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In Vitro Anti-arthritic activity of *Tecoma stans* (Linn.) Leaves

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Abstract: The World Health Organization (WHO) estimates 80% of the world population presently use herbal medicine for some aspects of primary health care. Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. There are many herbs which are described in Ayurveda for arthritis. The medicinal plant contains flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins phytoconstituents, having anti-inflammatory effects. *Tecoma stans* (Linn.) belongs to the family of Bignoniaceae. The root of *Tecoma stans* as diuretic, vermifuge and tonic. Pharmacological reports revealed that it is having antidiabetic, anticancer, antioxidant, anti-inflammatory activity, antimicrobial, and antifungal properties, and extensively used in the treatment of diabetes. Alcohol, Water, successive extracts of Petroleum ether, Chloroform, Methanol and Water extracts of *T. stans* leaves were tested for Antiarthritic activity using Diclofenac sodium as standard by *In-vitro* models like, Inhibition of protein denaturation and effect on membrane stabilization. Alcohol, Water and succesive Methanol extracts of *T. stans* leaves exhibited significant Antiarthritic activity. The results of the investigations justify us the folklore use of *T. stans* leaves in the treatment of inflammation during arthritis and the plant is worth for further chemical isolation and pharmacological investigations.

Keywords: *T. stans, Antiarthritic activity, Bignoniaceae*

I. Introduction

The World Health Organization (WHO) estimates 80% of the world population presently use herbal medicine for some aspects of primary health care. It is estimated that around 70,000 plant species have been used for medicinal purposes [1]. About 1% of the world's population is afflicted by rheumatoid arthritis, women three times more often than men. Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attack synovial joints [2, 3]. The production of auto antigens in certain arthritic diseases may be due to *in vivo* denaturation of proteins [4]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [5]. So, by controlling the production of auto antigen and inhibiting denaturation of protein and membrane lysis in rheumatic disease leads to anti-arthritic activity. Hence, inhibition of protein denaturation and membrane lysis were taken as a measure of the in vitro anti-arthritic activity. A larger number of herbal extracts are in vogue for the treatment of various types of arthritis. The root of *Tecoma stans* as diuretic, vermifuge and tonic. Pharmacological reports revealed that it is having antidiabetic, anticancer, antioxidant, antispasmodic, anti-inflammatory activity, antimicrobial, and antifungal, properties, and extensively used in the treatment of diabetes [6]. The leaves of the plant *Tecoma stans* have been traditionally claimed to be useful in inflammation conditions. By this reference the plant *Tecoma stans* is selected for scientific validation for the Antiarthritic activity.

II. Experimental Section

II.1. Collection and authentication of plant

Fresh leaves of *Tecoma stans* was collected from college campus of The M. L. Gandhi Higher Education Society, Modasa. Identification of plant was carried out by Dr. M. S. Jangid, Botanist, Sir P. T. Science College, Modasa. Voucher specimens [PHCOG/1224/2008/02] were deposited in Pharmacognosy museum, department of Pharmacognosy, Shri B M Shah College of Pharmaceutical Education and Research, Modasa.

II.2. Preparation of Extracts

Shade-dried and powdered leaves of *Tecoma stans* was extracted with (95%) ethanol and to get the alcoholic extract. The leaf powders were macerated with 400 ml distilled water and add few drops of chloroform for preventing microbial growth. Seven days with occasional shaking to get the aqueous extract. Another batch was also successively extracted first with petroleum ether (40-60), second chloroform, and third methanol in increasing order of polarity. Finally remaining marc was refluxed with water and to get successive water extract. Filter and take the filtrate. All the extracts were concentrated under reduced pressure using rotary evaporator and the residue was dried in desiccators over anhydrous calcium chloride.

II.3. Qualitative chemical tests

All the extracts like alcohol, water and succesive petroleum ether, chloroform, methanol, water extracts were tested in pharmacognosy & Phytochemical research laboratory in our college to know the different constituents present in them by the standard procedures. The extracts were tested for different phytoconstituents like sterols [7], alkaloids [8] triterpenes, saponins [9], flavonoids [10], carbohydrates [11].

II.4. Chemicals

Bovine serum albumin was procured from National chemicals, Vadodara, Gujarat, India. Isolated human red blood cells were collected from Ramani Blood Bank, Modasa, Gujarat.

