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Research Article

EXPLORING THE ETHANOLIC EXTRACT OF NEPHROLEPIS CORDIFOLIA LEAF FOR THE IN VITRO ACTIVITY OF MEMBRANE STABILIZATION AND PROTEIN DENATURATION ASSAY

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ABSTRACT

The present study deals with inspecting the inhibitory activity on ethanolic extract leaves of, Nephrolepis cordifolia. To inquire about the membrane stabilization and protein denaturation assay in the leaf of Nephrolepis cordifolia. Membrane stabilization and protein denaturation assay at varying concentrations. Denaturation of protein is one of the well-documented causes of Inflammation. Denaturation leads breakdown of various bonds that exist within a molecule and macromolecule. Membrane stabilization is the blocking action potential across nerve cells thereby producing a nerve block. The IC50 values of ethanolic leaf extract Nephrolepsis cordifolia was 68.97µg/ml and 7.48 µg/ml for protein denaturation and membrane stabilization assay. This study suggests ethanolic leaf extract Nephrolepsis cordifolia effectively produce Anti – Rheumatoid activity.

Key Words: Hyphenated Technique, Thin Layer Chromatography, Direct Bioautography, Estrogenic Compounds Etc.

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INTRODUCTION

Rheumatoid arthritis is a disease that causes chronic abnormal inflammation primarily affecting the joints. The most common sign and symptoms are pain, swelling, and stiffness of the joints. Rheumatoid arthiritis can also cause inflammation of other tissues and organs including the eyes, lungs, and blood vessels. (Carmona L, *et al.*2010) Rheumatic arthiritis is theorized to develop where a genetically susceptible individual experiences an external trigger eg (cigarette smoking, infection , trauma) that triggers an autoimmune reaction. (Howard R Smith) Rheumatic arthiritis causes damage mediated by cytokines, chemokines, and Metalloproteases. characteristically, peripheral joints [eg : wrists , metacarpophalangeal joints] are symmetrically inflamed , leading to progressive destruction of articular structures usually accompanied by systemic symptoms. Diagnosis is based on specific clinical, laboratory and imaging features. Treatment involves drugs, physical measure, sometime surgery. (Rheumatic arthritis Apostolos Kontzias) often the bone and cartilage of joints are destroyed, and tendons and ligaments weaken. All this damage to joints

cause deformities and bone erosion , usually very painful for a patient the onset of this disease is usually from the age of 35 to 60 years with remission and exacerbation. (Lee JE, *et al.* 2017) In a patient with inflammatory arthiritis the presence of a rheumatoid factor or anti citrullinated protein anti body, or elevated c – reactive protein level or erythrocyte sedimentation rate suggests a diagnosis of rheumatic arthiritis. Methotrexate is a typically first line drug for rheumatic arthiritis (AMY M, *et al.* 2011).

MATERIALS AND METHODS

Collection and identification of plant material:

The plant material was identified as leaves of Nephrolepsis cordifolia. The healthy matured leaves of Nephrolepsis cordifolia were collected from tamil nadu India during the month of oct 2020. The collected plant was authenticated by prof . p. Jayaraman , M.S.C ., ph.D ., Director institute of herbal botany plant anatomy research centre .

Preparation of the plant Extract:

The leaflets were air dried and pulverized into powder.The dried powder of the aerial and underground parts of Nephrolepsis cordifolia (100gm) was separately extracted with 50% (v/v) aqueous ethanol in a sealed apparatus at room temperature for 72hours. The filterate of the extracts of each plant part were combined and concentrated to remove the solvent using soxhlet apparatus at 20 ^{0}c until dryness.

Membrane Stabilization Assay

Preparation of Red Blood Cell (RBCS) Suspension

(Vasanthkumar T, *et al.* 2017). Fresh whole human blood (5ml) was collected in a heparinized tube and transferred to the centrifuge tube. Test tube were centrifuged at 3000 rpm for 10 min and washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 40% v/v suspension with isotonic solution (10 Mm sodium phosphate buffer).

