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TREATMENT OF BODILY DISARRAYS LIKE INFLAMMATION AND ARTHRITIS BY VARIOUS BIOLOGICAL EXTRACTS

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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 annuum, 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: *Artemisia maritima, Capsicum annuum, Juglans regia, inflammation, arthritis.*

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INTRODUCTION:

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. The commonly used drug for management of inflammatory conditions are no steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers. In the case of oxidative stress, reactive oxygen species are generated. These agents play an important role in causing various serious diseases such as ageing, cancer, coronary heart disease, Alzheimer's disease and inflammation. Hence, antioxidants that can scavenge these reactive oxygen species can be beneficial in the treatment of inflammatory disorders.

Rheumatoid arthritis (RA) is a disorder characterized by acute and chronic systemic inflammation that primarily involves the joints, but may also affect many tissues and organs, including blood vessels, heart, skin, lungs, and muscles. The onset and severity of disease is variable and usually insidious. RA initially presents with fatigue, musculoskeletal pain, and stiffness and only after weeks to months does it progress to involve joints. Generally, the small joints are affected first, particularly the small bones of the hands. Later larger joints are affected, becoming swollen, warm, and painful (1). Morning stiffness or stiffness upon inactivity are symptoms of RA and indicate active disease. The patient usually describes slowness or difficulty moving when getting out of bed or after staying in one position too long. Both sides of the body are involved and symptoms decrease with movement (2).

Currently, treatment strategies focus on alleviating active inflammation, because no curative treatment exists. The aim is to limit joint destruction. Recommended drug therapies include analgesics, NSAIDs, glucocorticoids, DMARDs, and anticytokines, used separately or in combination. Other conditions that affect individuals with RA include infections, renal impairment, lymphomas, and cardiovascular disease (3).

EXPERIMENTAL WORK:

Study area and plant collection

The concerned plant (*Artemisia maritima*) was collected from the Kishtwar region of Jammu and Kashmir. *Capsicum annuum* was collected from the local market of Dehradun. The plant parts were segregated shade dried and powdered in mixture separately. The powdered material was used for experiment.

Extraction

70 Gms of the each plant powder was weighed separately and accurately and then extracted in a Soxhlet Apparatus using thimble in order to get the best extract. Various solvents were used depending upon their polarity index with increasing polarity (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. After the Extraction with Acetone, the extract solution was subjected to filtration to remove the residue from extract. The filtrate was then collected and evaporated to remove the volatile solvent to its 1/4th volume on water bath at a suitable temperature. The whole filtrate was then made in solid form (powdered) after being kept in an oven. The residue was collected, and subjected to further extraction process.

Extraction B

The residue was then extracted with DMSO in a same manner as mentioned above, in extraction A.

Extraction C

The residue from extract B was subjected to Water extraction by decoction technique. In this technique the extract was dissolved in 500 ml of water. The whole solution was heated over water bath to remove all the water from the extract. Finally additional 500 ml of water was added to the extract, the extracted solution was finally evaporated to remove nearly 250ml of water. The solution was then subjected to filtration. The filtrate was then evaporated to remove nearly 1/4th of its volume. Finally the extract was dried in an oven at a temperature range 30-500C.

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

Extraction of Oil from the Wall nuts (*Juglans regia*)

Extraction of oil: For the extraction of oil from ***Juglans regia***, solvent extraction was performed and concrete & absolute oils were obtained. 75g of ***Juglans regia*** were used for the extraction of oil. The extraction process was carried out using water as a solvent by distillation process. When the entire aroma was taken out by solvent, the distillate was concentrated by heating process to get concrete oil. The apparatus used in each process was thoroughly washed and dried.

Concrete oil recovery: Dissolved organic residue in the water was collected in a flask and dried over by adding anhydrous $NaSO_4$. Concrete oil was taken in pre weighed 100ml flask and the weight of concrete oil was determined by again weighing the flask.

