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RESEARCH ARTICLE

Screening of solvent extracts of catharanthus roseus l. for isolation of anti-inflammatory compound

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ABSTRACT

Catharanthus roseus (L.) which is an important medicinal plant of the family Apocynaceae is used to treat many of the fatal diseases. *C. roseus* also possess good antioxidant potential. *Catharanthus roseus,* is a potent medicinal plant many of the pharmacological actions such as antimicrobial, antioxidant, anthelmintic, antifeedant, antisterility, antidiarrheal, antidiabetic effect etc. That is used to treat many of the fatal diseases. In the present study, the anti-inflammatory potential of solvent extracts of whole plant of *Catharanthus roseus* and isolation of anti-inflammatory compound from potent extracts was determined. Aqueous and Ethanolic solvent extracts of *Catharanthus roseus* were found to have significant anti-inflammatory activity at doses 250 and 300 mg/Kg during *in vitro* anti-inflammatory assay. During *in vivo* anti-inflammatory activity, the paw edema was reduced significantly in carrageenan induced albino rats through introduction of ethanolic extracts and aqueous extracts at a dosage 300 mg/Kg. Ethanolic and aqueous extracts showed potent anti-inflammatory activity in comparison to non polar solvent extracts. The extracts showed higher anti-inflammatory potential as the dose varies. Thus results showed that extracts showed significant anti-inflammatory activity in dose-dependent manner. The potent polar ethanolic extract of the plant was screened via combination of Column-TLC along with other chromatographic and spectroscopic techniques. The compound was isolated in the form of Quercetin as determined by UV light source at 254 nm on TLC glass plate.

Key words: *Catharanthus roseus*, solvent extracts, Quercetin, anti-inflammatory activity.

INTRODUCTION: Medicinal plants will continue to provide a source for generating novel drug compounds. Plants may become the base for the development of a new medicine or they may be used as phyto medicine for the treatment of disease [1]. It is estimated that plant materials are present in, or have provided the models for 50% Western drugs [2]. The primary benefit of using plant-derived medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments [3]. Many plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components. Today, the search for natural compounds rich in antimicrobial, antioxidant and anti-inflammatory properties is escalating due to their medicinal importance in controlling many related chronic disorders (cancer, diabetes, arthritis, hypertension etc). Ant-inflammatory activities and active principles from plants of North West Himalaya Garhwal region were determined [4-9]. Natural products derived from plants offer a new source of biological that may have a great impact overall human health [10]. Typical inflammatory diseases such as rheumatoid arthiritis, asthma, colitis and hepatitis are among the causes of death and disability in the world [11-13]. Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti inflammatory action [14]. Inflammatory response is a series of well coordinated dynamic mechanism consisting of specific vascular, humoral and cellular events that is characterized by the movement of fluids, plasma and inflammatory leukocytes (neutrophils, eosinophils, basophils and macrophages) to the site of inflammation [15, 16]. A variety of chemical mediators or signaling molecules such as histamine, serotonin, leukotrienes, prostaglandins and oxygen derived free radicals (O_2, OH, $ONOO^-$) are produced by inflammatory and phagocytic

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cells predominantly in the sequences which participates in onset of inflammation [17, 18]. *C. roseus* is extensively cultivated in northern India in order to meet their commercial and the ever increasing demand in the indigenous systems of the medicine also their need to the pharmaceutical industry. Traditionally, the plant has been used for relieving muscle pain, depression of the central nervous system and wasps stings. It is used in the cases of nose bleed, bleeding gums, mouth ulcers and sore throats. It has also been used internally for the treatment of the loss of memory, hypertension, cystitis, gastritis, enteritis, diarrhea and the raised blood sugar levels. Its application ranges widely from the prevention of cancer, cancer treatment, antidiabetic, stomachic etc. *Catharanthus roseus* is the highly exploited and studied medicinal plants as it was found to produce more than 100 monoterpenoid indole alkaloids (MIAs) that include the two major commercially important cytotoxic dimeric alkaloids that are used in the cancer chemotherapy [19]. *Catharanthus roseus* was also found to be a good source of the non-enzymatic and enzymatic antioxidants [20-21]. Other pharmacological uses of *C. roseus* include wound healing, analgesic, vasodilatory and hypoglycemic [22]. Hence, *Catharanthus roseus* (L.) G. Don is found to be the most extensively investigated medicinal plant that is known mainly for its pharmacologically important alkaloids [23]. In the present investigation we have screened polar and non polar solvent extracts of whole plant of *Catharanthus roseus* for anti-inflammatory activities. The potent extract was utilized to isolate anti-inflammatory compound.