Different concentration (50,100,200,400,800 and 1600 mg/ml) of the test sample **(NCE)** and reference standard (**diclofenac sodium)** was mixed with 0.1 ml of 40% **RBCs** suspension. The control sample consist of 0.1ml of RBC mixed with isotonic solution alone. The reaction mixture was incubated in a water bath at 56'C for 30min. at the end of the incubator, the tubes were cooled temperature. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was measured at 560 nm. Percentage of membrane stabilization activity was calculated by using the following formula,

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

Table 1: Membrane Stabilization Assay

Sample	Conc. (μg)	singlet	Duplicte	Triplicate	Singlet	Duplicte
Diclofenac Sodium	50	0.024	0.027	0.03	47.2222	53.0864
	100	0.1	0.118	0.115	87.3333	89.2655
	200	0.176	0.172	0.178	92.803	92.6357
	400	0.218	0.219	0.223	94.1896	94.2161
	800	0.333	0.33	0.334	96.0948	96.1616
	1600	0.436	0.442	0.44	97.0948	97.1342

Sample	Conc. (µg)	Singlet	Duplicate	Triplicate	Singlet	Duplicate
NCE	50	0.023	0.029	0.025	44.9275	56.3218
	100	0.037	0.034	0.03	65.7658	62.7451
	200	0.051	0.057	0.052	75.1634	77.7778
	400	0.092	0.097	0.099	86.2319	86.9416
	800	0.148	0.142	0.145	91.4414	91.0798
	1600	0.195	0.199	0.192	93.5043	93.6348

Concentration	Diclofenac Sodium	NCE
50	52.69547	50.19424
100	88.52813	62.09621
200	92.77419	76.19407
400	94.24187	86.79295
800	96.18847	91.26187
1600	97.11675	93.51396

Triplicate	Mean	SD	IC50 Value
57.7778	52.69547	5.288626	
88.9855	88.52813	1.044151	
92.8839	92.77419	0.126605	
94.3199	94.24187	0.068847	
96.2076	96.18847	0.02394	**7.48**
97.1212	97.11675	0.0294	

Triplicate	Mean	SD	IC50 Value
49.3333	50.19424	5.745729	
57.7778	62.09621	4.033333	
75.641	76.19407	1.392169	
87.2054	86.79295	0.503483	
91.2644	91.26187	0.180828	**36.19**
93.4028	93.51396	0.116335	

Table 2: Protein Denaturation Assay

Sample	Conc. (µg)	Singlet	Duplictate	Triplicate	% of inhibition Singlet	Duplicate
Diclofenac Sodium	50	0.134	0.139	0.137	37.8109	40.04796
	100	0.188	0.181	0.184	55.6738	53.95948
	200	0.332	0.332	0.337	74.8996	74.8996
	400	0.519	0.512	0.516	83.9435	83.72396
	800	1.007	1.003	1.002	91.7246	91.69159
	1600	1.563	1.561	1.558	94.6684	94.66154

Sample	Conc. (µg)	Singlet	Duplictate	Triplicate	% of inhibition Singlet	Duplicate
NCE	50	0.103	0.107	0.1	19.0939	22.11838
	100	0.13	0.134	0.138	35.8974	37.81095
	200	0.159	0.152	0.155	47.5891	45.17544
	400	0.175	0.17	0.173	52.381	50.98039
	800	0.336	0.339	0.332	75.1984	75.4179
	1600	0.36	0.362	0.368	76.8519	76.97974

