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EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF HYDROETHANOLIC EXTRACT OF *DELOSPERMA COOPERI* PLANT

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Keywords:

Delosperma cooperi, Anti-inflammatory activity, Protein denaturation, Hydroethanolic, Phytochemical

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ABSTRACT: Objective: The main objective of this study is to evaluate the anti-inflammatory activity of hydroethanolic extract of the entire plant except for the roots and flowers of *Delosperma cooperi* (Family: Aizoaceae), also known as hard ice-plant by *in-vitro* protein denaturation method. However, the anti-inflammatory effects of *D. cooperi* has not been studied. **Method:** The *D. cooperi* were extracted by the soxhlation method by using the hydroethanolic solution (60; 40) ratio. Then the extract was evaporated to dryness in order to yield dried crude extract of *D. cooperi*. The preliminary studies of phytochemicals were done for the confirmation of the presence of active constituents for the effective pharmacological activity. The anti-inflammatory activity was analyzed by using chicken's egg albumin denaturation method (*in-vitro*). The hydroethanolic extract was found to have a high ability to inhibit protein denaturation that was evaluated by using a UV spectrophotometer. **Result:** The extracts of *D. cooperi* exhibited concentration-dependent protein (Albumin) denaturation inhibition. The effect of the test sample was found to be good when compared with the diclofenac (standard). **Conclusion:** From the obtained results, it can be concluded that *D. cooperi* possessed a good anti-inflammatory activity against protein denaturation of egg albumin. The anti-inflammatory activity may be due to the presence of alkaloid as well as flavonoids. The anti-inflammatory activity of the extract can be established as a potential source for the development of new treatment against inflammation.

INTRODUCTION: Inflammation may be caused by various origins; ischemia, antigen-antibody reactions, infectious agents, physical or thermal shocks, and disturbance in physiological functions¹. At the time of tissue injury, inflammation is the normal body response to destroy or inactivate the invading organism and set the stage for tissue repair. It is activated by the secretion of chemical mediators from the migrating cells and injured tissue. It is a complex process associated with pain and appearance of protein denaturation, vascular permeability, and membrane alteration^{2,3}.

Non-steroidal anti-inflammatory drugs (NSAID) are the most frequently used drugs for the treatment of inflammatory conditions but has various adverse effects like arthritis and gastric ulcer formation due to their gastric irritation effects^{4,15}. Apart from this potent ADR, synthetic anti-inflammatory drugs have the greatest disadvantage, which is their toxicity as well as the reappearance of symptoms after discontinuation^{5,6}.

This has led us to find out natural alternative treatments. The natural product plays a vital role in the development of modern medicine. Nowadays, different traditional plant species and their active therapeutic metabolites are re-evaluated by large scale research through worldwide scrutiny. The plant kingdom is a rich source of newer compounds with significant anti-inflammatory activity. The benefit of taking herbal medicine is that its efficacy is optimum with a low incidence of toxicity and

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low cost. There are several constituents like alkaloid, glycoside, polysaccharides, resins, flavonoids, essential oils, phenolic compounds, fatty acids, etc. which are responsible for the anti-inflammatory activity⁷. There are about 229 scientific species of the genus *Delosperma* in which 175 are accepted species name¹⁶.

D. cooperi is also known as Hardy Ice-plant because of its water-holding capability. Alkaloid, flavonoids, glycosides, and dimethyl-tryptamine are chemical constituents which are present in this species which became a reason to select this plant for anti-inflammatory activity^{17, 18}.

MATERIALS AND METHODS:

Plant Material: The plants were procured in the month of February 2016 from the Garden of Devsthal Vidyapeeth College of Pharmacy, Lalpur, Rudrapur (U. S. Nagar). Remove their flowers and roots then was washed to remove earthy matters. Further, the plant was dried in the shade for 20-25 days and grounded to a fine powder for obtaining their hydroethanolic extract.

Chemical Used: Methanol, Diclofenac, Disodium hydrogen orthophosphate, Potassium dihydrogen phosphate, Sodium chloride (Finar reagents, Gujrat) and various reagents for phytochemical screening all are obtained from the laboratory of Devsthal Vidyapeeth College of Pharmacy, Lalpur, Rudrapur.

Preparation of Extract: 250 g of the dried plant was put in the Soxhlet apparatus & then it was subjected to soxhlation by using a mixture of water & ethanol (60:40) as the menstruum in order to obtain a hydro-ethanolic extract of the entire crude plant *D. cooperi*. The obtained hydroethanolic extract was filtered, and then the excessive solvent was evaporated by using a Vacuum Rotator evaporator under decreased pressure.