Percentage yield of concrete oil was calculated on the basis of dry weight of the plant.

Absolute oil recovery: Concrete oil was dissolved in minimum volume of absolute alcohol to remove the natural waxes present in the essential oil and was then filtered through a Whatmann filter paper # 43. Alcohol was removed by distillation Percent yield of absolute oil was also calculated on the basis of dry weight.

Anti-inflammatory Activity of various plant extracts

Inflammation is a complex physiological process mediated by a variety of signaling molecules produced by leukocytes, macrophages, and mast cells. Inflammation is a tissue response to injury characterized by increased blood flow to the tissue causing increased temperature, redness, swelling, and pain. Macrophages play an important role in inflammatory disease through the release of inflammatory mediators such as nitric oxide (NO), prostaglandin (PG) E2, and pro-inflammatory cytokines.

Preparation of Human Red Blood Cells (HRBC) Suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline Sakat S, Juvekar AR, Gambhire MN. – 2010 (4) Govindappa M, Naga Sravya S., Poojashri M. N., Sadananda T. S. and Chandrappa C. P. – 2011 (5)

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 37ºC 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

The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (250 mcg/ml) treated samples.

Observations and Results

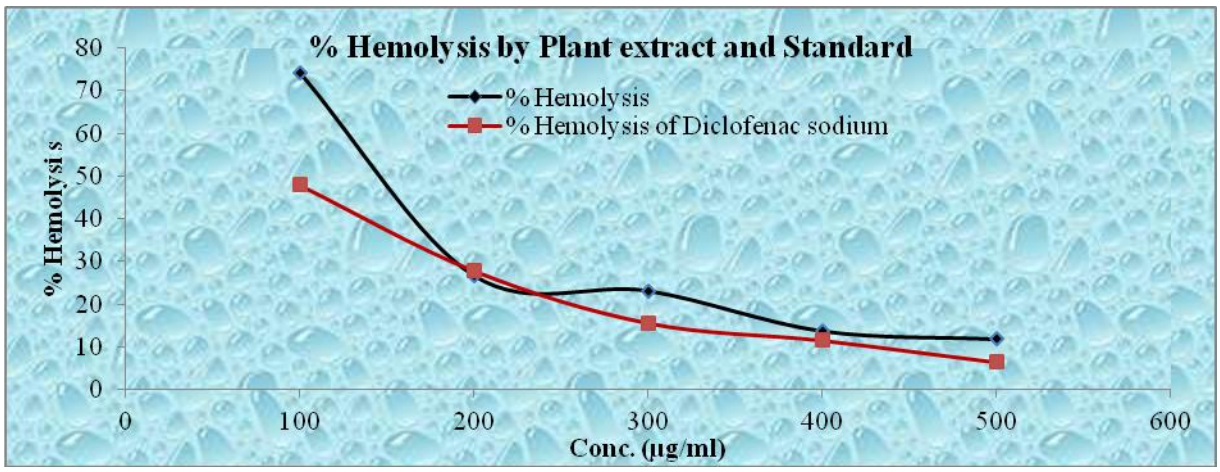
The anti-inflammatory properties of various extracts of *Artemisia maritima* have been carried out, in which the lysis of cell was done by hypotonicity. The percentage hemolysis and correspondingly the percentage stabilization against the membrane hemolysis were carried out. The percentage stabilization was found to increase against the concentration increase.