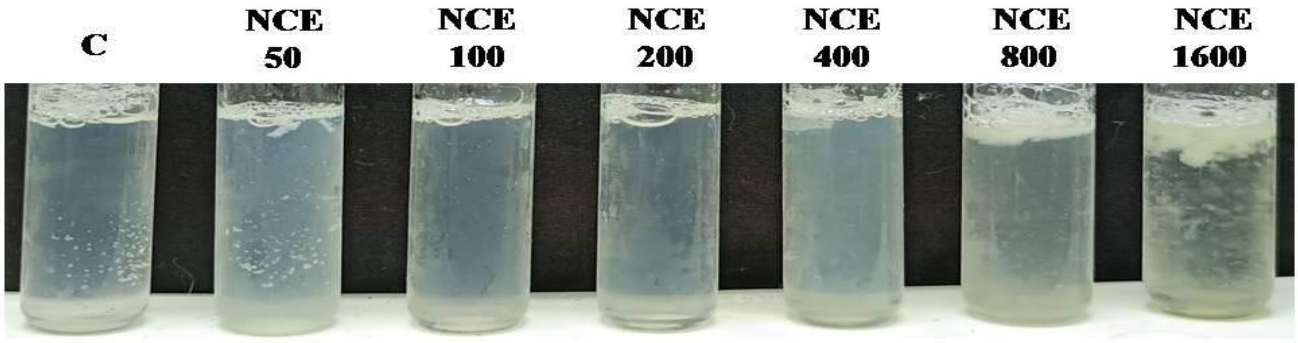
Conc.(µg) lofenac sod	
50	39.01055
100	54.78113
200	75.02373
400	83.83919
800	91.69983
1600	94.66039
50	39.01055

Triplicate	Mean	SD	IC50 value
39.1727	39.0105521	1.127294	
54.7101	54.78112938	0.859339	
75.272	75.0237349	0.215011	**68.97**
83.8501	83.83918953	0.110169	
91.6833	91.69982882	0.021845	
94.6513	94.66039228	0.008613	

Triplicate	Mean	SD	IC50 value
16.6667	19.29296595	2.731306	
39.6135	37.77396925	1.858321	
46.2366	46.33369876	1.209759	**263.10**
51.8304	51.7305959	0.705599	
74.8996	75.17196895	0.260159	
77.3551	77.06222216	0.261553	

Figure 1: Protein Denaturation Assay:

Sample :



Standard :

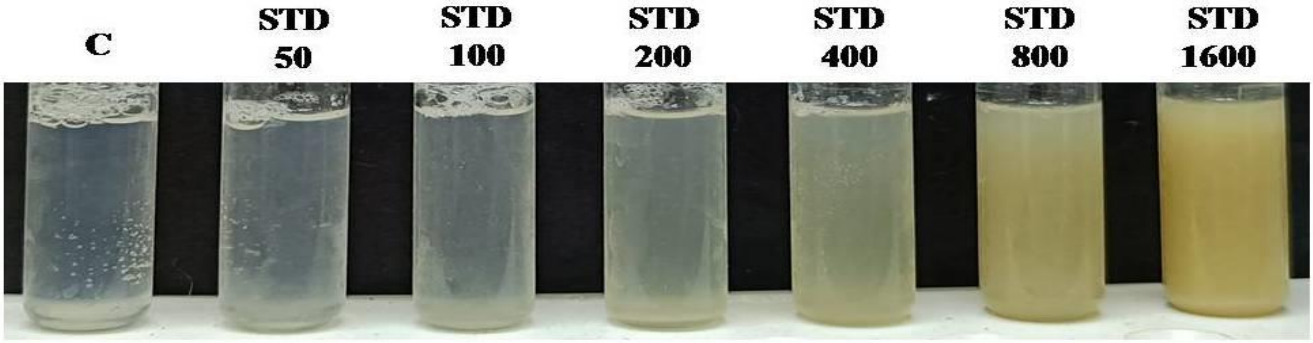
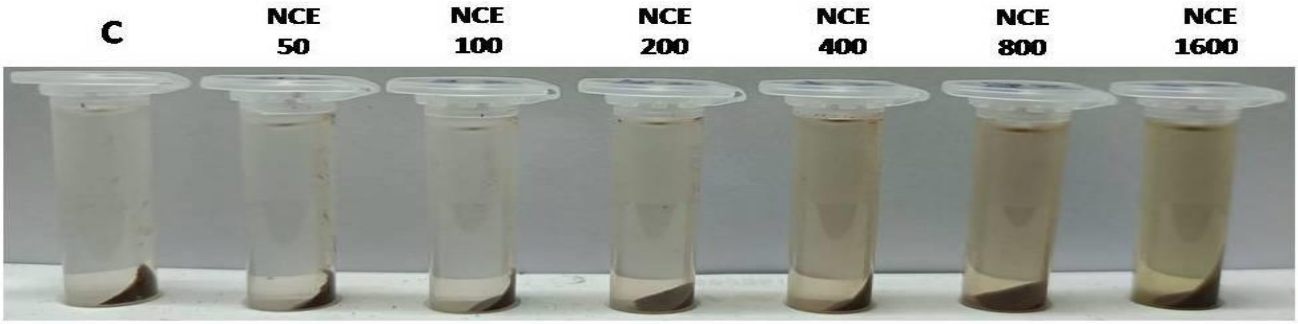


