diclofenac sodium (at the concentration range of 2mg to 10 mg/mL) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 1), however, the effect of diclofenac sodium was found to be more when compared with extract. This was further confirmed by comparing their IC<sub>50</sub> values. Leaf extract possessed IC<sub>50</sub> value 4 mg/mL whereas that of diclofenac sodium was found to be 2 mg/mL. (Table 1, Figure 2).

**Table 1. Inhibition of protein denaturation (In percentage)**

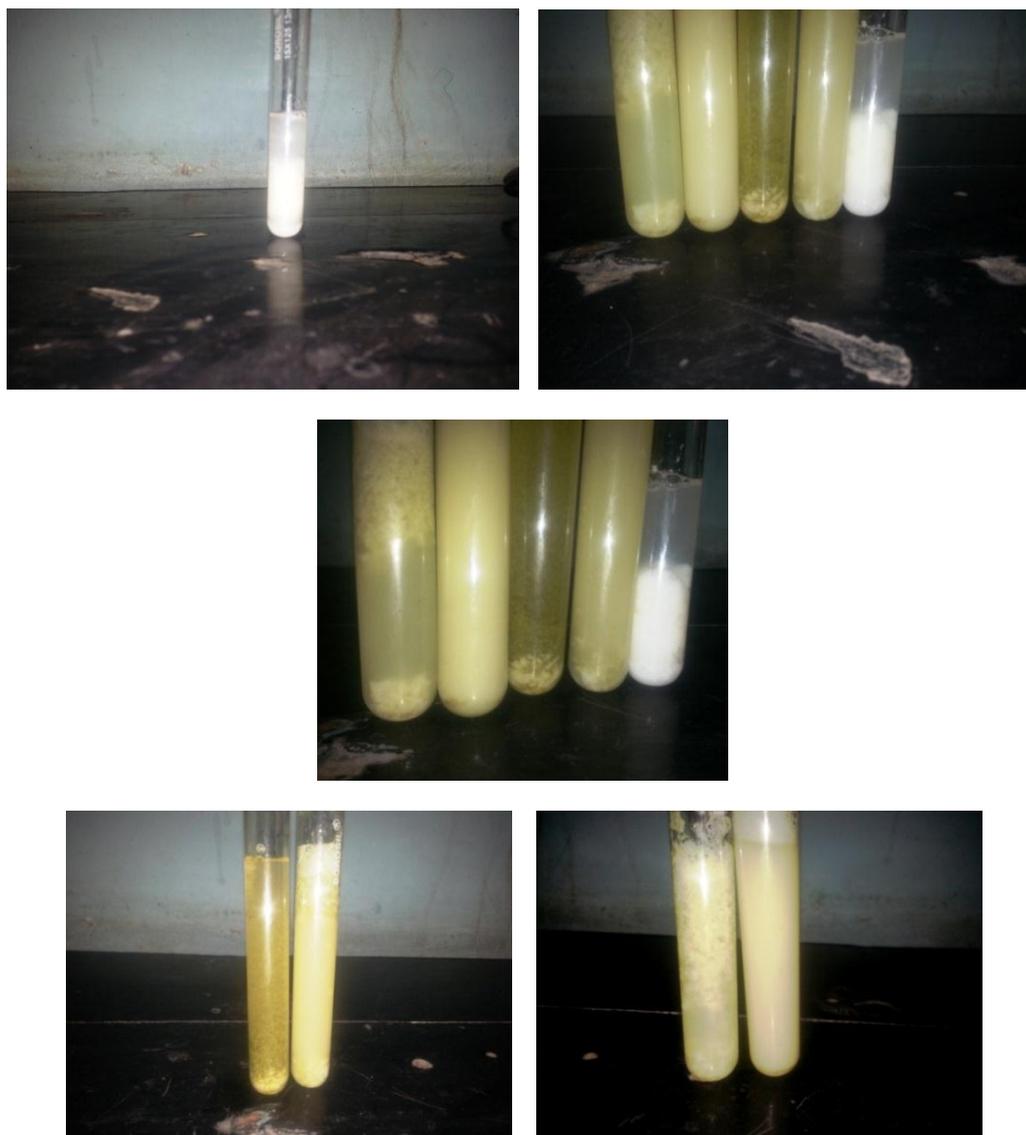
Concentration (mg/mL)	%Inhibition Extract Fraction)	%Inhibition (E Reference (Diclofenac)
1	56.5	83.3
2	78.8	211.1
3	100.0	238.8
4	213.3	248.8
5	218.0	255.5
6	220.0	331.1
7	243.3	333.3
8	240.4	334.4
9	237.7	368.8
10	250.1	371.1



**Figure 2. Inhibition of protein denaturation and IC<sub>50</sub>**

**Table 2: Phyto-chemical analysis of Crude extract and Fraction E**

Components	Crude extract	Fraction E
Alkaloids	+	++
Carbohydrates	+	+
Phenolic compounds	++	++
Flavanoids	++	++
Amino acids	+	-
Tanin	+	-



