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Effects of *Justicia Adhatoda* L. and *Clerodendrum wallichi* Merr. Crude Leaves Extracts on Protein Denaturation Inhibition for Potential Anti - Inflammatory Activity: *In Vitro*

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Abstract: ***The current study aimed to investigate the anti - inflammatory activity of methanolic and butanolic leaves extract of Justicia adhatoda L. butanolic extract (JABE), methanolic extract (JAME) and Clerodendrum wallichi Merr. Butanolic extract (CWBE), methanolic extract (CWME). The anti - inflammatory activity of these extracts was evaluated using in vitro - based assays: protein denaturation inhibition. Methanolic and butanolic extracts exhibited a concentration dependent inhibition of protein (egg albumin and BSA). Results showed that the percentage inhibition of protein denaturation at concentration of 500µg/ml of JABE has exhibited a significantly higher inhibition of 61.65% in egg and 81.21% in BSA, CWBE showed 80.33% in egg and 73.02% in BSA. Also, JAME exhibit a significant increase in protein inhibition of 59.69% in egg and 76.89% in BSA, CWME 76.1% in egg and 71.44% in BSA. In conclusion, results revealed that the studied leaves possess anti - inflammatory properties at different levels, and this could be due to the differences in the composition and concentration of bioactive compounds. The objective of the present study is to ameliorate in vitro conformation of potential inhibition of protein denaturation by using the methanolic and butanolic extracts of Justicia adhatoda L. and Clerodendrum wallichi Mer. The present study put forward that the crude extract of Justicia adhatoda L. and Clerodendrum wallichi Mer. act as potent in vitro anti - inflammatory activity.***

Keywords: Medicinal plant; anti - inflammatory activity; Egg albumin; denaturation; inhibition; Bovine serum albumin (BSA)

1. Introduction

The intricate and poorly understood interaction between humoral and cellular components is known as inflammation [1]. The complicated process of inflammation, which is commonly linked to pain, includes increased membrane permeability, increased protein denaturation, and increased vascular permeability. A natural defense mechanism against tissue damage brought on by toxic chemicals, microbes, or physical trauma is inflammation, the body's reaction to eliminate the irritants, inactivate or kill the invasive organism, and prepare the tissue for healing, causing chemical mediators released from damage tissue and migratory cell [3]. In vivo denaturation of proteins may be the cause of the generation of autoantigens in some arthritic conditions [4]. Electrostatic hydrogen, hydrophobic and disulfide bonding changes are most likely part of the denaturation mechanism [5]. Therefore, antiarthritic or anti - inflammatory effect is produced by regulating the generation of autoantigen and preventing denaturation of proteins and membrane lysis in rheumatic disease. Further, the in vitro anti - inflammatory effect was measured by protein denaturation. Nonsteroidal anti - inflammatory medicines (NSAIDs), which are frequently used to treat inflammatory disorder, have numerous side effects, most notable the development of gastric ulcer due to inflammation of the stomach [6, 7, 8]. There are two distinct forms of inflammation: acute inflammation and chronic inflammation. Acute inflammation is the body's broad response to any form of tissue injury, which could be chemical, thermal, or mechanical [9, 10]. The hallmarks of chronic inflammation are the concurrent breakdown and recovery of the tissues harmed by the process of inflammation and is a protracted inflammatory reaction that leads to a gradual shift in the type of cells near the site of inflammation [11]. Chronic inflammation is currently believed to be a risk factors for a number of age - related illness including cancer, diabetes, atherosclerosis and hypertension [12]. A low - grade inflammatory response can be caused and sustained by a number of factors, these include smoking, low sex hormones levels, age, and imbalance diet [13]. The development of traditional medicines has been benefited greatly from the used of natural products. Recent indepth studies on a wide range of plant species and their active therapeutic ingredients have caused the globe to re - evaluating conventional medicine. Finding and using natural compound as an alternative to manufacture drugs is made possible by the investigation on natural plant that have anti - inflammatory properties [14, 15]. Because of their therapeutic properties, plants have been used in traditional medicine for thousands of years. Organic anti - inflammatory analyzing plants can reveal molecules that, in comparison to synthetic drugs, may have lower toxicity profiles and fewer side effects [16]. Substances derived from plants are commonly used by people for a long period of time and are typically thought to be innocuous. This is especially important for chronic conditions when using anti - inflammatory drugs for and extended period of time may have detrimental effects on health. Among the many different types of bioactive compounds that are known to exist in natural plants are polyphenols, flavonoids, terpenoids, and alkaloids, these

