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SCREENING AND COMPARISON OF IN-VITRO ANTI ARTHRITIC ACTIVITY OF INDIVIDUAL CURCUMINOIDS WITH CHLOROFORM EXTRACT

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Abstract

The present study is aimed to evaluate the in-vitro anti arthritic activity of chloroform extract of *curcumalonga* rhizomes using inhibition of protein denaturation model and human red blood cell membrane stabilization model. Diclofenac sodium was used as a standard drug. Results evaluated that the chloroform extract of *curcumalonga* at different concentrations possessed significant anti arthritic activity as compared to standard drug used as Diclofenac sodium. The results obtained in the present investigations indicate that chloroform extract of *curcumalonga* rhizomes showed anti arthritic activity.

Key words: *curcumalonga* rhizomes, Anti arthritic, protein denaturation, membrane stabilization.

1. Introduction:

Rheumatoid arthritis is a chronic systemic inflammatory disease predominantly affecting the joints and particular tissue. Rheumatoid arthritis still remains a formidable disease, being capable of producing severe crimpling deformities and functional disabilities. Rheumatoid arthritis is classified as an inflammatory arthritis, the disease comprises of three basic inter related processes like inflammation, synovial proliferation and joint tissue destruction. The focus of Rheumatoid arthritis is the synovial lining. The Rheumatoid arthritis factor containing immune complexes found in the joints activate the pathological process. Tumor necrosis factor, alpha (TNF – alpha) is the product of macrophages, lymphocytes and plasma cells. The activated macrophages, lymphocytes and fibroblast produce a variety of cytokines that promote further synovial proliferation and inflammation. Synovial fluid in Rheumatoid arthritis contains various prostaglandins mainly PGE₂, Leucotriene B₄-TNF alpha, interleukins and other cytokines. It is now believed that manikins IL, IL6 and alpha are the central mediators of active rheumatoid processes.

2. Materials and Methods:

2.1 Collection of Plant material: The plant of *Curcuma longa* L. of the family zingiberaceae rhizomes were obtained from local supplier, Bangalore in the month of December 2013. The plant material was authenticated by Dr. Suresh, Advisor, SAMI Labs, Peenya, Bangalore.

2.2 Drugs and Chemicals:

Chloroform, methanol, methylene dichloride, Bovine serum albumin (5% w/v aqueous solution), PH 7.0, 1N HCl, Phosphate buffer (PH 6.3), Diclofenac sodium.

2.3 Preparation of Plant Extract:

The rhizomes were dried in shade and coarsely powdered with a blender. 1kg of the powder was subjected to continuous percolation using 5 liter Round bottom flask with the solvent chloroform for 24hrs, the solvent was recovered by distillation in rotary vacuum evaporator at 80⁰c. 46 Gms of chloroform extract was Loaded in column chromatography, eluted with chloroform; methanol (95:5), the residue obtained was characterized by TLC, HPLC, then the above residue was subjected to purification. Eluted with 100%MDC (Methylene dichloride). Three compounds were isolated & characterized by TLC, NMR, &IR the residue was stored in a desicator and used in further studies.

2.4 Assessment of in vitro Anti-arthritis Activity

Plant extracts: Isolated compounds of chloroform extracts and chloroform extract of plant curcuma longa. The 10, 50, 100, 200, 400, 800, 1000µg/ml concentrations of each compounds and extract were used

2.5 Inhibition of Protein Denaturation Method:

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Manilkara zapota* extract (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.

Percentage inhibition = (O.D of test solution – O.D of product control)/O.D of test control × 100

The control represents 100% protein denaturation .The results were compared with diclofenac sodium standard solution (200µg/ml)

3. Results and Discussion:

Denaturation of protein is one of the cause of rheumatoid arthritis is well documented(Mizushima,Y.1968).Production of auto antigen in certain arthritic disease may be due to denaturation of proteins (Brown,J.H.and Mackey,H.K.1986)

