



Studies on the Anti-Inflammatory Properties of Various extracts of *Hippophae rhamnoides*



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Submission: January 26, 2017; **Published:** February 28, 2017

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Abstract

The plant *Hippophae rhamnoides* have been analysed for the anti-inflammatory property and it had been found that the concerned plant possess a good potential against inflammation. Among the plant parts analysed, the fruit extracts have been found to possess high potential against inflammation followed by leaf extracts and root extracts possess the least effect against inflammation. Three different solvents have been used for extraction purpose viz, Dichloromethane, methanol and water. Among the various solvent extracts, the water extracts of all the three plant parts possess much more anti-inflammatory property followed by methanolic extracts, and lesser potential was noticed for DCM extracts.

Keywords: *Hippophae rhamnoides*; Anti-inflammatory; HRBC; Membrane hemolysis

Abbreviations: SBT: Sea Buckthorn; NO: Nitric Oxide; PG: Prostaglandin; HRBC: Human Red Blood Cells

Introduction

India and China are two of the largest countries in Asia which have the richest arrays of registered and relatively well known medicinal plants. India endowed with a rich wealth of medicinal plants is unique in the use of plants/plant parts by all sections of people either directly as folk medicine directly or indirectly in the pharmaceutical preparations of modern medicine. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different systems of medicines such as *Ayurveda*, *Unani* and *Siddha* in India. *Rigveda* and *Atharveda* which date back to 2000-1000 BC and several post Vedic treatises such as *Charakasamhita* (100 AD), *Sushruthasamhitha* (100-800 AD) and *Dhanwanthari Nighantu* (1200 AD) are the important ancient sources of information on medicinal plants. Sixty percent of the world population and 80% of the population in developing countries depend on traditional medicine, mostly plant drugs, for their primary health care needs. Out of the total known number of medicinal plants from India, approximately 46% are from the Himalayas. Out of the total medicinal plant species, 62 species of medicinal plants

are endemic to the Himalayas and 208 species have extended their distribution towards adjacent areas and are, therefore, considered as near endemic.

Over 200 species of Himalayan medicinal plants are consumed as raw, roasted, boiled, fried, cooked, or they are used in the form of oil, spices, jams or pickles. Sea Buckthorn (*Hippophae rhamnoides* L.) and its relatives in the family *Eleagnaceae* are shrubs and small trees having berries containing compounds with high nutritional and medicinal value Singh, V [1], Kalia RK et al. [2]. Sivaraj Anbarasu showed Sea Buckthorn (SBT) is distributed in India and the plant parts are used traditionally for several ailments. Medicinally, it has been proven to possess various pharmacological activities such as antioxidant, antimicrobial, antifungal, metabolic disorders, immune-stimulatory activity, hepato-protectant and anticancer activity. Yan-Jun Xu et al. [3] showed Sea buckthorn is a natural source of vitamins and several other bioactive compounds like carotenoids and flavonoids, which have been known to lower cholesterol, platelet aggregation, blood pressure and

blood sugar. In addition, sea buckthorn is a well known herb in treating tumors, stomach ulcers, skin diseases and arsenic poisoning. Alam Zeb [4] showed that Sea buckthorn juice is one of the imperative product obtained from the sea buckthorn berries, is commercially very important. The juice from the plant provides a nutritious beverage, high in suspended solids, and very high in vitamins especially in vitamin C and carotenoids. It contains many important nutrients and bioactive substances including vitamins, fatty acids, free amino acids and elemental components.

Materials and Method for anti-inflammatory analysis

The study, anti-inflammatory properties of "*Hippophae rhamnoides*" were carried out. All the chemicals and reagents used were of analytical grade. The concerned plant namely *Hippophae rhamnoides* was collected from Ladakh region of J & K. Dextrose, Sodium citrate, Citric acid, Sodium chloride, Phosphate buffer, Hypo saline, were purchased from Hi-media, methanol used were of analytical grade.

Experiment Work

Study area and plant collection

The concerned plant was collected from the Ladakh region of J & K. The plant parts were segregated shade dried and powdered in mixture. The powder was used for experiment.

Extraction

50 gms of the plant root, stem and leaf powder were weighed separately and accurately and then extracted in a Soxhlet Apparatus using thimble in order to get the best extract. Various solvents were used depending upon their polarity index with increasing polarity (DCM, Methanol and Water).

