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Phytochemical Screening and in vitro Anti-inflammatory Activity of Ficus rumphii extracts

Lalit Singh[1], Rohit Bhatia[1*], Neeraj Kumar[2], Ravindra K. Rawal[3]

[1]Department of Pharmaceutical Analysis, ISF College of Pharmacy, G.T. Road, Moga, Punjab, India

[2]Department of Devsthali Vidyapeeth College of Pharmacy Rudrapur, Udham Singh Nagar, Rudrapur, Uttarakhand, India

[3]Department of Chemistry, Maharishi Markandeshwar (Deemed to be University), Mullana, Haryana, India

[*]**Corresponding author:** Bhatia R, Department of Pharmaceutical Analysis, ISF College of Pharmacy, G.T. Road, Moga, Punjab, India; E-Mail: bhatiarohit5678@gmail.com

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Abstract

Inflammation is an important nonspecific defense reaction to tissue injuries, such as that caused by a pathogen or wound. Acute inflammation is the rapid response of the body to injury or cell death. There are several plants which possess significant anti-inflammatory properties and Ficus rumphii is one of them. Objective of the present study was to evaluate the anti-inflammatory activity of methanolic extracts of leaves, bark, and root of Ficus rumphii. The in-vitro anti-inflammatory activity was investigated by using the albumin denaturation assay method using aspirin as a reference drug. Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, saponins and carbohydrates in leaves; alkaloids, saponins, flavonoids, amino acid and carbohydrates in root; whereas carbohydrates, amino acid, saponins, flavonoids, and tannins were identified in bark. All the extracts (leaves, bark, and root) were tested at the concentration 50 to 500 µg/mL. Different extract of F. rumphii exhibited a concentration-dependent inhibition of albumin. From the results, it was concluded that phytochemicals (tannins, phenols, and saponins) present in the F. rumphii extract may be responsible for the anti-inflammatory.

Keywords: Inflammation, pathogen, Ficus rumphii, phytochemical analysis, flavonoids, saponins.

Introduction

Inflammation is the immediate defensive body response to foreign unfavorable spur such as trauma, pathogen or irritant chemicals which is characterized by its unique symptoms; redness, warmth, swelling and pain [1]. The primary target of inflammatory response is to confine and abolish the destructive stimuli as well as to isolate the ruined tissue components to culminate in healing of the affected tissues, organs, or system [2-4]. There are several kinds of anti-inflammatory drugs available in market to get an immediate response against inflammation [5]. Sadly, these classes of drugs are associated with mild to severe side effects such as liver disorders, gastric lesions, cardiovascular complications and sometimes renal failure [6,7]. Therefore, plant resources are the best choice to develop newer anti-inflammatory agents with least risk of side effects. Plants can be utilized to isolate and synthesize a wide verity of phytochemical compounds as secondary metabolites. It is evident that, huge numbers of photochemical have been used effectively to treat the various ailments for mankind. World Health Organization has identified and enlisted about 20,000 plant species which can be utilized to develop newer medicines against various human ailments. Most of the medicinal plant parts are used as raw drugs and they possess diverse biological properties [8]. Currently many plant derived anti-inflammatory principles are being used as medicines and are devoid of any toxic effects. The common types of inflammation observed in patients include appendicitis, bursitis, colitis, cystitis, dermatitis, phlebitis, rhinitis, tendonitis, tonsillitis, vasculitis etc. Acute inflammation is characterized by marked elevation in capillary intrusion, vascular permeability and abnormal activity of leukocytes whereas chronic inflammation can be symptomized by macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis), intrusion of immune cells and fibrosis [9]. Generally, tissue damage provokes an inflammatory response by the production of mediators and chemotactic factors. The reactive oxygen species (ROS) are also known to activate matrix metalloproteinase (e.g. collagenase) causing increased ruin of tissues e.g. collagenase damage seen in many arthritic reactions. These plant derived constituents scavenge these reactive oxygen species and are highly beneficial in the treatment of inflammatory disorders [10].