compounds have promising anti - inflammatory properties and can target variety of inflammatory pathways [17]. Novel molecules or chemical structures that could serve as the foundation for drug discovery and development can be found by examining naturally occurring anti - inflammatory plants. This could lead to the creation of more potent and precisely targeted anti - inflammatory medications. The main goal of the egg albumin denaturation assay is to ascertain whether certain substances or agents can prevent the denature state of egg albumin in specific conditions. The process by which a protein loses its biological activity and undergoes structural alterations is known as denaturation [18]. The experiment uses egg albumin as a model protein, and denaturation is achieved by subjecting it to extremes in pH, heat, or other denaturing agents. Denaturation alters the physical properties of egg albumin and results in the loss of its functional activity by upsetting its initial configuration. The egg albumin denaturation assay assesses a substance's anti - inflammatory properties by measuring its ability to stop or reduced egg albumin denaturation. The main advantages of herbal medication appear to be its low cost, low incidence of serious side effects, and perceived effectiveness [19]. This assay's implementation is justified by the fact that albumin protein denaturation produces antigens that trigger a type III hypersensitivity reaction, which in turn causes inflammation [20]. About 80% of human population rely on traditional medicine for primary health care. The selected plant *Justicia adhatoda* L. locally known as 'diengkthang' and *Clerodendrum wallichii* Merr. As 'hor - randieng' both found in Khasi Hills District, Meghalaya, India. These are perennial evergreen and highly branched shrubs, the tender leaves of these plants were use as a vegetables after cooking by the Khasi and Garo tribes of Meghalaya. They have been used traditionally for years as herbal medicine for treating cold, cough, skin diseases wounds, fever, headache and mostly used by the local for lowering blood pressure. Thus, it would be of great interest to evaluate these selected plants for their anti - inflammatory activity in vitro. Herbal medicines are cost effective, less - toxic and have minimum side effect. Furthermore, scientific evaluation for their biological activities of these plants could help to justify their used in health care system.

2. Material and Method

Sample collection: the leaves of *Justicia adhatoda* L. and *Clerodendrum wallichii* Merr. Were collected during the month of September - October following basing protocol, submitted to Botanical Survey of India, Eastern Regional Centre, Shillong Meghalaya, India for identification.

