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Invitro Anti Arthritic Activity of Citrus Peel

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ABSTRACT: Rheumatoid arthritic is a major ailment among rheumatic disorders. A large number for treatment of various type of rheumatoid disorders. Citrus peel (Linn). An Indian herb was reported to have antiarthritic activity as wellas anti-inflammatory as well as analgesic activity in-vitro as well as in-vivo. The present study deals with antiarthritic activity. Various in-vitro antiarthritic activity pharmacological model were studied, such as inhibition of protein denaturation effect of membrane stabilization and protein denaturation inhibitory action. Herbal extract (aq) with conc(100, 200, 500).There are various extract are used in the dried extract of peel. The study revealed that plant contains several physiological active phytochemicals such as phenols, saponin, flavonoid, tannins, steroid and glycoside. Crude methanol, ethyl acetate, and aqueous extracts of peel were evaluated for antiarthritic activity by protein denaturation and protein inhibition method.
Kewords: Citrus peel, Antiarthritic activity.

I. INTRODUCTION:

Rheumatoid arthritic is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage. It is a common disease having a peak incidence in 3rd to 4th of life with 3-5 times higher preponderance in female. Its prevalence depends upon age. Herbal drug constituent a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity. The factor responsible for the continued and extensive use of herbal remedies in Indian are their effectiveness easy availability, low cost, comparatively less toxic effect andshortage of practitioners and modern medicine in herbal areas. Number of synthetic medicine has been derived from medicinal herbs. Fruit are used to treat pulmonary disease. The seed are claimed to expel bladder and kidney stone and effective in rheumatism. Hence, the present study was undertaken to evaluate in-vitro antiarthritic activity of plant extract.

II. MATERIAL AND METHOD:

a) Collection of Plant:
The lemon were collected from in local market Sangola Village in Solapur, Maharashtra, India.

b) Authentication of Plant materials:
The Citrus peel was authenticated by, Department of Botany, Sangola College of Arts, Science and Commerce Sangola.
Citrus Peel cut into small pieces then it was dried shade shade dried for a period of 6-7 day at an ambient temperature 30℃. The lemon were grinded properly using a mortar pestel and later grinder to obtain the powdered form.

c) Preparation of extract:
Powder (20g) was homogenized with various solvent viz., ethanol, methanol, chloroform, and diethyl ether, according to high polarity of for two weeks and used as 100%concentrated extract against tested pathogen. The homogenized mixtures in different solvent were collected, filtered and evacuated for antiarthritic and antioxidant activities and phytochemical screening.

- **Assessment of In-vitro Antiarthritic Activity**

A) A Standardization of bovine serum albumin denaturation:
Inhibition bioassay:
Heat induced Bovine serum albumin denaturation. In-vitro inhibition bioassay described by chaterjee S et al was standardized using Diclofenac sodium as standard.
Materials: BSA, Nacl, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Methanol, Diclofenac sodium.

Reagent:
1. **5%Bovine serum albumin:** 5g of BSA was dissolved in 100ml of phosphate buffersaline (pH

2. **Phosphate buffer saline(pH6.3):** dissolved o.895g of dihydrogen phosphate and 0.68 g of potassium dihydrogen phosphate and 3.51g of Nacl in sufficient water to produce 1000ml. adjust to PH 6.3 by adding HCL.
3. **Preparation of standard solution:** Stock solution of 10,000 πg/ml of diclofenac sodium was prepared by using methanol solvent. From these stock solution 3 different concentration of 100, 200, 500, µg/ml were prepared using a methanol.

Procedure:

The reaction mixture 3ml contained 50µl of the Diclofenac sodium (100, 200, 500µg/ ml was prepared in methanol). 450µlof 5% w/v BSA was added to above the test tube. The test tube were incubated at 30℃ for 20 min and then heated at 57℃ for 3 min after cooling the test tube, 2.5 ml phosphate buffer saline (pH 6.3) was added to each test tube. The absorbance of these solution was determined by using spectrophotometer at the wavelength of 660 nm. A controlled were a number of a drug was added was also maintained. The protein denaturation inhibition was calculated as shown in below.

Calculation:

The % inhibition of denaturation was calculated from control where no drug was added.

% inhibition = (abs of control- Abs of treated)/ Abs of control ×100.

B) Standardization of In-Vitro trypsinase activity bioassay:

In-Vitro trypsinase bioassay inhibitory activity bioassay as an indicator of anti- arthritic activity, was standardized using diclofenac sodium as standard. The concentration of enzyme 0.06 mg/ml; 2% casein and 55 Trichloro acetic acid at pH 7.6 were optimized condition determined in 4.5.2 a above.

Reagent:

1. **25m M Tris -HCL (pH 7.4):** 3.0275g Tris in sufficient water to produce 100ml. Adjust the pH is necessary by using HCL.
2. **Casein (2%):** 2g of casein dissolved in 100ml tris buffer solution.
3. **Trichloro acetic acid solution:** 5g of trichloro acetic acid dissolved in 100ml in buffer.
4. **Enzyme Trypsin (0.06 mg/ml):** 6mg trypsin in 100ml tris buffer. From these solution take 1ml make up to 1o ml with tris buffer.