Figure 2: Membrane Stabilisation Assay :



Sample:

Standard:

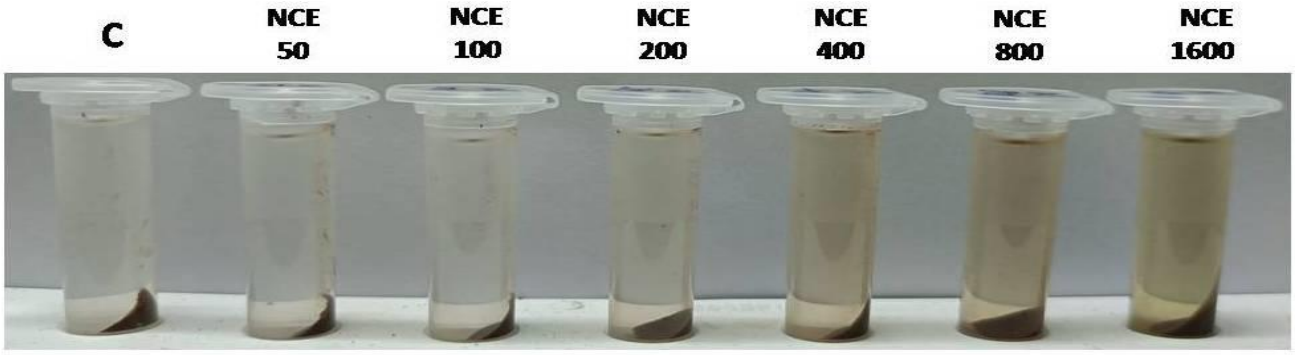
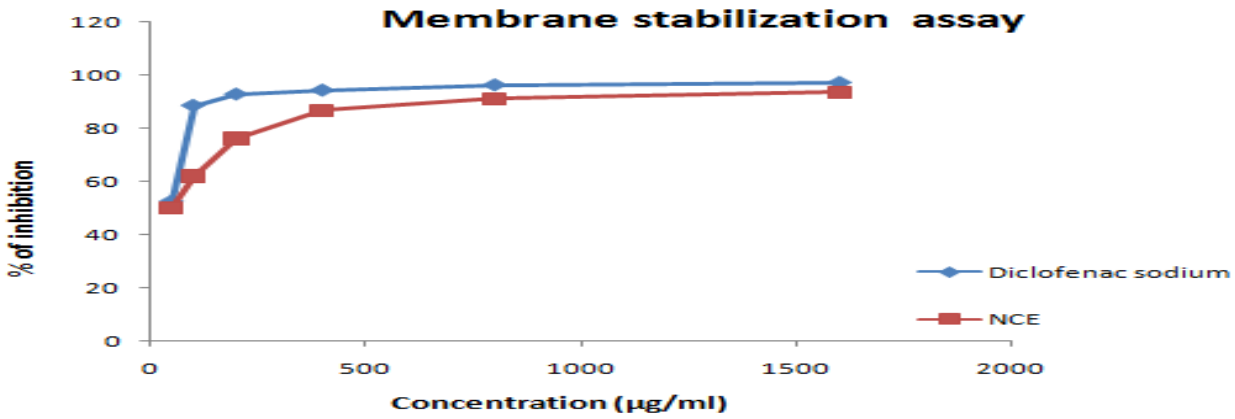