Sample extracts preparation

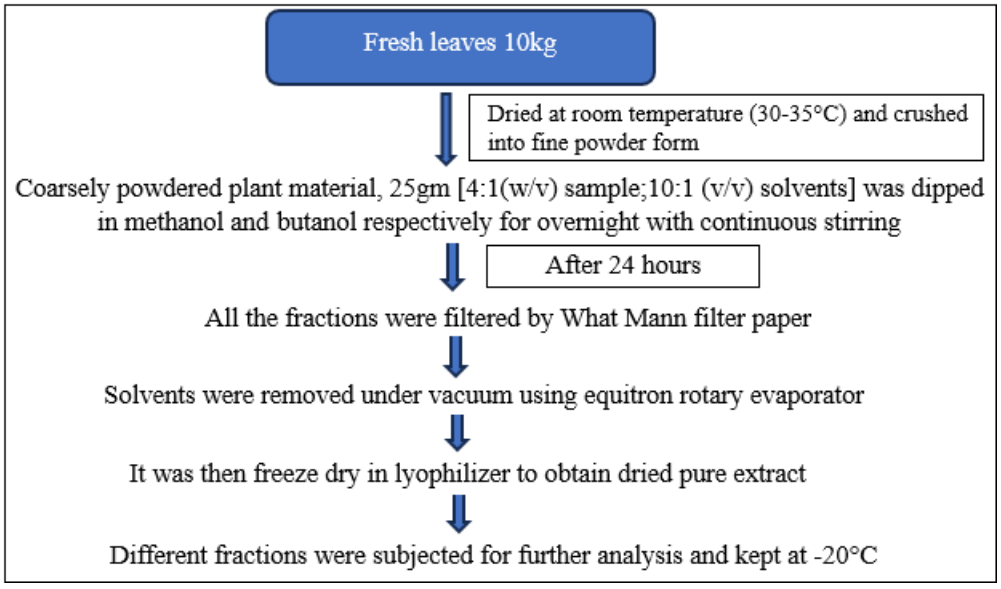


Figure 1: Flow chart for sample preparation

Chemicals: Chemical and Reagent
Fresh hen egg albumin solution, Bovine Serum Albumin (BSA), Phosphate buffered saline, Distilled water. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

In vitro anti - inflammatory activity: Inhibition of egg albumin denaturation
Preparation of reference drug (positive control)

Diclofenac sodium was used as reference drug. Diclofenac sodium was crushed into fine powder. About 10 mg of Diclofenac sodium powder was measured using a weighing analytical balance and was added to 10.0 ml of distilled water, respectively. The solution was mixed well using a vortex. [21]

Preparation of 1% egg albumin solution:
1% egg albumin solution was prepared using fresh hen eggs, to make a solution of egg - albumin. Fresh hen's egg, was break it gently, then add 1 mL of the translucent part to 100 mL of w/v distilled water and mix thoroughly. When making the solution, the water should be cold. Hot water will lead coagulation. [22]

To determine the percentage of inhibition of protein denaturation the following steps were followed:

For Control
2 ml of Milli - Q water, 2.8 ml of phosphate buffer saline (pH 6.4), and 0.2 ml of egg albumin.

Standard drug
2.8 ml of phosphate buffer saline (pH 6.4), 0.2 ml of egg albumin, and 2 ml of different concentrations of the typical standard drug Diclofenac sodium at concentrations.

For test sample,
2.8 ml of phosphate buffer (pH 6.4), 0.2 ml of egg albumin, and 2 ml of methanol and butanol leave extracts at concentrations of 100, 200, 300, 400 and 500 µg/ml. After 15 minutes of incubation at 37˚ C, the samples were heated for five minutes at 70˚ C. Following cooling, the UV - vis spectrophotometer's absorbance of turbidity was measured at 660 nm using Milli - Q water as the blank [23]. All the experiment were performed in triplicates. The equation below was used to determine the aforesaid % inhibition of protein denaturation [24].

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

Percentage inhibition I%= (Abs control−Abs sample) × 100/Abs of control

Where,
Abs sample= Absorbance of test sample,
Abs control= Absorbance of control

Protein denaturation using bovine serum albumin (BSA) [25, 26]
Preparation of 1% BSA
1g of BSA was added to 100 Milli - Q water (w/v)

The reaction mixture was consisting of 1% solution of bovine albumin, methanolic and butanolic extracts of *Justicia adhatoda* L. and *Clerodendrum wallichii* Merr. at different concentrations and the reaction mixture were incubated at 37°C for 20 min and then heated at 57°C for 20 min. After cooling the samples, the absorbance of turbidity was read at 660 nm using Milli - Q water as the blank. All the experiment were performed in triplicates.

