INVITRO ANTI INFLAMMATORY ACTIVITY OF PLANT EXTRACT OF PHYLLANTHUS RETICULATUS

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ABSTRACT

Phyllanthus reticulatus is valuable medicinal plant which has been valuable for centuries in Ayurveda medicine. The phytochemical analysis of phyllanthus reticulatus plant extracted (ethanol, benzene, aqeous) for the presence of various chemical compound such as flavonoids. Glycosides, alkaloids, starch, amino acid, lignin, volatile oil, fat and fixed oils, mucilage, pectin's, protein, steroids, and tritepeniods. Since flavonoids. Glycosides have remarkable anti-inflammatory activity. Our present work aims at In vitro anti-inflammatory activity of *phyllanthus reticulatus* by HRBC and protein denaturation method. Aspirin used in standard drug. The data of our studies suggests that phyllanthus reticulatus of plant showed significant anti-inflammatory activity.

KEYWORDS: *Phyllanthus Reticulatus,* Aqueous extract, Anti-inflammatory activity, HRBC method, Protein denaturation.

INTRODUCTION

Phyllanthus Reticulatus also known as the "Netted Phyllanthus" is a medicinal plant belonging to the family Phyllanthaceae. It is widely distributed in India, Bangladesh, Bhutan, china, etc. it has been traditionally used in Ayurveda medicine for the treatment of various ailments including inflammation, jaundice, and gastrointestinal disorders.

In recent years, there has been an increasing interest in pharmacological properties of *Phyllanthus Reticulatus,* including its potential anti-inflammatory activity. Inflammation is a complex physiological response to tissue damage, infection, or other irritants, and it plays a crucial role in the pathogenesis of several chronic diseases such as arthritis, asthma and inflammatory bowel disease.

Several studies have investigated the in-vitro anti-inflammatory activity of *Phyllanthus Reticulatus*. Plant extracts using different models. These studies have demonstrated that *Phyllanthus Reticulatus* plant extracts possess significant anti-inflammatory activity, which is attributed to the presence of various bioactive compounds such as flavonoids, alkaloids, and tannins.

The aim of this review to provide an overview of the articles published on the in-vitro anti-inflammatory activity of *Phyllanthus Reticulatus* plant extracts. The review will highlight the various experimental models used to evaluate the anti-inflammatory activity of *Phyllanthus Reticulatus* plant extracts and the underlying mechanisms of action. Additionally, the review will discuss the potential applications of *Phyllanthus Reticulatus* plant extracts as a natural anti-inflammatory agent for the treatment of inflammatory diseases.

Overall, the review of published articles on the in-vitro anti-inflammatory activity of *Phyllanthus Reticulatus* plant extracts suggests that this plant may be a promising source of natural anti-inflammatory agents. [1]

MATERIALS & METHOD

PLANT MATERIAL

The whole plant of *Phyllanthus Reticulatus* were collected from the area of sankari in salem district, Tamilnadu, India. The whole plant of Phyllanthus Reticulatus The plant material was authenticated by Dr. M. U. SHARIEF., Director, Botanical Survey of India, Coimbatore, Tamilnadu, India and a voucher specimen no:BSI/2022/577was deposited at the museum Sri Shanmugha College of Pharmacy, Sankari (637304) Tamilnadu, India.

EXTRACTION

Fresh Whole plant of *Phyllanthus Reticulatus* were collected cut into small pieces and dried under shade morning time for 10 days. The dried parts were passed through sieve (coarse 10/40) this powder was used for the preparation of solvent extraction and 500 gram of powder was extracted by soxhlet.

EXTRACTION OF PLANT MATERIAL

The rhizome material was dried in the shade for two month. Then shade dried plant was subjected to get coarse powder. The coarse powders were subjected to Soxhlet apparatus by using various solvent according to their polarity

BENZENE EXTRACT

The marc left after petroleum ether extraction was dried and extracted with 2-3 liters of benzene of (79-81^{o}C) by continuous hot percolation using Soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was then stored in a desiccators. A brown colour residue was obtained.

ALCOHOL EXTRACT

The marc left acetone extraction was dried and extracted with 2-3 liters of alcohol 95% by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. The extract was stored in desiccators. A light brown color residue was obtained.

