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# *In vitro* anti arthritic activity of *Opuntia stricta*

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**Abstract**
Rheumatoid arthritis (RA) is a chronic inflammatory and systemic auto immune disease affecting people predominantly between the ages of 20-50 years with unpredictable course. About 1% of the world's population is afflicted by rheumatoid arthritis and is two to three times more common in women than men. Allopathic medications have been prescribed to alleviate symptoms of this disease which results into associated side effects like heart attack, stroke, stomach ulcers, bleeding from the digestive tract, and kidney damage etc. Hence the use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines. The present study reveals that plant *Opuntia stricta* possess *in vitro* anti arthritic activity. *In vitro* anti arthritic activity done by protein denaturation method. The activity may be due to presence of secondary metabolites. The plant extracts showed anti arthritic activity in a concentration dependent manner and the activity was increased on increasing the concentration of extracts.

**Keywords:** *Opuntia stricta*, rheumatoid arthritis, *in vitro*, protein denaturation

## Introduction
Nature is a treasure of God, blessed us with enormous wealth of herbal plants which has a source of therapeutic agents for the precaution and cure of various ailments. According to WHO, world's 80% of population utilizes the herbal medicine for their primary health care (Kalaria P, *et al*., 2012) [1]. At present, researcher shows a great interest in medicinal agents that are derived from the plants, because presently available drugs has certain adverse effects or highly expensive. Since ancient time India uses herbal medicines in the alternative systems such as Ayurveda, siddha, unani & homeopathy (Kiran D, *et al*., 2011) [2]. In India about more than 2500 plant species which are used as a herbal medicines, now a days. By knowing traditional plant information and ayurvedic strategies one can discover new effective drugs with less side effects and cheaper drug. (Kasper DL, *et al*., 2005) [3].
Our body's immune system plays a crucial role, it's over activity may leads to fatal Condition. Generally, such disorders are autoimmune diseases i.e, the body's immune system attacks its own healthy cells and tissues (Chitme RH, *et al*., 2009) [4]. In that Rheumatoid Arthritis (RA) is a chronic, inflammatory systemic autoimmune disease which attacks the flexible (synovial) joints and cartilages. In most cases, RA starts infecting new joints and spread to other joints in the body, it progress rapidly (Chunxia C, *et al*., 2011) [5]. It is characterized by joint pain, swelling and destruction of cartilage and bones. Its non-specific symptoms include tiredness, soreness in and around the joints, fever, weight loss and poor appetite (Babushetty V, *et al*., 2012) [6].
Yet, its exact pathophysiology is unknown but release of certain free radicals such as nitrous oxide and superoxide radicals Produced as by-products of cellular metabolism. The release of such free radicals may induce the production of interleukins (IL) and tumor necrosis factor (TNF-α) from T-cells which ultimately influence the production of growth factors, cytokines and adhesive molecules on immune cells, thus it may cause tissue destruction and inflammation(Kasper DL, *et al*., 2005) [3]. RA affects mostly women and geriatric than men. Arthritis is not an infectious or contagious disease, it may cause due to certain factors such as, genetic factors, and lifestyle and trigger factors, it is more common in smokers. RA can be classified as, palindromic RA, Juvenile RA, Rheumatoid spondylitis and osteoarthritis (Chandrasekar R, *et al*., 2017) [7].
The current treatment of arthritis includes minimization of pain and inflammation using non-steroidal anti-inflammatory drugs (NSAIDs) as well as deceleration of disease progression using antirheumatic drugs (Kasper DL, *et al*., 2005 & Mazumder MP, *et al*., 2010) [3, 8] Due to adverse reactions of the NSAIDs and disease modifying ant rheumatic drugs, the arthritic patients tend to search for other treatments that are effective and less toxic. Therefore, complementary and alternative medicines are commonly preferred, which are safe, potent, cost effective and less toxic. Thus, based on traditional information, various medicinal plants extracts or active fractions are tested with experimental animal model of arthritis and inflammation.

## Materials and Methods
### Plant material
*Opuntia stricta* is a large sized species of cactus that can grow up to 2 meters in height and endemic in sub-tropical and tropical coastal areas of America and carriebean. This spiny shrub favors habitats such as rocky slopes, river banks and urban areas. The plant *Opuntia stricta* belongs to the family cactaceae. Synonym for the plants is cactus dillenii ker Gawl,

cactus strictus haw. The common names of plant are erect prickly pear and nopalestricto. Antiulcer, antioxidant, ant diabetic, anticancer, antiviral, neuroprotective and hepato protective effects of *Opuntia stricta* have been reported. But no specific studies are done on anti-arthritic activity of plant. Thus, an attempt was made to investigate anti-arthritic potential of extract of plant. The plant contains alkaloids, Flavonoids, Saponins, tannins, phenols and carbohydrates. Anti-arthritic activity of medicinal plants attributed to terpenoids, flavonoids, steroids, alkaloids, tannins and phenols.