I.Extraction A: The sample was extracted with a particular solvent (DCM) in a Soxhlet apparatus for a required period. After the Extraction with Petroleum ether, the extract solution was subjected to filtration to remove the residue from extract. The filtrate was then collected and evaporated to remove the volatile solvent to its 1/4th volume on water bath at a suitable temperature. The whole filtrate was then made in solid form (powdered) after being kept in an oven at 40-600C. The residue was collected, and subjected to further extraction process.

II.Extraction B: The residue was then extracted with Methanol in a same manner as mentioned above, in extraction A.

III.Extraction C: The residue from extract B was subjected to Water extraction by decoction technique. In this technique the extract was dissolved in 500 ml of water. The whole solution was heated over water bath to remove all the water from the extract. Finally additional 500 ml of water was added to the extract, the extracted solution was finally evaporated to remove nearly 250 ml of water. The obtained solution was subjected to filtration and then the filtrate was evaporated to remove nearly 1/4th of its volume. Finally the extract was dried in an oven at a temperature range 30-500C.

Anti-inflammatory activity of *Hippophae rhamnoides* plant extracts

Inflammation is a complex physiological process mediated by a variety of signaling molecules produced by leukocytes, macrophages, and mast cells. Inflammation is a tissue response to injury characterized by increased blood flow to the tissue causing increased temperature, redness, swelling, and pain. Macrophages play important role in inflammatory diseases through the release of inflammatory mediators such as nitric oxide (NO), prostaglandin (PG) E₂, and pro-inflammatory cytokines.

Preparation of human red blood cells (HRBC) suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline Sakat S, Juvekar AR et al. [5], Govindappa M et al. [6].

Heat induced hemolysis

The principle involved here is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The assay mixture composes 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with various concentrations of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 200, 300, 400, 500 µg) and control (distilled water in place of hyposaline to produce 100 % hemolysis) were incubated at 37oC for 30 min and then centrifuged. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm by using Patel DK, Prasad SK, Kumar R, Hemalatha S. - 2012 method.

The percentage of hemolysis of HRBC membrane can be calculated as follows:

$$\% \text{ Hemolysis} = (\text{Absorbance of Test Sample} / \text{Absorbance of Control}) \times 100$$

The percentage of HRBC membrane stabilization can be calculated as follows:

$$\% \text{ Protection} = 100 - [(\text{Absorbance by Test sample} / \text{Absorbance by Control}) \times 100]$$

Observation and Results

The anti-inflammatory properties of various extracts of *Hippophae rhamnoides* have been carried out by the lysis of cells which was carried out by hypotonicity. The percentage hemolysis and correspondingly the percentage stabilization against the membrane hemolysis were carried out. The percentage stabilization gets increased against the concentration increase.

Anti-inflammatory properties of leaf, fruit and root extracts of *Hippophae rhamnoides*

The anti-inflammatory properties of Leaf, Fruit and Root extracts have been carried out and it was found that the reference compound (Diclofenac sodium) have been found to possess the highest % stabilization as shown in all tables and figures (Tables 1-9) (Figures 1-18). Among the plant extracts it has been found that methanolic extract of fruit possesses the highest percentage stabilization followed by water extract of fruit, methanolic extract of root, water extract of leaf, ethanolic

extract of leaf, DCM extract of fruit, water extract of root, DCM extract of root and DCM extract of leaf respectively. The fruit extract (Figures 8,10,12) possesses the higher stabilization than root extracts (Figures 14,16,18), and least activity was observed for leaf extracts (Figures 2,4,6). The anti-inflammatory effect has been found to be concentration dependent, as the percentage inhibition increases with the increase in the concentration of plant extract. The graph was plotted between the concentration and the percentage stabilization and a straight line formation was observed.

