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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *COLDENIA PROCUMBENS*

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ABSTRACT

Objective: The objective of the study was to evaluate *in-vitro* Anti-inflammatory activity of Ethanol and aqueous extracts of whole plant of *Coldenia procumbens.* It is an annual herb belonging to Boraginaceae family. In the traditional system of medicine, the plant was used as anti-inflammatory, anti-microbial, analgesic, anti-diabetic, CNS depressant, hepato protective activity, anti-oxidant activity. **Methodology:** Ethanol and aqueous extracts of whole plant of *Coldeniaprocumbens* were used for Anti-inflammatory activity. The ethanol and aqueous extracts of the whole plant of *Coldeniaprocumbens* were studied for anti-inflammatory activity by protein denaturation, Human Red blood cell membrane stabilization and albumin denaturation method with reference to aspirin. **Results**: The anti-inflammatory activity of ethanol and aqueous extract of *Coldeniaprocumbens* with reference to aspirin was shown in the parameters. The percentage of inhibition of protein denaturation and percentage of membrane stabilization for ethanol extracts and aspirin was done at 100µg/ml. The ethanol extract of *C.procumbens* shows anti-inflammatory activity at concentration 1000 µg/ml shows 94% protection (inhibit protein denaturation) and 98% protection (Human red blood cell membrane stabilization). The aqueous extract of *C.procumbens* shows anti-inflammatory activity at concentration of 1000 µg/ml shows 89% (inhibition of protein denaturation) and 95% protection Human red blood cell membrane stabilization). **Conclusions:** The result concludes that the ethanol and aqueous extracts of *Coldeniaprocumbens* possess anti-inflammatory activity properties and further the plant can be explored for isolation of its active constituents.

KEYWORDS: *Coldeniaprocumbens*, Anti-inflammatory activity, Human Red blood cell membrane stabilization method, Albumin denaturation method.

INTRODUCTION

Inflammation is a complex natural response to harmful stimuli, such as physical trauma, noxious chemicals, bacteria and viruses. Inflammation is a defence reaction caused by an injury, and it is seen as a response to many diseases such as cancer, rheumatoid arthritis and diabetes.[1-3] In the injured tissue, activation of immune cells such as macrophages, monocytes, and neutrophils, and production of pro-inflammatory cytokines such as interleukins IL-1β, IL-6 and tumour necrosis factor (TNF)-α are observed. These responses are related to the development and progression of inflammation and autoimmune diseases.[4] IL-10 is the most important anti-inflammatory cytokine, and it is produced by activated immune cells and diminishes the production of inflammatory mediators.[5] Drugs used in the treatment of inflammation are non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal drugs, however, both can cause severe adverse side effects. Thus, the development of new, safe anti-inflammatory compounds is necessary. Plants used in traditional medicine for treating various inflammatory conditions are a source of biologically active compounds, and these plants have been used for a long time as crude material or pure compounds for the treatment of several diseases.[6]

ColdeniaProcumbens Linn (Boraginaceae) is an annual herb, common weed in India.[7-8] *ColdeniaProcumbens* is only species of its genus has a place both in the HortusBengalensis and Moon's Catalogue of ceylon plants.[9] In the traditional system of medicine, the plant was used as anti-inflammatory,[10] anti-microbial,[11] analgesic,[12] anti-diabetic.[13] CNS depressant.[14] hepatoprotective activity,[15] anti-oxidant activity.[16] Fresh leaves of *Coldeniaprocumbens* ground and applied to Rheumatic Swellings, equal parts of dried powder mixed with seeds of fenugreek causes Suppurations of boils.[17] The active constituent of plant is wedel lactone which is a derivative of coumestans,[18] This plant is widely used in traditional medicines in India, Africa, malaysia. Acetone, water, methanolic extract of dried aerial parts shows weak angiotensin-converting enzyme inhibition in vitro.[19-20] Present study made to investigate the anthelmintic potency of *ColdeniaProcumbens.* Considering the indigenous uses of the plant, the present

study deals with the investigation of in vitro anti-oxidant, anti-inflammatory and anti-arthritic activities in the leaves of the *C. procumbens.* The present study involves determination of anti-inflammatory activity of *C. procumbens* by Inhibition of albumin, protein denaturation and HRBC Membrane stabilization.