Anti-inflammatory Properties of *Artemisia maritima*

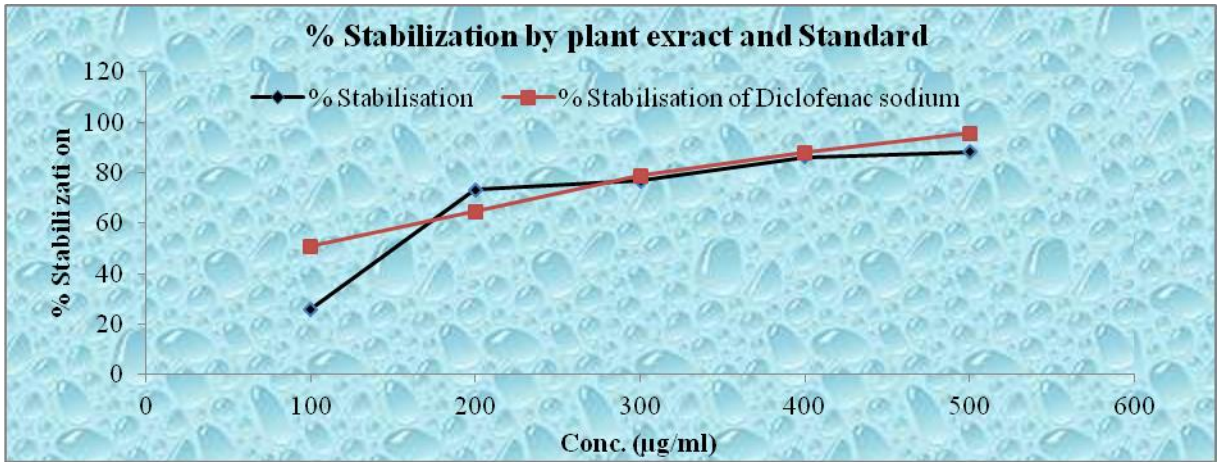
The anti-inflammatory properties of *Artemisia maritima* extracts have been carried out and it was found that the reference compound (**Diclofenac sodium**) have been found to posses the highest % stabilization as shown in all tables and figures**.** Among the plant extracts it has been found that water extract of *Artemisia maritima* posses the highest percentage stabilization followed by DMSO extract *Artemisia maritima* and least was found for Acetone extract respectively. The anti-inflammatory effect was found to be concentration dependent, as the percentage inhibition increases with the increase in the concentration of plant extract. The graph was plotted between the concentration and the percentage stabilization and a straight line formation was observed.

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(µg/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



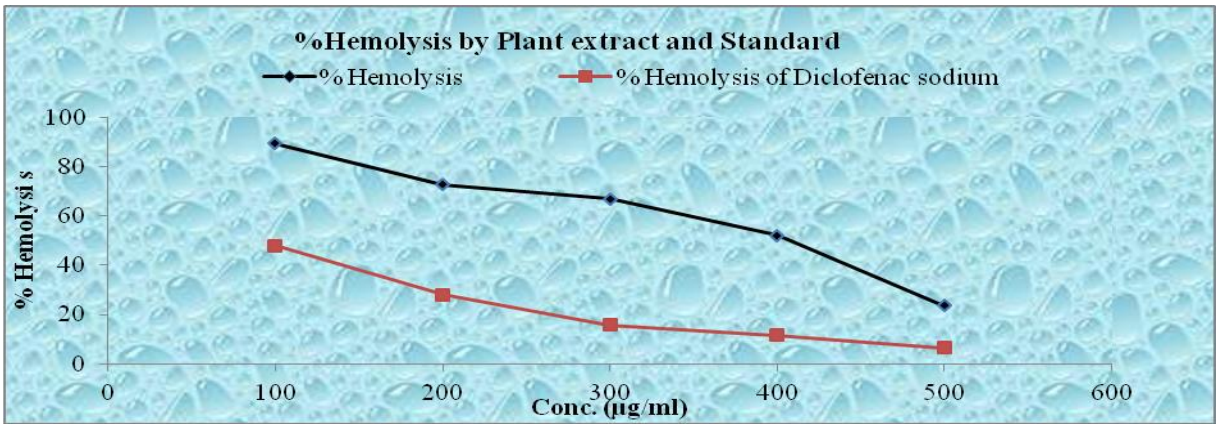
Graph 1: showing report of anti-inflammatory effect of Acetone extract of leaf of *Artemisia maritima* and Standard on HRBC membrane Hemolysis and membrane Stabilization