Table 1: Report of anti-inflammatory effect of DCM extract of leaf of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	41.56	58.44	47.97	50.9
1.0	40.41	59.59	27.89	64.6
1.5	39.31	60.69	15.63	78.97
2.0	38.65	61.35	11.54	87.99
2.5	32.65	67.35	6.44	95.52

Table 2: Report of anti-inflammatory effect of Methanolic extract of leaf of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	41.49	58.51	47.97	50.9
1.0	39.39	60.61	27.89	64.6
1.5	34.61	65.39	15.63	78.97
2.0	32.88	67.12	11.54	87.99
2.5	25.17	74.83	6.44	95.52

Table 3: Report of anti-inflammatory effect of water extract of leaf of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	21.14	78.86	47.97	50.9
1.0	20.00	80.00	27.89	64.6
1.5	20.01	79.99	15.63	78.97
2.0	18.92	81.08	11.54	87.99
2.5	18.11	81.89	6.44	95.52

Table 4: Report of anti-inflammatory effect of DCM extract of Fruit of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	39.88	60.12	47.97	50.9
1.0	37.17	60.83	27.89	64.6
1.5	35.54	62.46	15.63	78.97
2.0	34.66	63.34	11.54	87.99
2.5	26.87	70.32	6.44	95.52

Table 5: Report of anti-inflammatory effect of Methanolic extract of fruit of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	20.14	79.86	47.97	50.9
1.0	19.34	80.66	27.89	64.6
1.5	18.65	81.35	15.63	78.97
2.0	17.75	82.25	11.54	87.99
2.5	16.33	83.67	6.44	95.52

Table 6: Report of anti-inflammatory effect of water extract of Fruit of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	32.25	67.75	47.97	50.9
1.0	31.08	68.92	27.89	64.6
1.5	25.18	74.82	15.63	78.97
2.0	22.09	77.91	11.54	87.99
2.5	17.53	82.47	6.44	95.52

Table 7: Report of anti-inflammatory effect of DCM extract of Root of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	41.32	58.68	47.97	50.9
1.0	40.55	59.45	27.89	64.6
1.5	39.12	60.88	15.63	78.97
2.0	37.41	62.59	11.54	87.99
2.5	29.23	70.77	6.44	95.52

Table 8: Report of anti-inflammatory effect of Methanolic extract of Root of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	31.23	68.77	47.97	50.9
1.0	30.54	69.46	27.89	64.6
1.5	26.10	73.90	15.63	78.97
2.0	25.13	74.87	11.54	87.99
2.5	18.21	81.79	6.44	95.52

Table 9: Report of anti-inflammatory effect of water extract of root of *Hippophae rhamnoides* and standard on HRBC membrane hemolysis and membrane stabilization.

Conc. of Standard/ Plant Extract (µl/ml)	% Hemolysis	% Stabilisation	% Hemolysis of Diclofenac sodium	% Stabilisation of Diclofenac sodium
0.5	39.28	60.72	47.97	50.9
1.0	38.76	59.24	27.89	64.6
1.5	37.54	62.46	15.63	78.97
2.0	34.54	65.46	11.54	87.99
2.5	27.76	72.24	6.44	95.52

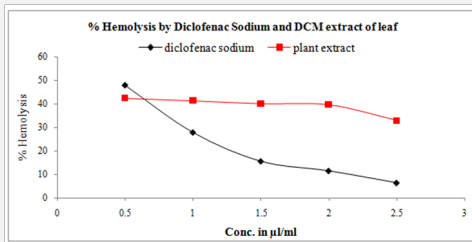


Figure 1: Graph represents HRBC Membrane Hemolysis by DCM extract of Leaf and Standard

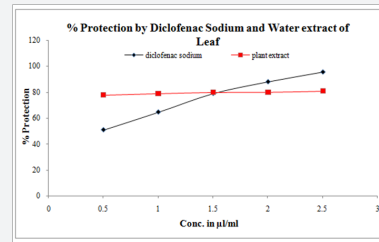


Figure 6: Graph represents the membrane % stabilization by Water extract of Leaf and the Standard.

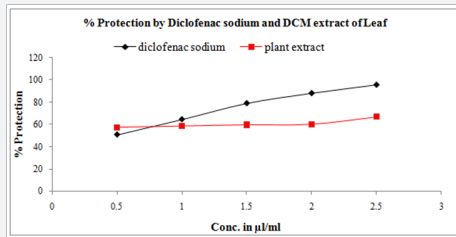


Figure 2: Graph represents the membrane % stabilization by DCM extract of Leaf and the Standard.

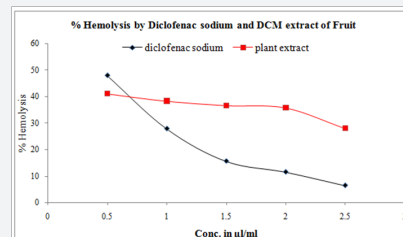


Figure 7: Graph represents HRBC Membrane Hemolysis by P DCM extract of Fruit and Standard.