Several reports are available in literature which describe the anti-inflammatory potentials of herbal drugs. Herbal drugs are frequently used due to their excellent potency, low range of side effects, easy availability and low cost. Medicinal plants are continuously being used since ancient time specially in developing countries for the management of inflammation. In recent years, there is an increasing consciousness about the importance of medicinal plants [11]. Ficus rumphii is a deciduous tree that can grow to a height of 20 meters [12,13]. The plant often begins life as an epiphyte, growing in the branch of another tree. This plant has been used since ancient time in the effective treatment of hypothermia, emetic, anti-cough, diuresis and CNS disorders [14,15]. The anti-inflammatory

potential of herbal drugs or extracts can be evaluated either by in vitro or in vivo models. Many protocols are described in each model to determine the anti-inflammatory activity. In this work the phytochemical evaluation of methanolic extracts of Ficus rumphii leaves, roots and barks was carried out and anti-inflammatory activity was evaluated through albumin denaturation assay method using aspirin as a reference drug.

Materials and Methods

Procurement of Plant Material

The fresh leaf, bark and root of Ficus rumphii were procured from the local herbal garden of Devsthali Vidyapeeth College of Pharmacy (DVCP). Pure, analytical grade chemicals and reagents were obtained from the chemical store of DVCP.

Extraction and preparation of extract

The dried barks, roots, leaves were pulverized and about 45.50g of bark, 61g of root and 50g of leaf powder was extracted with methanol in Soxhlet apparatus for 18 hours [16]. The solvent was evaporated with the help of rota-evaporator and further crude extract was obtained by heating on water bath. The yield of the methanol extract of Ficus rumphii leaves, bark, root was found to be 30.02%, 40.032%, 50.12%.

Preparation of Phosphate Buffer 6.4

Weighed 2.5g of potassium dihydrogen phosphate, 2.5g of disodium hydrogen orthophosphate and 8.2g of NaCl and were dissolved in sufficient quantity of distilled water to make up the volume 950mL with distilled water [17].

Phytochemical analysis

The methanolic extracts of leaves, bark and roots were evaluated for the presence of various phytoconstituents by employing various chemical tests

In vitro Anti-Inflammatory activity

2 ml of varying concentrations (50,100,200,300,400,500 µg/ml) of plant extract was mixed with 0.2 ml of egg albumin. PH was adjusted to 6.4 by addition of 2.8 ml of phosphate buffer. 5 ml of double distilled water was taken as control and further the mixture was incubated at 37o ± 2oC in a BOD [biological oxygen demand] incubator for 15 min. After incubation the mixture was heated at 70o C for 5 minutes followed by cooling. Then their absorbance was measured at 660 nm by using distilled water as blank. Aspirin was taken as a reference standard for comparison of results [18].

% Inhibition = (Abs control – Abs sample)/ Abs control x 100

Results and Discussion

Utilization of animals for evaluation of pharmacological activity is associated with some problems such as non-availability of suitable models, ethical considerations and lack of rationale. Therefore, in our study we have chosen in vitro albumin denaturation assay method to evaluate the anti-inflammatory activity of the methanolic extracts of root, bark and leaves as. It is evident that denaturation of certain tissue proteins is the significant cause of many inflammatory diseases. Therefore, development of newer therapeutic agents which are able to prevent protein denaturation may serve as drug candidates for the treatment of inflammation.

Phytochemical Analysis

The results of phytochemical analysis have been summarized in table 1. All the abstracts showed a difference in presence of various phyto-constituents. The results revealed the presence of flavonoids, alkaloids, tannins, saponins, and carbohydrates in leaves; whereas alkaloids, saponins, flavonoids, amino acid, and carbohydrates in root, whereas carbohydrates, amino acid, saponins, flavonoids, and tannins in bark.

