In Vitro Anti-Rheumatoid Arthritis Activity of *Polyalthia Korinti* Extracts

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Abstract-Rheumatoid arthritis, the common human autoimmune disease is characterized by chronic inflammation in joints followed by pannus formation within filtered lymphocytes and fibrinoid joints of a synovial membrane with concomitant destruction of cartilage and bone. Denaturation of tissue proteins is a marker for inflammatory and arthritic diseases since most biological proteins lose their biological function when denatured. As a result, agents that prevent protein denaturation may be a promising target for anti-arthritis drug production. With this idea in mind, an in vitro test was conducted to determine the existence of anti-arthritic properties in plant extracts. In the present study, the protein denaturation bioassay was selected as one of the in *vitro* assessment of the anti-arthritic property of *P.korinti* leaves and bark extracts with a wide range of dose concentrations. *Polyalthia korinti* extracts prevented heat-induced protein denaturation in our sample. Out of all the extracts of *P.korinti* leaves methanol extract act as most potent inhibitor to prevent protein denaturation. This study promising target for anti-arthritis drug production.

Key words: *Polyalthia korinti*, Rheumatoid arthritis, chronic inflammation, Protein denaturation

I. INTRODUCTION

Rheumatoid arthritis is the most common of the rheumatic diseases. Rheumatoid arthritis strikes people during their most active years. Rheumatoid pannus is characterized by articular inflammation and the development of inflammatory and invasive tissue, which ultimately leads to joint destruction. Anti-inflammatory drugs and analgesics, such as steroids, are commonly used to relieve symptoms, while disease-modifying antirheumatic drugs (DMARDs), as well as newer treatments such as anti-CD20 therapy and anti-tumor necrosis factor (TNF) therapy, are often used to suppress or prevent the underlying immune response. Long-term use of both of these chemicals, however, has been attributed to gastrointestinal toxicity, cardiovascular toxicity, and other toxicity [1-2]. In certain arthritic diseases, in-vivo protein denaturation can be responsible for the production of autoantigens. Changes in electrostatic, hydrogen, hydrophobic, and disulfide bonding are thought to induce denaturation [3]

Anti-arthritic activity is achieved in rheumatoid arthritis by inhibiting protein denaturation and controlling autoantigen growth. The current study will look into the inhibition of protein denaturation using bovine serum albumin and egg albumin as a measure of in vitro antiarthritic activity. Plant extracts are increasingly being used to treat a wide range of arthritis symptoms. In the treatment of arthritic disorders, modern medicine has a high success rate. Although there are a number of existing drugs that can be used to treat these disorders, long-term use of these medications can have significant side effects. As a result, there is a strong desire to find new therapeutic agents with as few side effects as possible [4]

II. MATERIALS AND METHODS

Collection and Identification of Plant material

The leaves and bark material of *Polyalthia korinti* (Dunal.) Thaw. was collected from the Seshachalam Hills. Seshachalam hill ranges of Eastern Ghats lie between 13°38' to 13°55' N latitudes and 79° 07 to 79° 24’ E longitudes and spread over two districts viz., Chittoor and Kadapa of Southern Andhra Pradesh.

The Plant was authenticated by taxonomic expert Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University (SVU), Tirupati, and Andhra Pradesh. Where the voucher specimens were deposited (Herbarium voucher No 566).

The required quantity of plant parts was collected and separated from undesirable materials. Washed with running water followed by distilled water to remove the contaminants. Chopping process was carried out and they were allowed to dry under shade [5] The dried material was ground into a coarse powder with the help of a suitable pulverizer. The powder was stored in an airtight container and kept in a cool, dark and dry place

Extraction Technique

The dried powder of the leaves and bark was extracted sequentially [6]using a soxhlet apparatus[7] with various solvents based on their polarity, such as hexane, chloroform, methanol, and water. Using a rotary evaporator, the extracts were concentrated and solvent-free under reduced pressure. The dried crude concentrated extracts were weighed and stored in an airtight bottle until used for analysis.

Anti-Rheumatoid arthritis Activity:

The aim of this study was to evaluate *P.korinti*'s anti-rheumatoid activity by following methods-inhibition of protein denaturation method using bovine albumin and inhibition of protein denaturation method using egg albumin.