Formula
Percentage of inhibition of protein denaturation was calculated as follows:
Percentage inhibition I % = (Abs control - Abs sample) X 100/ Abs Control

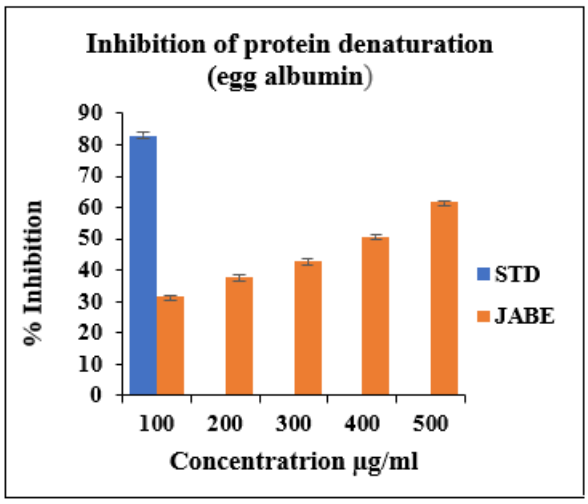
Where,
Abs sample= Absorbance of test sample,
Abs control= Absorbance of control

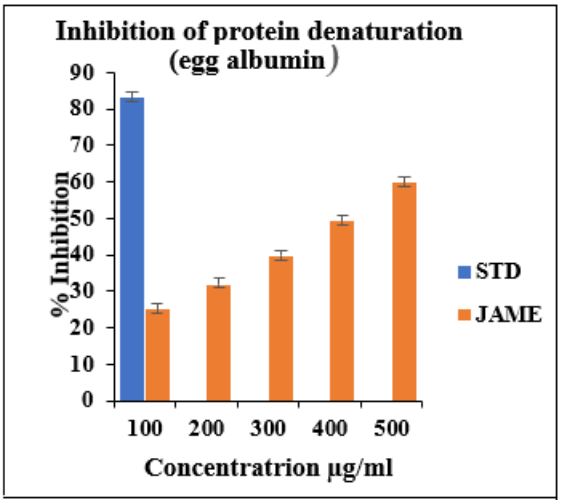
Statistical Analysis
As a statistical analysis tools Excel, GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used. To calculate the mean and the standard error of the mean, all results (absorbance) in triplicated.

3. Result & Discussion

The impact of *Justicia adhatoda* L. and *Clerodendrum wallichii* Merr. were assessed against the denaturation of egg albumin and BSA. The results was summed up in figure 2 & 3 below:

In vitro Anti - Inflammatory Activity using Egg Albumin;