S.No.	Phytochemical constituent	Chemical test	Bark extract	Leaf extract	Root extract
1	Carbohydrate	Molish Test	+	+	-
		Fehling Test	+	-	+
2	Proteins	Million's Test	-	-	-
		Biuret's Test	-	-	-
		Xanthoprotein Test	-	-	-
3	Amino acid	Ninhydrin Test	+	-	+
4	Steroid	Salkowaski Reaction	-	-	-
		Liberman-Burchard Reaction	-	-	-
		Liberman's Reaction	-	-	-
5	Triterpenoid	Thionyl Chloride Test	-	-	-
6	Glycoside	Legal Test	-	-	-
		Keller killiani Test	-	-	-
7	Saponin Glycoside	Foam Test	+	+	+
8	Flavonoid	Shinoda Test	+	+	+
9	Alkaloid	Dragendroff's Test	-	+	+
		Mayer's Test	-	-	-
		Wagner's Test	-		

10	Tannins and phenolic constituents	Lead Acetate Test	-	+	+
		Ferric Chloride Test	+	+	+

In vitro anti-inflammatory activity

In the present investigation, the in vitro anti-inflammatory effect of methanolic extract of root bark and leaves was evaluated against denaturation of egg albumin. The results are summarized in Table 2. The present findings exhibited a concentration dependent inhibition of protein albumin denaturation by methanolic extract of root, bark and leave throughout the concentration range of 100 to 500 µg/mL (Figure 1). Aspirin, at a concentration 200 µg/mL, was used as a reference drug. The change in absorbance of sample with respect to control is an indicator of protein stability i.e., inhibition of protein (albumin) denaturation or anti-denaturation effect by the test extracts and the reference drug aspirin. In current work, in-vitro anti-inflammatory test, the 500µg/ml of crude methanol extract of Ficus rumphii leaves, bark, root, showed mean inhibition of protein denaturation 95.3±.016%, 67±.0036%, 94.87±.01% and whereas for 200µg/ml of Aspirin showed the maximum inhibition 75.89±.56%.

It is well established that polyphenolic compounds of natural origin possess wide range of promising biological properties [19]. Hence it can be concluded that the in vitro anti-inflammatory effect displayed by the methanolic extracts, may be due to their polyphenols contents or may be synergistic effects of two or more constituents instead of single constituent.

Test Sample	Concentration (µg/ml)	% Inhibition Albumin Denaturation
Methonolic Extract of Ficus rumphii Leaves	100	15±.046%
	200	42±.02%
	300	86.3±.03%
	400	92.5.016%
	500	95.3±.016%
	100	3.5±.0046%
Methonolic Extract of Ficus rumphii Bark	200	15±.0020%
	300	25±0025%
	400	51.75±.0037%
	500	67±.0036%
Methanolic Extract of Ficus rumphii root	100	12±.06%
	200	45.87±.05%
	300	72.62±.05%
	400	82.00±.01%
	500	94.87±.01%
Aspirin	200	78.38±.01%

Table 2: Effect of methanol extract of Ficus rumphii on albumin denaturation

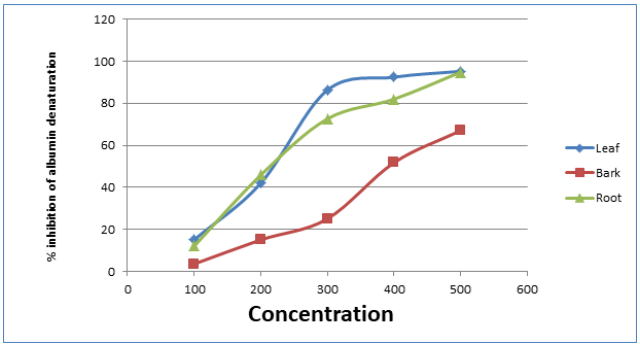

Figure1: Effect of methanol extract of Ficus rumphii on albumin denaturation

Conclusion

It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH. The current investigation revealed that Ficus rumphii had marked anti-inflammatory effect against the denaturation of protein in vitro. It is well evident that inflammatory and arthritic disease can be caused by the denaturation of tissue proteins. Therefore, it is suggested that anti-inflammatory effect of this plant should be further evaluated in other experimental models in pursuit of newer phototherapeutic agents against inflammation.

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