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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 functions [1]. 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].

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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

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 activity [7]. There are about 229 scientific species of the genus Delosperma in which 175 are accepted species name [16].

*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 Devsthali 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 Devsthali 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 Vaccum 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-

% Yield = Weight of extract (gm) / Weight of dry powder (gm) × 100

**Phytochemical Analysis:** The hydroethanolic extracts were subjected to preliminary phyto-chemical 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 ºC for 15 min in a BOD incubator and then heated to 70 ºC 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 manner [11]. The percentage inhibition of protein denaturation was calculated by the following formula:

Percentage inhibition = (Abs Control – Abs Sample) / Abs control × 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 | |
| | 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 | 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 hydro-ethanolic extract of *D. cooperi* efficiently reduces the denaturation of proteins in terms of percentage inhibition ($IC_{50}$- 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 |

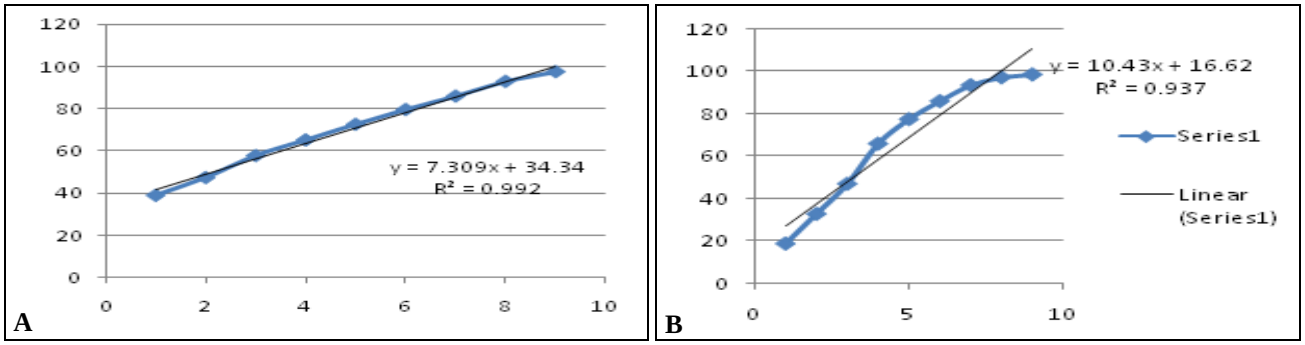


**FIG. 1A: INHIBITION OF PROTEIN DENATURATION BY DICLOFENAC USED AS A STANDARD DRUG, B: INHIBITION OF PROTEIN DENATURATION BY HYDROETHANOLIC EXTRACT OF *D. COOPERI* USED AS A TEST DRUG**

**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 $IC_{50}$ of 3.2 mg/Kg was evaluated, whereas standard diclofenac was evaluated to have an $IC_{50}$ 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 activities [14]. 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.

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