The present study involves determination of anti-inflammatory activity of *C. procumbens* by Inhibition of albumin, protein denaturation and HRBC Membrane stabilization.

MATERIALS AND METHODS

Collection and authentication

Coldeniaprocumbens was collected from in and around Chembarambakkam, Chennai India. The plant was identified and authenticated by the taxonomist. The authenticated specimen was deposited in the Department of Pharmacognosy, SreeSastha Pharmacy College. The authentication specimen number is SSPC/P.COG /001/2019. The aerial parts were dried in room temperature for 2 months. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preparation of extract

The plant material (whole plant) was shade dried and coarsely powdered with it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to preclinical screening.

Preparation of the Ethanol extract

Ethanol extract was prepared according to the methodology of Indian pharmacopoeia 1996. The coarse powder material was subjected to Soxhlet Extraction separately and successively with 210ml ethanol and 90ml distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40◦C – 50◦C). The paste form of the extracts was put in air tight container and stored in refrigerator.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of *Coldeniaprocumbens* was studied by using inhibition of albumin denaturation technique which was studied according to[21-22] followed with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples the turbidity was measured at 660nm. The Percentage inhibition of albumin denaturation was calculated as follows

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

Inhibition of albumin denaturation

The test was performed according to the modified method of Oyedepo et aland.[23] the reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mMTrisHCl buffer (pH 7.4) and 1 ml test sample of different concentrations (7.5 – 1000 µg/ml). The mixture was incubated at 37oC for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% per chloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X100/ Abs control

Human Red blood Cell Membrane Stabilization Method

The HRBC membrane stabilization was used as a method to evaluate the anti-inflammatory activity (Gandhisan et al 1991).. The blood was collected from healthy human volunteer who has not taken any NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment, `mixed with an equal volume of sterilized Alsever medium (2%, (w/v) dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride in water). The blood was further centrifuged at 3000 rpm for 10 min, and the packed cells were washed with isosaline (0.85%, pH 7.2) and finally 10% (v/v) suspension was made with isosaline. The assay mixture contained the secondary metabolite from the plat extract, 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL hyposaline (0.36%), and 0.5 mL HRBC suspension. Diclofenac was used as a reference drug. Instead of the hyposaline, 2 mL distilled water was used as the control. The assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm for 10 min. The hemoglobin content in the supernatant was estimated using a UV–Visible spectrophotometer at 560 nm (Anosike et al., 2012). The percentage hemolysis was calculated using the following equation

Percentage inhibition =100- (Abs sample) / (Abs control) X100

RESULTS AND DISCUSSION

Anti- inflammatory activity

Aromatic and medicinal plants are very important sources of secondary metabolites, which have a range of applications in control of human and plant diseases, pharmaceutical industry and cosmetics.(Pandeyand Tripathi, 2011) The ethanol extracts of the whole plant of *Coldeniaprocumbens* was studied for anti-inflammatory activity by protein denaturation, HRBC membrane stabilization and albumin denaturation method. The anti-inflammatory activity is concentration dependent, with increase in concentration the activity also increases. The anti-inflammatory activity of ethanol and aqueous extract of *Coldeniaprocumbens* with reference to aspirin was shown in Table 1 and 2. The percentage of inhibition of