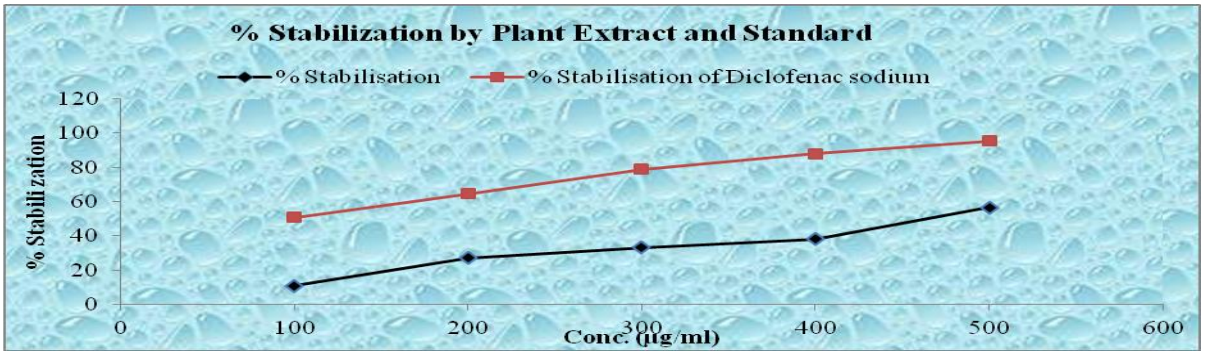
Graph 2: showing report of anti-inflammatory effect of Acetone extract of leaf of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization

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(µg/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



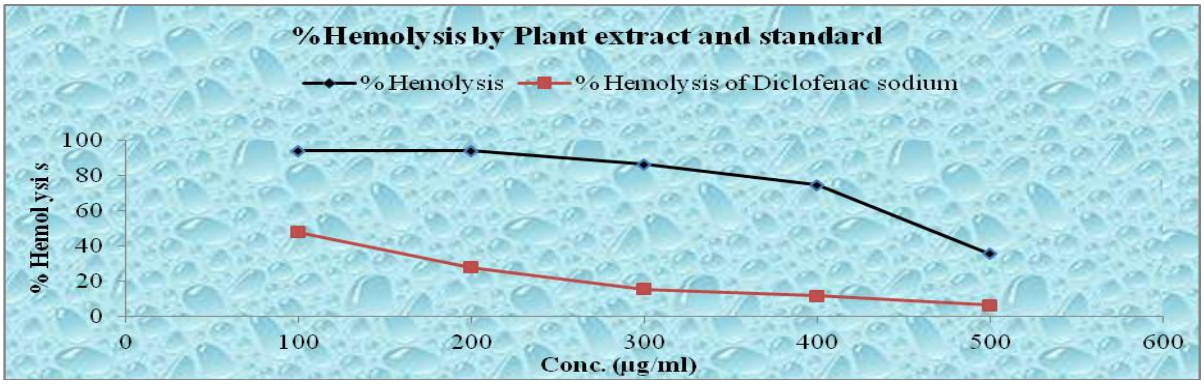
Graph 3: showing report of anti-inflammatory effect of DMSO extract of leaf of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization



