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PHARMACOLOGICAL POTENTIAL OF *EXCOECARIA AGALLOCHA* L. ON RHEUMATOID ARTHRITIS

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ABSTRACT
Rheumatoid arthritis is a chronic inflammatory disorder of autoimmune origin that preferentially attacks smaller synovial joints of legs and hands to later period it will develops and affect body tissues and organs that causing permanent disability. Recently, there is no complete curable treatment for Rheumatoid arthritis but symptoms are suppressed on an individual basis. This study is aimed to evaluate the potential activity of *Excoecaria agallocha* L. (Euphorbiaceae) against Rheumatoid arthritis. *Excoecaria agallocha* is a mangrove plant. It is widely present in the coastal region mainly in Tamilnadu (Adirampattinam, Chidambaram). It has potential pharmacological activity against several diseases particularly for pain related disease. Ethno medically it has anti-rheumatism,anti-paralysis, anti-epileptic, anti-leprosy and anti-psoriatic activity. In order to perform this study, 70% hydroalcoholic extract was obtained by using Soxhlet extraction method. In-vitro studies were performed for anti-inflammatory and anti-arthritic activity using membrane stabilization and protein denaturation method. Both assays give significant activity against inflammation and arthritis. The inhibitory concentration of membrane stabilization (anti-inflammatory assay) and protein denaturation (anti-arthritic assay) were found to be IC50 220.92 µg/mlandIC50 436.56 µg/ml respectively. The in-vivo study was performed for 21 days by using formalin induced rheumatoid arthritis in rats. The percentage inhibition of paw volume was recorded for both 200mg/kg and 400mg/kg of HAEEA and it was found to be 30.12 % and 41.34 % respectively. Hence *Excoecaria agallocha* has shows good activity against rheumatoid arthritis.

Keywords: Excoecaria agallocha L., Rheumatoid arthritis, Anti-inflammatory, Anti-arthritic, Formalin induced arthritis.

INTRODUCTION

The *Excoecaria agallocha* L. (Euphorbiaceae) is one type of mangrove plant. It has been distributed throughout of the coastal region of the world mainly tropical Africa, North Australia and Asia [1,2,3]. The global distribution of mangrove forest is about 1,81,000 sq.km. The Excoecaria genus nearly about 70 species of 27 genera have been reported [2]. It has ecologically great influence against cyclone, tsunami and act as an energy source of the marine food chain [4]. It is ethno botanically valuable medicine to treat rheumatism, leprosy and paralysis [3].

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It is traditionally used to treat ulcer, sores, conjunctivitis, dermatitis and hematuria. The bark smoke is used to treat leprosy [4].

It has been traditionally used as an anti-rheumatoid drug in Siddha system of medicine but the exact formulation procedure, formulation type method of administration is not given. They are simply given that it is used to treat 64 types of pain related disease. The people who living near by the coastal region the major problem is joint swelling and body pain. It is may be due to environmental condition. The villages people have been overcome the above problem by using aged leaves of *Excoecaria agallocha*. The aged leaved boiled with water and poured to painful area. It reduces pain and swelling of the joints. There is more number of chemical constituents

present in this plant, like triterpenoids, diterpenoids, flavonoids, phenolic compounds, tannins and alkaloids [5].

Rheumatoid arthritis is an auto immune disease, our body cells mistakenly attacks the own body tissues which leads to synovial bones and cartilage destruction finally producing inflammation and swelling of joints [6]. It affects globally one percentage of people and women three times more susceptible than men [7, 8]. The main pathogenesis of rheumatoid arthritis is the activation of B-cells, T-cells, plasma cells, neutrophils, dendritic cells and macrophages which actively release pro-inflammatory cytokines like TNF-α, IL-1β and IL-6.These pro-inflammatory cytokines produce joint damage and permanent disability. These pro-inflammatory cytokines finally enters into the blood stream results to the systemic inflammation. The causes of the disease were not clearly identified. It may be the features including abnormal body immune response, genetic, biologically triggered viral infections and hormonal changes [8]. This disease can be diagnosed by using modern methods like RA factor analysis, C-reactive protein test, anti-CCP test and X-ray. There is no specially framed medicine for rheumatoid arthritis disease and the drugs are produced more number of severe side effects. Currently, there is no curable treatment for this auto immune disease so the newer type of medicine should be developed for reducing the side effects [9].

