

Treating Diseases like Inflammation and Arthritis with Different Biological Extracts to Help the Body

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Abstract

Inflammation and the arthritis are the two most irremovable diseases which affect human life at the time of old age. Different preventive measures from all parts of the world are taken into consideration, so that effective and constructible care and a balanced medicine could be made against the concerned bodily arrays. As per the allopathic medicines are taken into consideration they furnish some relief, but only up-to the use of the medicines. But nowadays scientists showed their interest towards the utilisation of medicinal plants against almost all types of diseases. Similarly the present study was carried out about the three medicinal plants viz, Artemisia maritima, Capsicum annum, Juglans regia against inflammation and arthritis. Among the three plants Juglans regia oil was found most effective against inflammation and arthritis followed by capsicum annum. Also among various extracts of the concerned plants more polar solvents extracts proved to be more effective as compared to less polar solvent extracts.

Keywords: Arthritis, Capsicum Annum, Juglans Regia, Artemisia Maritima, Inflammation

INTRODUCTION

Inflammation is the body's natural protective response to tissue damage caused by physical trauma, toxic

chemicals, or infectious pathogens. No steroidal anti-inflammatory medicines are a frequent treatment for inflammatory disorders, but they have a number of side

effects, including stomach irritation, which can contribute to the formation of gastric ulcers. Reactive oxygen species are produced in the presence of oxidative stress. These molecules have a key role in the development of a number of major diseases, including ageing, cancer, coronary heart disease, Alzheimer's disease, and inflammation. As a result, antioxidants that can scavenge reactive oxygen species may be useful in the treatment of inflammatory diseases. Rheumatoid arthritis (RA) is a systemic inflammatory disease that primarily affects the joints, but can also affect other tissues and organs such as the blood vessels, heart, skin, lungs, and muscles. The disease's onset and severity are unpredictable, and it's usually subtle. RA begins with fatigue, musculoskeletal discomfort, and stiffness, progressing to joint involvement only after weeks or months. Tiny joints, particularly the small bones of the hands, are usually the first to be damaged. Larger joints are subsequently impacted, becoming swollen, heated, and painful. Symptoms of RA include morning stiffness and stiffness after inactivity, both of which suggest ongoing disease. When getting out of bed or after being in one position for too long, the patient generally complains of slowness or trouble moving. Both sides of

the body are affected, and symptoms improve as you walk about.

Because there is no cure, therapeutic techniques currently focus on reducing active inflammation. The goal is to keep joint destruction to a minimum. Analgesics, NSAIDs, glucocorticoids, DMARDs, and anticytokines are all recommended pharmacological treatments, which can be used alone or in combination. Infections, renal impairment, lymphomas, and cardiovascular disease are some of the other problems that afflict people with RA.

EXPERIMENTAL WORK:

Study area and plant collection

The plant in concern (*Artemisia maritima*) was picked in Jammu and Kashmir's Kishtwar district. *Capsicum annum* was obtained from the Dehradun local market. Separately, the plant pieces were shade dried and ground in a combination. For the experiment, powdered substance was employed.

Extraction

To obtain the optimum extract, 70 grammes of each plant powder were weighed separately and accurately, and then extracted in a Soxhlet Apparatus using a thimble. Various solvents were

utilised, with increasing polarity index, depending on their polarity index (Acetone, DMSO and Water).

Extraction A

The sample was extracted with a particular solvent (Acetone) in a Soxhlet apparatus for a required period, till no extract was coming out of the sample, as being examined by taking a small amount of the extracted solvent from the main chamber of the Soxhlet apparatus over the watch glass for the appearance of precipitate. The extract solution was filtered after the acetone extraction to eliminate the residual from the extract. On a water bath at an appropriate temperature, the filtrate was collected and evaporated to eliminate the volatile solvent to 1/4th volume. After being stored in an oven, the entire filtrate was converted into a solid shape (powdered). The leftovers were collected and treated to a second extraction process.

Extraction B

After that, the residue was extracted with DMSO in the same way as in extraction A.

Extraction C

Water extraction by decoction procedure was used to remove the residue from extract B. The extract was dissolved in 500 mL of water in this method. To eliminate

all of the water from the extract, the entire solution was boiled over a water bath. Finally, 500 mL of water was added to the extract, followed by the extraction solution being evaporated to remove almost 250 mL of water. Following that, the solution was filtered. After that, the filtrate was evaporated to remove approximately a quarter of its volume. Finally, the extract was dried in an oven at temperatures ranging from 30 to 500 degrees Celsius.

