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Research Article

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Evaluation of *in vitro* anti-inflammatory potential of the methanolic extract *Clematis buchaniana* plant

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Abstract The main goal of the present study was to evaluate the *in-vitro* anti-inflammatory activity of the methanolic extract of *Clematis buchaniana* plant. For this purpose the entire aerial parts of *Clematis buchaniana* were extracted by soxhelation in methanol. Then the solvent was evaporated to dryness in order to yield the dried crude extract of *Clematis buchaniana*. Then the extract was subjected to preliminary phytochemical screening in order to determine the active constituents for effective pharmacological activity. *In-vitro* anti-inflammatory activity was evaluated by using protein denaturation model in the albumin of fresh chicken's egg. Methanolic extract was found to be more potent when compared with the control.

Keywords Anti-inflammatory, Protein denaturation, *Clematis buchaniana*, methanolic extract, phytochemical screening

Introduction

The use of traditional herbs for medicine has been a common practice from Stone Age. From years ago man has always scrutinized his biotic world for medicines which mainly contains plants as the most precious store for medicines [1]. Keeping it in view familiarity increased with the various species of Clematis (Rananculaceae) which made it an essential garden plant in today's period. *Clematis* is a genus of about 300 species within the buttercup family Ranunculaceae. Their garden hybrids have always been popular among gardeners, beginning with *Clematis jackmanii*, a garden since 1862; more hybrid cultivars are being produced constantly [2-3]. They are mainly obtained from Chinese and Japanese origin. Some other species of Clematis has been evaluated for its anti-inflammatory, cytotoxic, and antimicrobial effects but *Clematis buchaniana* has not been studied much. The whole plant of *Clematis buchaniana* was traditionally used in Napalese medicinal plants for stronger immunity, cooling effect & asthma [4].

So due to its traditional uses and other established pharmacogical activities on the different species of Clematis and minimal pharmacological data on *Clematis buchaniana* made it an intense area for studying anti-inflammatory activity and to perform screening of phytochemicals making use of various reagents [5].

So the main purpose of this anti-inflammatory activity on *Clematis buchaniana* was actually to determine its inhibitory concentration required in the inhibition of denaturation of proteins found in albumin of fresh hen's egg which is detected by the UV spectrometer [6].

Materials and Methods

All the chemicals were used for analytical grade and obtained from laboratory of Devsthali Vidyapeeth College of Pharmacy, Lalpur, Rudrapur. The chemicals and equipment used during the study are enlisted below:

Table 1: List of chemicals used

S. No.	Chemicals Used	Manufacturer
1.	Methanol	Finar reagents, Gujrat
2.	Diclofenac	Finar reagents, Gujrat
3.	Disodium hydrogen orthophosphate	Finar reagents, Gujrat
4.	Potassium dihydrogen phosphate	Finar reagents, Gujrat
5.	Sodium chloride	Finar reagents, Gujrat

Table 2: List of Instruments

S. No.	Instruments Used	Manufacturer
1.	Electronic Weighing Machine	Citizen
2.	UV-Visible spectrometer	Shimadzu, Japan
3.	Ultrasonic bath sonicator	Scientech
4.	Vaccum rotatory evaporator	Scientech
5.	Hot air oven	Scientech
6.	Water bath	Scientech
7.	BOD Incubator	Scientech

Collection and Authentification of Plant Material

The entire plant of ***Clematis buchaniana*** were collected from the village of Kotma Kalimath (Kedarnath region) hilly areas of Garhwal and then further identified and authenticated by the Botanical Survey of India, Dehradun. The voucher specimen no. 115904 was deposited in herbarium. Further the identified plant was washed to remove any dust and other earthy matter, further was shade dried and powdered with laboratory mill. The crushed plant was subjected for extraction.