Preparation of solution:

Stock solution of 10,000 µ/ml of diclofenac sodium was prepared by using methanol as solvent. From thes stock solution 3 different concentration of 100, 200, 500µg/ml were prepared by using methanol as solvent.

Procedure:

The reaction mixture 3 ml contained 100µl of trypsine, 350µl 25mM Tris- HCL Buffer (ph-7.4) and 50µl of the solution of diclofenac sodium (100,200,500µg/ml was prepared in methanol). The mixture were incubated at 37℃for 5min. then 500πl of 2%W/V casein was added. The mixture were incubated at 37℃ for 20 min. 2ml of 5% Trichloro acetic acid was added to terminate the reaction. Cloudy suspension was centrifuged at 5000 rpm for 5 min. absorbance of the supernatant was read at 280nm. A control were compensated the volume of standard was also measured.

Calculation:

The % inhibition was calculated from equation below.

% inhibition =(Abs of control – abs of treated)/ Abs of control×100

III. RESULT AND DISCUSSION:

Antiarthritic effect of various extract of citrus peel was studied significantly by using in-vitro inhibition of protein denaturation model. The effect of ethanolic extract of citrus peel on inhibition of protein denaturation shown in table no 1. Extract of citrus peel at three different concentration (dose level) provided, significant protection against denaturation of proteins. Most of investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritic. Production of auto- antigen in certain rheumatoid disease may be due to in- vitro denaturation of proteins. Mechanism of denaturation probably involves alteration in bonding obtained data stated that citrus peel could be used as potent anti-arthritic agent.

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The ability of Diclofenac sodium to inhibit heat induced protein denaturation is show in table no: 1

Table no: 1 Inhibition of Heat induced protein denaturation by Diclofenac sodium In- vitro.

SI no	Standard drug	Concentration µg/ml	% inhibition	Mean ±SD
1	Diclofenac sodium	100	62.90%	0.0100±0.0010***
		200	72.61%	0.0076±0.0005***
		500	77.38%	0.006±0.0005***

Table no 1.The % protein denaturation inhibition of the different extracts of Citrus peel at different concentration is shown in Table.

SI no	Plant extract	Concentration µg/ml	% inhibition	Mean ± SD
1	Petroleum ether(60-80%)	100	66%	0.0093±0.005***
		200	58.14%	0.0116±0.0005***
		500	58.24%	0.1553±0.0011***
2	Chloroform extract	100	60.57%	0.1467±0.0005***
		200	53.67%	0.1383±0.0005***
		500	62.81%	0.1723±0.0005***
3	Ethyl acetate	100	63.45%	0.5183±0.0011***
		200	66.26%	0.4790±0.0***
		500	75.28%	0.3503±0.0005***
4	Aqueous extract	100	56.63%	0.1613±0.0005***
		200	50.80%	0.1830±0.0***
		500	60.57%	0.1467±0.0005***
5	Control	_	_	0.0286±0.0011***

The result are expressed as mean ±SD.*P<0.05, **P<0.01, P*<0.001difference with control.**

Graph no:1 Graphical representation of In- vitro protein denaturation inhibition activity of the different extract of Citrus peel and standard diclofenac sodium.

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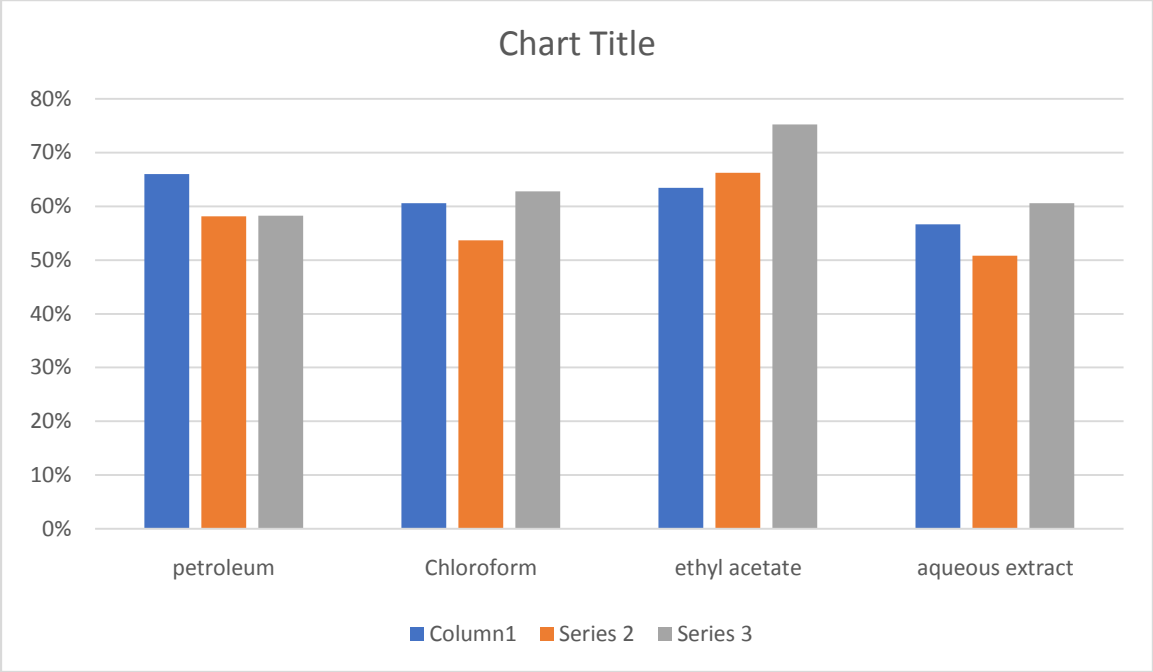