Materials and methods:

Authentication of the plant

The mangrove plant of *Excoecaria agallocha* L. was authenticated by DR Stephen, dept of botany, American college, Madurai-20.

Collection of plant materials

The fresh twigs of *Excoecaria agallocha L.* was collected in the period of October – November at coastal region of Adirampattinam (Tamil nadu). The collected plant material was wash properly with water and removes the debris, dry the twigs at shade area for two weeks. The dried zone should free from dust and moisture after drying process the twigs material was powdered by using mixer grinder and sieve at 40 mesh sizes. Finally, the finely powdered material was subjected to extraction process by using Soxhlet apparatus.

Procedure for extraction

The finely powdered *Excoecaria agallocha* twigs material was under go the soxhlet extraction method by using hydroalcoholic solvent (70% ethanol and 30% water) for a duration of three successive days (72hrs) the temperature should not exceeding more than 100^{o}C. the dark brown colour hydroalcoholic extract was collected, filtered and the filtrate was subjected to concentrated under reduced pressure using rotary vacuum evaporator. The concentrated product was freeze dried and the product was finally used for further experimental studies [1].

***In-vitro* evaluation of anti-inflammatory study (Membrane stabilization method)**

Preparation of RBC suspension

Raw human blood 5 ml was taken in small heparinized tube and centrifuged the sample at 3000 rpm for 10 to 15 minutes and washed with normal saline for three times. The blood volume was measured and prepared as 40% suspension with 10mM sodium phosphate buffer to make isotonic solution.

Heat induced hemolytic assay

Various concentration of HAEEA (test) and Diclofenac sodium (standard) were prepared (50, 100, 200, 400, 800, 1600µg\ml). Each dilution has to make the following procedure. Add 0.1 ml of 40% RBC's suspension. The test and standard solution were incubated at 56^{o}C to 60^{o} C for 30 minutes. After the whole process, the sample tubes were cooled to room temperature. the mixture was finally centrifuged at 2500 rpm for 5 minutes from these mixture the absorbance were taken by using supernatant liquid at 560 nm. The percentage membrane stabilization activity was calculated by using following formula [10].

% of inhibition = {(OD of test – OD of control)/OD of test}*100

***In-vitro* anti-arthritic evaluation (Protein denaturation method)**

The *in-vitro* experiment was performed with doing smaller modification (gnana et al., 2011). The HAEEA and standard drug (Diclofenac sodium) was separately dissolved with little amount of dimethyl sulfoxide and diluted with phosphate buffer (0.2M and PH-7.4) final concentration of DMSO in all the solution was less than 2.5%.

The test solution 4ml containing various concentration of HAEEA was mixed with 1ml of 1mM albumin solution in phosphate buffer and the mixture were incubated at 37^{o} C for 15 minutes. The denaturation of protein was performed by maintaining the reaction mixture at 60^{o}C in water bath for 15 minutes. The same procedure has been followed for standard drug.

After cooling the reacting mixture, the turbidity of the test and standard sample was measured at 660 nm. The protein denaturation was calculated by following formula [11].

% of inhibition = {(OD of test – OD of control)/OD of test}*100

Experimental animals

Swiss albino rats 200g to 250g were used to this experiment. The animals were maintained in standard condition temperature (23-25^{o}C) and humidity (55%) with light (12 hrs) and dark (12 hrs) cycle. The rats were fed with diet pellet and tap water. The total *in-vivo* was based on the norms of animal ethical committee (CPCSEA) and the approval was given by institutional animal ethical

committee (CPCSEA) member and the proposal number is AKCP/IAEC\27/20-21.

***In-vivo* evaluation of anti-Rheumatoid arthritic activity of HAEEA**

Method: formaldehyde induced arthritis in rats (non-immunological induced arthritis)

Experiment was performed by using albino rats. Animals were divided in to 5 groups and each groups as following 6 animals.

Group 1- Normal control (3ml/kg i.p normal saline)
Group 2- Arthritic control (0.1 ml formalin 2%v/v)
Group 3- HAEEA(1) 200 mg/kg, orally.
Group 4- HAEEA(2) 400 mg/kg, orally.
Group 5- Standard control Diclofenac sodium 10 mg/kg, orally.