The % yield of all the extracts were determined as w/w.

Oil Extraction from the Wall Nuts (*Juglans regia*)

Extraction of oil: For the extraction of oil from *Juglans regia*, solvent extraction was done and concrete & absolute oils were obtained. The oil was extracted using 75g of *Juglans regia*. By distillation, the extraction procedure was carried out utilising water as a solvent. When the complete scent was wiped out by solvent, the distillate was concentrated by heating procedure to get concrete oil. The apparatus used in each procedure was carefully cleansed and dried.

Concrete oil recovery: In a flask, dissolved organic residue in water was collected and dried by adding anhydrous

NaSO₄. Concrete oil was placed in a 100ml flask that had been pre-weighed, and the weight of the concrete oil was determined by weighing the flask again.

On the basis of the plant's dry weight, the percentage yield of concrete oil was estimated.

Absolute oil recovery: Concrete oil was diluted in a small amount of pure alcohol to eliminate the natural waxes included in the essential oil, and then filtered using a Whatmann filter paper # 43. Distillation was used to extract the alcohol. On the basis of dry weight, the percent yield of absolute oil was also computed.

Anti-inflammatory Activity of various plant extracts

Inflammation is a complex physiological process mediated by leukocytes, macrophages, and mast cells, which produce a range of signalling chemicals. Increased blood flow to the tissue causes increased temperature, redness, swelling, and discomfort, which is a tissue reaction to damage. Through the secretion of inflammatory mediators such as nitric oxide (NO), prostaglandin (PG) E₂, and pro-inflammatory cytokines, macrophages play a significant role in inflammatory illness.

Human Red Blood Cells (HRBC) Suspension Preparation

Fresh whole human blood was drawn and combined with an equal volume of sterilised Alsever solution (2% dextrose, 0.8 percent sodium citrate, 0.05 percent citric acid, and 0.42 percent sodium chloride in water). The packed cells were washed three times with isosaline after being centrifuged at 3000 rpm for 10 minutes (0.85 percent , pH 7.2). The volume of blood was measured and reconstituted as a 10% v/v isosaline suspension. Sakat S, Juvekar AR, Gambhire MN, Sakat S, Juvekar AR, Gambhire MN, Sakat S, Juvekar AR, Gambhire M (4) M. Govindappa, S. Naga, M. N. Poojashri, T. S. Sadananda, and C. P. Chandrappa – 2011

Heat Induced Hemolysis

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with various concentrations of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 200, 300, 400, 500 µg) and control (distilled water instead of hyposaline to produce 100 % hemolysis) were incubated

at 37°C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm, Patel DK, Prasad SK, Kumar R, Hemalatha S. – 2012 (6).

The percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = (Absorbance of Test Sample

/ Absorbance of Control) x 100

The percentage of HRBC membrane stabilization can be calculated as follows:

% Protection = 100 – [(Absorbance by Test sample / Absorbance by Control) X 100]

Inhibition of protein denaturation method by Deshpande V, Jadhav VM and Kadam V J. 2009 (7) The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Artemisia maritima* extracts (100 and 250 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660

nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percent inhibition= 100- (O.D. of test – O.D. of product control) X 100/ O D of control

100 percent protein denaturation is represented by the control. The results were compared to samples treated with acetyl salicylic acid (250 mcg/ml).

OBSERVATIONS AND RESULTS

The anti-inflammatory activities of several *Artemisia maritima* extracts were investigated, with cell lysis induced by hypotonicity. The percentage hemolysis was measured, as well as the % stabilisation against membrane hemolysis. The percentage stability was discovered to rise in tandem with the concentration rise.

