IN VITRO ANTI-INFLAMMATORY ACTIVITY OF *VACHELLIA NILOTICA (L)* AND *HIBISCUS LILIIFLORUS* AGAINST THE PROTEIN DENATURATION

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ABSTRACT:

Objective: To evaluate the anti-inflammatory effects of ethanolic extracts of *Vachellia nilotica (L)* and *Hibiscus liliiflorus* leaves extract against protein denaturation assay. Method: To evaluate the extract's anti-inflammatory properties, it was incubated with BSA solution at various doses under carefully monitored experimental circumstances. The different concentractions (100 µg/ml, 200 µg/ml, and 500 µg/ml) of extracts as well as standard drug were examined. The extract's absorbance was then measured. Diclofenac sodium is used as the standard drug for comparison. Result: Significant effect for Anti-inflammatory activity was shown by both the plants. Synergistic effect also studied on the both plants. Conclusion: From the present study we can concluded that *Vachellia nilotica (L)* and *Hibiscus liliiflorus* plants had notable anti-inflammatory activity.

Keywords - Anti-inflammatory, Protein denaturation, *Vachellia nilotica (L)*, *Hibiscus liliiflorus*, synergistic, etc.

I. INTRODUCTION

Inflammation is a biological response to harmful stimuli, such as pathogens or tissue injury, involving immune cells, blood vessels, and molecular mediators. The process is characterized by the release of pro-inflammatory cytokines and the activation of various signaling pathways, which can result in tissue damage if uncontrolled. One of the consequences of inflammation is the denaturation of proteins, which disrupts their structure and function, contributing to the inflammatory process. Protein denaturation refers to the alteration of a protein's natural structure, leading to the loss of its biological function. This phenomenon can be triggered by various factors, including heat, pH changes, and oxidative stress, all of which are prevalent during inflammatory responses. Inhibition of protein denaturation is therefore considered a potential therapeutic strategy to mitigate inflammation.

Herbal plants are rich sources of bioactive compounds, including flavonoids, saponins, tannins, and alkaloids, which have demonstrated anti-inflammatory properties. The protein denaturation method provides a reliable in vitro assay to evaluate the anti-inflammatory potential of these extracts. By assessing the ability of herbal extracts to prevent protein denaturation, researchers can identify promising candidates for the development of natural anti-inflammatory agents.

The protein denaturation method typically involves exposing a protein solution (often bovine serum albumin) to heat or other denaturing conditions in the presence of various concentrations of herbal extracts. The extent of protein denaturation is measured spectrophotometrically, allowing for the calculation of percentage inhibition of denaturation.

This method not only provides insights into the anti-inflammatory potential of the extracts but also helps in understanding the mechanisms by which these herbal compounds exert their effects.

In conclusion, the investigation of anti-inflammatory activity in herbal plants through the protein denaturation method presents a promising avenue for the discovery of natural therapeutic agents. This approach aligns with the growing interest in phytotherapy as a safer alternative to conventional anti-inflammatory drugs, which often come with significant side effects.

Vachellia nilotica (L.) commonly known as prickly acacia or Indian gum arabic tree , is a species belonging to the Fabaceae family, specifically the sub-family Mimosoideae. This plant is native to arid and semi-arid regions across Africa, the Arabian Peninsula, and the Indian subcontinent, including countries such as India, Pakistan, and parts of Southeast Asia like Myanmar. Vachellia nilotica typically manifests as a scrambling shrub or small tree, characterized by its thorny branches and twice-compound leaves. The plant produces globular flower clusters, which are predominantly yellow, and its fruit is elongated, containing several seeds.

This species serves for its medicinal properties, having been used in traditional Ayurvedic medicine due to its antioxidant and various therapeutic activities. Furthermore, *Vachellia nilotica* is a source of gum arabic, which has applications in food and pharmaceutical industries. However, it can also become invasive in certain regions, impacting local biodiversity.The study of Vachellia nilotica is significant for understanding its ecological interactions, potential agricultural benefits, and its role in traditional medicine. Research can also focus on its adaptability to climate change and its utility in sustainable practices, particularly in regions facing desertification and land degradation.

Hibiscus liliiflorus, commonly known as the lily-flowered hibiscus, is a species of flowering plant in the family Malvaceae. The plant has a branched taproot and an aerial, erect, green, cylindrical, and branched stem. *Hibiscus liliiflorus* is characterized by its simple, petiolate leaves with alternate phyllotaxy. The leaf shape is ovate with an acute tip and a serrated margin. The venation is unicostate reticulate, and the leaf surfaces are glossy. *Hibiscus liliiflorus* is investigating the plant's phytochemical constituents and potential pharmacological activities could lead to the discovery of novel therapeutic applications.

II. MATERIALS AND METHODS

- **Drugs & Chemicals :**

BSA, NaCl, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Methanol, Standard diclofenac sodium all chemicals were procured from Research lab, Mumbai, Maharashtra, India.

- **Plant Material:**

Leaves of *Hibiscus liliiflorus* and *Vachellia nilotica (L)* plants was collected from native areas of Sangli (Maharashtra, India) and dried. Dried leaves were ground mechanically and converted into coarse powder.

- **Extraction method:**

Extraction is carried out with the help of soxhlet apparatus method. The Sequential solvent extraction method is used. In this method solvents are used with lower polarity to greater polarity. The solvents were used in series as pet ether, chloroform and ethanol, respectively.

50 gm of plant powder is extracted by using 150 ml of pet ether and temperature is maintained at 60^{O}C for 72 hrs. Extracted powder is dried and used for next solvent i.e. chloroform and temperature is maintained as 55^{O}C for next 72 hrs. and the same powder is used for ethanol and temperature is maintained at 70^{O}C for 72 hrs. All the extracts are collected and stored in amber colored bottles.