The groups were divided and named as like the above following procedure. The group 1, only receiving normal saline 3ml/kg orally and it served as normal control, group 2 only receiving sub plantar injection of 0.1 ml 2% v/v formaldehyde solution and it served as arthritic control, group 3 receiving HAEEA (1) 200 mg/kg orally, group 4 receiving HAEEA (2) 400 mg/kg orally, group 5 serve as standard control it receiving standard Diclofenac sodium 10 mg/kg, orally. This study was performed for about 21 days. On 1st day the drug was administered after 30 minutes 0.1 ml of formaldehyde was injected to the right hind paw of all group animals except group 1 animals. The paw volume was measured by using plethysmometer. The formalin should injected with 5 intervals from day 1 to day 21 and the paw volume also measured with 5 intervals such as 4th, 7th,10th,14th and 21st day. Finally, plot the percentage of inhibition [12].

Results and discussion

The IC_{50} value of the given sample 97911 (**HAEEA)** and the standard drug **Diclofenac sodium** was found to be **220.9267 µg/ml** and **21.4784µg/ml**, respectively

Evaluation of *in-vitro* anti-arthritic activity of HAEEA extract.

The IC_{50} value of the given sample 97911 (**HAEEA)** and the standard drug **Diclofenac sodium** was found to be **436.56 µg/ml** and **18.47 µg/ml**, respectively.

Values are expressed as mean±SEM; *n*=6; One-way ANOVA followed by Dunnett's test used and $P < 0.05$ was considered as statistically significant when compared with arthritic control group

Drug (HAEEA) treatment with two doses, i.e., 200 and 400 mg/kg when administered before formaldehyde has shown significant and dose-dependent percentage inhibition of paw volume from the 4thday to 21th day of the experimental study.

The percentage inhibition of paw volume is also recorded for both 200mg and 400mg treatment showed the 1st and 21th days of the study for low dose was found to be 30.12% and for high dose 41.34% and for the standard drug inhibition has been noted as 49.03% respectively.

TABEL 1: Anti-inflammatory study of standard Diclofenac sodium.

Conc (µg)	Absorbance at 560nm			% of Inhibition			Average±SD
	1º	2º	3º	1º	2º	3º	
50	0.071	0.072	0.074	49.765	50.463	51.801	50.676±1.034
100	0.138	0.136	0.131	74.154	73.774	72.773	73.567±0.713
200	0.235	0.235	0.233	84.822	84.822	84.692	84.779±0.075
400	0.813	0.815	0.819	95.613	95.623	95.645	95.627±0.016
800	1.091	1.092	1.095	96.730	96.733	96.742	96.735±0.006
1600	1.101	1.103	1.108	96.760	96.766	96.781	96.769±0.010

Table: 2 Anti-inflammatory study of HAEEA extract:

Conc (µg)	Absorbance at 560nm			% of Inhibition			Average±SD
	1º	2º	3º	1º	2º	3º	
50	0.045	0.043	0.044	20.740	17.054	18.939	18.911±1.843
100	0.048	0.046	0.047	25.694	22.463	24.113	24.090±1.615
200	0.068	0.063	0.065	47.549	43.386	45.128	45.354±2.090
400	0.081	0.087	0.085	55.967	59.003	58.039	57.670±1.551
800	0.687	0.689	0.683	94.823	94.823	94.777	94.803±0.023
1600	1.057	1.052	1.053	96.625	96.609	96.612	96.616±0.008

Table: 3 Anti-arthritic study of standard Diclofenac sodium:

Conc (µg)	Absorbance at 660nm			% of Inhibition			Average±SD
	1º	2º	3º	1º	2º	3º	
50	0.091	0.092	0.094	49.81	50.36	51.41	50.53±0.814
100	0.188	0.186	0.181	75.70	75.44	74.76	75.30±0.484
200	0.249	0.255	0.253	81.66	82.09	81.94	81.90±0.219
400	0.357	0.355	0.359	87.20	87.13	87.27	87.20±0.071
800	0.485	0.481	0.484	90.58	90.56	90.56	90.55±0.040
1600	0.688	0.685	0.681	93.36	93.33	93.29	93.32±0.034