AQUEOUS EXTRACT

The marc left after alcohol extraction was dried and macerated with 2-3 liters of chloroform water (0.25%) in mouthed bottle for three days. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was then store in desiccators. A black colour residue was obtained. All the above extracts were used for identification of constituents by phytochemical tests. From the weight of drug, the extract content was calculated. [2]

$$\text{Extractive Value (\%)} = \frac{wt.of\ extractive}{wt.of\ drug} \times 100$$

PHARMACOLOGICAL SCREENING

Evaluation of in-vitro anti-inflammatory activity

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 (2% dextrose,0.8% sodium citrate,0.5% citric acid and 0.42% NaCl) and centrifuged at 3000rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various Concentrations of extract were prepared (20, 60,80 & 100µg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension were added. It was incubated at 370 c for 30min and centrifuged at 3000rpm for 20min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm Aspirin (100µg/ml) was used as reference standard and a control was prepared by omitting the extract. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula.[5]

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

Abs control =Absorbance of control

Abs sample = Absorbance of sample

MEMBRANE STABILIZAION TEST

PREPARATION OF RBC SUSPENTION:

Fresh whole human blood (10ml) was collect and transferred to the haparinized centrifuged tube. The tubes were centrifuged at 3000 rpm for 10 min and were washed three time with equal volume of normal saline. The volume of the blood was measured and reconstituted at 10%v/v suspension with normal saline.

HEAT INDUCED HEMOLYSIS:

The reaction mixture (2ml) consisted of 1ml of test drug solution and 1ml of 10% HRBC suspension. Instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing mixture were incubated in a water bath at 56o C for 30 mints. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 mints and the absorbance of the supernatants was taken at 560nm.The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by using the formula, [6 7]

100 x (Vt / Vc – 1)

Where,

Vt = Absorbance of test sample

Vc = Absorbance of control

Inhibition of albumin denaturation:

The anti-inflammatory activity of test drug was studied by using inhibition of albumin denaturation technique. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm.(UV Visible Spectrophotometer).

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

RESULT

PHYTO – CHEMICAL INVESTIGATION:

The powder and various extracts of the plant were subjected to chemical tests for identification of its active constituents.[3,4]

Table 1. Preliminary Phyto Chemical Test of *Phyllanthus Reticulatus*

CHEMICAL TEST	EXTRACTS			
	BENZENE	ETHANOL	AQUEOUS	PHYLLANTHUS RETICULATUS
ACIDIC COMPOUNDS	-	+	-	+
ALEURONE GRAINS	+	+	+	+
ALKALOIDS	+	+	+	+
AMMINO ACIDS	-	+	+	+
CARBOHYDRATES	–	–	–	–
CELLULOSE	_	_	_	_
LIGNIN	+	_	_	+
VOLATILE OIL	+	+	+	+
FATS &FIXED OILS	+	+	_	+
FLAVONOIDS	+	+	+	+
GLYCOSIDES	_	+	_	+
INULIN	-	-	-	-
MUCILAGE	+	+	+	+
PECTINS	+	-	-	+
TANNINS	-	+	-	+
PROTEINS	+	+	+	+
STARCH	-	-	+	+
STEROIDS &TRITERPENOIDS	+	+	-	+
NAPTHOQINONES	-	-	-	-

1. Inhibition of protein denaturation:

The in vitro anti-inflammatory effect of *Phyllanthus Reticulatus* was evaluated by denaturation of bovine albumin. The results are summarized in Table: 2 and Fig No: 1. The in vitro anti-inflammatory effect of *Phyllanthus Reticulatus* was performed by inhibition of protein denaturation method. *Phyllanthus reticulatus* showed significant anti-inflammatory activity in a concentration dependant manner.

Ethanol extract of *Phyllanthus Reticulatus* at concentration of 20, 60, 80 and 100 µg/ml showed 23%, 28%, 48% and 56% inhibition.

Aqueous extract of *Phyllanthus Reticulatus* at concentration of 20, 60, 80 and 100 µg/ml showed 18%, 20%, 39% and 51% inhibition.

Benzene extract of *Phyllanthus Reticulatus* at concentration of 20, 60, 80 and 100 µg/ml showed 28%, 40%, 62% and 69% inhibition.

All the results were compared with standard drug Aspirin at 100µg/ml which showed 72% of protein denaturation respectively.

Table 2. *Invitro* Anti Inflammatory activity of Plant Extraxt of *Phyllanthus Reticulatus* On inhibition of Protein Denaturation.