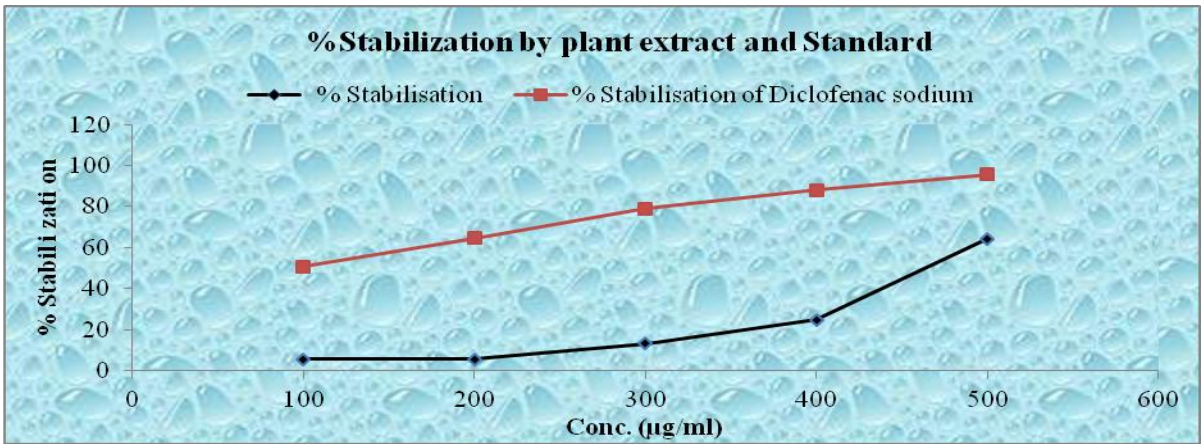
Graph 4: showing report of anti-inflammatory effect of DMSO extract of leaf of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization

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 (µg/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



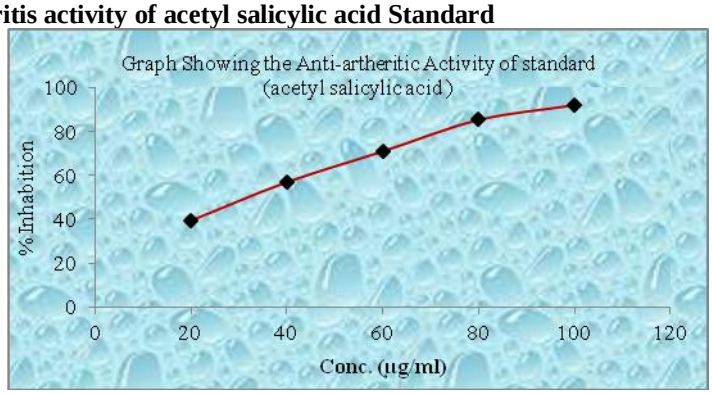
Graph 6: showing report of anti-inflammatory effect of water extract of leaf of *Artemisia maritima* and Standard on HRBC membrane hemolysis and membrane stabilization

Anti-arthritis activity of capsicum

The **Anti-arthritis activity** of various capsicum extracts Viz (Acetone, DMSO and Water) have been analyzed, and it was found that **(water extract)** posses the highest anti-arthritis activity followed by **(DMSO extract)**. The percentage inhibition by plant extracts was found to be concentration dependent, percentage inhibition increases with the increase in the concentration of the plant extracts. The **IC_{50}** value was determined from the straight line graph. The **IC_{50}** value of all the plant extracts was found lesser than the reference compound **(acetyl salicylic acid).** The **IC_{50}** value of various plant extracts follows the order **(water extract, DMSO extract and finally Acetone Extract)** was found to be **(56, 72, 80)** respectively and are presented in **(Tables).**

Table 4: Showing Anti-arthritis activity of acetyl salicylic acid Standard

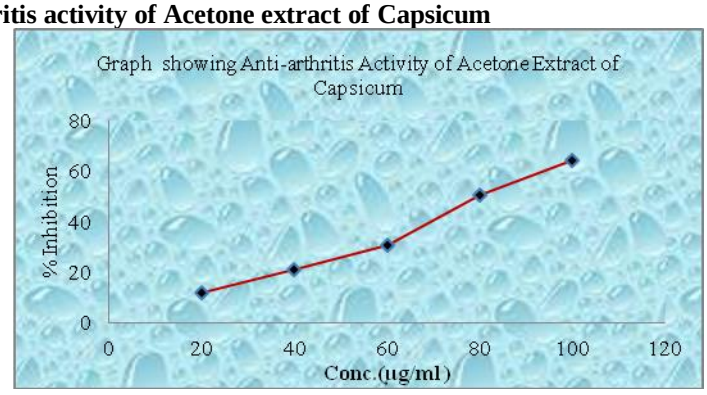
S. No	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.545	39.61	
2	40	0.456	57.12	
3	60	0.312	71.11	32.5
4	80	0.198	85.43	
5	100	0.071	92.17	