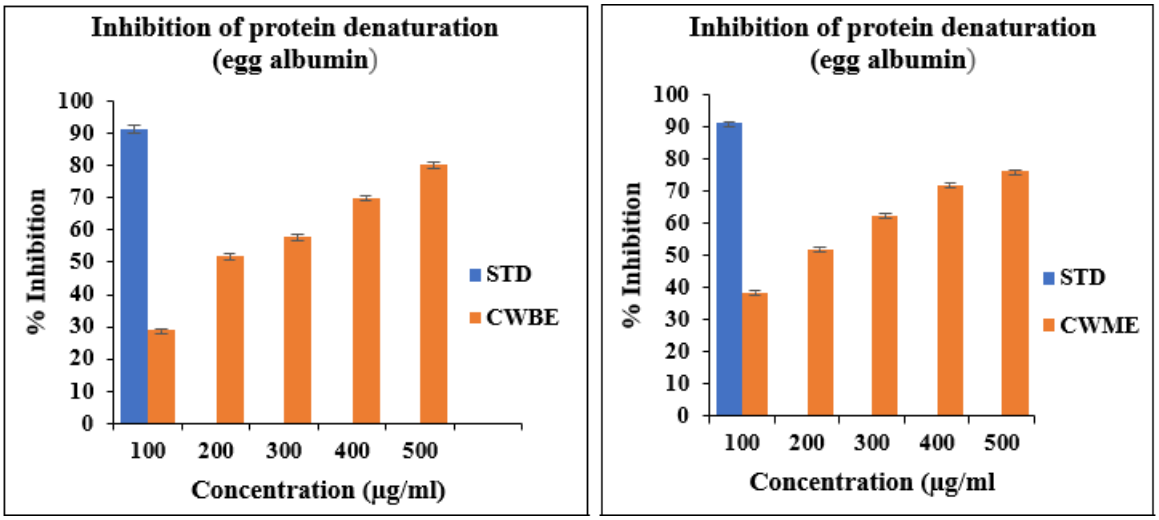


Figure 2: *In vitro* anti - inflammatory activity of *Justicia adhatoda* L. butanolic (JABE) and methanolic (JAME) and *Clerodendrum wallichii* Merr. Butanolic (CWBE) and methanolic (CWME) on protein denaturation (Fresh egg albumin); Diclofenac sodium (STD); Values are presented as M ± SEM, M: Mean; SEM: Standard error of mean.

In - vitro anti - inflammatory activity using bovine serum albumin (BSA);