Materials and Methods

The chemicals and reagents used were of Analytical Grade and were procured from Ranbaxy and CDH. The animal house used was ethical committee approved in Bundelkhand University, Jhansi (U.P), India.

Collection of Plant material

The whole plant of *Catharanthus roseus* belonging to Apocynaceae family was selected for the study. The herbariums of plant material were prepared and were further identified by Dr. Ajai Swami, Chinmaya Degree College, Haridwar (U.K), India. The whole plant was dried under shade and ground to form the fine powder.

Preparation of Solvent Extracts

The powdered plant material was soaked in approximately 400 ml of ethanol, water, hexane and chloroform separately on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whatmann filter paper No. 1. The filtrate was then concentrated on a rotary evaporator at 50^oC to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4^0C. The extracts doses were optimized by determination of LD_{50} of the dose. The dose at which death of the 50 % of the animal population in the group occurs is known as LD_{50}.

***In vitro* studies for determination of anti-inflammatory potential**

(A) The human red blood cell (HRBC) membrane stabilization method

The method as prescribed [24] was adopted with some modifications 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 (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric 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 (25 and 50 mg/ml) using distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37^0C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection was calculated using the following formula:

Percent Protection (%) = (100- OD of drug treated sample/OD of Control) X 100

(B) Inhibition of Albumen Denaturation

Method as prescribed [25] was followed with minor modifications. The reaction mixture was consisting of test extracts (at concentrations 500 and 600 mg/ml) and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows:

Percent inhibition (%) = (OD of Control-OD of Sample/OD of Control) X100

(C) Heat induced hemolysis

The reaction mixture (2 ml) consisted of 1 ml of test sample solution (at concentrations 500 and 600 mg/ml) and 1 ml of 10 % RBCs suspension, instead of test sample

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only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 minutes. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent hemolysis was calculated by the formula mentioned in the above procedure.

In vivo studies for determination of anti-inflammatory potential

Animals

Extracts of whole plant of *Catharanthus roseus* were evaluated. Male albino rats (180–200 g) were used taking into account international principles and local regulations concerning the care and use of laboratory animals [26] The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22 ± 1ºC with a 12 h light/dark cycle. The *in vivo* anti-inflammatory activity was performed in Bundelkhand University, Jhansi (U.P), India. The institutional animal ethical committee has approved the protocol of the study.

Carrageenan-induced edema in rats

For screening *in vivo* anti-inflammatory activity for each of the extracts, 7 Groups of five animals each were used.

Group I: Treatment with Vehicle/Control (Distilled water); 10 ml/Kg

Group II: Treatment with Vehicle/Control (Ethanol); 10 ml/Kg

Group III: Treatment with Ethanolic extract of whole plant of *Catharanthus roseus* (Test); 250 & 300 mg/Kg

Group IV: Treatment with Aqueous extract of whole plant of *Catharanthus roseus* (Test); 250 & 300 mg/Kg

Group V: Treatment with Hexane extract of whole plant of *Catharanthus roseus* (Test); 250 & 300 mg/Kg

Group VI: Treatment with Chloroformic extract of whole plant of *Catharanthus roseus* (Test); 250 & 300 mg/Kg

Group VII: Treatment with Standard drug, Diclofenac Sodium (10 mg/Kg)

Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile carrageenan in saline into the right hind paw. The solvent extracts of plant at dose of 250 and 300 mg/kg were administered orally 60 minutes before carrageenan injection. Diclofenac Sodium (10 mg/kg) was used as reference drug. Control group received the vehicle only (10 ml/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0, 1, 2, 3, and 4 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Phytochemical screening of the extract

The portions of the dry extracts were subjected to the Phytochemical screening using the method as adopted [27-29] Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars, cardiac glycosides and anthraquinones.