Graph 7: Showing Anti-arthritis Activity of Standard (acetyl salicylic acid)

Table 5: Showing Anti-arthritis activity of Acetone extract of Capsicum

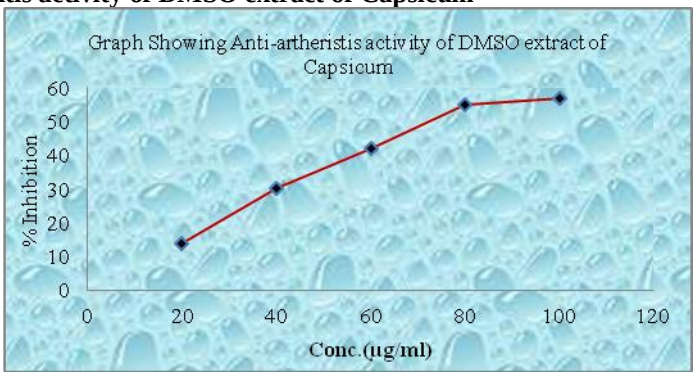
S. No.	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.725	12.12	
2	40	0.653	20.84	
3	60	0.572	30.66	80
4	80	0.409	50.42	
5	100	0.294	64.36	



Graph 8: Showing Anti-arthritis Activity of Acetone Extract of Capsicum

Table-6: Showing Anti-arthritis activity of DMSO extract of Capsicum

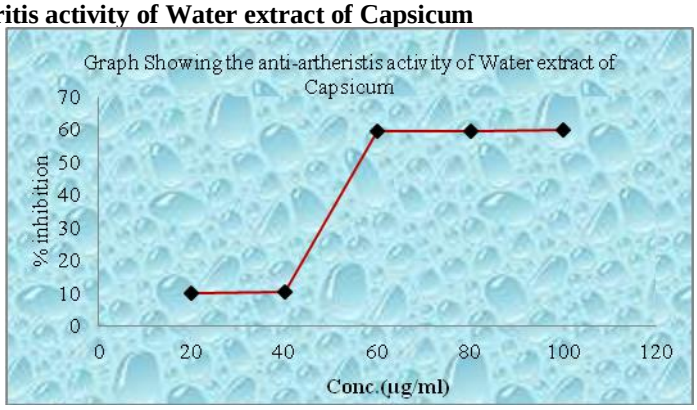
S. No	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.685	13.83	
2	40	0.554	30.31	
3	60	0.461	42.01	72
4	80	0.357	55.09	
5	100	0.341	57.10	



Graph 9: Showing Anti-arthritis Activity of DMSO extract of Capsicum

Table -7: Showing Anti-arthritis activity of Water extract of Capsicum

S. No	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.889	9.74	
2	40	0.882	10.45	
3	60	0.400	59.39	56
4	80	0.399	59.49	
5	100	0.396	59.79	



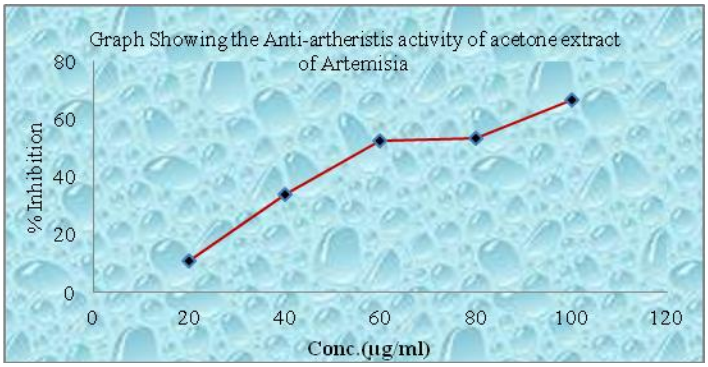
Graph 10:Showing Anti-arthritis Activity of Water extract of Capsicum