Artemisia maritima's Anti-Inflammatory Properties

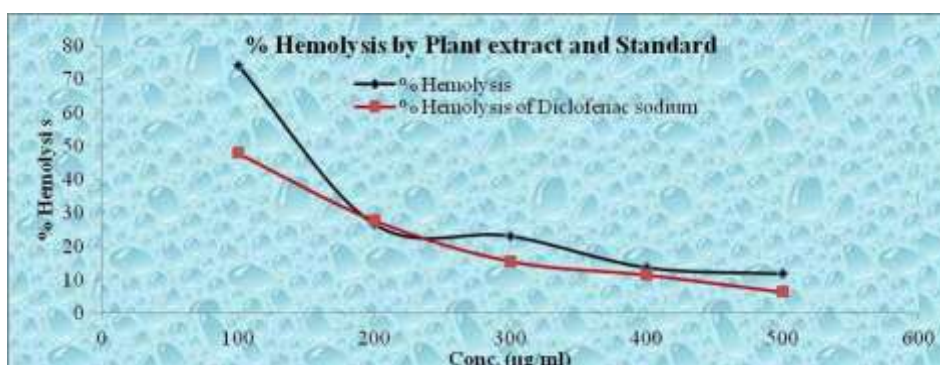
Anti-inflammatory activities of *Artemisia maritima* extracts were investigated, and it was discovered that the reference compound (Diclofenac sodium) had the highest percent stabilisation, as demonstrated in all tables and figures. The maximum percentage stabilisation was

observed in the water extract of *Artemisia maritima*, followed by DMSO extract of *Artemisia maritima*, and the lowest was found in Acetone extract, respectively. The percentage inhibition increases as the concentration of plant extract increases,

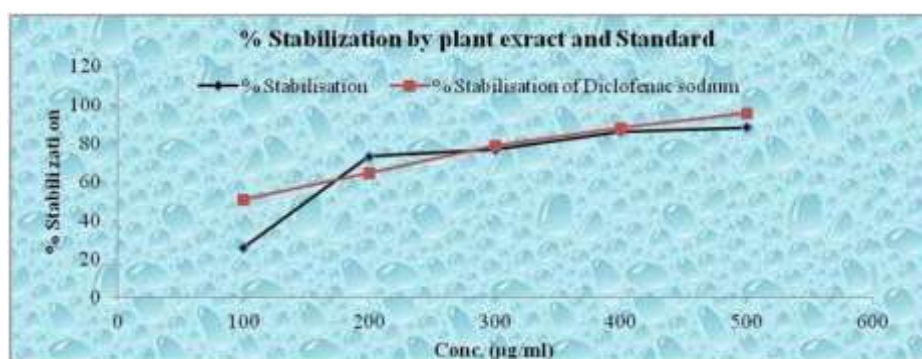
indicating that the anti-inflammatory activity is concentration dependent. A straight line formation was found when the graph was plotted between the concentration and the percentage stabilisation.

Table-1: Report of anti-inflammatory effect of Acetone extract of leaf of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization

Conc. of standard plant extract($\mu\text{g/ml}$)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
100	74.21	25.79	47.97	50.9
200	26.74	73.26	27.89	64.6
300	23.13	76.87	15.63	78.97
400	13.73	86.27	11.54	87.99
500	11.80	88.20	6.44	95.52



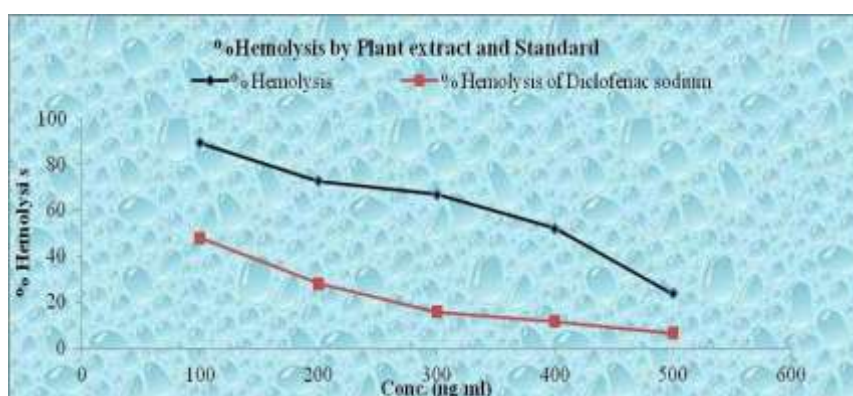
Graph 1: showing report of anti-inflammatory effect of Acetone extract of leaf of *Artemisia maritima* and Standard on HRBC membrane Hemolysis and membrane Stabilization