Table no:B The Trypsinase inhibitory activity of diclofenac sodium In-vitro are shown in Table no:1
Table no:1Trypsinase inhibitory activity of diclofenac sodium In-vitro.

SI no	Standard drug	Concentration µg/ml	% inhibition	Mean ±SD
1	Diclofenac sodium	100	71.50%	0.1060±0.0112***
		200	62.81%	0.1383±0.0005***
		500	55.37%	0.1660±0.0043***

Table no:2 Trypsinase inhibitory activity of Citrus peel Linn extracts In-vitro.

SI no	Plant extract	Concentration µg/ml	% inhibition	Mean ±SD
1	Control	-	-	0.3723±0.0005***
2	Petroleum ether	100	53.68%	0.1723±0.0005***
		200	67.29%	0.1217±0.0005***
		500	70.07%	0.11113±0.0011***
3	Chloroform	100	56.19%	0.6480±0.0242***
		200	45.49%	0.7733±0.00111***

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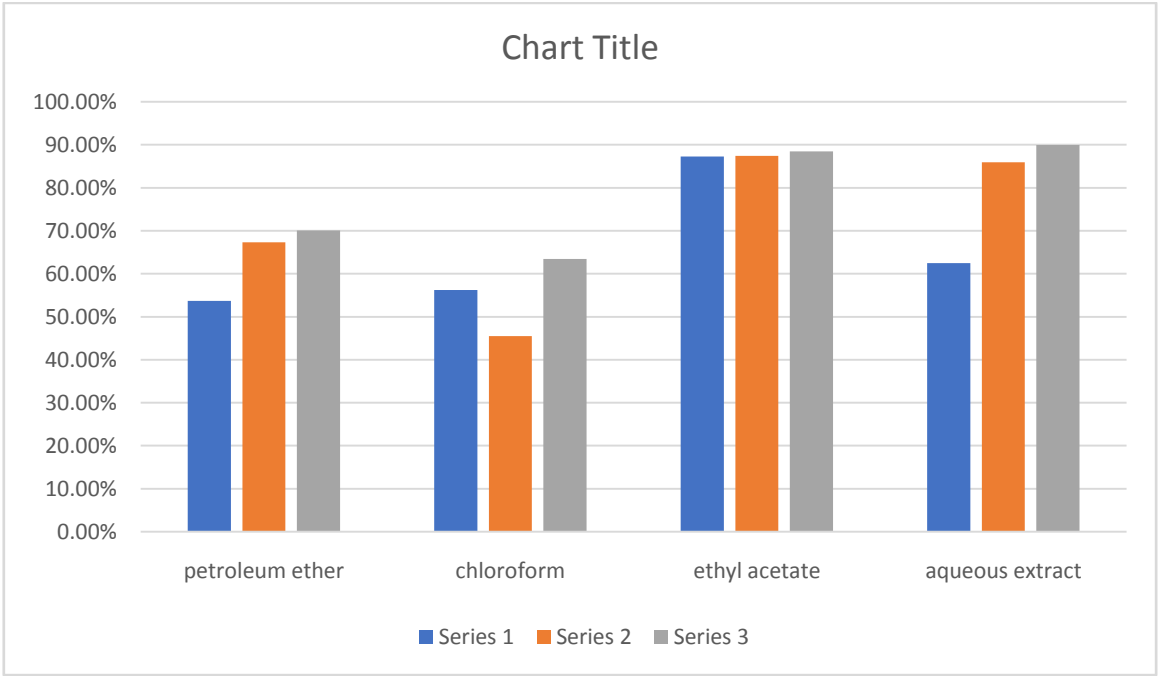
		500	**63.45%**	**0.5183±0.0011*** **
4	**Ethyl acetate**	**100**	**87.25%**	**0.1807±0.0005*** **
		200	**87.39%**	**0.1738±0.0005*** **
		500	**88.45%**	**0.1633±0.0011*** **
5	**Aqueous extract**	**100**	**62.45%**	**0.1397±0.0005*** **
		200	**85.93%**	**0.0523±0.0005*** **
		500	**89.96%**	**0.0373±0.0005*** **

The result are expressed as mean ±SD.*P<0.05, **P<0.01, P*<0.001difference withcontrol.**

Graphical Representation of In-vitro Trypsinase inhibitory activity of different extracts of Citrus peel



IV. CONCLUSION:

From the result obtained in the present study, it may be concluded that citrus peel possess significant antiarthritic activity, Hence it could be beneficial for further work as active antiarthritic agent.

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