Inhibition of Protein Denaturation Method Using Bovine albumin:

The inhibition of albumin denaturation technique developed by Mizushima et al (1968) [8]and Sakat et al (2010) [9], with minor modifications, was used to monitor *Polyalthia korinti* leaves and bark extracts for anti-rheumatoid arthritic function. The reaction mixture consisted of various concentrations of test extracts and 1% aqueous solution of bovine albumin fraction, pH (6.3) adjusted with a small amount of 1N HCl. The sample extracts were incubated at 37ºC for 20 min and then heated to 51º C for 30 min, after cooling the samples, 1ml of Phosphate buffer saline (pH 6.3) was applied to each sample tube. The turbidity was measured using spectrophotometry at 660 nm. The experiment was repeated three times. The percentage of protein denaturation inhibition was determined as follows.

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

Inhibition of Protein Denaturation Method Using Egg albumin:

Each reaction mixture contained 0.2 mL fresh hen's egg albumin, 2.8 mL phosphate-buffered saline (PBS, pH 6.4), and 2 mL *P.korinti* leaves and bark extracts at various concentrations. As a control, a similar amount of double-distilled water was used. Then the mixtures were incubated at (37±2) oC in a biochemical oxygen demand (BOD) incubator for 15 min and then heated at 70ºC for 5 min. After cooling, their absorbance was determined at 660 nm. As a control, Diclofenac sodium was used [10]. The formula below was used to measure the percentage inhibition of protein denaturation.

Percentage of inhibition = (Abs control-Abs test sample) x 100/ Abs control.

Each experiment was repeated three times, and the average was taken. The dose-response curve was used to assess the extract concentration for 50% inhibition (IC_{50}).

III. RESULTS AND DISCUSSION

Anti-Rheumatoid arthritis Activity:

Arthritis is a type of joint disease characterized by inflammation of one or more joints, resulting in discomfort, stiffness, and loss of joint function. Denaturation of the protein is one of the major causes of arthritis. Because of the denaturation of the protein in certain arthritic diseases, an auto antigen is produced. Denaturation is thought to be caused by changes in electrostatic hydrogen, hydrophobic, and disulfide bonding[11].

Inhibition of Protein Denaturation Method Using Bovine albumin

The results of albumin denaturation of the *P.korinti* extracts were displayed in table 1 and 2. The present findings exhibited a concentration-dependent inhibition of protein (albumin) denaturation by leaves and bark extracts throughout the concentration range of 50- 250 µg/ml (Figure 1 and 2). Diclofenac sodium (at the concentration range of 50-250 µg/ml) was used as the standard drug, which also exhibited concentration-dependent inhibition of protein denaturation. All extracts exhibited albumin denaturation with percentage inhibition values between 42.33±1.53 % to 85.00±1.00% at a concentration of 250µg/ml. When compare with bark,

leaves extracts show more potent inhibitory activity against the denaturation of proteins. Inhibition of albumin denaturation at 250µg/ml was found to be highest in methanol extract of leaves followed by methanol extract of bark, water extract of leaves, water extract of bark, chloroform extract of leaves, chloroform extract of bark, hexane extract of leaves, hexane extract of bark and the values were 85.00±1.00, 77.00±1.00, 70.33±1.53, 65.67±1.53, 61.33±1.53, 53.00±1.00 and 47.00±1.00 respectively. Percentage inhibition value for the standard diclofenac sodium was found to be 95.00±1.00% at a concentration of 250µg/ml.

Table: 1 Inhibition of Albumin Denaturation by *Polyalthia korinti* Leaves extracts

Conc. (µg/ml)	Hexane Extract	IC50	Chloroform Extract	IC50	Methanol Extract	IC50	Water Extract	IC50	Standard Diclofenac	IC50
50	12.67±0.58		17.67±1.15		38.67±0.58		23.67±1.15		41.67±1.53	
100	24.00±1.00		32.67±1.15		56.33±1.53		40.67±0.58		60.00±2.00	
150	30.67±1.15	259.87±7.75	44.33±1.53	186.94±4.16	70.67±0.58	81.40±2.58	52.33±0.58	149.16±3.06	74.00±1.00	69.36±5.95
200	41.33±1.53		54.00±1.00		83.67±1.53		64.00±1.00		87.00±1.00	
250	47.00±1.00		61.33±1.53		90.67±1.15		70.33±1.53		95.00±1.00	

* Mean ±SD (n=3) is used to describe each value.