Table: 4 Anti- arthritic activity of HAEEA

Conc (µg)	Absorbance at 660nm			% of Inhibition			Average ±SD
	1º	2º	3º	1º	2º	3º	
50	0.046	0.047	0.046	0.724	2.836	0.724	1.428± 1.21
100	0.053	0.055	0.054	13.836	16.969	15.432	15.412±1.56
200	0.062	0.065	0.063	26.344	29.743	27.513	27.866±1.72
400	0.075	0.074	0.073	39.111	38.288	37.442	38.280±0.83
800	0.187	0.186	0.198	75.579	75.448	76.936	75.987±0.82
1600	0.209	0.211	0.229	78.149	78.357	80.058	78.855±1.04

Table 5: in-vivo Anti- rheumatoid arthritic activity of HAEEA extract

S.NO	GROUPS	TREATMENT	Paw volume in ml (mean±standard deviation)					
			0 day	4th day	7th day	10th day	14th day	21st day
1	Group 1	Normal control	0.0	0.02±0.06	0.03±0.03	0.02±0.01	0.02±0.01	0.04±0.02
2	Group 2	Arthritic control	1.02	1.86±0.02	2.32±0.02	2.60±0.01	2.98±0.03	3.12±0.02
3	Group 3	HAEEA extract 200mg/kg	0.98	1.70±0.01	2.02±0.0	2.12±0.02	2.25±0.03	2.18±0.01
4	Group 4	HAEEA Extract 400mg/kg	0.9	1.64 ±0.04	1.91 ±0.03	1.95 ±0.02	2.04 ±0.02	1.83 ±0.01
5	Group 5	Standard control	0.6	1.62±0.04	1.72±0.03	1.75±0.05	1.80±0.02	1.59±0.02

Table 6: percentage of inhibition of paw volume

S. NO.	Groups	Treatment	(% of inhibition)					
			0 day	4th day	7th day	10th day	14th day	21st day
1	Group 1	Normal control	-	-	-	-	-	-
2	Group 2	Arthritic control	-	-	-	-	-	-
3	Group 3	200 mg/kg	-	8.60%	12.93%	18.46%	24.49%	30.12%
4	Group 4	400 mg/kg	-	11.82%	17.67%	25%	31.54%	41.34%
5	Group 5	Standard control	-	12.90%	25.86%	32.16%	39.59%	49.03%

Figure 1: Membrane Stabilization assay for standard drug (Diclofenac sodium)