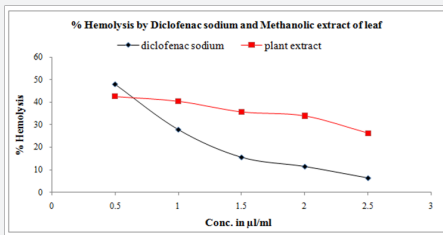


Figure 3: Graph represents HRBC Membrane Hemolysis by Methanol extract of Leaf and Standard.

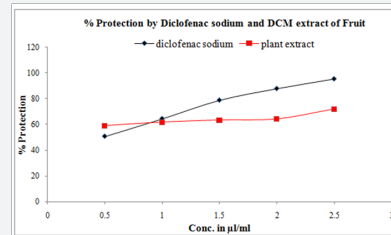


Figure 8: Graph represents the membrane % stabilization by DCM extract of Fruit and the Standard.

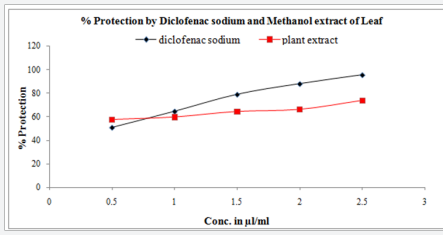


Figure 4: Graph represents the membrane % stabilization by Methanol extract of Leaf and the Standard.

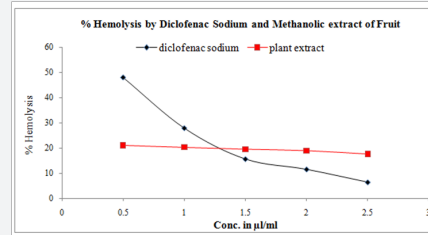


Figure 9: Graph represents HRBC Membrane Hemolysis by Methanolic extract of Fruit and Standard.

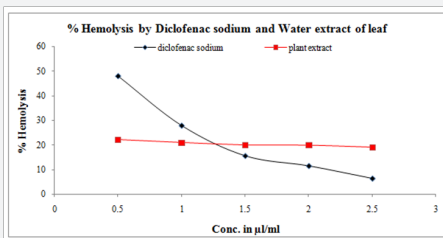


Figure 5: Graph represents HRBC Membrane Hemolysis by Water extract of Leaf and Standard.

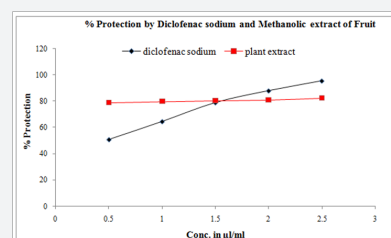


Figure 10: Graph represents the membrane % stabilization by Methanolic extract of Fruit and the Standard.

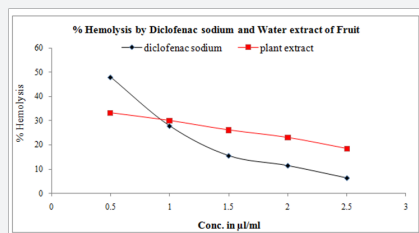


Figure 11: Graph represents HRBC Membrane Hemolysis by Water extract of Fruit and Standard.

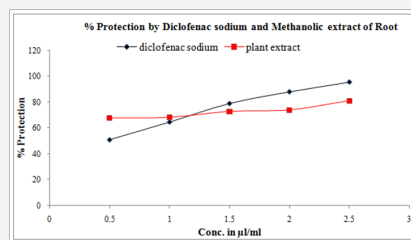


Figure 16: Graph represents the membrane % stabilization by Methanolic extract of Root and the Standard

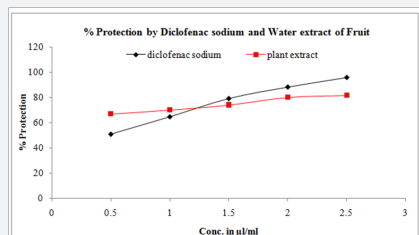


Figure 12: Graph represents the membrane % stabilization by Water extract of fruit and the Standard.