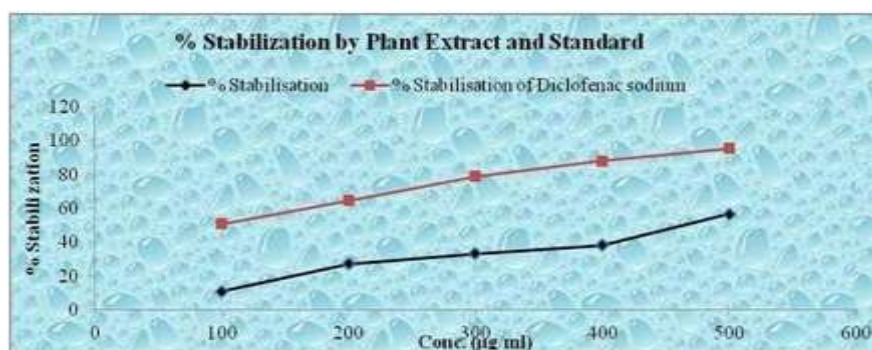
Graph 2: showing report of anti-inflammatory effect of Acetone extract of leaf of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization

Table-2: Report of anti-inflammatory effect of DMSO extract of leaf of Artemisia and Standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of standard plant extract($\mu\text{g/ml}$)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
100	89.32	10.68	47.97	50.9
200	72.81	27.19	27.89	64.6
300	66.99	33.01	15.63	78.97
400	52.01	37.99	11.54	87.99
500	23.57	56.43	6.44	95.52



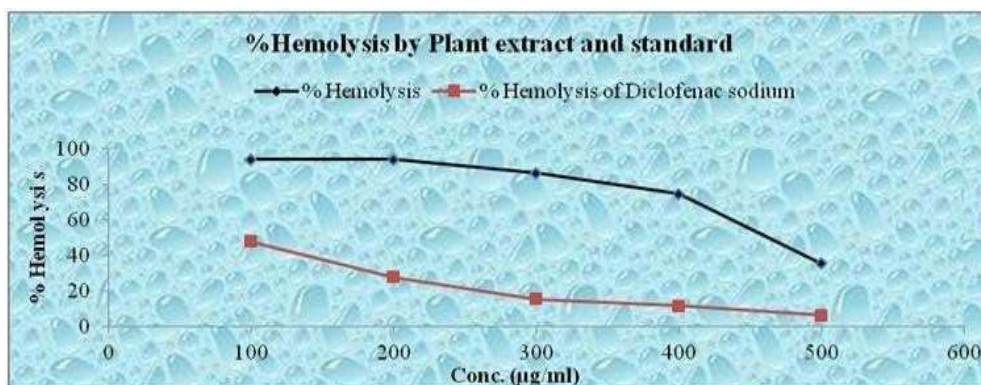
Graph 3: showing report of anti-inflammatory effect of DMSO extract of leaf of Artemisia maritima and Standard on HRBC membrane hemolysis and membrane stabilization



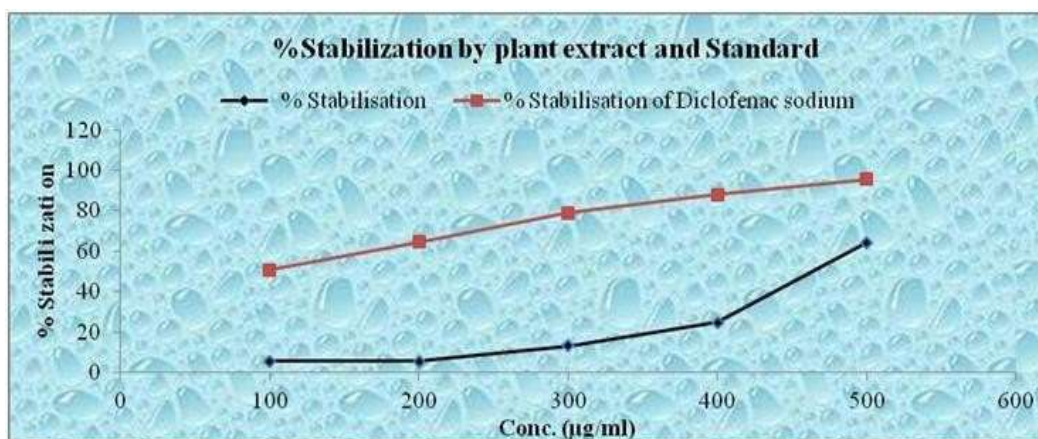
Graph 4: showing report of anti-inflammatory effect of DMSO extract of leaf of Artemisia maritima and Standard on HRBC membrane hemolysis and membrane stabilization