protein denaturation and percentage of membrane stabilization for ethanol extracts and aspirin was done at 100µg/ml. It shows anti-inflammatory activity at concentration 1000 µg/ml shows 94% (inhibit protein denaturation) and 98% protection membrane stabilization). The aqueous extract of *Coldeniaprocumbens* shows anti-inflammatory activity at concentration of 1000 µg/ml shows 89% (inhibition of protein denaturation) and 95% protection membrane stabilization). With the increasing concentration protein denaturation is decreased as shown in Figure 2 and membrane stabilization protection is increased as shown in Figure 2. From the results, it is concluded that the combination of ethanol extracts of *Coldeniaprocumbens* possess greater anti-inflammatory activity than individual plant extract. Here, the anti-inflammatory activity was assessed by in vitro screening methods such as protein denaturation and HRBC method. Denaturation of proteins is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured, and production of auto antigen in certain arthritic disease is due to denaturation of the protein,[24] The mechanism of denaturation involves alteration in electrostatic hydrogen, hydrophobic, and disulphide bonding,[25] The inhibition of protein denaturation with ethanol extract 94% was with aqueous extract 89% was and concentration of 1000 µg/ml. During inflammation, lysosomal hydrolytic enzymes are released which causes damages of the surrounding organelles and tissues with variety of disorders. The erythrocyte membrane is analogous to the lysosomal membrane,[26] and its stabilization implies that the extract may aswell stabilize lysosomal membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release.[27] It is reported that the ethanol extract of showed 98%and aqueous extract of *Coldeniaprocumbens* showed 95% and protection at a concentration of 1000 µg/ml. The inhibitory effect of different concentration of *Coldeniaprocumbens* on protein denaturation as shown in Table 3 and Figure 3.*Coldenia procumbens*at a concentration range of 7.5, 15.5,31.2,62.5, 125,250,500,1000 µg/ml and standard aspirin 100ug/ml showed significant inhibition of denaturation of egg albumin in concentration dependent manner.It is reported that the ethanol extract of showed 94% and aqueous extract of *Coldeniaprocumbens* showed 89% protection at a concentration of 1000 µg/ml.

Table 1: Anti-inflammatory activity of Ethanol and Aqueous extract of *Coldeniaprocumbens* by Protein denaturation method.

S. No	Concentration(µg/ml)	Ethanol extract		Aqueous extract	
		Optical density	% Inhibition	Optical Density	% Inhibition
1	7.5	0.52±0.17	80	0.72±0.24	52
2	15.5	0.48±0.16	81	0.63±0.21	63
3	31.2	0.45±0.15	82	0.54±0.18	68
4	62.5	0.36±0.12	86	0.54±0.17	69
5	125	0.27±0.09	89	0.49±0.16	71
6	250	0.21±0.07	91	0.45±0/15	73
7	500	0.18±0.06	93	0.36±0.12	78
8	1000	0.09±0.63	94	0.18±0.06	89
9	Aspirin 1000	0.22±0.07	92	0.21±0.07	87

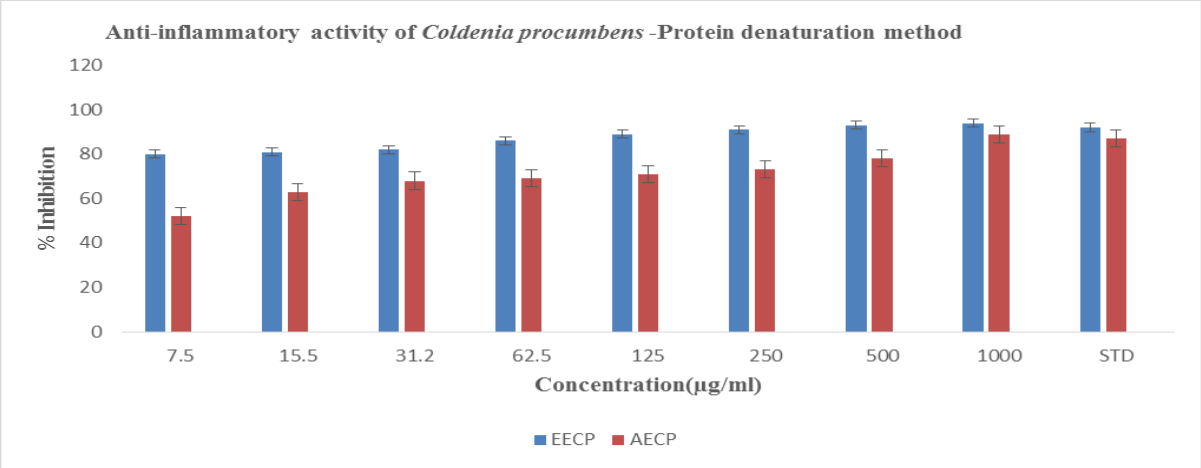

Fig. 1: Anti-inflammatory activity of Ethanol and Aqueous extract of *Coldenia procumbens* by Protein denaturation method.