Table: 2 Inhibition of Albumin Denaturation by *Polyalthia korinti* Bark extracts

Conc. (µg/ml)	Hexane Extract	IC50	Chloroform Extract	IC50	Methanol Extract	IC50	Water Extract	IC50	Standard Diclofenac	IC50
50	10.00±1.00		15.33±1.15		25.33±1.53		21.00±1.00		41.67±1.53	
100	20.67±0.58		26.67±0.58		45.00±1.00		35.00±1.00		60.00±2.00	
150	26.33±1.53	300.16±11.55	35.67±0.58	230.00±3.76	59.33±1.53	129.63±3.99	48.00±1.00	167.19±3.43	74.00±1.00	69.36±5.95
200	34.00±1.00		44.67±0.58		69.33±1.53		60.67±2.08		87.00±1.00	
250	42.33±1.53		53.00±1.00		77.00±1.00		65.67±1.53		95.00±1.00	

* Mean ±SD (n=3) is used to describe each value.

Fig: 1 Inhibition of Albumin Denaturation By *Polyalthia korinti* Leaves extracts

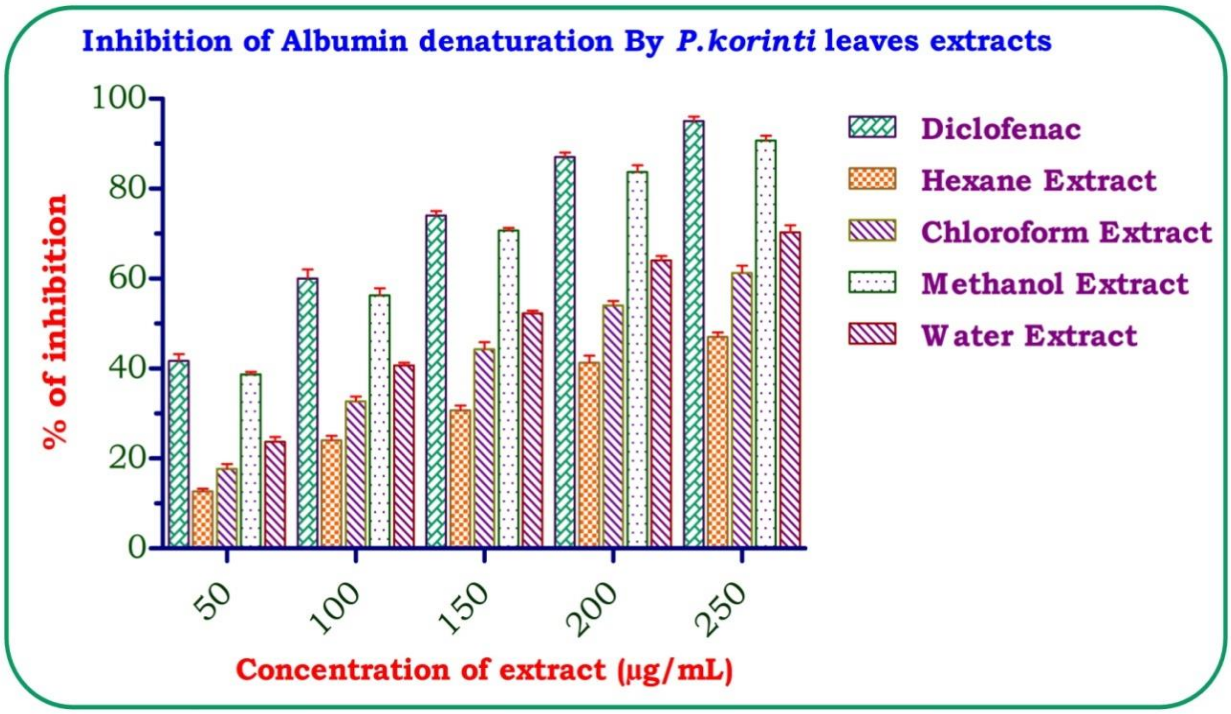
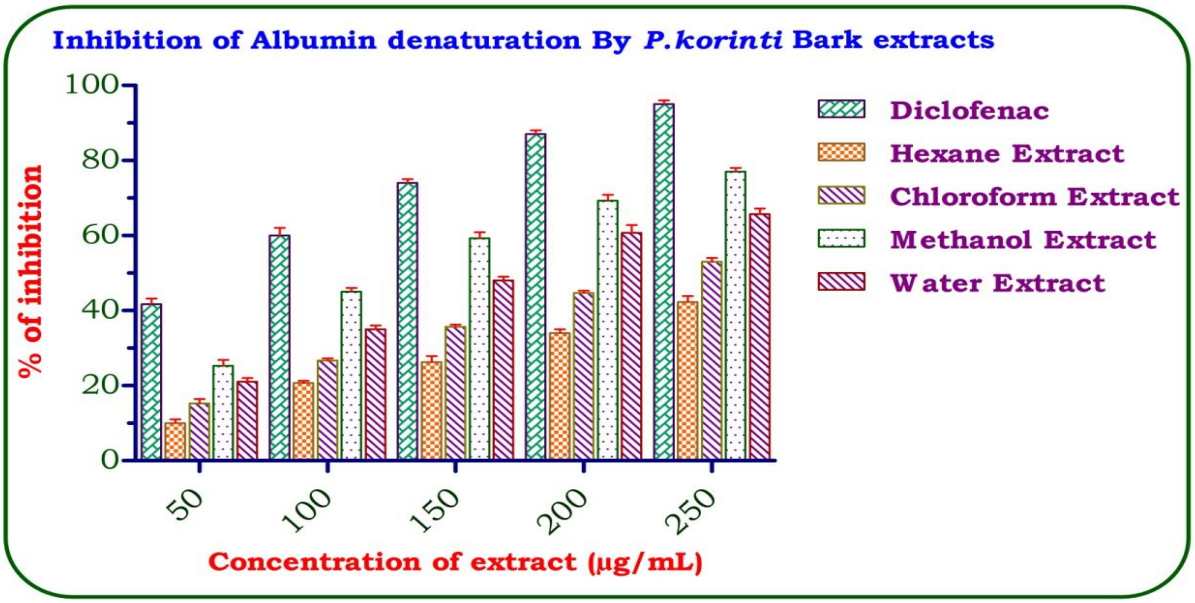