Test for Alkaloids

The 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Wagner's reagent Reddish orange colored turbidity or precipitation was taken as indicator for the presence of alkaloids.

Test for Tannins

About 0.5 g of the sample was dissolved in 10 ml of boiling water and was filtered. Few ml of 6% $FeCl_3$ was added to the filtrate. Deep green color appeared confirmed the presence of Tannins.

Test for Flavanoids

About 0.2 gm of the extract was dissolved in methanol and heated for some time. A chip of mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was Indicator of the flavanoids.

Test for Saponin

About 0.5 g of the plant extract was stirred with water in the test tube. Frothing persists on warming was taken as a evidence for the presence of saponin.

Test for Steroids

Salkowski's method was adopted for the detection of steroids. About 0.5 g of extract was dissolved in 3 ml of chloroform and filtered. To the filtrate, conc. $H_2 SO_4$ was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring [29].

Test for Cardiac glycosides

About 0.5 g of the extract was dissolved in 2ml of glacial acetic acid containing 1 drop of 1% $Fecl_3$. This was under laid with conc. $H_2 SO_4$. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

Test for reducing Sugars

1ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The

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production of a brick red precipitate indicated the presence of reducing sugars.

Test for Anthraquinones

5ml of the extract solution was hydrolyzed with diluted/conc. H_2SO_4. 1 ml of dilute ammonia was added to it. Rose pink colour confirmed the presence of anthraquinones.

Isolation and characterization of anti-inflammatory compound from the potent extract via chromatographic and spectroscopic techniques

The potent polar solvent extracts (ethanolic extract) of the plant were screened via combination of Column-TLC along with other chromatographic techniques.

Column-TLC:

The potent fractions obtained from Column chromatography of the extracts were determined for potent molecule by TLC. The solvent system used in TLC was Toluene: Ethyl Acetate: methanol (5: 3: 2, v/v). Chromatograms were developed at room temperature (24°C) in glass twin-trough chambers (100 mm × 100 mm, with metal lids) previously saturated with mobile phase vapor for 30 minutes. Ascending mode was used for development of thin layer chromatography. Further, the TLC plate was dried in a current of air with the help of an air dryer at 110°C for 10 minutes, and immediately. The presence or absence of the investigated compounds was determined according to their Rf values with the corresponding spot of expected standards. Calculations for percentage were done considering standard and sample Rf, AUC and dilution factor**.**

HPLC:

Chromatographic analysis was carried out by C18v reversed phase column (250 x 4.6 mm) packed with 5µm diameter particles. The mobile phase was methanol: acetonitrile: water (60:20:20 v/v/v). The mobile phase was filtered through a 0.45 µm membrane filter. Further deaerated ultrasonically prior to use. Flow rate and injection volume were 1.1 ml /min and 10 µl. The Chromatographic peaks of the analytes were confirmed by comparing their Retention time and UV Spectra with those of the reference standards.

Preparation of Standard Solution

About 5 mg of given Quercetin standard was accurately weighed and taken into 10 ml volumetric flasks and dissolved in 10 ml mobile phase. To prepare 0.5 mg/ml sample solution. The volumetric flask contains standard solution kept for sonication for 10 minutes. Then standard solution was filtered with 0.45 µm membrane filter paper with sample filter

Results and Discussion

Aqueous and Ethanolic solvent extracts of *Catharanthus roseus* were found to have significant anti-inflammatory activity at doses 250 and 300 mg/Kg during *in vitro* anti-inflammatory assay. During *in vivo* anti-inflammatory activity, the paw edema was reduced significantly in carrageenan induced albino rats through introduction of ethanolic extracts and aqueous extracts at a dosage 300 mg/Kg. Ethanolic and aqueous extracts showed potent anti-inflammatory activity in comparison to non polar solvent extracts. The extracts showed higher anti-inflammatory potential as the dose varies. Thus results showed that extracts showed significant anti-inflammatory activity in dose-dependent manner.