Anti-arthritis activity of Artemisia

The **Anti-arthritis activity** of various **Artemisia** extracts Viz (Acetone, DMSO and Water) have been analyzed, and it was found that **(DMSO extract) (Fig)** posses the highest anti-arthritis activity followed by **(water extract)**. The percentage inhibition by plant extracts was found to be concentration dependent, percentage inhibition increases with the increase in the concentration of the plant extracts. The **IC_{50}** value was determined from the straight line graph. The **IC_{50}** value of all the plant extracts was found lesser than the reference compound **(acetyl salicylic acid).** The **IC_{50}** value of various plant extracts follows the order **(DMSO extract, water extract and finally Acetone Extract)** was found to be **(40, 51.5, 57)** respectively and are presented in **(Tables).**

Table -8: Showing Anti-arthritis activity of Acetone extract of Artemisia

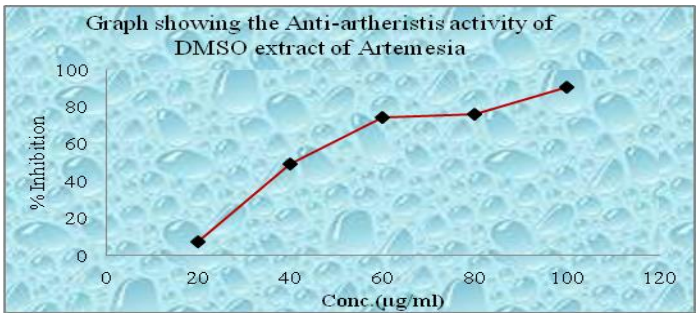
S. No	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.860	10.88	
2	40	0.640	33.67	
3	60	0.459	52.43	57
4	80	0.450	53.36	
5	100	0.321	66.73	



Graph 11: Showing the Anti-arthritis Activity of Acetone extract of Artemisia

Table -9: Showing Anti-arthritis activity of DMSO extract of Artemisia

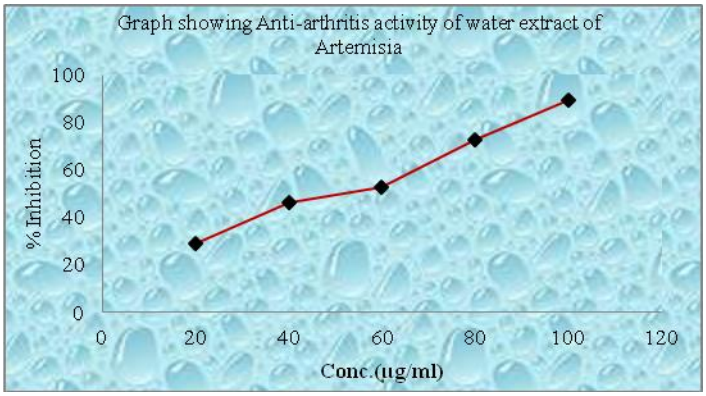
S. No	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.785	7.64	40
2	40	0.432	49.17	
3	60	0.220	74.11	
4	80	0.202	76.23	
5	100	0.081	90.47	



Graph 12:showing the Anti-arthritis activity of DMSO extract of artemesia

Table -10: Showing Anti-arthritis activity of Water extract of Artemisia

S. No	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.681	29.06	51.5
2	40	0.516	46.25	
3	60	0.455	52.60	
4	80	0.262	72.70	
5	100	0.102	89.37	

