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In vitro antiarthritic screening of few herbal plants extract

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Abstract
Inflammatory illnesses like arthritis are treated with non-steroidal anti-inflammatory medicines (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs). Most of these produces serious side effects. A growing number of people throughout the world are turning to herbal formulations for their equivalent efficacy, less side effects, and general acceptability. Now a day's 60 percent to 90 percent of arthritis patients turn to non-conventional methods of pain relief. The efficacy of three plant components' aqueous extracts on *In vitro* anti-arthritic activity is being studied. The aqueous extracts of *Withania somanifera* Linn, *Curcuma Longa* and *Cedrus deodara* were studied for their *In vitro* anti-arthritic activity. All the extracts were showed good anti-arthritic activity (inhibition of protein denaturation and stabilization of membrane) at the concentration of 1mg/ml compared with standard diclofenac sodium.

Keywords: *Curcuma Longa*, *Withania somnifera*, *Cedrus deodara*, anti-arthritic activity

Introduction
Arthritis is a musculoskeletal system disorder following mechanical and biological events that destabilize normal coupling between degradation and synthesis within articular cartilage [1, 9]. Some forms of arthritis such as RA and lupus can infect multiple organs and cause widespread symptoms. Arthritis exhibits infection of the joint with age and causes severe pain or trauma to the joint. For whatever reason, the immune system attacks the body rather than defending it. Systemic symptoms including inflammation and synovial hyperplasia are common in RA [Iain B, 2011]. RA induced by prolonged inappropriate inflammatory responses is one of the most prevalent of all chronic inflammatory joint diseases. Bone erosion is a central feature of RA. It begins in the joints with the inflammation of the synovium [2, 10]. There is no cure available for RA. However, regular medications and surgery be needed at a later stage. Oxygen-derived free radicals are known to play an important role in the aetiology of tissue injury in RA [11]. The goals of currently used antirheumatic drugs are to reduce pain and swelling, delay the progression of the disease, minimize the disability, and ultimately improving patient life and expectancy. Most of these objectives are achieved by combination of non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs, corticosteroids, and biological agents [12]. The different NSAIDs are basically equally effective, but their effect will vary from person to person. Further NSAIDs can cause stomach ulcers when used over a long period of time. The worsening condition of arthritis requires proper therapy for arthritis along with better economical consideration for chronic treatment. As synthetic molecules have not been proven to provide adequate therapy due to toxicity, side effects, or reappearing of symptoms on discontinuation, there is urgent need to have alternative options for arthritis, Research has indicated that people suffering from chronic pain and those dissatisfied with current treatment are very likely to seek alternative treatments. Among the various there has been an exponential growth in the field of herbal medicine, and these drugs are gaining popularity in both developing and developed countries because of their natural origin, reduced risk of side effects, effective with chronic conditions, lower cost, and widespread availability.
Withania somnifera root [3]: *Withania somnifera* contains with anolides, a class of polymers that has gained attention for its purported therapeutic properties, in its roots. Rheumatoid arthritis (RA), polyarthritis (PA), and other arthritic conditions have all been connected to the usage of this drug.
Curcuma Longa rhizome [4, 5]: The colouring principle of turmeric was insulated in the 19th century and was named curcumin, which was uprooted from the rhizomes of *C. longa*., with unheroic colour and is the major element, being responsible for the anti-inflammatory

Goods [13]. In old Hindu drug, is considerably used for the treatment of sprains and bumps caused by injury.
Cedrus deodara wood [6]: The stem bark of this plant, once air-dried, can be extracted in water, yielding a substance with anti-inflammatory and anti-arthritic effects.

Materials and Methods

All selected 3 herbal plants collected from agricultural field of Maharashtra and were authenticated by Pharmacognosy Dept., Adarsh College of Pharmacy, Vita. The collected plants were sorted, washed, ground, and sieved through no. 40 mesh to produce a powder. Maceration with water and chloroform was then used for extraction (16:1). Concentrated extracts were stored in airtight glass jars [7].