**Drug and chemicals**

Diclofenac sodium was used as a standard drug.

Ethanol, Bovine serum albumin and Egg albumin and all the reagents procured analytical grade.

**Experimental method**

**Extraction**

Fresh whole plant of *Opuntia stricta* was collected, cut into small pieces dried under room temperature for 45-60 days. In soxhlet extractor, the dried powdered *Opuntia stricta* was extracted with solvent ethanol. Then, the extract was concentrated to a distillation apparatus. The extract were used for *in vitro* anti arthritic test. (Sumithra singh and Nidhi Sharma, 2016) [14].

**Preliminary phytochemical screening of *Opuntia stricta***

The ethanol extract of the plant were used for preliminary phytochemical screening to detect the presence and absence of alkaloids, flavanoids, triterpenoids, glycosides, steroids, tannins, essential oils and saponins.

***In vitro* anti arthritic activity**

**Inhibition of protein denaturation (bovine serum albumin)**

Denaturation of tissue protein is one of the causes of inflammatory and arthritic diseases. Production of auto antigen in certain arthritic diseases may be due to denaturation of protein. Hence, *in vitro* agent which prevent protein denaturation could be worthwhile during anti-arthritis drug development. (Lavanya R *et al*., 2010, Srividhya S, Sridevi G, 2016) [9, 10].

The following three solutions were used.

**Test solution**

0.5 ml of test solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of extracts in various concentrations (100, 200, 300, 400, and 500 µg/ml).

**Test control solution**

0.5 ml of test control solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of distilled water.

**Standard solution**

0.5 ml of standard solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of diclofenac sodium solution (100 µg/ml).

The pH of the above solutions is adjusted to 6.3 using a small amount of 1N HCl. Incubate the samples at 37°C for 20 min and heat it at 57°C for 3 min which were cooled, and 2.5 ml of phosphate buffer (pH 6.3) is added to it. Control represents 100% proteins. After cooling, measure absorbance at 660 nm using pure blank. (Vaijayanthimala P, *et al*., 2019) [11]

Diclofenac sodium (standard drug) is used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation is calculated as follows:

Percentage inhibition = (OD control-OD sample/OD control)*100

**Inhibition of albumin denaturation (egg albumin)**

**Methodology**

The following three solutions are used.

**Test solution**

5 ml of test solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and 2 ml of in various concentrations of extracts (100, 200, 300, 400, and 500 µg/ml).

**Test control solution**

5 ml of test control solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffered saline and 2 ml of distilled water.

**Standard solution**

5 ml of standard solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and diclofenac100 µg/ml. The pH of the above solutions is adjusted to 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heat it at 70°C for 5 min denaturations, and the results are compared with standard diclofenac sodium (Mizushima Y, Kobayashi M, 1968, Chandra S *et al*., 2012) [12, 13]

After cooling, measure absorbance at 660 nm using pure blank. Diclofenac sodium (standard drug) is used as reference. The percentage inhibition of protein denaturation is calculated as follows:

Percentage inhibition = (OD control - OD sample / OD control)*100

**Results**

**Preliminary phytochemical screening of *Opuntia stricta***

Preliminary phytochemical screening of the extracts revealed the presence of essential oil, flavonoids, tannins, alkaloids, glycosides, saponins and steroids. The ethanolic extract showed more active constituents. So that the ethanolic extract was selected for the anti-arthritic study.

**Inhibition of protein denaturation (Bovine serum albumin)**

The extracts were found to be effective as anti-arthritic agent and showed significant activity as compared to the standard drug. The anti-arthritic activity was also shown in a concentration dependent manner and the activity was increased on increasing the concentration of extracts. Hence, maximum activity was reported at the highest concentration taken for evaluation. Ethanol extract was found to be more

effective which showed 78.82% inhibition of protein denaturation at the concentration of 500µg/ml which was the highest concentration evaluated. The percentage inhibition of protein denaturation by bovine serum albumin method and egg albumin method by the extracts at different concentration and their comparison with the standard drug are shown below the table.