***In vitro* studies for determination of anti-inflammatory potential**

The human red blood cell (HRBC) membrane stabilization method:

Amongst aqueous and ethanolic extracts, the ethanolic extracts and aqueous extracts at a concentration of 300 mg/ml showed 82.5 ±0.06 % and 81.13±0.06 % respectively protection activity of HRBC in hypotonic solution in comparison to hexane extracts (63.34 ±0.06 %). The results were compared with standard Diclofenac Sodium which showed 83.54 ±0.06 % protections at 300 mg/ml. *Catharanthus roseus* whole plant extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The results are shown in **Table 1** and **Figure 1**.

Table 1: Percent activity of HRBC membrane stabilization

S. No.	Groups	Concentration of Extracts of *Catharanthus roseus*	Percent Protection± SD
I	Control	250 mg/ml	-
		300 mg/ml	-
II	Ethanolic extract	250 mg/ml	70.12 ±0.06
		*300 mg/ml	82.5±0.06
III	Aqueous extract	250 mg/ml	78.5 ±0.06
		*300 mg/ml	78.34 ±0.06
IV	Standard (Diclofenac Sodium)	25 mg/ml	72.34 ±0.06
		*50 mg/ml	83.54 ±0.06

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***, potent activity**

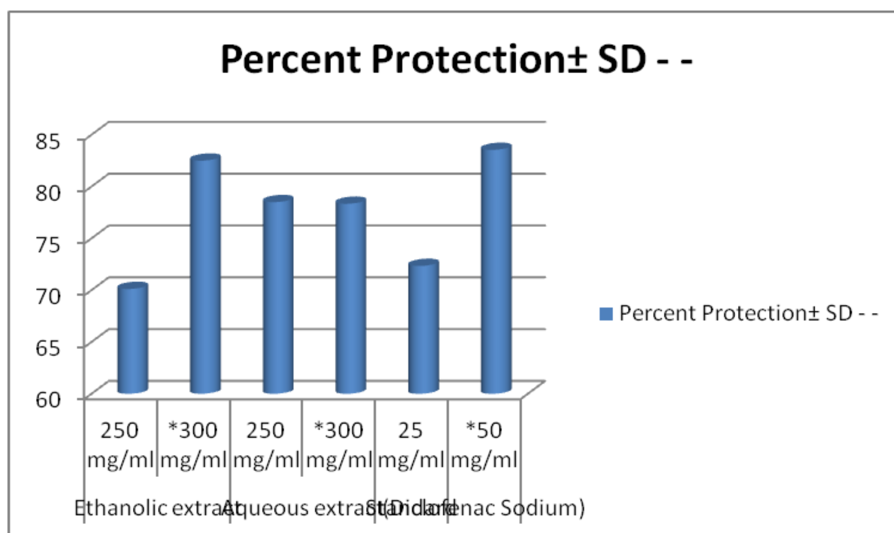

Figure 1: Percent activity of HRBC membrane stabilization

Inhibition of Albumen Denaturation:

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of extract protein denaturation was studied. The ethanolic and aqueous extracts were found to be effective in inhibiting heat induced albumin denaturation in comparison to aqueous extracts at a dose of 300 mg/ml. Maximum inhibition 83.31±0.06 was observed from ethanolic and aqueous extracts equally followed by hexane extracts (63.31±0.06). The results were compared with standard Diclofenac Sodium which showed 93.54 ±0.06 % inhibition in albumen denaturation at 300 mg/ml. Since during inflammation condition, protein of the cell gets denatured, thus here albumen protein is used as a model whose protection in denaturation by plant extracts was studied. The results are shown in **Table 2** and **Figure 2**.

Table 2: Percent inhibition of albumen denaturation

S. No.	Groups	Concentration of Extracts of *Catharanthus roiseus*	Percent Protection± SD
I	Control	250 mg/ml	-
		300 mg/ml	-
II	Ethanolic extract	250 mg/ml	76.2 ±0.06
		*300 mg/ml	83.31±0.06
III	Aqueous extract	250 mg/ml	72.0 ±0.06
		*300 mg/ml	78.01±0.06
IV	Standard (Diclofenac Sodium)	*50 mg/ml	93.54 ±0.06