Table 2: Anti-inflammatory activity of Ethanol and Aqueous extract of *Coldeniaprocumbens* by HRBC membrane stabilization method.

S. No	Concentration(µg/ml)	Ethanol extract		Aqueous extract	
		Optical	% Inhibition density	Optical	% Inhibition density
1	7.5	0.39±0.13	80	0.25±0.08	79
2	15.5	0.27±0.09	86	0.22±0.07	80
3	31.2	0.23±0.08	88	0.23±0.08	81
4	62.5	0.17±0.06	91	0.17±0.06	85
5	125	0.14±0.04	93	0.14±0.05	88
6	250	0.13±0.04	94	0.11±0.03	90
7	500	0.06±0.02	97	0.08±0.02	93
8	1000	0.04±0.01	98	0.05±0.01	95
9	Standard 1000(Aspirin)	0.12±0.04	96	0.13±0.04	89

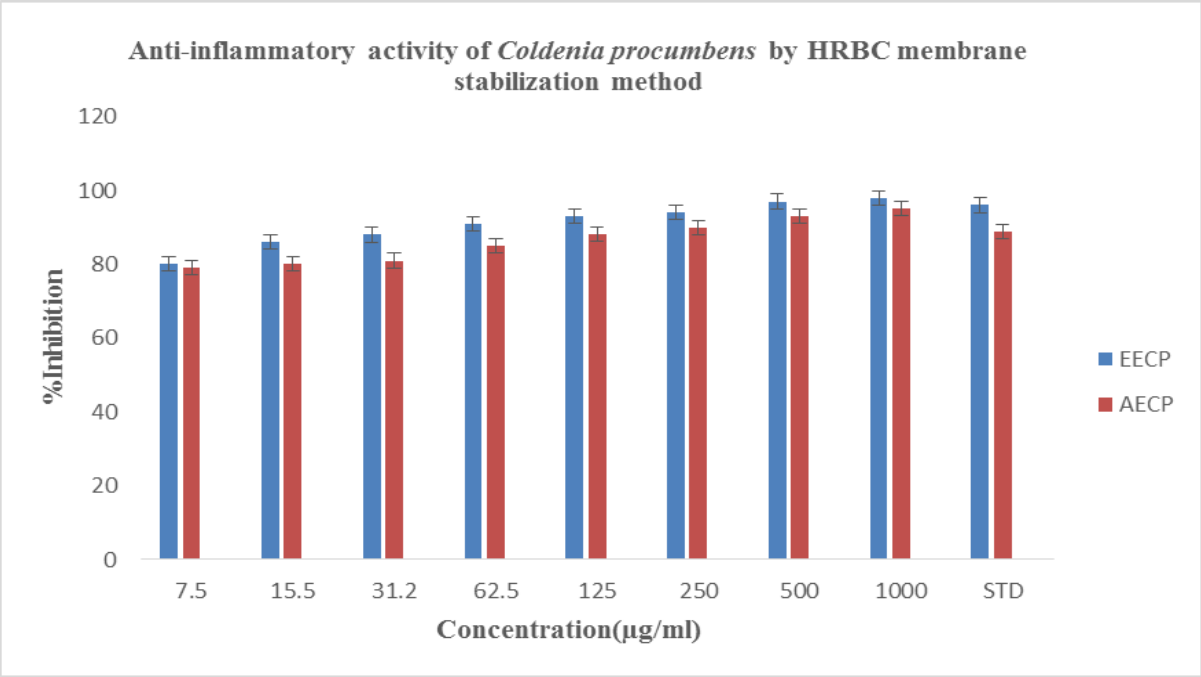

Fig 2: Anti-inflammatory activity of Ethanol and Aqueous extract of *Coldeniaprocumbens* by HRBC membrane stabilization method.

