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INVITRO ANTI -INFLAMMATORY ACTIVITY OF COLDENIA PROCUMBENS

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ABSTRACT

The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. Therefore, there is a necessity to explore their uses and to ascertain their therapeutic properties. The plant *Coldenia procumbens* is used commonly in Indian system of medicine for various ailments. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Coldenia Procumbens* for the selected area for anti-inflammatory activity. Ethanolic and aqueous extract of whole plant of *Coldenia Procumbens* were subjected for *invitro* anti-inflammatory activity. The results obtained indicate that the extracts possessed significant level of activity; the highest concentration of extract was high effective as an anti-inflammatory agent. However, these effects need to be confirmed using *in vivo* models and clinical trials for its effective utilization as therapeutic agents.

KEYWORDS: Anti-inflammatory, Protein denaturation, Coldenia Procumbens.

INFLAMMATION

Inflammation is a normal protective response to tissue injury, and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair.[1] When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Symptoms of inflammation include redness, swelling and pain, joint stiffness, loss of joint function as a result of infection, irritation, or injury. Plants may become the base for the development of a new medicine, or they may be used as phytomedicine for the treatment of disease.[2] In this growing interest,

many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities.[3] Most of the anti-inflammatory drugs available in the market, having a wide range of problems such as efficacy and undesired effects including gastrointestinal tract disorders and other unwanted effects, gastrointestinal disturbances, renal damages, respiratory depression.[4] This situation highlights the need for advent of safe, novel, and effective analgesic and anti-inflammatory compounds.[5]

Coldenia Procumbens Linn (Boraginaceae) is an annual herb, common weed in India.[6,7] *Coldenia Procumbens* is only species of its genus has a place both in the Hortus Bengalensis and Moon's Catalogue of ceylon plants.[8] In the traditional system of medicine, the plant was used as anti inflammatory[9], anti microbial[10], analgesic[11], anti diabetic[12], CNS depressant[13] hepatoprotective activity[14] anti-oxidant activity.[15] Fresh leaves of *Coldenia procumbens* ground and applied to Rheumatic Swellings, equal parts of dried powder mixed with seeds of fenugreek causes Suppurations of boils.[16] The active constituent of plant is wedelolactone which is a derivative of coumestans[17] 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.[18,19] Present study made to investigate the anthelmintic potency of *Coldenia Procumbens*. 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.

Collection and preparation of plant material

The plant species namely *Coldenia Procumbens* plant was collected by in around Adichapuram, Tiruvarur District, Tamil nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli **(Voucher number of the specimen, 001)** (Gamble,1997).

Preparation of the aqueous 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

Ethanolic extract was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 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 Enicostemma axillare was studied by using inhibition of albumin denaturation technique which was studied according to[20][21] 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 al[21] and.[22] The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 – 500 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% perchloric 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[21],[23]

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 and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed

three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

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

RESULTS AND DISCUSSION

Anti- inflammatory activity

The ethanol extracts of the whole plant of *Coldenia procumbens*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 antiinflammatory activity of ethanol and aquousextract of *Coldenia procumbens* with reference to aspirin wasshown in Table 1 and 2. The percentage of inhibition of protein denaturation and percentage ofmembrane stabilization for ethanolic 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 *Coldenia procumbens*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 ethanolic extracts of *Coldenia procumbens* 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 autoantigen in certain arthritic disease is due to denaturation of the protein.[24] The mechanism of denaturation involves alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding.[25] The inhibition of protein denaturation with ethanolic 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 as

well 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 ethanolic extract of showed 98%and aqueous extract of *Coldenia procumbens* showed 95% and protection at a concentration of 1000 µg/ml. The inhibitory effect of different concentration of *Coldenia procumbens* 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 ug/ml and standard aspirin 100ug/ml showed significant inhibition of denaturation of egg albumin in concentration dependent manner.It is reported that the ethanolic extract of showed 94% and aqueous extract of *Coldenia procumbens* showed 89% protection at a concentration of 1000 µg/ml.

Table 1: Ethanol and Agueous extract of coldenia procumbens Protein denaturation method.

S.No	Concentration (µg/ml)	Ethanol Extract		Aqueous Extract	
		Optical dencity 560nm	% of production	Optical dencity 560nm	% of Production
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.	Standard100 (Aspirin)	0.22 ±0.07	92	0.21± 0.07	87

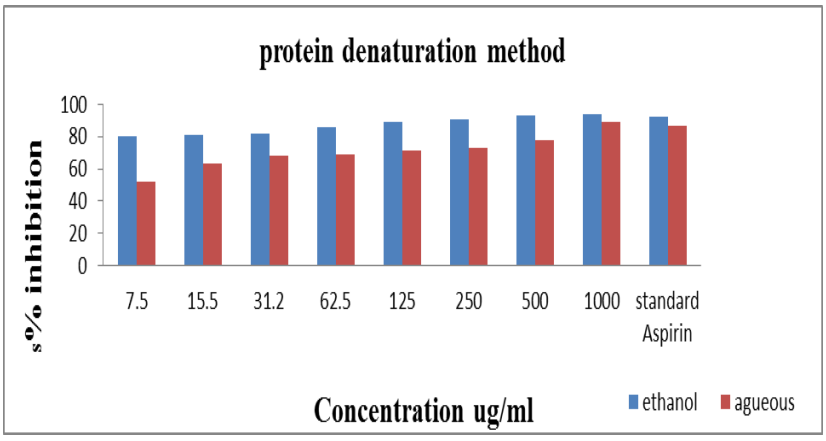

Figure 1: Ethanol and Agueous extract of *coldenia procumbens* Protein denaturation method.

