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Anti-inflammatory and Antioxidant activities of *Polyscias filicifolia* saponins

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Abstract

The plant Polyscias filicifolia, commonly known as fern leaf Panax is rich in triterpenoid saponins. The present study orients in the anti- inflammatory and antioxidant activity of these constituents. The saponin fraction obtained from the leaves and roots of *Polyscias filicifolia* were tested for its anti inflammatory and anti-oxidant activities. Carrageenan and formaldehyde induced paw edema and cotton pellet granuloma methods were used to study the effect of their saponins in acute, sub acute and chronic inflammatory conditions. Ibuprofen was used as a reference standard in this study. The extracts were screened also for their antioxidant and COX-2 inhibitory activities. Results indicated that *P.filicifolia* saponins effectively reduced the carrageenan, formaldehyde induced edema and cotton pellet induced granuloma. The saponin extracts from these plants were found to be a potent inhibitor of hydroxyl, superoxide, peroxide and nitric oxide radicals in invitro studies. In the present study it was observed that *Polyscias filicifolia* inhibit the oxygen free radicals like hydroxyl, superoxide, peroxide and nitric oxide which is believed to be involved in the development of inflammatory disorders, the saponin extract significantly inhibited cylco-oxygenase II enzyme activity in, invitro studies.

Key words: *Polyscias filicifolia* saponins, anti inflammatory activity, oxygen radical scavenging and cyclo oxygenase inhibitory activities.

Introduction

P.filicifoia (Family: Araliaceae) are available throughout the warmer parts of India, especially in Kerala [1]. The plant is grown as an evergreen shrub in tropical regions and is

known as fern leaf panax. Phytochemical investigations [2] proved that these plant leaves and roots are very rich in triterpenoid saponins. There was no report in the literature on any systematic phytopharmacological screening studies of these plants. The plants having triterpenoids are the most widely used for the treatment of inflammation in the traditional medicine of different cultures [3]. Another report indicated that triterpenoids with oleanolic acid as the basic moiety were selective inhibitors of cyclo oxygenase. (COX-2) and nitric oxide synthase activity[4]. COX-2 plays an important role in the patho physiology of inflammatory conditions. In the light of the above findings, we have decided to study the anti- inflammatory and antioxidant activity of these plant saponins.

Materials and Methods

Plant material

P.filicifolia leaves and roots were collected during the month of August from North Paravoor, Kerala and authenticated at the institute of forest genetics and tree breeding, Coimbatore, Tamilnadu. Voucher specimens were deposited in the herbarium of the Pharmacognosy laboratory, College of Pharmaceutical sciences, Medical College, Trivandrum.

Preparation of the leaf and root saponins extracts [6]

The fresh leaves and roots of *P. filicifolia* were dried in shade and ground to a coarse form. This was soxhleted with chloroform – methanol (2:1). These extracts were designated as PFiL & PFiR for *P.filicifolia* leaf and root saponins. The yield obtained for PFiL was 17.5%, and PFiR was 16.8% respectively.

Acute Toxicity Study:

The acute toxicity study [7] was carried out using Swiss albino mice. The extracts were administrated orally in doses of 0.1, 0.25, 0.5, 2.0 & 2.5 g/ kg body weight to different groups of mice of six each. Animals were observed at regular intervals of 1h for a period of 72 h and no toxic symptoms were observed up to a dose of 2.5 g / kg body weight. Based on this study, doses of 100 and 250mg/ kg were arbitrarily selected for animal experiments.

Anti- Inflammatory studies:

a) **Carrageenan induced acute inflammation** [8]

Wistar male albino rats (100-120g) were used in this study. Carrageenan was injected into the sub plantar aponeursis of the right hind paw of rats. An hour before carrageenan injection the animals received the drug or ibuprofen (100mg/kg) or vehicle (0.5% carboxy methyl cellulose) orally.

Table 2. Effect of *P. filicifolia* saponins to carrageenan induced paw edema

Drug group	Dose mg / kg	Mean paw volume ± S.D	Percentage inhibition of paw edema (%)
Control	0.5% CMC	0.833 ± 0.34	-
PFiL	250	0.402 ± 0.034	51.9%
PFiR	250	0.39 ± 0.007	53.18 %
Ibuprofen	100	0.286 ± 0.042	65.67%*

n = 5, Student 't' test, P value <0.05*,
PFiL – *P. filicifolia* leaf saponins
PFiL – *P. filicifolia* root saponins

b) Formaldehyde induced arthritis:

In this study [9], formaldehyde (2% V/V, 0.1ml) was injected sub cutaneously under the sub plantar aponeurosis of the left hind paw. The plant extracts at doses of 250 mg/kg were given orally for 10 days to four groups of animals of six each. The reduction in inflammation was assessed plethysmometrically on a daily basis. The percentage inhibition was calculated and tabulated in table 1.