*Potent Extracts/Drug

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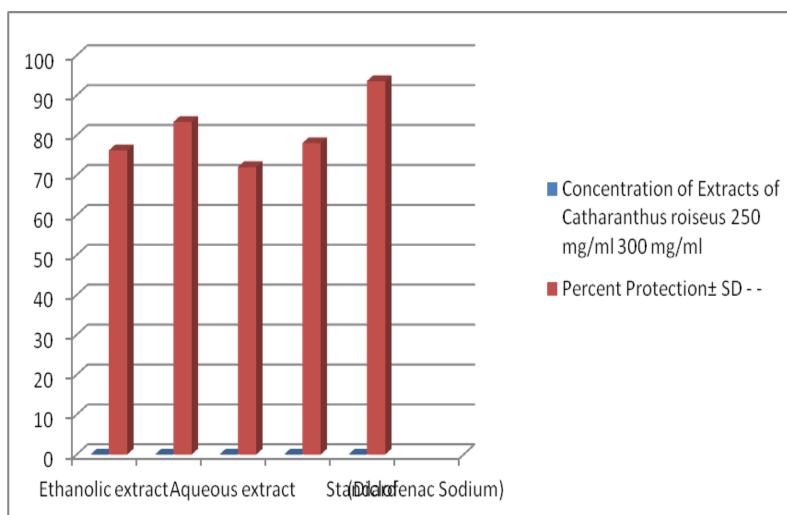

Figure 2: Percent inhibition of albumen denaturation

Heat induced hemolysis:

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different polar extracts of the plant. Both the extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree. The maximum inhibition was recorded 88.82±0.06% equally from ethanolic and aqueous extracts followed by hexane extracts (56.25±0.05 %). The results were compared with standard Diclofenac Sodium which showed the maximum inhibition 85.92 ±0.05 % at 120 mg/ml. Heat induced hemolysis method is another method depicting HRBC membrane stabilization. The results are shown in **Table 3** and **Figure 3**.

Table 3: Percent Inhibition of Heat Induced Hemolysis

S.No.	Groups	Concentration of Extracts of *Catharanthus roseus*	Percent Protection± SD
I	Control	250 mg/ml	-
		300 mg/ml	-
II	Ethanolic extract	250 mg/ml	72.2 ±0.06
		*300 mg/ml	88.82±0.06
III	Aqueous extract	250 mg/ml	62.0 ±0.06
		*300 mg/ml	78.81±0.05
IV	Standard (Diclofenac Sodium)	25 mg/ml	78.54 ±0.06

, potent extract

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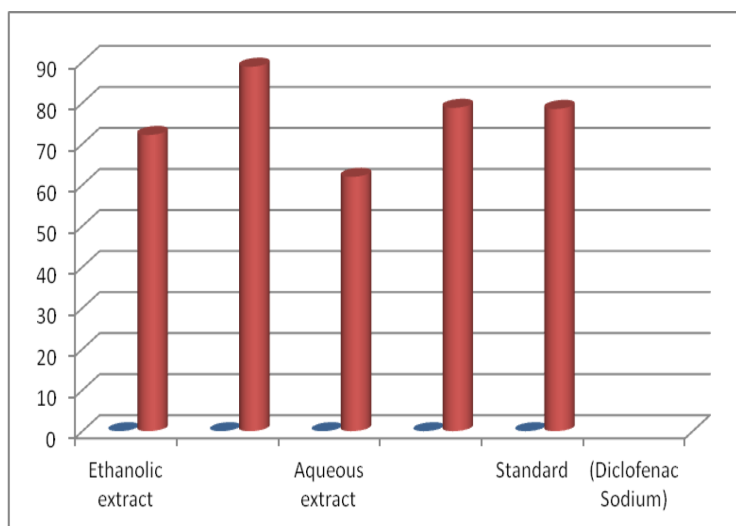

Figure 3: Percent Inhibition of Heat Induced Hemolysis

In vivo studies for determination of anti-inflammatory potential

Carrageenan-induced edema in rats

The anti-inflammatory activities of extracts were found to have effect in dose-dependent manner. There was a gradual increase in edema paw volume of rats in the control group. However, in the test groups, ethanolic extract and aqueous extract (300 mg/kg) showed a significant reduction in the edema paw volume. There was no reduction in inflammation found in case of rats treated with ethanol and distilled water. The results showed that ethanolic fractions of the plant causes significant reduction in inflammation i.e. 85 % (300 mg/kg) followed by aqueous extract i.e. 83 % (300 mg/kg) compared to standard anti-inflammatory drug, Diclofenac Sodium i.e. 87 % (10 mg/kg). The values of reduction in paw volume, 0.10 ± 0.05, 0.12 ± 0.05 and 0.16 ± 0.05 were found significantly of ethanol extract, aqueous extract and Diclofenac sodium, respectively at 4 h after carrageenan administration. The results are shown in **Table 4**.