Treatment	Concentration	Absorbance at 660 nm	% Inhibition
Control	-	0.37	-
Ethanol	20	0.26	23
	60	0.25	28
	80	0.19	48
	100	0.16	56
Aqueous	20	0.30	18
	60	0.28	20
	80	0.21	39
	100	0.18	51
Benzene	20	0.30	18
	40	0.29	21
	80	0.22	40
	100	0.20	36
Aspirin	100	0.10	72

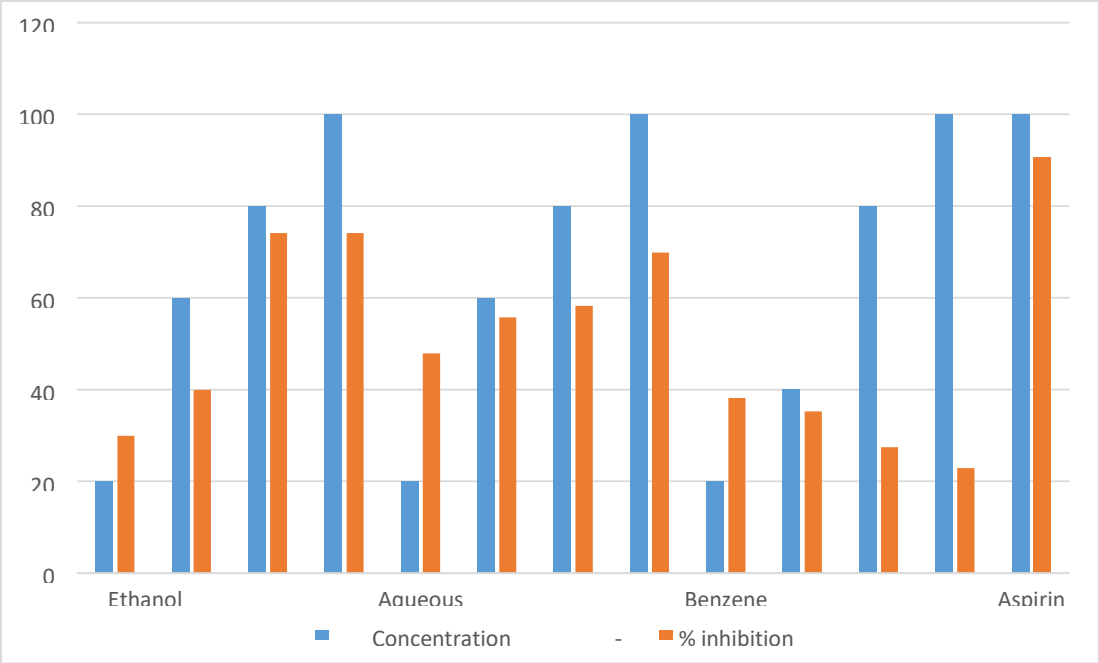

Fig.No: 1 *Invitro* Anti Inflammatory activity of Plant Extract of *Phyllanthus Reticulatus* On inhibition of Protein Denaturation

2. Human red blood cell membrane:

The result of in vitro anti-inflammatory activity of *Phyllanthus Reticulatus* On human red blood cell membrane were given in Table: 3 and Fig. No: 2. the in vitro anti-inflammatory activity of *Phyllanthus Reticulatus* was performed by using human red blood cell membrane stabilization method. *Phyllanthus Reticulatus* showed significant anti-inflammatory activity in a concentration dependant manner.

Benzene extract of *Phyllanthus Reticulatus* at concentration of 20, 60, 80 and 100 µg/ml showed 38%, 35%, 27% and 22% inhibition.

Ethanol extract of *Phyllanthus Reticulatus* at concentration of 20, 60, 80 and 100 µg/ml showed 30%, 40%, 56% and 74% inhibition.

Aqueous extract of *Phyllanths Reticulatus* at concentration of 20, 60, 80 and 100 µg/ml showed 48%, 56%, 58% and 70% inhibition.

All the results were compared with standard drug Aspirin at 100 µg/ml which showed 91% inhibition of Human red blood cell membrane.

Treatment	Concentration	Absorbance at 660 nm	% Inhibition
Control	-	1.412	-
Ethanol	20	0.9893	29.91
	60	0.8481	39.91
	80	0.6193	56.24
	100	0.3651	74.08

Aqueous	20	0.7355	47.84
	60	0.6246	55.73
	80	0.5887	58.20
	100	0.4256	69.85
Benzene	20	0.8723	38.15
	40	0.9142	35.23
	80	1.021	27.43
	100	1.088	22.86
Aspirin	100	0.1321	90.62

Table.3 *Invitro* Anti Inflammatory activity of Plant Extract of *Phyllanthus Reticulatus* On human red blood cell membrane

