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IN VITRO ANTI-INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF LEAF AND BARK OF HOLOPTELIA INTEGRIFOLIA PLANCH

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ABSTRACT: The different extracts of leaf and bark of the plant *Holoptelia integrifolia* were investigated for *in-vitro* anti-inflammatory activity by human red blood cell membrane stabilization (HRBC) method. The HRBC membrane stabilization activity of the petroleum ether extract of leaf at the concentration of 200 mg/ml showed 68.12+/-1.05 % and petroleum ether extract of bark at the concentration of 200 mg/ml showed 71.25+/-1.72 %. Inhibition of denaturation in hypotonic solution with standard Diclofenac sodium 100 mg/ml showed 79.25%. Therefore, these extracts showed equipotent activity to Diclofenac sodium.

INTRODUCTION: Herbal medicine present in many local varieties depending on the regional flora and many modern drugs were originally extracted from plant sources, even if they are now made synthetically and many other drugs are descended from plant substances.

The inflammatory response involves a complex enzyme activation, mediator release, fluid cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease condition.

Diclofenac is the original non-steroidal anti-inflammatory drug (NSAID).

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Currently much interest has been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent.

The *Holoptelia integrifolia* (family - Ulmaceae) tree locally known as Chirabilva, is a large road side tree distributed throughout India, up to an altitude of 600 m and grows up to 15-18 m height. A specimen of *Holoptelia integrifolia* measuring 33.1 m tall and of girth at breast height 6.91 m near Udaipur (Rajasthan) believed to be 300 years old by considering ethanopharmacological data, in this work, the various extracts of leaf and bark were studied for its *in vitro* anti-inflammatory activity[1-6].

MATERIALS AND METHODS:

Extraction Process: The dried leaves and bark subjected to size reduction and then extracted by petroleum ether, ethyl acetate, ethanol by using

Soxhlet apparatus (successive solvent extraction method).

Then collected extracts were filtered by using filter paper, concentrated under vacuum. Finally ash values and extractive values calculated and are presented in **Table 1 & 2.**

Qualitative phytochemical analysis: The preliminary chemical tests were carried out for the all extracts of leaf and bark of *Holoptelia integrifolia* to identify the presence of various phytoconstitutents [7, 8].

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

The human red blood cell (HRBC) membrane Stabilization method: The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution

RESULT AND DISCUSSION:

Ash Value: (2% dextrose, 0.8% sodium citrate, 0.5% critic acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100 and 200 mg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension were added. It was incubated at 370C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560 nm. Diclofenac (100 and 200 g/ml) was used as reference standard and a control was prepared by omitting the extracts [9-12].

%Protection = (100-optical density of drug treated sample/ optical density of control) x 100.

TABLE 1: DIFFERENT ASH VALUES OF LEAF AND BARK OF *HOLOPTELIA INTEGRIFOLIA*

S. No.	Types of ash value	Observations (%) w/w
1	Total ash	5.7 % (L), 7.6% (B)
2	Acid insoluble ash	5.0 % (L), 6.1% (B)
3	Water soluble ash	0.97% (L), 1.2% (B)

The ash value of the *Holoptelia integrifolia* leaf and bark was calculated and the total ash, acid insoluble ash, and water soluble ash was found out to be 5.7% (L), 5.0% (L). 0.97% (L), 7.6% (B), 6.1% (B) and 1.2% (B) respectively.

Extractive value: The extractive value of leaves and bark of *Holoptelia integrifolia* was calculated and the extractive value of petroleum ether, ethyl acetate, and ethanol was found out to be 2.1% (L), 1.5% (L), 3.2% (L), 1.9% (B), 0.8% (B) and 3.6% (B) respectively.

TABLE 2: DIFFERENT EXTRACTIVE VALUES OF *HOLOPTELIA INTEGRIFOLIA*

S. No.	Extract	Weight of drug (gm)	Extractive value (%) w/v (Leaf)	Extractive value (%) w/v (Bark)
1	Petroleum ether	5	2.1	1.9
2	Ethyl acetate	5	1.5	0.8
3	Ethanol	5	3.2	3.6

***In-vitro* Anti-inflammatory activity of *Holoptelia integrifolia* leaf and bark by HRBC Membrane Stabilization Method:** The investigation is based to identify the newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. *Holoptelia integrifolia* has significant anti-inflammatory activity which may be due to presence of flavonoids, Tri-Terpenoids, Flavonones, steroids and Phenolic compounds. The HRBC Membrane stabilization method was used for the in-vitro anti-inflammatory activity of the petroleum ether, ethyl acetate and ethanol extracts of leafs and bark of *Holoptelia integrifolia* . The HRBC Membrane stabilization activity of the petroleum ether extract of the leaf at the concentration of 200 mg/ml showed 68.12+/-1.05 % and petroleum ether extract of the bark at the concentration of 200 mg/ml showed 71.25 +/-1.72 % inhibition of denaturation in hypotonic solution while standard Diclofenac 100 mg/ml showed 79.25% inhibition of denaturation **(Table 3).**

TABLE 3: %INHIBITION OF DIFFERENT EXTRACTS OF THE *HOLOPTELIA INTEGRIFOLIA*

S. No.	Type of Extract	Concentration (mg/ml)	%inhibition of denaturation
1	Control	-	-
2	Petroleum ether (L)	100	36.25+/-1.23
3	Petroleum ether (L)	200	68.12+/-1.05
4	Petroleum ether (B)	100	41.25+/-1.65
5	Petroleum ether (B)	200	71.25+/-1.72
6	Ethylacetate (L)	100	29.23+/-1.21
7	Ethylacetate (L)	200	46.24+/-1.7
8	Ethylacetate (B)	100	49.29+/-1.82
9	Ethylacetate (B)	200	60.83+/-1.24
10	Ethanol (L)	100	27.23+/-1.21
11	Ethanol (L)	200	30.26+/-1.52
12	Ethanol (B)	100	29.82+/-0.96
13	Ethanol (B)	200	62.25+/-1.34
14	**Diclofenac**	100	78.25+/-1.69

CONCLUSION: Petroleum ether extract of *Holoptelia integrifolia* leaf and bark (200 mg/ml) exhibited membrane stabilization or heat induced hemolytic effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogues to the lysosomal membrane 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 constituent of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage from the above study it was concluded that the petroleum ether extract of leaf and bark (200mg) *Holoptelia integrifolia* has significant membrane stabilization property.

In-vitro anti-inflammatory studies of this plant cause the suppression of both inflammation and arthritis. One of the causes of rheumatoid arthritis is denaturation of proteins and inhibition denaturation is one of the *in vitro* tests to screen anti-inflammatory drugs. From the preliminary screening study, it shows the presence of Flavonones, Flavones, Tri-Terpenoids, steroids, flavonoids and Phenolic compounds. Anti-inflammatory activity of flavonoids has been recognized long back in rodents and reviewed extensively. Some examples include quercetin, silymarin apigenin, daidzein, genistein etc., Hence proper isolation of the active constituents might help in the finings of new lead compounds in the files of anti-inflammatory drug research.

Studies related to active constituent enzyme expression (COX2, lipoxygenase) are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity.

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