Table 2: Ethanol and Agueous extract of *coldenia procumbens* of HRBC membrane stabilization method.

S.No	Concentration (µg/ml)	Ethanol Extract		Aqueous Extract	
		Optical dencity at560nm	% of Production	Optical dencity at560nm	% of Production
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 100 (Aspirin)	0.12± 0.04	96	0.13 ±0.04	89

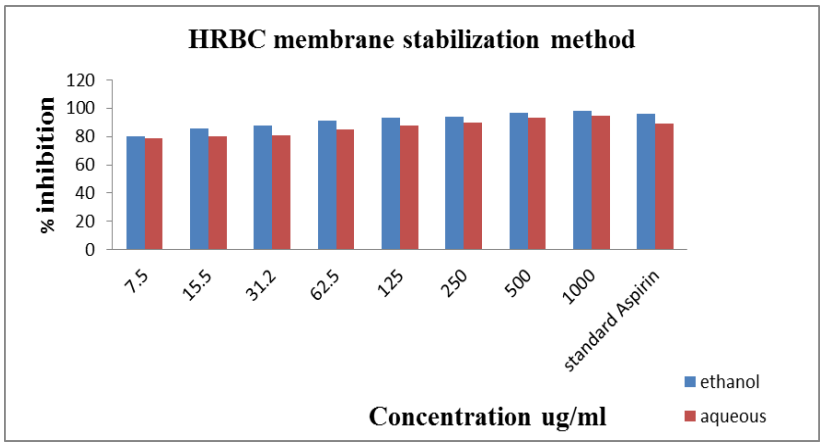

Figure 2: Ethanol and Agueous extract of *coldenia procumbens* of HRBC membrane stabilization method.

Table 3: Ethanolic and Agueous extract of *Coldenia procumbens* of Albumin denaturation method.

S.No	Concentration (µg/ml)	Ethanol Extract		Aqueous Extract	
		Optical dencity at560nm	% of Production	Optical dencity at560nm	% of Production
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.	Standard100 (Aspirin)	0.54±0.18	73	0.63±0.21	68

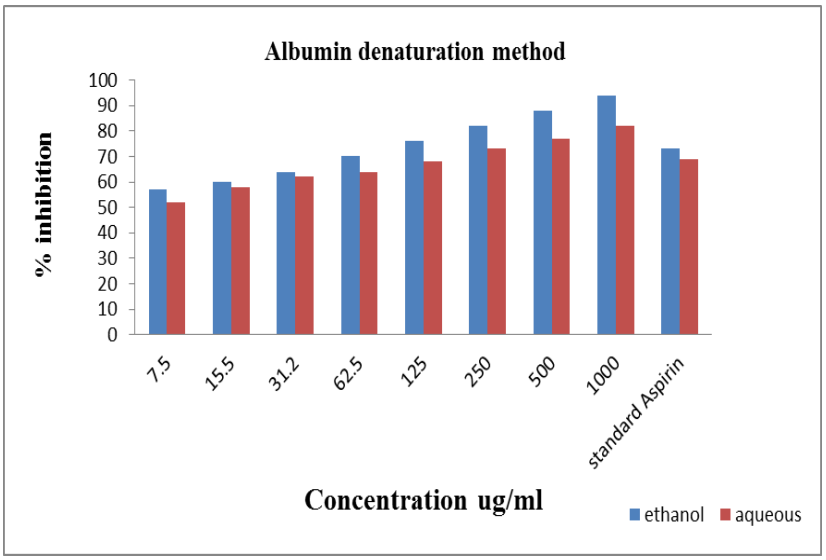

Figure 3: Ethanolic and Agueous extract of *Coldenia procumbens* of Albumin denaturation method.

Aspirin in the concentration of 100ug/ml used as standard also offered protection of effect on protein denaturation method. The inhibitory effect of different concentration of *coldenia procumbens* is presented in.

CONCLUSION

Coldenia procumbens was screened for its anti-inflammatory activity by albumin denaturation, protein denaturation and HRBC membrane stabilization model. Aqueous and ethanolic 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 ethanolic and aquous extracts of *Coldenia procumbens* 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 *Coldenia procumbens* the highest anti-inflammatory activity when compared with extract.

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