Table 1: Effect of *P.filicifolia* leaf & root saponins on formaldehyde induced arthritis in albino rats

Drug groups	Dose mg/ kg	Mean edema (ml) of paw ± S.D.									% reduction in paw volume
		2	3	4	5	6	7	8	9	10	
Control		0.92 ±0.03	1.03 ±0.01	1.15 ±0.01	1.22 ±0.02	1.14 ±0.03	1.12 ±0.02	1.12 ±0.03	1.08 ±0.016	1.02 ±0.03	10.86%
PFiL	250	0.62 ±0.017	0.67 ±0.012	0.65 ±0.026	0.58 ±0.007	0.52 ±0.011	0.44 ±0.027	0.37 ±0.0206	0.36 ±0.0096	0.35 ±0.014	43.5%
PFiR	250	0.65 ±0.016	0.64 ±0.037	0.61 ±0.03	0.57 ±0.014	0.52 ±0.011	0.492 ±0.02	0.42 ±0.012	0.39 ±0.011	0.36 ±0.027	44.6%*
Ibuprofen	100	0.58 ±0.012	0.42 ±0.02	0.58 ±0.037	0.55 ±0.03	0.5 ±0.04	0.45 ±0.04	0.4 ±0.03	0.37 ±0.02	0.28 ±0.02	51.72%**

c) Cotton Pellet granuloma induced chronic inflammation:

In this method [10] cotton pellets weighing around 50± 1 mg were first sterilized by autoclaving and implanted sub cutaneously on either side in the dorsal region of the animals under light ether anesthesia.

PFiL & PFiR at doses of 100 & 250 mg/kg were given to four groups of rats for ten days starting from the day of pellet implantation. To one group, Ibuprofen (100mg/kg) was given as a reference standard. On the 10^{th} day the cotton pellets were dissected out, cleaned from extraneous tissues and dried in a Petri dish at 60^0C. The dry weight of each pellet was determined. The granuloma weight was calculated by subtracting the dry cotton pellet weight from the 9^{th} day weight. The percentage inhibition was calculated and tabulated in table 3.

Table 3. Anti- inflammatory studies of *P. filicifolia* saponins by Cotton pellet granuloma method

Drug group	Dose mg / kg	Dry weight of granulaoma ± S.D	% reduction of granuloma weight (compared to control)
Control (0.5%cmc)	1ml/kg	85.2 ± 0.8	
PFiL	100	58.4± 3.42	31.45%
PFiL	250	42.6 ± 2.32	46.5%*
PFiR	10	59.5 ± 1.125	30.16%
PFiR	250	51.4 ± 0.616	39.7%

Oxygen radical scavenging activity

a) Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside. In this study [16] 5mM solution of sodium nitroprusside was mixed with various concentrations of crude drug extract PFiL, PFiR (0.1 mg/ml-1mg/ml) and incubated at 25^0C for 5h. The Griess reagent was prepared by mixing a 1% solution of sulphanilamide, 2% solution of orthophosphoric acid, and 0.1%

solution of napthyl ethylene diamine dihydrochloride, added to the plant extracts and absorbance was measured at 546nm. The result is tabulated in table 4.

Table 4. Nitric oxide radical scavenging activity of Polyscias saponins

Drug group	Concentration mg/ml	Absorbance at 546nm ± S.D	% inhibition ± S.D
Control	-	0.7410.027	-
PFiL, PFiR	0.1	0.62 ± 0.07, 0.63 ± 0.042	16.21 ± 0.12, 14.09 ± 0.18
PFiL, PFiR	0.4	0.28 ± 0.036, 0.33 ± 0.05	62.16 ± 0.42, 55.4 ± 0.36
PFiL, PFiR	0.6	0.26 ± 0.72, 0.32 ± 0.6	64.8 ± 2.12, 56.8 ± 1.08
PFiL, PFiR	1.0	0.215 ± 0.036, 0.24 ± 0.12	69 ± 0.36**, 67.56 ± 1.09**

b) Hydroxyl radical scavenging activity

This study [11] was conducted by measuring the inhibition of deoxyribose degradation in presence of the test drug extracts. Hydroxyl radical was generated by Fe EDTA and H_2O_2 in presence of ascorbic acid. PFiL & PFiR were added in various concentrations (250 µg/ml-1000µg/ml) to a reaction mixture containing deoxyribose (3mM); $FeCl_3$ (0.1 mM), EDTA (0.1mm), ascorbic acid (0.1mM) H_2O_2 (2mM) in phosphate buffer(20 mM/pH- 7.4) to make a final volume of 3ml. To this mixture tri- chloroacetic acid and thiobarbituric acid (0.5m each) were added and measured the absorbance at 532nm. The percentage of hydroxyl radical inhibition and IC50 were determined by the method of Halliwell *et.al* [11]. Copper Sulphate solution (0.01mm) was used as a reference standard. The results are tabulated in table 5.