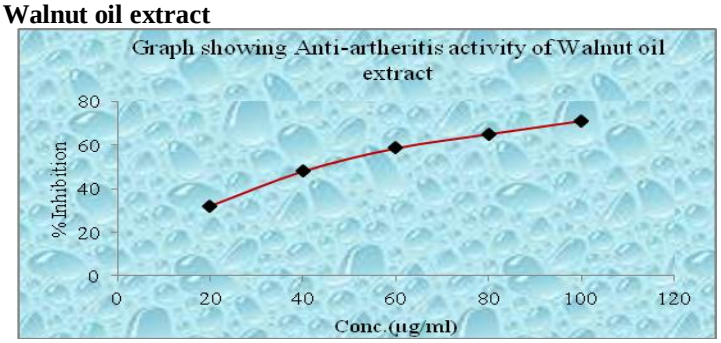


Graph 13: Showing the Anti-arthritis activity of Water extract of Artemisia

Anti-arthritis activity of Walnut oil

Table-11: Showing Anti-arthritis activity of Walnut oil extract

S. No.	Conc. (µg/ml)	Absorb	% Red	IC_{50} Value
1	20	0.668	32.12	43
2	40	0.660	48.05	
3	60	0.570	58.61	
4	80	0.504	65.12	
5	100	0.394	71.19	



Graph 14: Showing Anti-arthritis activity of Walnut oil extract

DISCUSSION:

Anti- inflammation refers to the property of a medicine or medicinal like product to inhibit the process of inflammation or swelling. Anti-inflammatory drugs make half of the analgesics, reducing pain by reducing inflammation at a particular body part of an organism.

Artemisia maritima, capsicum extracts were used against inflammation and the results obtained were found to possess a high level of effect against inflammation. The anti-inflammatory effect of plant is due to the presence of various secondary phytochemicals like flavonoids, alkaloids etc. the most enhancing capacity against the inflammation was found to be due the hemeoxygenase. So the plant could be used as best anti-inflammatory source. Flavonoids have been found present to a large extent in Ginkgo, these flavonoids may include caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside like substance have a potent anti-inflammatory effect as reported by Williams CA, Goldstone F, Green ham J-1996 (8) The anti-inflammatory effect of all the plant extracts have been carried out in reference to standard diclofenac sodium (anti-inflammatory drug). The inflammation was done by hypotonicity induced membrane lysis. The stabilization against membrane induced hypotonicity was found to be concentration dependent, and the stabilization percentage in all the plant extracts was found less than the reference compound (Diclofenac sodium). Among the plant extracts the highest stabilization was found in case of water extracts of Artemisia, capsicum fallowed by DMSO extracts of the concerned plants and the least

results were obtained by utilizing the Acetone extracts of the concerned plants.

Among the all plant extracts it has been found that the polar solvent extracts posses' highest anti-inflammatory activity than the less polar solvent extracts. The results get agreed as most of the amino acids are anti-inflammatory in nature so are the polar plant extracts. These compounds have direct influence upon the inflammation and the plant extracts fulfill the job in highly influenced manner.

Protein denaturation or arthritis 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. **[**24**]** Denaturation of protein is one of the cause of rheumatoid arthritis was documented. Production of auto antigen leads to denaturation of protein in certain arthritic disease. 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 effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on 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. Water extract of Artemisia and capsicum have pronounced effect against arthritis, followed by DMSO and acetone extracts. W*alnut oil*, showed maximum inhibition of protein denaturation than other extracts. The activity may be due to the presence of phyto-compounds with anti-arthritic activity.

CONCLUSION:

In conclusion it could be concluded that inflammation and the arthritis are the main two problems which get more raised with the progress of age. Actually both the bodily disarrays are due to the change in the structure of the proteins. So the continues use of allopathic medicines cannot fulfill the job against the concerned disorders. Body exercises give some relief against these concerned disorders. But as per the study in reference the continued use of plant extracts or phytochemicals can do the job to a large extent. Also the types of medicines are free from side effects. So the plants in references should be completely utilized against the diseases in reference and also for other body disarrays.

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