Fig: 2 Inhibition of Albumin Denaturation By *Polyalthia korinti* Bark extracts



Out of the all extracts, methanol extract of leaves and bark showed the lowest IC50 109.68±1.94 and 129.63±3.99 µg/ml, whereas hexane extracts of leaves and bark showed highest IC50 259.87±7.75 and 300.16±11.55 µg/ml, respectively. The lowest IC50 indicates the strongest ability of the extracts to act as an inhibitor of protein denaturation. In view of that, the study has shown that the methanol extract of leaves acts as a strong inhibitor for the denaturation protein.

Inhibition of Protein Denaturation Method Using Egg albumin:

Results for denaturation of egg albumin inhibitory activity of *P.korinti* leaves and bark extracts are shown in the table 3 and 4. Inhibition of egg albumin denaturation levels was within the range of 12.33±0.58 - 81.33±1.15% within the concentrations of 50-250µg/ml (Figure 3 and 4). The methanol extract of *P.korinti* leaves showed an improved ability to inhibit the denaturation of egg albumin (81.33±1.15%) followed by methanol extract of bark (73.00±1.73%)at 250µg/ml concentration. Whereas hexane extract of *P.korinti* bark has shown the least inhibitory activity (12.33±0.58 %) at the concentration of 50µg/ml. The order of the egg albumin denaturation inhibitory activity of *P.korinti* leaves and bark extracts was methanol > water > chloroform > Hexane. From these results, the strongest inhibition was obtained by methanol extract of leaves at a concentration of 250µg/ml. The standard Diclofenac showed 94.33±1.53% of inhibition at a concentration of 250µg/ml. Out of the all extracts, methanol extract of leaves and bark showed the lowest IC_{50} 102.72±3.97 and 133.18±7.48 µg/ml, whereas hexane extracts of leaves and bark showed highest IC_{50} 277.77±4.90 and 315.86±8.93 µg/ml, respectively. The lowest IC_{50} indicates the strongest ability of the extracts to act as an inhibitor of the denaturation of egg albumin. In view of that, the study has shown that the methanol extract of leaves acts as a strong inhibitor to inhibit the denaturation of egg albumin.

Table: 3 Inhibition of Egg Albumin Denaturation By *Polyalthia korinti* Leaves extracts

Conc. (µg/ml)	Hexane Extract	IC50	Chloroform Extract	IC50	Methanol Extract	IC50	Water Extract	IC50	Standard Diclofenac	IC50
50	15.00±1.00		18.67±1.15		36.33±0.58		25.33±1.53		38.67±1.15	
100	24.00±1.00		29.33±0.58		53.67±1.53		36.33±1.53		56.33±1.53	
150	30.33±1.53	277.77±4.90	39.67±0.58	205.40±2.26	69.00±1.00	90.19±1.51	48.67±0.58	163.94±2.85	72.67±1.15	80.52±4.50
200	38.33±0.58		50.00±1.00		82.33±1.53		58.00±2.00		86.00±1.73	
250	45.67±1.15		57.67±0.58		90.00±1.00		67.00±1.73		94.33±1.53	

* Mean ±SD (n=3) is used to describe each value.