Preparation of crude plant extracts

The plant for its essential components was extracted by following procedure:

200g of the dried plant was kept in the soxhlet apparatus (borosil) and then it was subjected to soxhlation by using methanol as the menstrum in order to obtain methanolic extract of the entire crude plant Clematis buchaniana. The obtained methanolic extract was filtered and the excessive solvent was evaporated using Vaccum Rotator evaporator under reduced pressure. After evaporation of solvent the crude extract was placed in the dessicator for removal of remaining moisture from the extract to dry it completely. Then, the percentage yield of the dried crude extract was evaluated using the given formula that yielded to about 52 gm of crude dry extract [6-9].

% Yield = Weight of extract (g)*100/Weight of dry powder (g)

Phytochemical Screening

The methanolic extract of *Clematis buchaniana* was now subjected to preliminary phytochemical investigation performed in accordance with the procedure given below [10-12]:

Pharmacological Studies

***In-vitro* Anti-inflammatory Activity**

The anti-inflammatory activity was performed by the *in-vitro* method of egg albumin denaturation. At some instances of inflammation sometimes denaturation of proteins becomes the cause of inflammation that further exaggerates and produces autoantigens that further becomes the initial cause of inflammatory diseases. Those drugs or agents which inhibit or prevent this denaturation of proteins can be further used to prevent inflammation leading to the production of an anti-inflammatory drug [13-14].

Evaluation of *in-vitro* anti-inflammatory activity

The varying concentrations of test sample and that of standard were prepared mainly 10, 20, 30, 50, 100, 200, 300, 400 and 500 µg/ml by using 5000 µg/ml as stock {prepared by dissolving 50 mg of material(test/sample) upto 10ml with distilled water}. Then the reaction mixture was prepared (5 ml) in triplicate that consisted of 0.2 ml of egg

albumin obtained from fresh hen's egg, 2.8 ml of phosphate buffer (pH-6.8) and 2 ml of varying concentrations of above extract and standard drug separately. Similar volume of double distilled water was used as control.

The mixture containing was kept in incubator for incubation at 37±2 °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min .Then cooled the above mixtures and the absorbance was measured at 660 nm using UV Shimadzu 1800 and taking vehicle as blank. During entire experiment diclofenac was used as the standard drug against which the reduction in denaturation of proteins was measured by similar method of measuring absorbance. The %inhibition of protein denaturation was calculated by using given formula [13-14]:

$$\% \text{ Inhibition} = Abs_{control} - Abs_{test} / Abs_{control} \times 100$$

Results

Phytochemical screening

The distribution of phytochemical constituents in methanolic extract of whole plant *Clematis buchaniana* was evaluated qualitatively. The presence of phytocompounds such as flavanoids, Carbohydrates, proteins has been confirmed in selected plant [10-15].

Table 3: Results of phytochemical Screening

S. No.	Test	Methanolic Extract
1. Alkaloid	Dragendroff's test	-
	Mayer's test	-
	Wagner's test	-
	Hager's test	-
2. Carbohydrates	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
3. Proteins	Lead acetate test	+
4. Saponins Glycosides	Foam test	-
5. Cardiac Glycosides	Killer-killani test	+
6. Flavanoids	Lead acetate test	+

In vitro Anti inflammatory activity

Denaturation of protein is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of methanolic solvent of plant extract protein denaturation was studied. From the result, it is evident that the methanolic extract of *Clematis buchaniana* efficiently reduces the denaturation of protein in terms of percentage inhibition (IC_{50}: 3.58 mg/kg) whereas as that of standard diclofenac was found to be 2.14 mg/kg [13-15].