**Figure 3. In-Vitro Antiinflammatory activity of *Lawsonia inermis* by egg albumin assay at different concentrations**

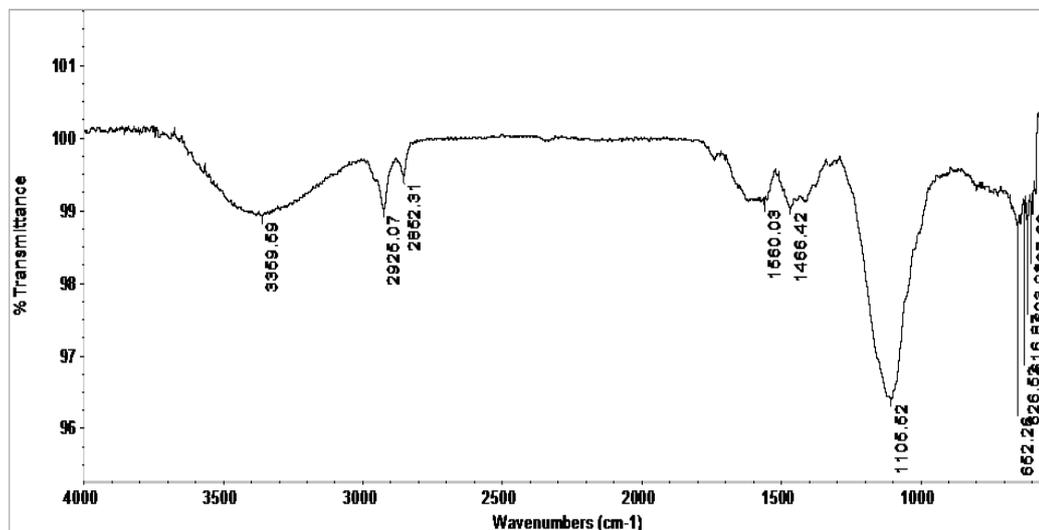


Figure 4. FT-IR Spectra of *Lawsonia inermis* Active Fraction 'E'

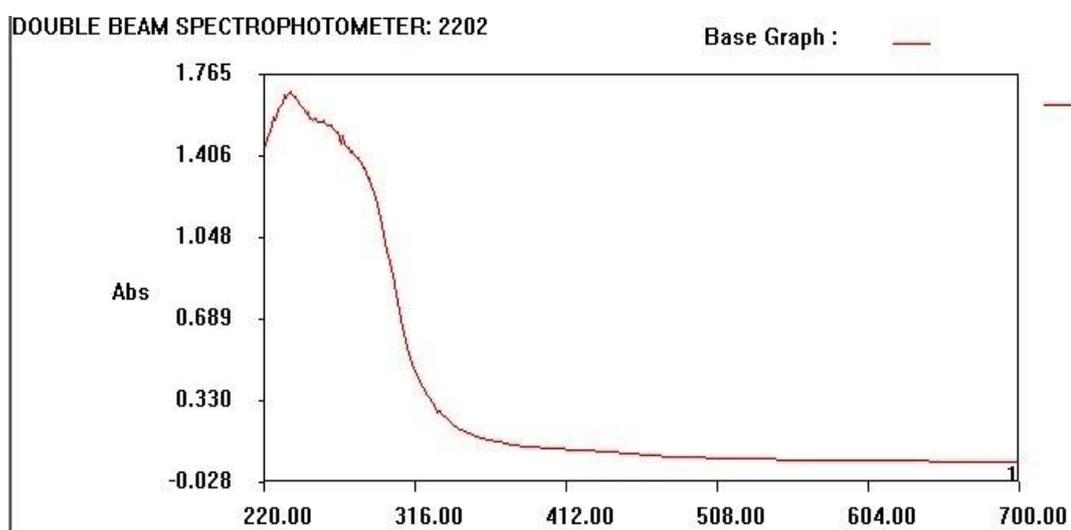


Figure 5. UV Spectra of Spectra of *Lawsonia inermis* Active Fraction 'E'

## CONCLUSION

From the results and observations it is evidently seen that *Lawsonia inermis* has exhibited high potential *In-vitro* anti-inflammatory activity at concentration of 5 mg/ml (243.3 % prevention of protein denaturation).

From Table 1 and Figure 3, it is clear that activity of suspected 'lawsone' (Figure 4-5) is lower than that of standard diclofenac. But taking in-consideration the side effects of allopathic drugs, natural remedies from such herbs would be more preferable in making pills or ointments for better results.

## ACKNOWLEDGEMENT

The authors are thankful to the Principal D.B.F Dayanand College of Arts and Science, Principal V.G.Shivdare College of Arts, Commerce and Science, Solapur (M.S.), India, for providing all the essential laboratory facilities to do present research work.

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