Table 4: Anti-inflammatory activities of different extracts of _Catharanthus roseus_

Paw volume (ml) ± SD

Experiment	Control	Diclofenac Sodium (10 mg/kg orally)	Ethanol extract (300 mg/kg)	Aqueous extract (300 mg/kg)	Ethanol (300 ml/kg)	Distilled water (300 ml/kg)
er treatment	0.25±0.05	0.21±0.05	0.23± 0.05	0.28±0.003	0.20±0.05	0.34±0.05
2h after treatment	0.25±0.05	0.18±0.05	0.20± 0.05	0.24±0.05	0.15±0.05	0.34±0.05
4h after treatment	0.25±0.05	0.16±0.05	0.10± 0.05	0.12±0.05	0.30±0.05	0.34±0.05

±, S.D, Standard Deviation (P<0.05).

Phytochemical screening:

Different conventional methods were followed to determine qualitatively the presence of phytochemical constituents present in the potent extracts. The ethanol and aqueous extracts of the plant possessed alkaloids, steroids, saponin, reducing sugars, tannins, cardiac glycosides and anthraquinones while only flavanoids were found to be absent. The results are indicated in **Table 5.**

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Table 5: Phytochemical Screening of the active constituents

	Phytoconstituents							
Solvent extracts	**Alkaloids**	**Tannins**	**Flavanoids**	**Saponin**	**Steroids**	**Cardiac glycosides**	**Reducing sugars**	**Anthraquinones**
Ethanolic extract	+	+	_	+	+	+	+	+
Aqueous extract	+	+	_	+	-	+	+	+

*+, present; -, absent

Isolation of anti-inflammatory compound from ethanolic extract of *Catharanthus roseus*

The potent polar solvent extracts (ethanolic extract) of the plant were screened via combination of Column-TLC along with other chromatographic and spectroscopic techniques.

Column-TLC:

Further, the TLC plate was dried in a current of air with the help of an air dryer at 110°C for 10 minutes, and immediately. The presence or absence of the investigated compounds was determined according to their Rf values with the corresponding spot of expected standards. Calculations for percentage were done considering standard and sample Rf, AUC and dilution factor. The compound was isolated in the form of Quercetin as determined by UV light source at 254 nm.

Figure 4: TLC chromatogram as viewed in UV light of the compound extracted (Quercetin) along with standard

HPLC:

RP-HPLC separation of standard Quercetin was carried out at 262 nm. Flow rate and injection volume were 1.1 ml /min and 10 µl. The Chromatographic peaks of the analytes were confirmed by comparing their Retention time and UV Spectra with those of the reference standards. Quercetin was determined out by the integration of the peak using external standard method with reference to the standard. All chromatographic operations were carried out at ambient temperature. The results are shown in **Figure 5(a)** & **(b).**