Membrane stabilizing activity [8, 14-18]

The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC (human red blood cell) membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by the extracts to predict the anti-inflammatory activity *In vitro*.

Procedure

The HRBC method used was used for the estimation of anti-arthritic activity *In vitro*. Blood was collected from healthy human volunteers who did not consume NSAID for two weeks was obtained. Then it was mixed with equal volume of sterilized Alsever solution. This blood solution was centrifuged at 3000 rpm and the packed cells separated. The packed cells were washed with isosaline solution and 10%v/v suspension was made with isosaline. The reaction mixtures 4.5ml consists of 2ml hypotonic saline (0.25% NaCl) + 1ml 0.15M phosphate buffer (pH 7.4) + 100 µg/ml)+ 0.5ml of 10% rat RBC in normal saline. The mixture was incubated at 56 ºC for 30 minutes. The tubes were cooled under running tap water for 20 minutes were cooled under running tap water for 20 minutes. The mixture was centrifuged for 3000 rpm for 10min and the absorbance of the supernatant was measured by spectrophotometer (Equiptronics) at 560 nm. Diclofenac sodium was used as standard drug. The percentage haemolysis was estimated by the assuming the haemolysis produced for in the control as 100%.

$$\textbf{Percentage Stabilization} = 100-(\textbf{O.D.of Test} - \textbf{O.D.of Sample}) / \textbf{O.D.of Control X 100}$$

Protein denaturation assay [16, 17]

Principle: Denaturation of proteins is one of the phenomenon's that results in the disturbance of stability and structure of the protein. The chemistry of proteins has always been important owing to the abundance of these biomolecules in the living system.

Procedure

In vitro anti-arthritic activity carried out by protein denaturation method. The reaction mixture (1 mL) consisted of 0.1 mL of egg albumin (from fresh hen's egg), 0.5 mL of Phosphate buffered saline (PBS, pH 6.4) and 0.4 mL of Sample A and Sample B Sample C, at the concentration 1mg/ml. Similar volume of double-distilled water served as a control. Then the mixtures were incubated at (37 ºC ±2) in an incubator for 15 min and then heated at 70 ºC for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration (1 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,

% inhibition = absorbance of control – absorbance of test / absorbance of control x 100**.**

Result & Discussion

Anti-inflammatory activity of different formulation by membrane stabilizing study

The percentage membrane stabilization of aqueous extracts of barks of *Withania somnifera* Linn was found to be 82.31% at the concentration range of 1000 µg/ml. The percentage membrane stabilization of aqueous extracts of *Curcuma Longa* Linn was found to be 82.99% at the concentration range of 1000 µg/ml. The percentage membrane stabilization of aqueous extracts of *Cedrus deodara* was found to be 80.27 %at the concentration range of 1000 µg/ml. The percentage membrane stabilization of standard Diclofenac sodium was found to be 90.47% at the concentration range of 1000 µg/ml.

Table 1: Anti-inflammatory activity of different formulation by membrane stabilizing study.

Compounds	Concentration	O.D.	Mean	% inhibition
Blank		1.50 1.45 1.48	1.47	
Standard (Diclofenac sodium)	1000µg/ml	0.13 0.14 0.15	0.14	90.47
Sample: A (*Curcuma Longa*)	1000µg/ml	0.25 0.26 0.24	0.25	82.99
Sample: B (*Withania somnifera*)	1000µg/ml	0.23 0.29 0.28	0.26	82.31
Sample: C (*Cedrus deodera*)	1000µg/ml	0.28 0.29 0.32	0.29	80.27

It was shown that 3 plant aqueous extracts have anti-arthritic properties when evaluated for their ability to inhibit hypotonicity-induced membrane lysis in HRBCs. Aqueous extracts have membrane stabilisation percentages ranging

from 80 to 83%. Percent at 1mg/ml. Since the lysosomal membrane is a good surrogate for HRBC when evaluating anti-arthritic activity *In vitro*, it was hypothesized that the extract would have the same effect. Activated neutrophil lysosomal contents, such as bactericidal enzymes and proteases, can induce more tissue inflammation and injury if the lysosomal membrane is damaged. Hypotonicity-induced haemolysis can result from the osmotic loss of intracellular electrolytes and fluids.