**Table 1:** Anti arthritic activity of ethanol extract of *opuntia stricta* by bovine serum albumin method

| Effect of herbal extracts in different concentrations | | Absorbance at 660nm average | % Inhibition |
|---|---|---|---|
| Control | | 0.85 | ---------- |
| Diclofenac (100 µg/ml) | | 0.09 | 89.41% |
| Ethanol extract | 100 µg/ml | 0.49 | 42.35% |
| | 200 µg/ml | 0.40 | 52.94% |
| | 300 µg/ml | 0.32 | 62.35% |
| | 400 µg/ml | 0.24 | 71.76% |
| | 500 µg/ml | 0.18 | 78.82% |

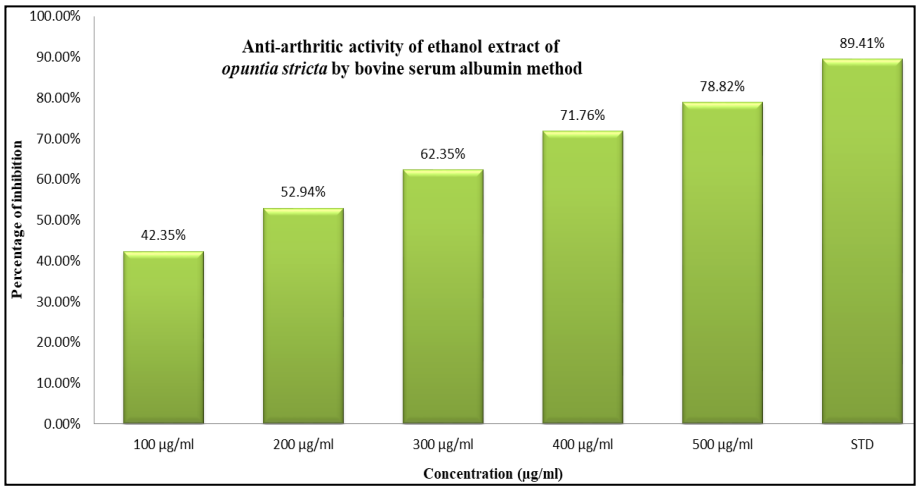


**Fig 1**

**Table 2:** Anti arthritic activity of ethanol extract of *opuntia stricta* by egg albumin method

| Effect of herbal extracts in different concentrations | | Absorbance at 660nm average | % Inhibition |
|---|---|---|---|
| Control | | 0.85 | ---------- |
| Diclofenac (100 µg/ml) | | 0.09 | 89.41% |
| **Ethanol extract** | 100 µg/ml | 0.76 | 10.58% |
| | 200 µg/ml | 0.69 | 18.82% |
| | 300 µg/ml | 0.58 | 31.76% |
| | 400 µg/ml | 0.34 | 60.00% |
| | 500 µg/ml | 0.20 | 76.47% |

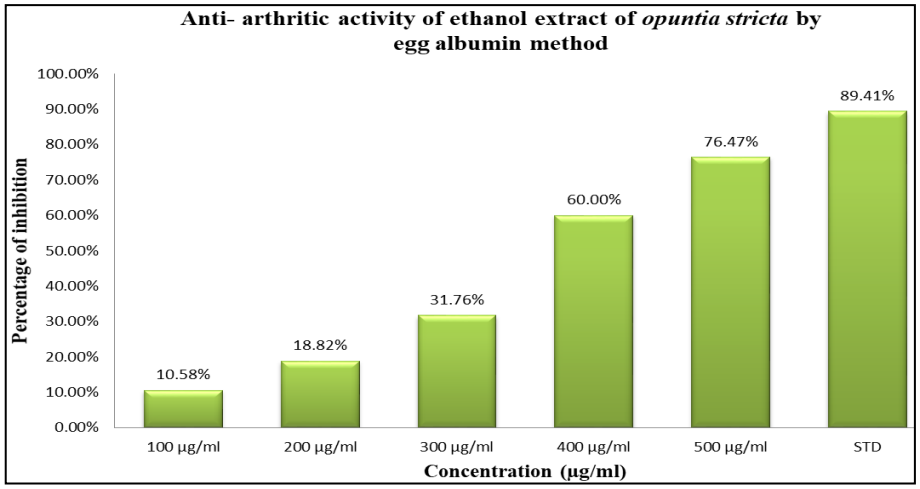


**Fig 2**

**Discussion**

Based on the results, it was evidently proven that the ethanolic extract of different concentrations was more effective in inhibiting the protein denaturation.

From this data, in bovine serum albumnin method 500 µg/ ml of ethanolic extract of *Opuntia stricta* showed 78.82% of maximum inhibition which was compared to diclofenac standard, which exhibited 89.41% inhibition at 100 µg/ml. In egg albumin method 500 µg/ml of ethanolic extract of *opuntia stricta* showed 76.47% of maximum inhibition which was compared to diclofenac standard, which exhibited 89.41% inhibition at 100 µg/ml.

**Conclusion**

This finding justifies the usefulness of *opuntia stricta* in the management and treatment of inflammation associated disease like arthritis. From the results obtained in the present studies, it may be concluded that *Opuntia stricta* possess significant anti arthritic activity which is compared to synthetic anti arthritic agents. This activity may be due to the presence of secondary metabolites. The present study indicates that the extract of *Opuntia stricta* exhibit strong anti arthritic property which could be a potential source of anti arthritic property.

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