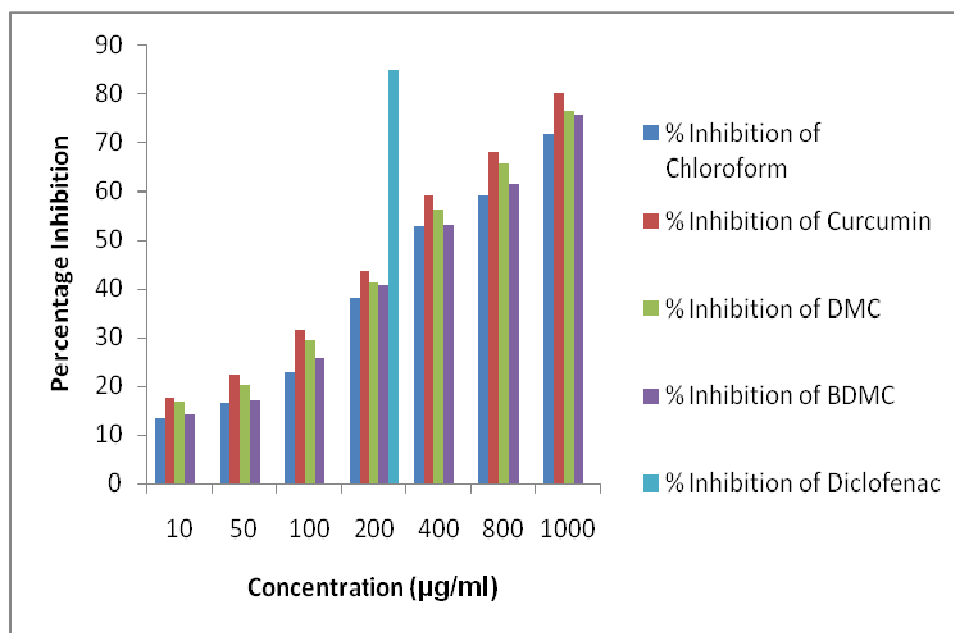
The mechanism of denaturation of probably involves alteration in electrostatic hydrogen, hydrophobic and disulphide bonding .From the results of the present study it can be stated that chloroform extract and isolated compounds, i.e., Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin (chloroform extract) are capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins and its effect was compared with standard drug Diclofenac sodium.Curcumin showing maximum activity. The effect is represented as follows Curcumin >Demethoxy curcumin> Bisdemethoxycurcumin> Chloroform extract.

The above order suggests that the relative properties of the active compounds in the extract are definitely May small. Hence the effectiveness of extract is less compare to isolated compounds.

Table 1: Percentage inhibition of protein denaturation of the chloroform extract, isolated compounds and the standard Diclofenac Sodium are tabulated as below.

Concentration (µg/ml)	Percentage inhibition of chloroform extract	Percentage inhibition of Curcumin	Percentage inhibition of Demethoxy curcumin	Percentage inhibition of Bis demethoxy curcumin	Percentage inhibition of Diclofenac sodium
10	13.32	17.61	16.72	14.33	
50	16.47	22.29	20.91	17.11	
100	22.86	31.34	29.43	25.85	
200	38.12	43.17	41.17	40.47	84.74
400	52.93	59.11	56.28	53.14	
800	59.26	67.87	65.73	61.54	
1000	71.64	79.94	76.16	75.73	

Figure 1: Percentage inhibition of protein denaturation of the chloroform extract, isolated compounds and the standard Diclofenac Sodium are tabulated as below.



4. Discussion:

The in vitro pharmacological activities were performed for the chloroform extract and isolated compounds of the Curcuma Longa Rhizome.

Protein denaturation produces auto antigens which may be a cause for the development of rheumatoid arthritis. The study of anti arthritic activity of the chloroform extract and isolated compounds (chloroform extract) showed a dose dependant inhibition of protein denaturation and was comparable to that of the standard. While comparing these isolated compounds with chloroform extract, the effect represented as below. Curcumin > Demethoxy curcumin > Bisdemethoxycurcumin > Chloroform extract.

The above order suggests that the relative proportions of the active compounds in the extract are definitely May small. Hence the effectiveness of extract is less compare to isolated compounds.

5. Conclusion: From the above studies, the chloroform extract and isolated compounds exhibit anti arthritic activity by inhibition of protein denaturation .This active molecules may be taken up for further studies to enable them to be explored as adjunct in arthritis therapy.

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