Table: 4 Inhibition of Egg Albumin Denaturation By *Polyalthia korinti* Bark extracts

Conc. (µg/ml)	Hexane Extract	IC50	Chloroform Extract	IC50	Methanol Extract	IC50	Water Extract	IC50	Standard Diclofenac	IC50
50	12.33±0.58		15.67±1.15		28.67±0.58		20.67±1.15		38.67±1.15	
100	20.33±0.58		24.00±1.00		46.33±1.53		30.33±1.53		56.33±1.53	
150	27.33±1.53	315.86±8.93	33.33±1.53	259.93±7.55	55.33±1.53	133.18±7.48	42.00±1.00	188.59±5.53	72.67±1.15	80.52±4.50
200	33.00±2.00		40.67±1.15		63.33±1.53		53.67±1.15		86.00±1.73	
250	12.33±0.58		47.67±1.15		73.00±1.73		62.33±1.53		94.33±1.53	

* Mean ±SD (n=3) is used to describe each value.

Fig: 3 Inhibition of Egg Albumin Denaturation by *Polyalthia korinti* Leaves extracts

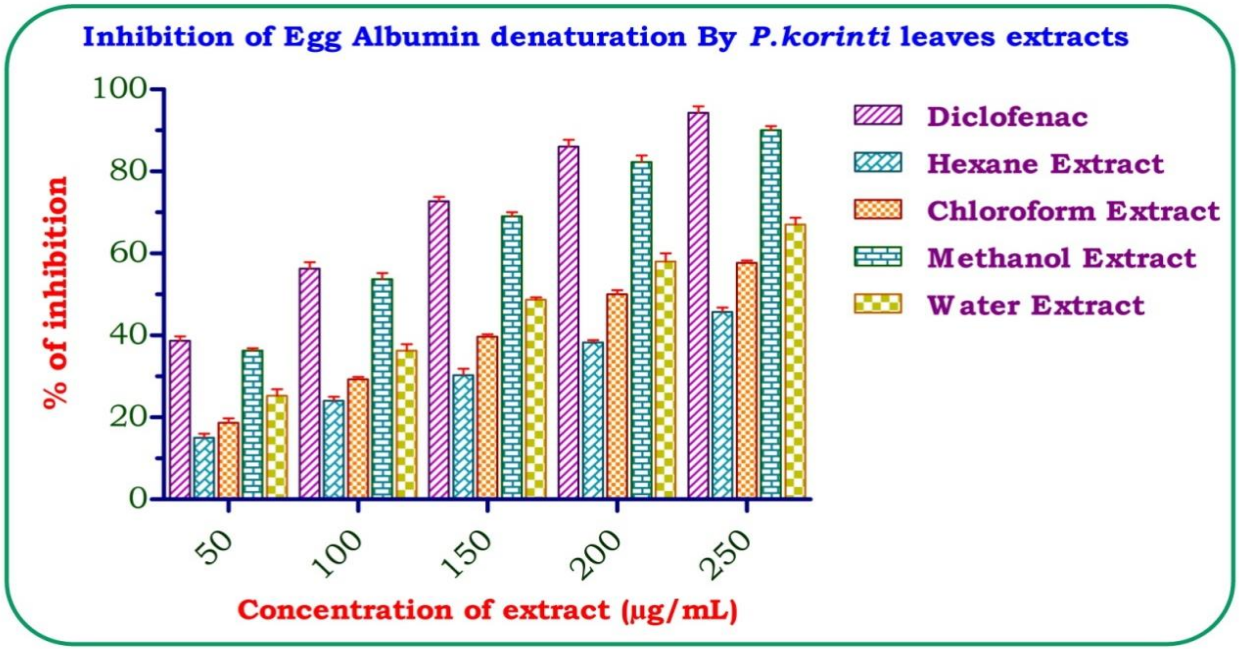
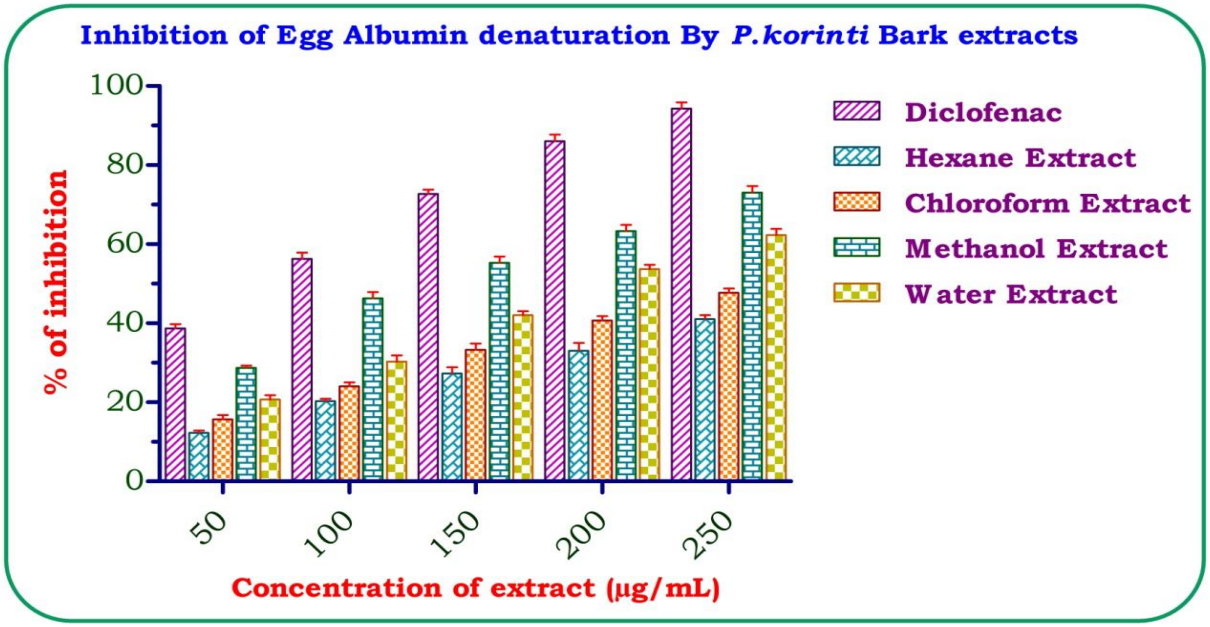