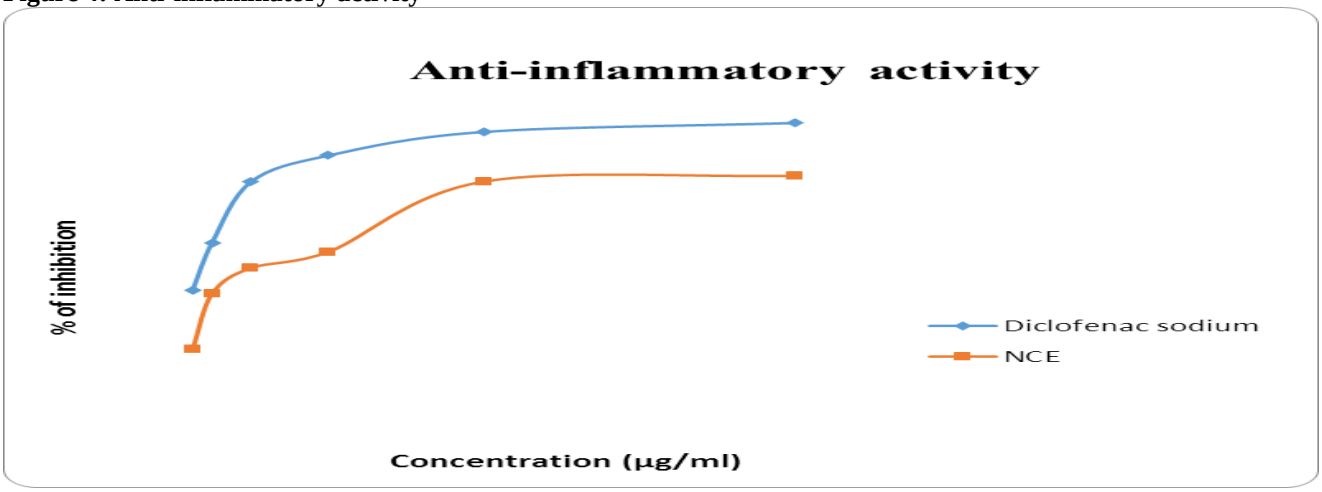
Figure 3: Membrane Stabilization assey



				Mean
Control	0.012	0.011	0.015	0.0126

Control	**singlet**	**Duplicate**	**Triplicate**	**Mean**
	0.085	0.081	0.084	0.08333

Figure 4: Anti-inflammatory activity



RESULT AND DISCUSSION

In vitro activity of rheumatoid arthritis was determined by ethanolic extract leaves of Nephrolepis cordifolia by using membrane stabilization Nephrolepisn denaturation assay. Nephrolepis cordifolia proved inhibitory activity on membrane stabilization and protein denaturation. For both activity diclofenac sodium is a reference standard drug.In membrane stabilization activity absorbance of supernatant was measured at 560nm.In protein denaturation assay turbidity was measured at 600nm. Membrane stabilization involves two membrane which are the erythrocyte membrane and the lysosomal membrane is maintained by anti-inflammatory drugs by stabilizing the membrane. Different concentration [50,100,200,400,800,600 mg/ml] of test sample and standard is used to determine the final value. Membrane stabilization and protein denaturation of ethanolic extract was plotted as the graph shown in fig. For membrane stabilization assay the maximum percentage of inhibition was 93.513 obtained at the concentration 0f 1600µg/ml, whereas the standard drug shows 97.116 at the concentration of 1600µg/ml. The IC50 value of the given test sample (NCE) and reference standard (diclofenac sodium) was found to be 36.19 µg/ml and 7.48 µg/ml.

CONCLUSION

The study clearly explained that the ethanolic extract of Nephrolepis cordifolia effectively inhibited the denaturation of protein and membrane stabilization assay. It is more effective than the reference standard anti-inflammatory drug, diclofenac. By the natural presence of some chemical constituents in the ethanolic extract leaves of Nephrolepis cordifolia are effective in the inhibitory effect of membrane stabilization and protein denaturation. It can therefore be concluded, these extracts possess significant anti-rheumatic activity.

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