Table 4: Results of Anti inflammatory activity

Conc.	Absorbance (diclofenac)	Control	Abs. Ext 1	Abs. Ext 2	Abs. Ext 3	% inhibition Absorbance diclofenac	% inhibition Ext 1	% inhibition Ext 2	% inhibition Ext3	Average
10	0.413	0.679	0.567	0.524	0.598	39.17526	16.49485	22.82769	11.92931	17.08395
20	0.356	0.679	0.413	0.409	0.429	47.56996	39.17526	39.76436	36.81885	38.58616
30	0.286	0.679	0.386	0.382	0.375	57.87923	43.15169	43.7408	44.77172	43.88807
50	0.236	0.679	0.294	0.256	0.252	65.243	56.70103	62.2975	62.8866	60.62838
100	0.186	0.679	0.233	0.228	0.229	72.60677	65.68483	66.42121	66.27393	66.12666
200	0.139	0.679	0.222	0.222	0.188	79.82872	67.30486	67.30486	72.31222	68.97398
300	0.096	0.679	0.087	0.074	0.059	85.86156	87.18704	89.10162	91.31075	89.1998
400	0.049	0.679	0.061	0.052	0.066	92.78351	91.0162	92.34168	90.27982	91.21257
500	0.018	0.679	0.03	0.027	0.025	97.34904	95.58174	96.02356	96.31811	95.97447

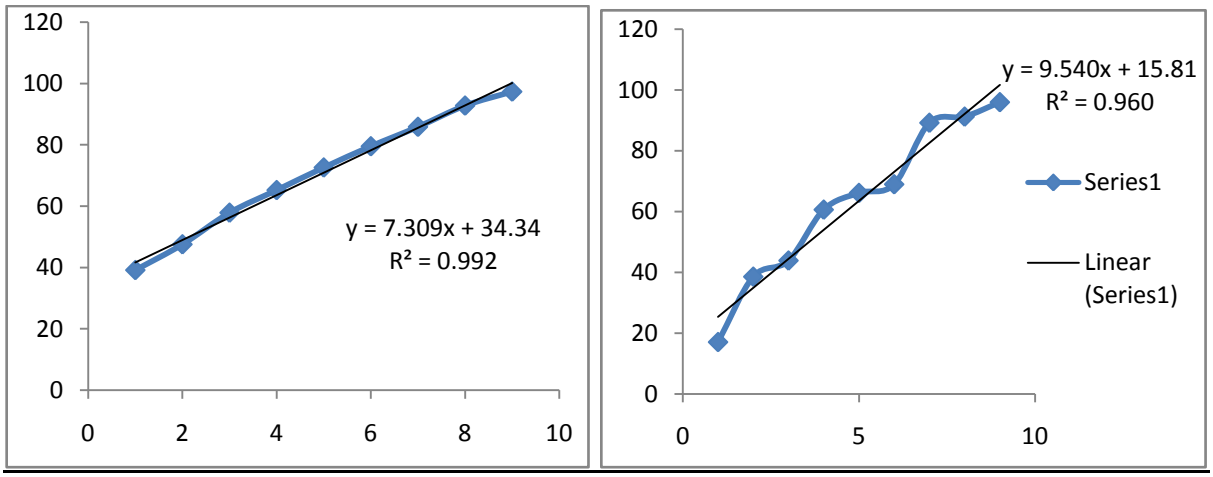

Figure 1: Inhibition of protein denaturation, A: inhibition by diclofenac (standard drug), B: inhibition by methanolic extract of Clematis buchaniana.

Discussion and Conclusion

The present study has been subjected to following aspects of an important herbal medicinal plant *Clematis buchaniana (*family Rananculaceae) the study included *in vitro* anti-inflammatory activity via protein denaturation method.

The *in-vitro* anti-inflammatory activity performed by protein denaturation method inferred that the extract of *Clematis buchaniana* was used in the concentration range of 10-500 µg/ml & it showed a significant concentration dependent inhibition of protein denaturation providing us an IC_{50} of 3.58 mg/Kg where as that of standard diclofenac was found to be 2.14 mg/Kg. Hence, on future grounds this herb can be used as a highly potent anti inflammatory agent. The anti-inflammatory and anti-angiogenic activity of *Clematis buchaniana n*eed further more detailed study in the path of isolation and authentication of screened phytochemicals responsible for above activities of this medicinal drug [10-15].

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