II. 4 *In Vitro* Antiarthritic activity

II.4.1. Inhibition of protein denaturation

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of different extracts of *Tecoma stans* (100 to250 µ g/ml). The pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37oC for 20 min and then heated at 57^{o} C for 3min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Take absorbance spectrophotometrically at 416 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The control represents 100% protein denaturation [12]. The results were compared with Diclofenac sodium (100-250 µg/ml). The percentage inhibition of protein denaturation was calculated as follows.

$$\textbf{Percentage inhibition} = 100 - [(AT - APC)/Ac] \times 100 \quad (1)$$

Where; AT is the Absorbance of test; APC is the Absorbance of product control
Ac is the Absorbance of Control

II. 4.2. Effect on Membrane Stabilization

II.4.2.1. Preparation of Red Blood cells Suspension

Fresh whole human blood (10 ml) was collected and transferred to the heparin zed centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal

volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

The assay mixtures contain 1ml phosphate buffer (pH 7.4, 0.15M), 2ml 0.15 M hypotonic saline (0.36 % NaCl), (pH 7.4), 0.5 ml of Human red blood cells (10% v/v) and 0.5 ml test solution and standard drug Diclofenac sodium (250, 500, 750, 1000µg/0.5 ml) in normal saline. For control tests, 1 ml of isotonic saline was used instead of test solution while product control tests lacked red blood cells. The mixtures were incubated at 56^{o}C for 30 minutes. The tubes were cooled under running tap water for 20 minutes. The mixtures were centrifuged and the absorbance of the supernatants read at 560 nm [13, 14]. Percentage membrane stabilizing activity was carried out using equation.

$$\textbf{Percentage stabilization= [(AT-APC)/Ac] x 100} \quad (2)$$

Where; AT is the Absorbance of test; APC is the Absorbance of product control, Ac is the Absorbance of Control.

II. 5 Statistical analysis

Comparison between control and drug treated groups were analyzed by one way Anova. $P<0.05$ and $P<0.01$ were considered to be significant.

III. Results and Discussion

III.1. Preliminary Phytochemical investigation of *T. stans* linn leaves

Alcohol extract showed the presence of alkaloids, carbohydrates, flavonoids, amino acids, Triterpenoids, glycosides and tannins. Aqueous extract contains carbohydrates, flavonoids, amino acids, glycosides and tannins, succesive petroleum ether extract showed presence of Triterpenoids, succesive chloroform extract contains alkaloids and Triterpenoids, succesive Methanol extract contains flavonoids, glycosides and tannins, succesive aqueous extract contains carbohydrates.

III.2. Inhibition of protein denaturation

The alcohol, water, successive extracts of petroleum ether, chloroform, methanol and Diclofenac sodium had exhibited dose dependent percentage inhibition in protein denaturation, but successive Water extracts had not inhibit protein denaturation in the concentration tested. Results suggest that alcohol, water and successive methanolic extracts had extremely significant antiarthritic activity.

Table 1: Inhibition of protein denaturation of T. stans leaves

Extracts/ Standard	**Percentage Protein Denaturation**			
Concentration µg/0.5ml	**100**	**150**	**200**	**250**
Diclofenac Sodium	69.12±0.22	78.09±0.33	85.50±0.11	94.01±0.11
Alcohol**	25.4± 0.55	40.1±0.33	57.2±0.11	60.2±0.55
Water**	35.2±0.022	46.1±0.98	58.3±0.33	64.1±0.55
Su. Pet. Ether	4.5 ±0.55	6.7 ±0.33	8.3±0.22	9.2±0.46
Su. Chloroform*	12.4±0.66	17.5±0.66	26.6±0.57	33.7±0.44
Su. Methanol*	27.5±0.44	39.3±0.03	53.3±0.66	59.2±0.55
Su. Water	--	--	--	--

** Extremely significant (P<0.01), * Significant (p< 0.05), ns- Not significant (P> 0.05)

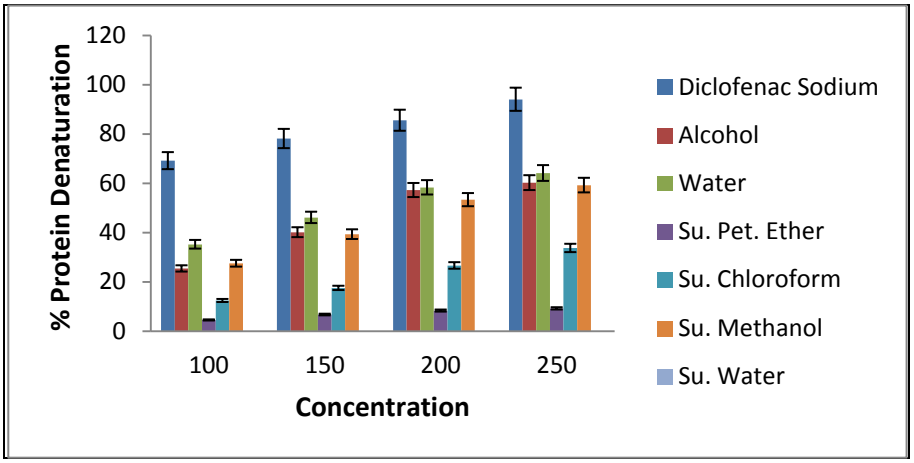

Figure 1: Inhibition of protein denaturation of T. stans leaves

III.3. RBC membrane stabilization activity

The alcohol, water, successive extracts of petroleum ether, chloroform, methanol extracts and Diclofenac sodium had shown dose dependent percentage inhibition of RBC stabilization, but successive Water extracts does not give inhibition of stabilization of RBC. Results suggest that alcohol, water and successive methanolic extracts had extremely significant antiarthritic activity.