Table 3: Anti-inflammatory activity of Ethanol and Aqueous extract of *Coldeniaprocumbens* by Albumin denaturation method.

S. No	Concentration(µg/ml)	Ethanol extract		Aqueous extract	
		Optical	% Inhibition density	Optical	% Inhibition density
1	7.5	0.72±0.24	57	0.86±0.28	52
2	15.5	0.63±0.21	60	0.79±0.26	58
3	31.2	0.54±0.18	64	0.76±0.25	62
4	62.5	0.45±0.15	70	0.71±0.23	64
5	125	0.36±0.04	76	0.63±0.21	68
6	250	0.27±0.09	82	0.54±0.18	73
7	500	0.18±0.06	88	0.45±0.15	77
8	1000	0.09±0.63	94	0.36±0.12	82
9	Standard 1000(Aspirin)	0.54±0.18	73	0.63±0.21	68

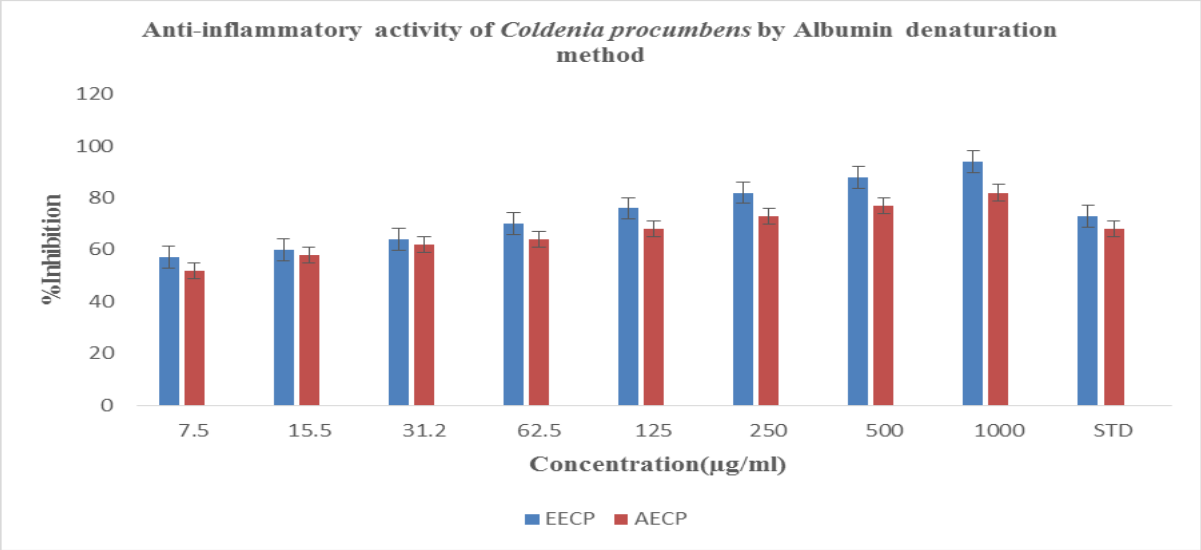

Fig. 3: Anti-inflammatory activity of Ethanol and Aqueous extract of *Coldeniaprocumbens* by Albumin denaturation method.

CONCLUSION

Coldeniaprocumbens was screened for its anti-inflammatory activity by albumin denaturation, protein denaturation and HRBC membrane stabilization model. Aqueous and ethanol extract at the concentration 1000ug/ml showed significant anti -inflammatory activity was compared to that aspirin. In the present investigation, the results indicate that the ethanol and aqueous extracts of *Coldeniaprocumbens* possess anti-inflammatory activity properties. The protective effect against protein denaturation and membrane stabilization is known to be a good index of anti-inflammatory activity. From the present study, it is concluded that combination of possesses *Coldeniaprocumbens* the highest anti-inflammatory activity when compared with extract.

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Competing interests

Author has declared that no competing interests exist.

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