Table-3: Report of anti-inflammatory effect of WATER extract of leaf of Artemisia and Standard on HRBC membrane hemolysis and membrane stabilization

Conc. of standard plant extract ($\mu\text{g/ml}$)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
100	94.35	5.65	47.97	50.9
200	94.23	5.77	27.89	64.6
300	86.55	13.45	15.63	78.97
400	74.91	25.01	11.54	87.99
500	35.70	64.30	6.44	95.52



Graph 5: showing report of anti-inflammatory effect of water extract of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization



Graph 6: showing report of anti-inflammatory effect of water extract of leaf of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization

DISCUSSION

The ability of a medicine or medicinal-like agent to stop the process of inflammation or swelling is referred to as anti-inflammatory. Anti-inflammatory medications make half of the analgesics, lowering pain by reducing inflammation at a particular body area of an organism.

The extracts of *Artemisia maritima* and *capsicum* were used to treat inflammation,

and the results showed that they had a significant anti-inflammatory impact. The anti-inflammatory impact of plant is related to the existence of numerous secondary phytochemicals like flavonoids, alkaloids etc. the most boosting power against the inflammation was determined to be due the hemeoxygenase. As a result, the plant could be employed as the most effective anti-inflammatory agent.

Flavonoids have been discovered in abundance.

In Ginkgo, these flavonoids may include caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside similar material have a powerful anti-inflammatory action as described by Williams CA, Goldstone F, Green ham J-1996. All of the plant extracts were tested for anti-inflammatory effects in comparison to normal diclofenac sodium (anti-inflammatory drug). Membrane lysis caused by hypotonicity caused the inflammation. The stabilisation against membrane induced hypotonicity was found to be concentration dependant, and the stabilisation percentage in all the plant extracts was found less than the reference chemical (Diclofenac sodium) (Diclofenac sodium). Among the plant extracts the highest stabilisation was observed in case of water extracts of Artemisia, capsicum followed by DMSO extracts of the relevant plants and the least results were produced by employing the Acetone extracts of the concerned plants.

Among the total plant extracts it has been found that the polar solvent extracts posses' greater anti- inflammatory activity than the less polar solvent extracts. The results are consistent because most amino acids, as well as polar plant extracts, are

anti-inflammatory in nature. These substances have direct influence upon the inflammation and the plant extracts accomplish the job in highly affected manner.

Protein denaturation or arthritis is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or substance, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. When biological proteins are denatured, they lose their biological function. Enzymes, for example, lose their activity when their substrates are no longer able to bind to the active site. Protein denaturation has been identified as one of the causes of rheumatoid arthritis. In some cases of arthritic illness, the production of auto antigen causes protein denaturation. Modulation of electrostatic, hydrogen, hydrophobic and disulphide bonding leads to the denaturation of proteins, which is the main mechanism of protein denaturation. This anti-denaturation action was further substantiated by the change in viscosities. It has been shown that the viscosities of protein solutions rise following denaturation. From the result of the present study, it can be stated that all the extracts of Artemisia, capsicum are capable of controlling the production of

auto – antigen and thereby it inhibit the denaturation of proteins of both fresh egg albumin and bovine albumin in dose dependent manner and its effect was compared with the standard drug diclofenac sodium. Artemisia and capsicum water extracts show a strong anti-arthritis effect, followed by DMSO and acetone extracts. Walnut oil inhibited protein denaturation the most out of all the extracts tested. The existence of phyto-compounds with anti-arthritic activity could explain the activity.

CONCLUSION

It may be stated that inflammation and arthritis are the two most common disorders that become more prevalent as people age. In reality, both bodily disarrays are caused by changes in protein structure. As a result, continuing to utilise allopathic treatments will not be enough to combat the illnesses in question. Exercises for the body provide some relief from the above-mentioned conditions. However, according to the study, sustained use of plant extracts or phytochemicals can help to a great amount. In addition, the drugs have no negative effects. So the plants in references should be entirely employed against the ailments in reference and also for other body disarrays.

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