Result of Anti-arthritic activity by Protein denaturation assay

At 1 mg/ml, aqueous extracts of *Withania somnifera* Linn roots inhibited protein denaturation by 85.03%. At 1 mg/ml, aqueous extracts of *Curcuma Longa* inhibited protein denaturation by 82.99%. At 1 mg/ml, aqueous extracts of *Cedrus deodara* Linn roots inhibited protein denaturation by 76.19%. At 1 mg/ml, standard Diclofenac sodium inhibited protein denaturation by 90.47%.

Table 2: Anti-inflammatory activity of different formulation by protein denaturation method.

Compounds	Concentration	O.D.	Mean	% inhibition
Blank		1.50 1.45 1.48	1.47	
Standard (Diclofenac sodium)	1mg/ml	0.13 0.14 0.15	0.14	90.47
Sample A (*Curcuma Longa*)	1mg/ml	0.23 0.26 0.28	0.25	82.99
Sample B (*Withania somnifera*)	1mg/ml	0.20 0.23 0.24	0.22	85.03
Sample C (*Cedrus deodara*)	1mg/ml	0.35 0.36 0.34	0.35	76.19

Three plants had their aqueous extracts tested for anti-arthritic activity using the protein denaturation assay for bovine serum albumin exhibited a reduction of protein denaturation by 60% or more. Despite the fact that 20% protein denaturation inhibition is the minimal threshold for possible anti-arthritic agents, this is not the case. Arthritis and other inflammatory diseases are commonly associated with protein denaturation in the tissue. Auto-antigens may be produced in response to the degradation of proteins seen in arthritic diseases. According to a number of studies, protein denaturation is an important factor in the generation of auto-antigens in inflammation. By losing their tertiary structure and secondary structure, proteins can be denatured by an external stress or chemical. There is a pathogenic aspect to protein denaturation because it causes protein conformation to be lost, which leads to functional loss. This makes the BSA protein denaturation test appropriate for determining anti-arthritic activity since it reduces protein denaturation. Experiments were carried out in the range of 6.2 to 6.5, which corresponds to the pH at which supposedly heat-treated BSA is stabilised (denaturation is blocked) by numerous medicines.

Summary & Conclusion

In the globe, arthritis affects a vast number of people, making it one of the most frequent ailments. Physical inactivity, a sedentary diet, and long hours in front of a computer are all factors that contribute to the development of arthritis in today's society. The consequences for younger generations are dire. NSAIDs, steroids, and disease-modifying antirheumatic medicines (DMARDs) are just a few of the therapies available to help manage the symptoms and keep the illness under control. These drugs, however, have a number of serious side effects. Because of this, a growing number of people are turning to herbal medicines in quest of less hazardous side effects and reduced prices. Herbal extracts or polyherbal formulations may be used alone or in combination with allopathic drugs to treat arthritis in a synergistic manner.

There is currently no treatment for arthritis that can prevent joint damage. Existing therapies, on the other hand, are ineffective and have undesirable side effects. A wide number of rheumatoid arthritis treatments are available, but each one has significant side effects. Inflammatory and arthritis-related illnesses are most often treated with anti-inflammatory drugs such NSAIDs and DMARDS. NSAIDS may cause ulcers, cancer, digestive issues, and allergic reactions if used over a long period of time. Complementary and alternative medicine, such as herbal medicines, are essential at this point in time. Traditional and medical herbs and plant components are increasingly being used to treat arthritic pain, due to a growing number of herbal medicines. In conclusion, all drugs used for *In vitro* anti-arthritic activity by using protein denaturation inhibition assay at the concentration 1000 ug/ml showed good result compared to standard Diclofenac. In conclusion, all drugs used for *In vitro* anti-arthritic activity by using membrane stabilization inhibition assay at the concentration 1000 ug/ml showed good result compared to standard Diclofenac.

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Conflict of Interest

There are no conflicts of interest among the authors.

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