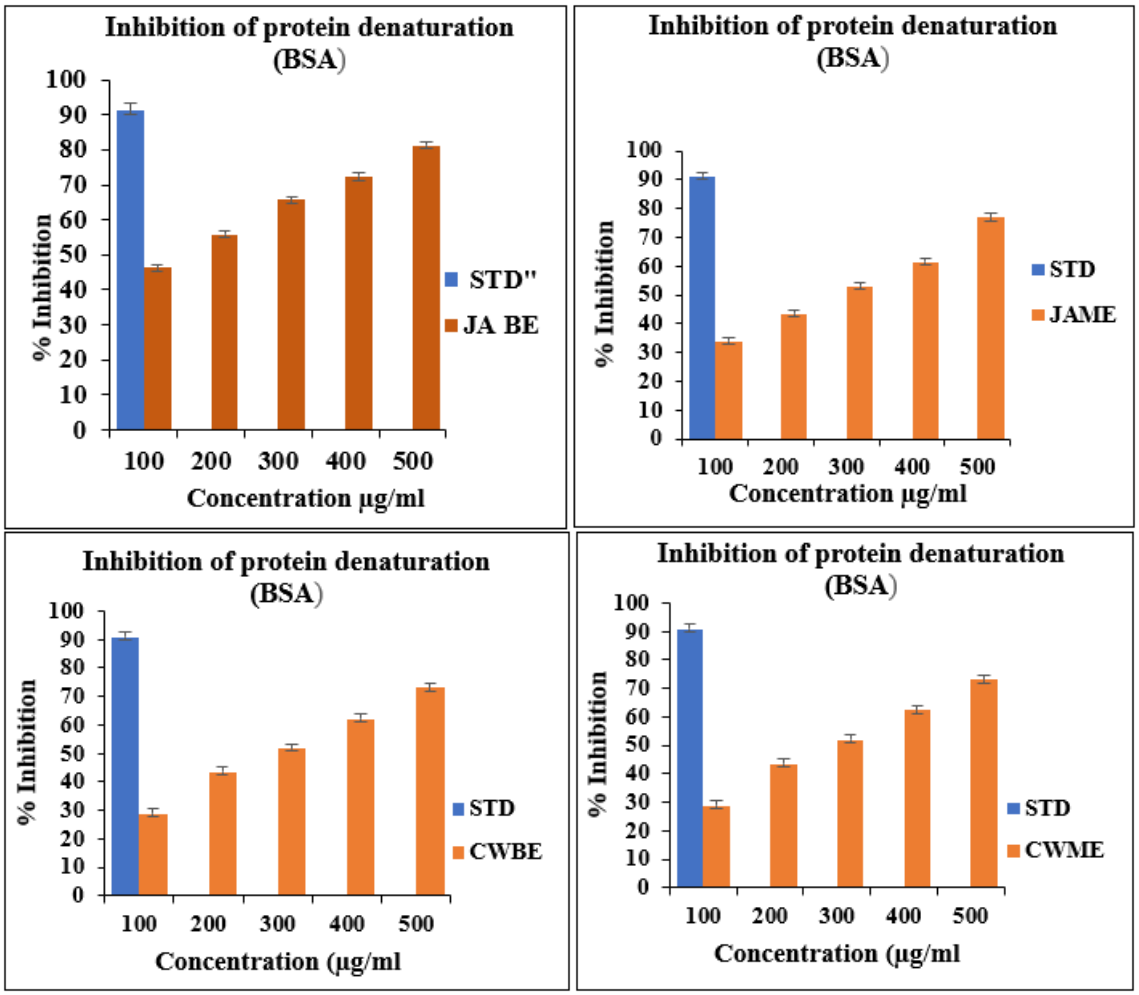


Figure 3: *In vitro* anti - inflammatory activity of *Justicia adhatoda* L. butanolic (JABE) and methanolic (JAME) and *Clerodendrum wallichii* Merr. Butanolic (CWBE) and methanolic (CWME) on protein denaturation (Bovine Serum Albumin); Diclofenac sodium (STD). Values are presented as M ± SEM, M: Mean; SEM: Standard error of mean;

By inhibiting protein denaturation, JABE, JAME, CWBE, CWME extracts demonstrated notable anti - inflammatory efficacy at 100 - 500 µg/ml herbal extract's impact was examined by contrasting it with that of regular diclofenac sodium. Diclofenac sodium at concentration of 100 µg/ml demonstrates, 82.98% inhibition of egg albumin denaturation (figure 2). Numerous studies show that, one of the causes of inflammation is protein denaturation, which leads to the

generation of autoantigens in inflammation. [27] Denaturation of proteins is a process wherein proteins lose their secondary and tertiary structures due to the application of external stressors or compounds, such as heat, an organic solvent, a concentrated inorganic salt, or a strong acid or base. When denatured, the majority of biological proteins cease to function biologically. One of the well - established causes of inflammation is the denaturation of tissue proteins. [28] The test extract and the reference medication diclofenac sodium inhibited protein (albumin) denaturation or had an anti - denaturation effect, as seen by the test sample's decreased absorbance as compared to the control [29]. At concentration 500 µg/ml JABE exhibit highest percentage inhibition 61.65%, in egg and 81.21% in BSA, JA ME showed 59.69% in egg and 76.89 % in BSA, also CWBE showed 80.33% in egg and 73.03 in BSA, CWME 76.1% in egg and 71.44% in BSA *in vitro* assay. Comparatively, in both of these plants, butanol extract outperformed the methanol extract. At 500µg/ml, the highest percentage suppression of protein denaturation was observed. While standard, diclofenac sodium at concentration 100 µg/ml showed percentage inhibition of 91.17% in egg 90.71% in BSA respectively. Both *Justicia adhatoda* L. and *Clerodendrum wallichi* Merr. Butanol and methanol leaves extracts demonstrated a significant inhibitory impact and concentration - dependent suppression of protein denaturation. Additionally, *Justicia adhatoda* L and *Clerodendrum wallichi* Merr. plant extracts showed findings that were equivalent to standard in this case, the typical anti - inflammatory medication, diclofenac sodium. Moreover, natural therapies made from these plants would be better choice for better outcomes when taking into account the side effects and cost of commercial drugs. Therefore, studying and analysing natural plants might result in the identification of a new bioactive substances with significant anti - inflammatory potential. One known cause of inflammation is protein denaturation. The capacity of plant extracts to prevent denaturation was examined as part of the investigation into the mechanism behind the anti - inflammatory response [30].

4. Conclusion

Based on the current study's findings, it can be concluded that, the results of the *in vitro* investigation on these two medicinal plants can suppress protein denaturation in inflammation investigation and regulate autoantigen synthesis. Therefore, *Justicia adhatoda* L. and *Clerodendrum wallichi* Merr. shown effective anti - inflammatory activity and can therefore be utilized to manage inflammatory conditions. To fully clarify the active principles and the precise mechanism of action, pharmacological and biochemical research is required.

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