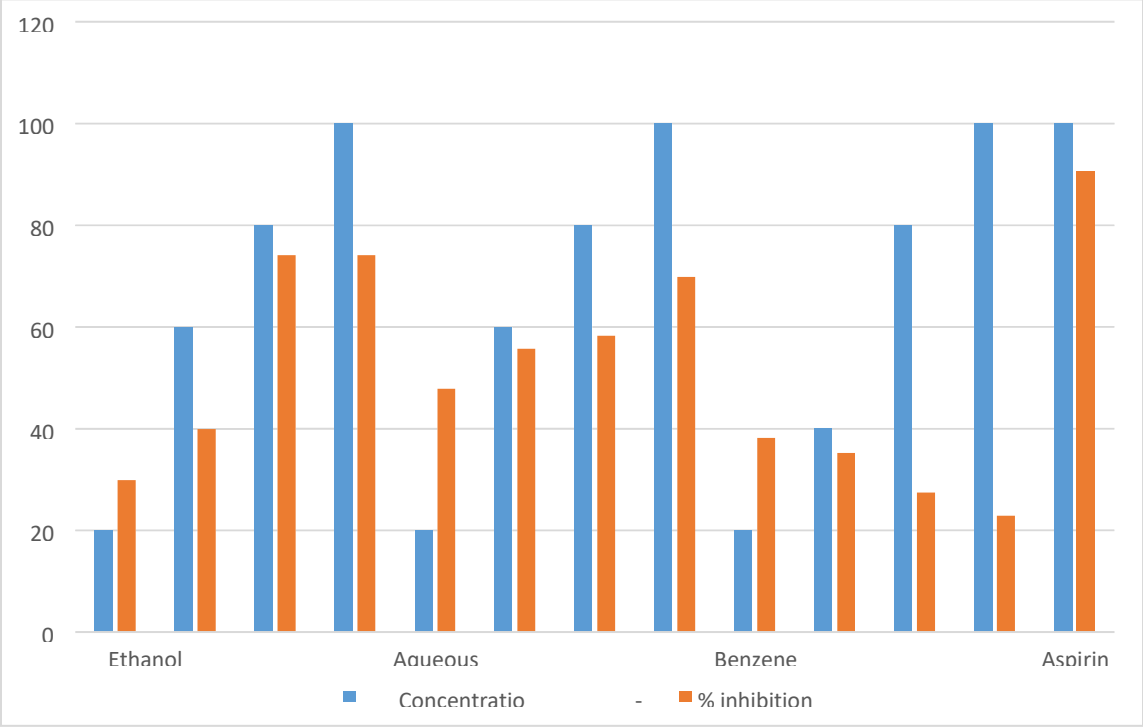

Fig.No:2. *Invitro* Anti Inflammatory activity of Plant Extract of *Phyllanthus Reticulatus* On human red blood cell membrane

DISCUSSION

The various concentration of compound *Phyllanthus Reticulatus* ranging from 20µg/ml to 100µg/ml were tested for its protein denaturation and HRBC method. The results were clearly demonstrated that the compound *Phyllanthus Reticulatus* at different concentration have anti denaturation activity.

Maximum percentage of inhibition 56% was observed from ethanol extracts followed by aqueous 51% at the maximum concentration & benzene 40% at the maximum concentration of 100 µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition 72% at the concentration of 100µg/ml.

In HRBC method ethanol extract of maximum concentration100µg/ml shows 74%, aqueous

extract 70% followed by benzene shows 38%. Aspirin, a standard drug showed the maximum inhibition of 91% at the concentration of 100µg/ml.

Literature suggest that, the anti-denaturation property of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine and aliphatic threonine and lysine residue regions of the BSA. They have also reported that therapeutic molecules could be activating the tyrosine motif rich receptor dually with threonine that regulates signal transduction biological pathways for their overall biological action. [8 9 10]

Compounds interacting with the aliphatic region around the lysine residue on the BSA could be interesting as anti-oxidant with anticancer activity such as polyphenols, phenylpropanoids and the disulphides. However the isolated compound *Phyllanthus Reticulatus* is phenolic in nature; hence they may be the region for its possible anti-denaturation activity. [8 10 11 12]

CONCLUSION

Phyllanthus Reticulatus is a valuable medicinal plant which has been valuable for centuries in ayurvedic medicine. Phytochemical analysis of *Phyllanthus Reticulatus* whole plant extracts revealed the presence of various bio chemical compounds such as flavonoids, glycosides, alkaloids, aleurone, starch, amino acids, lignin, volatile oil, fats and fixed oils, mucilage, pectins, proteins, steroids and triterpenoids. Since glycoside and flavonoids have remarkable anti-inflammatory activity. Our present work aims at evaluating the in vitro anti-inflammatory activity of *Phyllanthus Reticulatus* by protein denaturation and HRBC method. Denaturation of protein is a well-documented cause of inflammation and rheumatoid arthritis. The data of our studies suggests that *Phyllanthus Reticulatus* of whole plant extract showed significant anti-inflammatory activity when compared with standard aspirin.Therefore our studies support the isolation and use of active constituents of *Phyllanthus Reticulatus* in treating inflammation.

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