- **Assessment for Anti-inflammatory activity:**

In separate test tubes, 0.9 ml of 5% aqueous solution of bovine serum albumin and 0.1 ml of a test solution containing extracts at varying concentrations (100 µg/ml, 200 µg/ml, and 500 µg/ml produced in methanol) were used for the experiment. Instead of using the test solution, 0.1 ml of distilled water was utilized as the control. Instead of the test solution, 0.1 ml of diclofenac sodium was employed (various concentrations were produced in methanol, viz. 100, 200, and 500 µg/ml).After 5 minutes of incubation at 37 ^{0}C, the mixture was heated to 55 ^{0}C for 3 minutes, and the test tubes were cooled. Each test tube received 2.5 ml of phosphate buffer saline with a pH of 6.3 after cooling. The absorbance was measured at 660 nm spectrophotometrically.

- **Calculation :**

The inhibition to the protein denaturation was measured as

$$\text{Percentage inhibition} = \frac{\text{Abs. of control} - \text{Abs. of treated}}{\text{Abs. of control}} \times 100$$

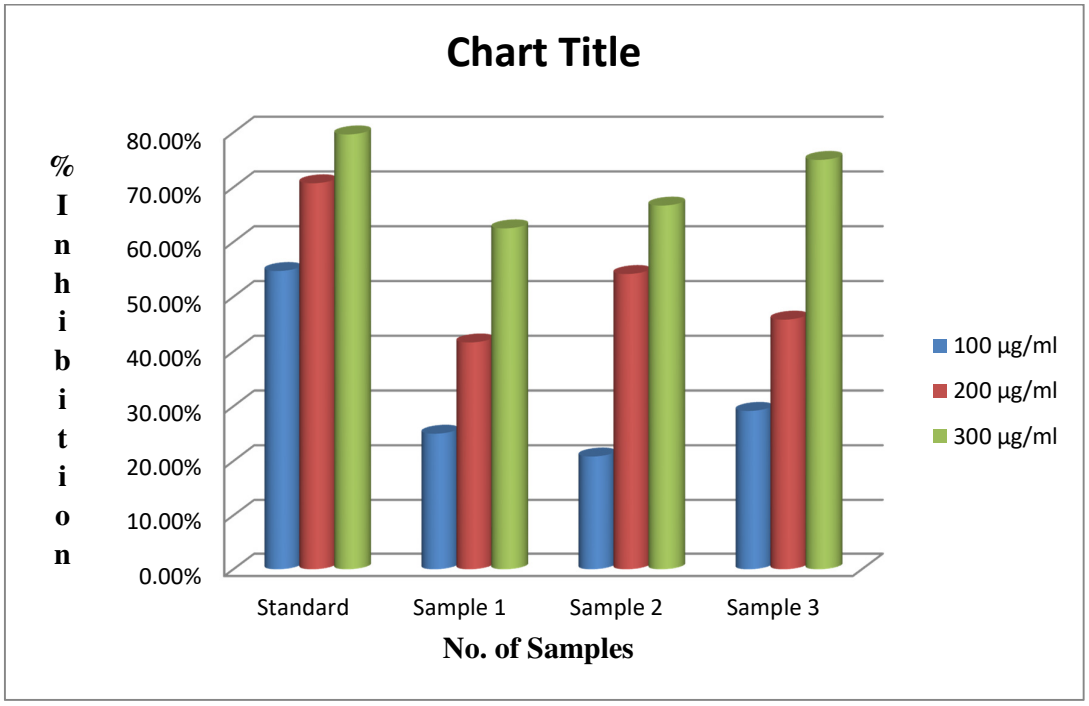
III. RESULT:

In the present investigation, the in vitro anti-inflammatory activity was evaluated by using protein denaturation method. The results are recorded in table 1. The present study was done on three samples: sample1, sample2 and sample3. Different concentrations (100 µg/ml, 200 µg/ml, and 500 µg/ml) of samples and standard drug (Diclofenac Sodium) are evaluated.

Table No. 1: Anti-inflammatory Activity on Sample1, 2 & 3

Sr. no	Sample	Conc.	Absorbance	% inhibition
01	**Control**	-	0.024	**-**
02	**Standard (Diclofenac Sodium)**	100	0.118	54.71 %
		200	0.082	70.73 %
		500	0.053	79.66 %
03	**Sample 1**	100	0.018	25 %
		200	0.014	41.66 %
		500	0.009	62.50 %
04	**Sample 2**	100	0.019	20.83 %
		200	0.011	54.16 %
		500	0.008	66.66 %
05	**Sample 3**	100	0.017	29.16 %
		200	0.013	45.83 %
		500	0.006	75%

Graph No. 1: Percentage inhibition of different concentrations of sample for Anti-inflammatory assay.



IV. Discussion:

In the present study the protein denaturation bioassay was done for in vitro assessment of anti-inflammatory property of ethanolic extracts of *Vachellia nilotica (L)* and *Hibiscus liliiflorus* leaves. Protein denaturation refers to the alteration of a protein's natural structure, leading to the loss of its biological function. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. By assessing the ability of herbal extracts to prevent protein denaturation, researchers can identify promising candidates for the development of natural anti-inflammatory agents. Sample 1 is extract of *Vachellia nilotica (L)*, sample 2 is extract of *Hibiscus liliiflorus* and sample 3 is extract mixture of sample 1 and 2 in (1:1) ratio. All three samples show significant anti-inflammatory activity. Sample 1 has percentage inhibition is 62.50 % , sample 2 gives % inhibition is 66.66 % and sample 3 shows 75% percentage inhibition in protein denaturation assay. All three samples have good anti-inflammatory effects, but sample 3 has the best effect. This suggests that it works even better when combined with something else, showing a synergistic effect.

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