After evaporation of the solvent, the crude extract was placed in the desiccator for the removal of excess moisture from the extract for making it completely dry. Different concentrations of extract were freshly prepared to form the dry extract with distilled water at the time of anti-inflammatory activity. Then, the percentage yield of the dried crude extract was calculated by using the following formula-

$$\% \text{ Yield} = \frac{\text{Weight of extract (gm)}}{\text{Weight of dry powder (gm)}} \times 100$$

Phytochemical Analysis: The hydroethanolic extracts were subjected to preliminary phytochemical screening for the determination of major chemical groups. The plant extract was taken and tested for flavanoids, alkaloids, carbohydrates, proteins, and cardiac glycosides following standard protocol^{8, 19}.

Inhibition of Egg Albumin Denaturation: The anti-inflammatory activity of *D. cooperi* was evaluated by using inhibition of egg albumin denaturation methods^{9, 10} followed with minor modifications. Preparing various concentrations of test and standard drugs. The reaction mixture (5 ml) was prepared that consisted of 2 ml test extract, 0.2 ml of egg albumin solution, 2.8 ml of phosphate buffer (pH: 6.4) was used to adjust the pH of the reaction mixture. The samples were incubated at 37 °C for 15 min in a BOD incubator and then heated to 70 °C for 5 min; after cooling the mixture, the turbidity was measured at 660 nm (UV 1800, SHIMADZU). Diclofenac sodium was used as a standard drug. The experiment was performed in triplicate manner¹¹. The percentage inhibition of protein denaturation was calculated by the following formula:

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

RESULTS: The extracts were subjected to preliminary phytochemical analysis to determine the presence of various phytoconstituents, and results are tabulated in **Table 1**.

TABLE 1: RESULTS OF PHYTOCHEMICAL SCREENING OF PLANT *D. COOPERI*

S. no.	Test	Hydroethanolicextract
1	Alkaloid	
	Dragendorff'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	Cardiac Glycosides	
	Killer-Killani Test	+
5	Flavanoids	
	Lead Acetate Test	+

Preliminary phytochemical analysis of the extracts of *D. cooperi* revealed the presence of sugar, protein, and carbohydrates as major constituents. The evaluation of secondary metabolites showed the hydroethanolic extract of *D. cooperi* contains; alkaloids, flavonoids, and glycosides.

Inhibition of Egg Albumin Denaturation: By the application of external stress, heat, or compounds like strong acid or base, organic solvent, and concentrated inorganic salts, the proteins lose their secondary and tertiary structure, and most of them lose their biological function, which is termed as protein denaturation.

This denaturation of protein causes inflammation^{12, 13}. In this investigation, the anti-inflammatory activity or the ability of plant extract to inhibit the process of protein denaturation was studied by the *in-vitro* egg albumin method due to the problems associated with the ethical issues in order to make use of animals in research when other suitable methods are available.

From the result, it is evident that the hydroethanolic extract of *D. cooperi* efficiently reduces the denaturation of proteins in terms of percentage inhibition (IC₅₀- 3.2 mg/kg). The results are indicated in **Table 2**.

TABLE 2: RESULTS FOR THE IN-VITRO ANTI-INFLAMMATORY ACTIVITY BY IN-VITRO INHIBITION OF EGG ALBUMIN DENATURATION BY TEST AS WELL AS STANDARD DRUG

Conc.	Abs. of (Diclofenac)	Control	Abs. Ext 1	Abs. Ext 2	Abs. Ext 3	% inhi. Abs. Diclofenac	% inhi. Ext 1	% inhi. Ext 2	% inhi. Ext3	Avg.
10	0.413	0.679	0.516	0.586	0.549	39.17526	24.00589	13.69661	19.1458	18.94944
20	0.356	0.679	0.415	0.458	0.492	47.56996	38.88071	32.54786	27.5405	32.98969
30	0.286	0.679	0.327	0.358	0.391	57.87923	51.84094	47.27541	42.41532	47.17722
50	0.236	0.679	0.242	0.233	0.215	65.243	64.35935	65.68483	68.33579	66.12666
100	0.186	0.679	0.177	0.154	0.123	72.60677	73.93225	77.31959	81.88513	77.71232
200	0.139	0.679	0.111	0.091	0.078	79.82872	83.65243	86.59794	88.51252	86.2543
300	0.096	0.679	0.058	0.039	0.031	85.86156	91.45803	94.25626	95.43446	93.71625
400	0.049	0.679	0.021	0.019	0.012	92.78351	96.90722	97.20177	98.2327	97.44723
500	0.018	0.679	0.012	0.007	0.005	97.34904	98.96907	98.2327	99.26362	98.8218