Table 2: Effect on membrane stabilization of T. stans leaves

Extracts/ Standard	**Percentage Inhibition Membrane Stabilization**			
Concentration µg/0.5ml	**250**	**500**	**750**	**1000**
Diclofenac Sodium	84.32±2.33	87.19±2.34	90.20±1.13	92.41±2.22
Alcohol**	65.1± 1.77	69.2±2.08	75.2±1.03	80.7±0.33
Water**	45.5±2.44	50.2±1.22	58.2±2.10	61.2±0.44
Su. Pet. Ether	4.5±2.01	8.2±2.35	14.5±2.23	19.2±1.44
Su. Chloroform	27.2±1.35	35.2±1.50	40.2±0.99	45.3±2.55
Su. Methanol**	54.1±1.20	60.3±0.76	66.3±1.14	75.3±0.36
Su. Water	--	--	--	--

** Extremely significant (P<0.01), * Significant (p< 0.05), ns- Not significant (P> 0.05)

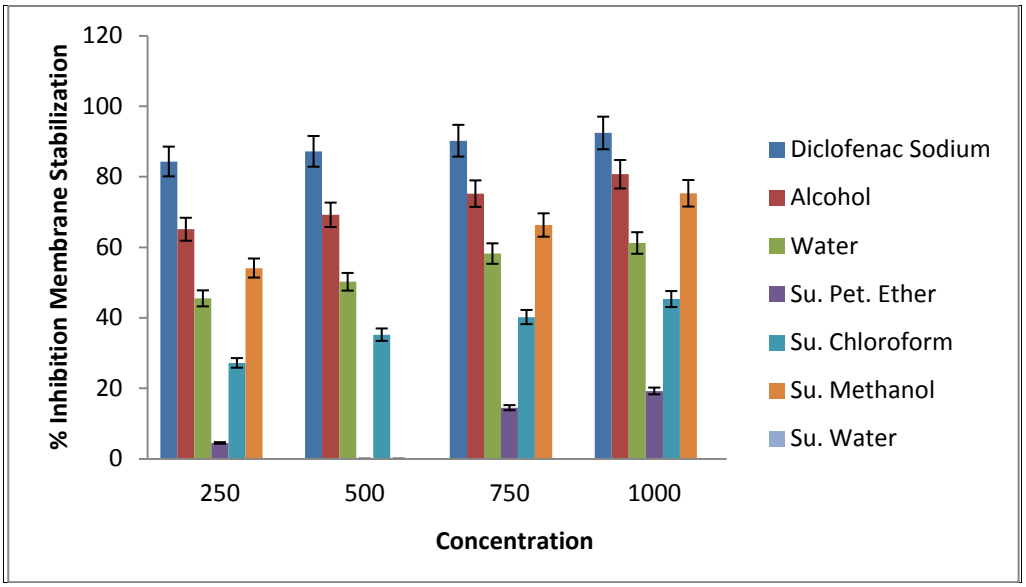

Figure 2: Effect on membrane stabilization of T. stans leaves

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site [15]. Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and disulphide bonding. The increments in absorbance of plant extract and reference drug with respect to control indicated the stabilization of albumin protein [16]. From the results (Tables 1 and 2) of our present study, it can be stated that Alcohol, Water and Su. Methanolic extracts are capable to inhibit denaturation of protein and membrane lysis in rheumatic disease. Alcohol extract showed the presence of alkaloids, carbohydrates, flavonoids, amino acids, Triterpenoids, glycosides and tannins. Aqueous extract contains carbohydrates, flavonoids, amino acids, glycosides and tannins; Methanol extract contains flavonoids, glycosides and tannins. Flavonoids, glycosides and tannins are common phytoconstituents alcohol, water and succesive methanol extracts.

IV. Conclusion

Inhibition of protein denaturation and membrane stabilization was studied to establish the mechanism of anti-arthritic effect of *T. stans* leaves Protective effect on heat and hypotonic saline-induced erythrocyte lysis is known to be a very good index of anti-inflammatory activity of any agent. Since the membrane of RBC is structurally similar to the lysosomal membrane, the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane. Therefore, our present in-vitro studies on *T. stans* leaves extracts demonstrated the significant anti-arthritic activity. Due to the presence of active principles such as Flavonoids, glycosides and tannins may responsible for this activity. Hence, *T. stans* leaves can be used as a potent anti-arthritic agent.

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