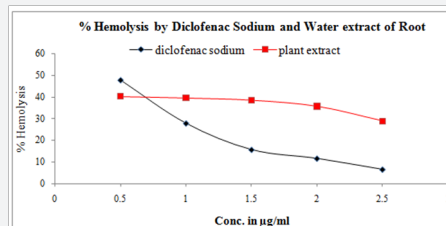


Figure 17: Graph represents HRBC Membrane Hemolysis by Water extract of Root and Standard.

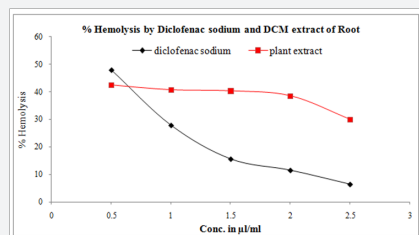


Figure 13: Graph represents HRBC Membrane Hemolysis by DCM extract of Root and Standard.

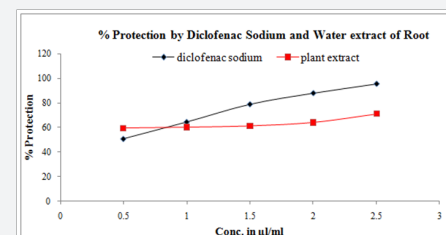


Figure 18: Graph represents the membrane % stabilization by Water extract of Root and the Standard.

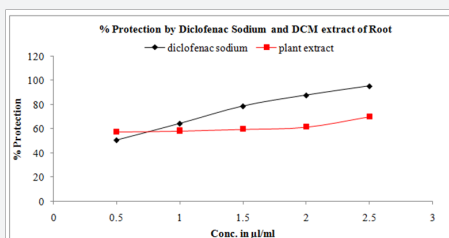


Figure 14: Graph represents the membrane % stabilization by DCM extract of Root and the Standard.

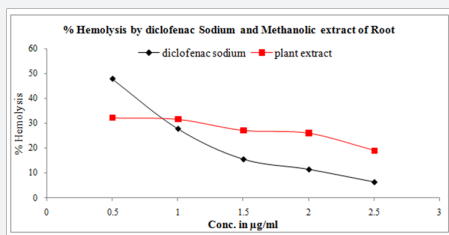


Figure 15: Graph represents HRBC Membrane Hemolysis by Methanolic extract of Root and Standard.

Discussion

Anti-inflammation refers to the property of a medicine or medicinal like product to inhibit the process of inflammation or swelling. Anti-inflammatory drugs make half of the analgesics, reducing pain by reducing inflammation at a particular body part of an organism. *Hippophae rhamnoides* plant is a well known herb used as folk medicine in North America and as home recipes for a variety of benefits, and is also used as a nontoxic herb in traditional Chinese medicine. *Hippophae rhamnoides* plant extracts were analysed for their anti-inflammatory effect and results obtained were found to possess a high level of effect against inflammation. The anti-inflammatory effect of plant is due to the presence of various secondary phytochemicals like flavonoids, alkaloids etc. the most enhancing capacity against the inflammation was found to be due the hemoxygenase. So the plant could be used as best anti-inflammatory source. The anti-inflammatory effect of all the plant extracts have been carried out in reference to standard diclofenac sodium (anti-inflammatory drug). The inflammation was done by hypotonicity induced membrane lysis. The stabilisation against membrane induced hypotonicity was found to be concentration dependent,

and the stabilisation percentage in all the plant extracts was found less than the reference compound (Diclofenac sodium). Among the plant extracts the highest stabilization was found in case of methanolic extract of fruit (Figure 10) followed by water extract of fruit (Figure 12). Among the all plant extracts it has been found that the polar solvent extracts possess highest anti-inflammatory activity than the less polar solvent extracts. The results get agreed as most of the amino acids are anti-inflammatory in nature so are the polar plant extracts. These compounds have direct influence upon the inflammation and the plant extracts fulfill the job in highly influenced manner. Thus *Hippophae rhamnoides* plant extracts can be used as best anti-inflammatory medicines in addition to its already recognized properties.

Conclusion

Anti-inflammatory activity of the concerned plant is due to the presence of various types of phytochemical species. An anti-inflammatory response is the action against inflammation. From the concerned study it can be concluded that the plant bear a good potential to overcome inflammation. The anti-inflammatory activity determined was found more in the polar solvents which could be due to the polar compounds extracted

from the plant extracts. So overall it can be concluded that nature furnishes every source against any type of disorder.

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DOI:10.19080/JPCR.2017.02.555584

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