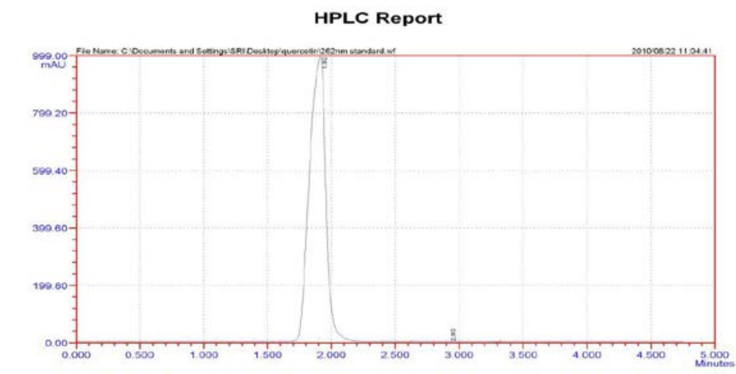

Figure 5 (a): HPLC chromatogram of Quercetin standard

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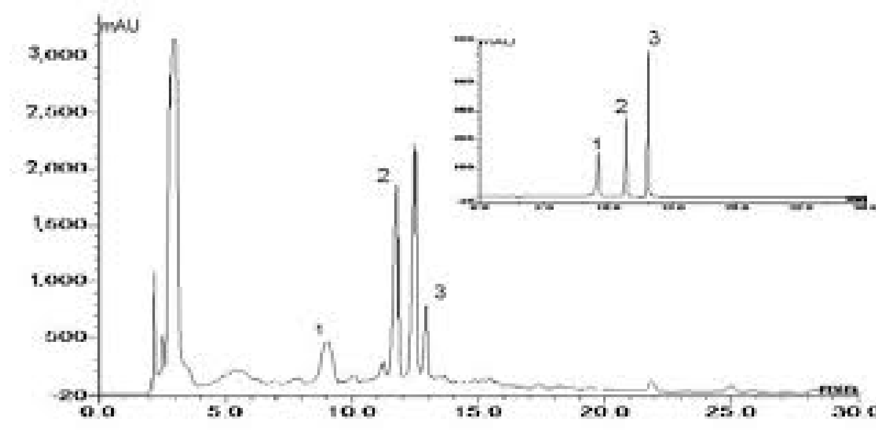

Figure 5 (b): HPLC chromatogram of Quercetin extracted from C. roseus

In the present investigation carried out to evaluate the anti-inflammatory potential of solvent extracts of *Catharanthus roseus* (Family: Apocynaceae) through *in vitro* and *in vivo* procedures, the results were found to be very surprising and promising. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane [15] 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 constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage [18]. From the above study it was concluded that the ethanolic extract of *Catharanthus roseus* has significant membrane stabilization property compared to the aqueous extract of the same plant and it was comparable to the standard drug Diclofenac Sodium. Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation. From the above study it was concluded that the ethanolic extract of *C.roseus* had maximum albumen denaturation protection property as compared to the aqueous extract and other non polar solvent extracts of the plant. The results were found in reference to the standard drug Diclofenac Sodium. The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The precise mechanism of this membrane stabilization is yet to be elucidated; it is possible that the *Catharanthus roseus* produced this effect by reducing the surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins. The above findings also confirmed that ethanolic extracts of *Catharanthus roseus* possessed maximum protection activity of RBC membrane by heat induction in comparison to aqueous extracts. This can be due to the reduction in heat induced by inflammation (associated with any disease) by the effect of plant extracts. When the extracts were evaluated for in vivo anti-inflammatory activity on carrageenan induced albino rats, the ethanolic and aqueous extracts also showed the similar pattern as that of in vitro studies. In the test groups, ethanolic extract and aqueous extract (300 mg/kg) showed a significant reduction in the edema paw volume. There was no reduction in inflammation found in case of rats treated with ethanol and distilled water. The results showed that ethanolic fractions of the plant causes significant reduction in inflammation i.e. 85 % (300 mg/kg) followed by aqueous extract i.e. 83 % (300 mg/kg) compared to standard anti-inflammatory drug, Diclofenac Sodium i.e. 87 % (10 mg/kg). The values of reduction in paw volume, 0.10 ± 0.05, 0.12 ± 0.05 and 0.16 ± 0.05 were found significantly of ethanol extract, aqueous extract and Diclofenac sodium, respectively at 4 h after carrageenan administration. The non polar solvent extracts viz. hexane and chloroform extract showed much minimal anti-inflammatory potential in comparison to polar ethanol and aqueous extracts. The pure anti-inflammatory compound isolated from the potent ethanolic extract of the plant, *Catharanthus roseus* was Quercetin as revealed from HPLC and TLC profile. It was found that the compound showed potent reduction (85 %) in paw edema in comparison to standard drug (70 %) ($P<0.05$).

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(Uttarakhand) India for HPLC and analysis of the isolated fraction.

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