Figure 2: Membrane stabilization assay for HAEEA

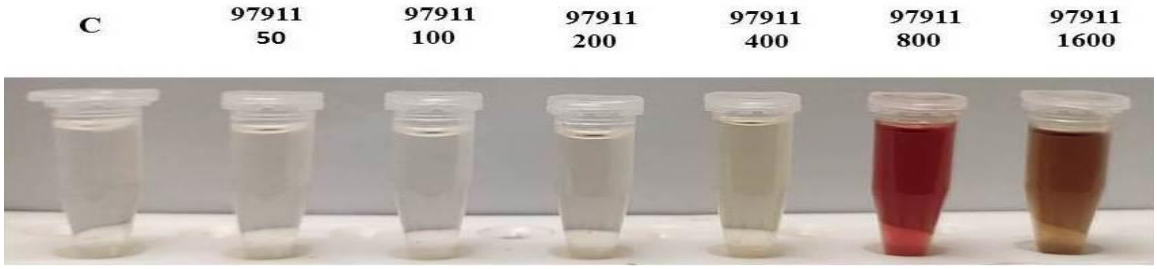


Figure 3: Anti-inflammatory assay graphical representation

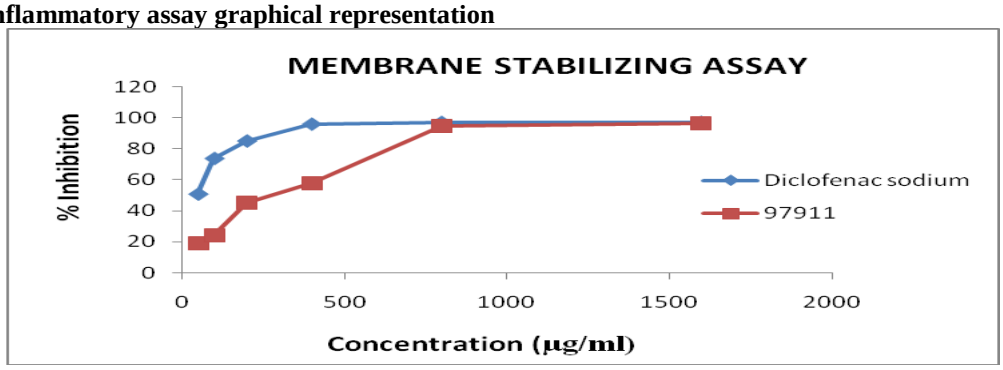


Figure: 4 protein denaturation for standard (Diclofenac sodium)

Figure 5: Protein denaturation for test sample (HAEEA)