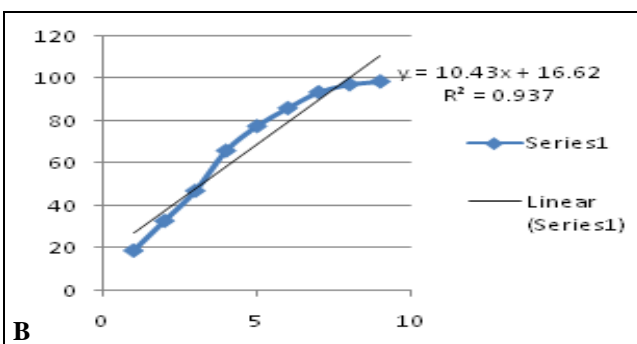
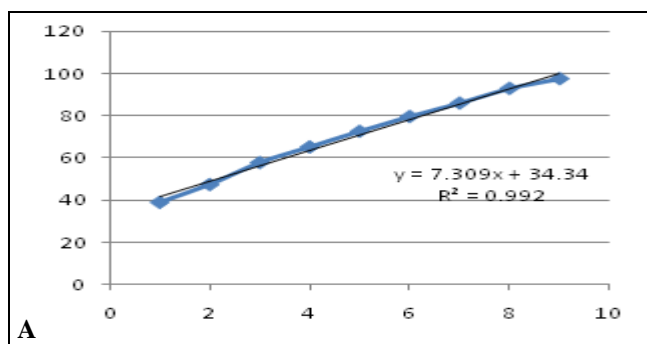


FIG. 1A: INHIBITION OF PROTEIN DENATURATION BY DICLOFENAC USED AS A STANDARD DRUG, B: INHIBITION OF PROTEIN DENATURATION BY HYDROETHANOLIC EXTRACT OF *D. COOPERI* USED AS A TEST DRUG

DISCUSSION: The preliminary phytochemical screening of hydro-ethanolic extract of *D. cooperi* confirmed the presence of various phytochemical compounds such as carbohydrates, flavonoids, proteins, cardiac glycosides. The *in-vitro* anti-inflammatory activity of *D. cooperi* was done by protein denaturation method in which various concentrations (10-500 µg/ml) of the test sample were compared with the same concentration of standard diclofenac sample. The results showed significant concentration-dependent inhibition of protein denaturation with an IC₅₀ of 3.2 mg/Kg was

evaluated, whereas standard diclofenac was evaluated to have an IC₅₀ of 2.14 mg / Kg. Hence, in the future, this herb can be used as a potent anti-inflammatory agent as per the *in-vitro* study.

CONCLUSION: Biological assays using isolated compounds revealed that flavonoids exhibit a wide range of effects like antimicrobial, antiviral, anti-neoplastic, anti-hepatotoxic, anti-ulcerogenic, anti-allergic, and hypolipidemic activities¹⁴. However, future studies are required for the anti-inflammatory activity of *D. cooperi* furthermore

detailed in the path of isolation and authentication of screened phytochemicals, which may be responsible for the above activities of this plant.

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Author Contribution: Mahak Arora contributes to the implementation of the research, to the result analysis, and to the writing of the manuscript.

REFERENCES:

- Osadebe P and Okoye F: Anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. J of Ethnopharmacology 2003; 89(1): 19-24.
- Gunawardena D: Determination of anti-inflammatory activities of standardised preparations of plant-and mushroom-based foods. European Journal of Nutrition 2014; 53(1): 335-43.
- Chatterjee P: Evaluation of anti-inflammatory effects of green tea and black tea: a comparative *in-vitro* study. Journal of Advanced Pharmaceutical Technology & Research 2012; 3(2): 136.
- Tripathi K: Jaypee Brothers Medical Publishers (P) Ltd., edition 7 2013.
- Shen T: Toward more selective antiarthritic therapy. Journal of Medicinal Chemistry 1981; 24(1): 1-5.
- Chawla AS, Handa SS, Sharma AK and Kaith BS: Plant Anti-inflammatory agents. Journal of Scientific and Industrial Research 1987.
- Beg S: Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. Pharmacognosy Reviews 2011; 5(10): 120.
- Khatun R., Roy S, and Rahman MAA: *In-vitro* comparative evaluation of anti-inflammatory and thrombolytic activity of three Mikania species available in Bangladesh. Journal of Pharmacognosy and Phytochemistry 2017; 6(5): 1007-11.
- Mizushima Y and Kobayashi M: Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology 1968; 20(3): 169-73.
- Sakat S, Juvekar AR and Gambhire MN: *In-vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci 2010; 2(1): 146-55.
- Chandra S: Evaluation of anti-inflammatory effect of ashwagandha: a preliminary study *in-vitro*. Pharmacognosy Journal 2012; 4(29): 47-49.
- Umapathy E: An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plants Res 2010; 4(9): 789-95.
- Leelaprakash, G and Dass SM: *In-vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. International J of Drug Deve and Research 2011; 3(3): 189-96.
- Di Carlo G: Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Scienc 1999; 65(4): 337-53.
- Bennett PN and Brown MJ: Clinical pharmacology. New Delhi Churchill Livingstone 2005.
- <http://www.theplantlist.org/1.1/browse/A/Aizoaceae/Delosperma/> last seen in 15 october 2019.
- Peter W: Jeffers, the alkaloids sceletzum alkaloids. ISBN 1981; 1-3.
- Trout & friends Sacred Cacti Ed 3rd A Bettter Days Publication. 2004; 8.
- Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy. Edition 14, Nirali Prakashan 2010.

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