Fig: 4 Inhibition of Egg Albumin Denaturation by *Polyalthia korinti* Leaves extracts



Protein denaturation happens when proteins lose their tertiary and secondary structures due to the use of a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Denaturation of tissue proteins is a marker for inflammatory and arthritic diseases since most biological proteins lose their biological function when denatured [12]. As a result, agents that prevent protein denaturation may be a promising target for anti-arthritis drug production. With this idea in mind, an in vitro test was conducted to determine the existence of anti-arthritic properties prior to conducting an in vivo test. In the present study, the protein denaturation bioassay was selected as one of the in *vitro* assessments of the anti-arthritic property of *P.korinti* leaves and bark extracts with a wide range of dose concentrations.

Rheumatoid arthritis, the most prevalent human autoimmune illness, is characterized by persistent inflammation in joints, followed by pannus development inside filtered lymphocytes and fibrinoid synovial membrane joints, with simultaneous cartilage and bone degradation. Protein denaturation, according to certain research, is one of the causes of rheumatoid arthritis [13-14]. In some rheumatic diseases, in-vivo denaturation of proteins can lead to the production of autoantigens. Changes in electrostatic, hydrogen, hydrophobic, and disulfide bonding are thought to cause denaturation. Several anti-inflammatory medicines have been demonstrated to prevent thermally driven protein denaturation in a dose-dependent manner [15]. *Polyalthia korinti* extracts prevented heat-induced protein denaturation in our sample, which may be one of the reasons for their anti-arthritic properties.

IV. CONCLUSION

The anti-arthritic activity of *P.korinti* extracts were carried out by inhibition of protein denaturation method using Bovine albumin and Egg albumin. From this study it is concluded that *P.korinti* extracts were exhibited concentration-dependent inhibition of protein denaturation. When compare with bark, leaves extracts show more potent inhibitory activity against the denaturation of proteins. The maximum percentage of inhibition was shown in methanol extract of leaves followed by methanol extract of bark. Out of all extracts, methanol extract of leaves and bark showed the lowest $IC_{50.}$ In view of that, the study has shown that the methanol extract of leaves acts as a strong inhibitor for the protein denaturation. Further research needed to isolate and characterize the metabolites responsible for anti-arthritic activity.

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