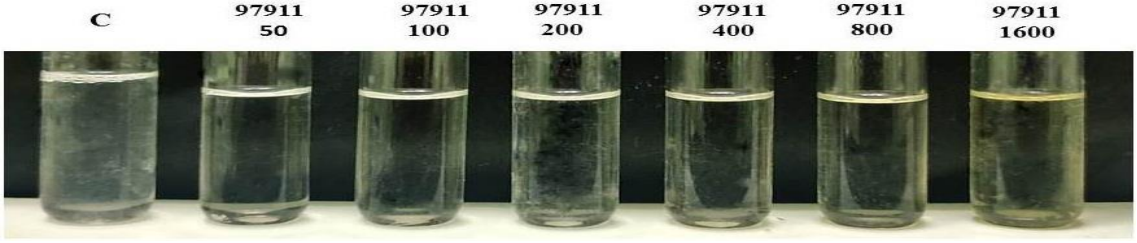


Figure 6: Anti- arthritic assay graphical representations

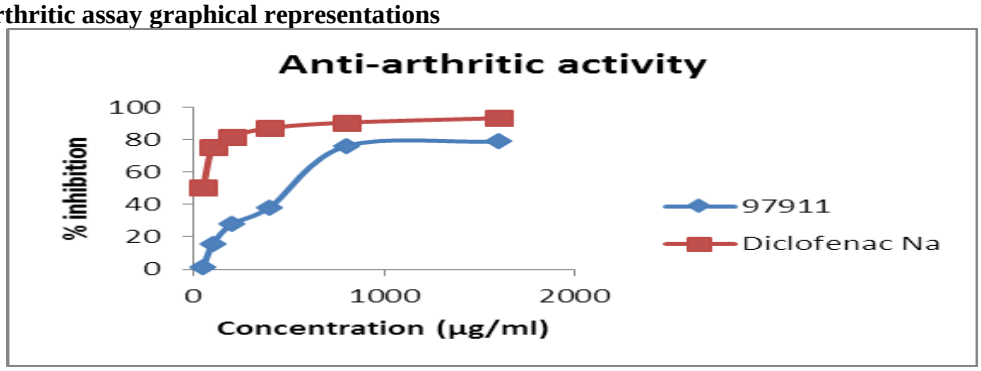


Figure 7: Anti- rheumatoid arthritic activity graphical representation

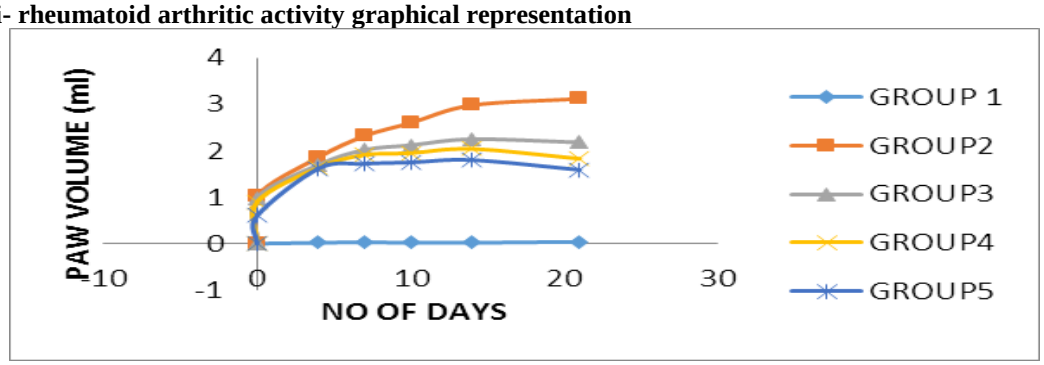
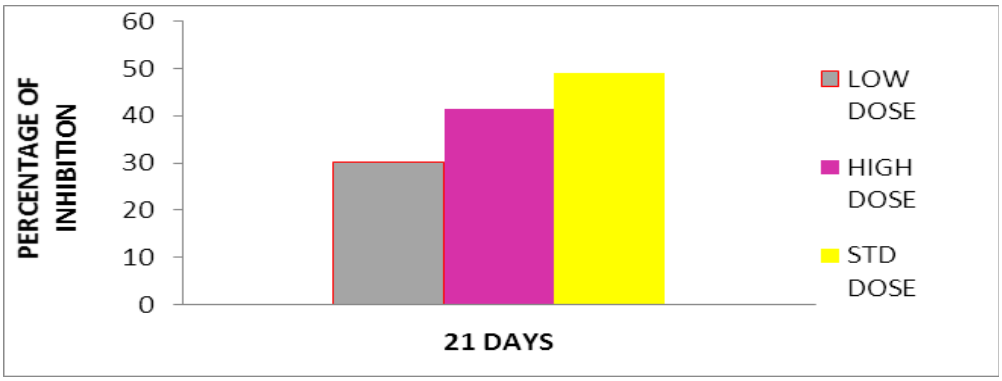


Figure 8: Percentage of inhibition of paw volume (Graphical representation)



DISCUSSION:

In rheumatoid arthritis the major problem is joint inflammation and destruction of cartilage. The destruction of cartilage is happen because of denaturation of protein. So the inhibition of protein denaturation was determined by using *in-vitro* protein denaturation method and the inhibition of inflammation was evaluated by using *in-vitro* membrane stabilization method.

Formaldehyde-induced paw edema in rats is one of the most suitable test procedures to screen anti-Rheumatoid arthritic activity. Anti-Rheumatoid arthritic activity was reported to be mediated either by inhibition of phospholipase-A2 of prostaglandins from arachidonic acid and also by blocking the release of proinflammatory cytokinins such as Tumor necrosis factor α (TNF-α), Interleukin 1β (IL-1β) and Interleukin 6 (IL-6), thereby reducing the systematic inflammation. The main aim of the study is to reduce the pain, swelling and inflammation in the joints using these drugs is to prevent degradation in joints, inflammation and function restore of disabled joints. Several side effects were reported by the allopathic drugs such as gastrointestinal disease, immune deficiencies and hormonal disturbances. The treatments for RA, primary goal is to reduce side effects with increased therapeutic activity. Nowadays Ayurvedic systems and Siddha systems are playing pivotal role for treating rheumatoid arthritis.

CONCLUSION

The Present work is an attempt to evaluate pharmacological activity of *Excoecaria agallocha* L. against Rheumatoid arthritis. *In-vitro* anti-arthritic activity (Protein denaturation method) and *In-vitro* anti-inflammatory activity (Membrane stabilization assay) were evaluated it shows significant activity against inflammation and arthritis and IC_{50} were found to be 220.92µg/ml,436.56µg/ml respectively. *In vivo* Anti-rheumatoid arthritis activity was studied for HAEEA extract at 200 mg/kg and 400 mg/kg and this study revealed that significant dose dependent activity. The percentage of inhibition of paw volume for 200 mg/kg and 400 mg/kg of HAEEA extract was found to be 30.12% and 41.34% respectively. According to the above studies it can be concluded that HAEEA extract possesses good activity against Rheumatoid arthritis.

Future scope:

Future research studies may be extended in order to identify and isolate which phytoconstituents is responsible for Anti-Rheumatoid arthritis activity.

Formulation can be designed and developed from HAEEA extract which could be used in the treatment of Rheumatoid arthritis, Osteoarthritis and joint inflammation related problems.

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