Table 5. Hydroxyl radical scavenging activity Polyscias saponins

Drug Groups	Concentration mcg/ml	% hydroxyl radical scavenging activity (Absorbance at 532nm in bracket)	IC_{50} Value (mcg/ml)	
Control	-	0.398 ± 0.15 (absorbance at 532nm)	PFiL	PFiR
PFiL, PFiR	1000	76.4 ± 0.24, 74.3 ± 0.012 (0.095 ± 0.012) (0.102 ± 0.011)		
PFiL, PFiR	750	55.45 ± 0.32, 52.2 ± 0.16 (0.177 ± 0.021) (0.195 ± 0.22)	485.5	674.6
$CuSO_4$	0.01mM	54.46 ± 1.63		
PFiL, PFiR	500	76.4 ± 0.24, 74.3 ± 0.012		
PFiL, PFiR	250	35.4 ± 0.012, 32.0 ± 0.016		
PFiL, PFiR	125	20.5 ± 0.012, 18.42 ± 0.32		

c) Superoxide radical scavenging activity

Superoxide radical scavenging activity was studied according to the literature [12]. Alkaline DMSO (1% in 5mM NaOH) was added to the reaction mixture containing nitro-blue tetrazolium (NBT 0.1mg) and the test drugs (PFiL & PFiR) at various concentrations. The absorption was determined at 560nm. The reduction of NBT by the superoxide radical generated was calculated in presence and absence of the test drugs. In this study, thiourea (20mM) was used as the reference standard. The results are tabulated in Table 6.

Table 6. Superoxide radical scavenging activity of Polyscias saponins

Drug Groups	Concentration mcg/ml	% superoxide radical scavenging activity	IC_{50} Value (mg/ml)	
Control	-	1.639 ± 0.29	PFiL	PFiR
PFiL, PFiR	1000	62.4 ± 0.21, 58.2 ± 0.112	525.5	634.4
PFiL, PFiR	500	39.8 ± 0.11, 41.4 ± 0.22		
PFiL, PFiR	125	14.5 ± 0.22, 11.4 ± 0.312		
Thio urea	20 mM	55.06 ± 0.306		

d) Effect of PFL, PFR, PFiL & PFiR on lipid peroxidation

In this study [13] the liver tissue homogenate was adjusted to 10mg/ml. The effect of PFiL & PFiR on lipid peroxides was estimated as malondialdehyde by thiobarbituric acid (TBA) method. To the reaction mixture containing PFiL & PFiR at various concentrations, 1ml of liver tissue homogenate and 1 ml of HCl- trichloroacetic acid – thiobarbituric reagent was added. The mixture was heated for 5 minutes in a water bath at 37^0C and after cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant liquid was measured at 532 nm against blank and the lipid peroxide content was determined using the extinction coefficient $1.5\lambda\ 10^{-6}m^{-1}\ cm^{-1}$. The final result was expressed as nanomoles of malondialehyde per mg of protein. Vitamin E at a concentration of 50 mcg/ml was used for comparing the results. The results are tabulated in the table No: 7.

Table 7. Effect of Polyscias saponins on lipid peroxidation

Drug group	Concentration (mcg/ml)	nanomoles of malondialdehyde/mg of protein	% scavenging activity
Control	-	3.965 ± 0.02	-
PFiL, PFiR	1000	1.61** ± 0.78, 1.66* ± 0.012	59.39**, 58.13*
PFiL, PFiR	750	1.76 ± 0.011, 2.27 ± 0.108	55.6, 55.10
PFiL, PFiR	500	1.87 ± 0.011, 2.27 ± 0.108	52.8, 42.7
PFiL, PFiR	250	2.32 ± 0.14, 3.27 ± 0.166	41.4, 17.5
PFiL, PFiR	125	3.36 ± 0.124, 3.6 ± 0.081	15.2, 9.2
Vitamin C	50	1.91 ± 0.27	51.82 ± 0.92

COX – 2 inhibitory activity

The experimental protocol described by Padikkale et al [15] was followed. Fresh blood from healthy volunteers who had not taken any NSAIDS for at least seven days prior to blood extraction was collected in heparinized tubes. Platelet rich plasma was prepared by centrifugation at 2500 rpm. The platelet count was adjusted to 25000/µl and suspended in Hepes – Thyroid buffer (pH-7.4).

Table 8: Effect of Polyscias saponins on COX-2 inhibitory activity

Drug group	Concentration mg/ml	n.Moles of MDA/ml of Protein Rich Plasma	% inhibition
Control	-	86.6 ± 5.02	-
PFiL	500	55.64 ± 0.99	35.75 %
PFiR	500	42.47 ± 1.216	50.95 %
Ibruprofen	100	31.9 ± 2.14	63.16 %**

The reaction system contains 1ml of platelet suspension and the saponin extract at 50 mcg/ml. The mixture was incubated for 10 minutes, and then diethylmaleimide (10 µl, 10 µM) solution was added and incubated again for 1h at 37^0C. At the end of the incubation period, malondialdehyde (MDA) formed in the reaction mixture was measured by thiobarbituric acid method [13]. The percentage inhibition was then calculated from the absorbance at 532 nm compared to the control. The results are tabulated in the table 8.

Results and Discussion

The acute toxicity studies indicated that all the plant extracts taken, in this study showed no toxic symptoms upto a dose of 2.5g/kg body weight. The plant extracts PFiL, PFiR showed effective inhibition of hydroxyl and superoxide radical formation, in *in-vitro* studies at higher doses. On lipid peroxidation, all the saponin extracts showed a dose dependent inhibitory effect in malondialdehyde formation.The saponins obtained from the leaves and roots exhibited significant COX-2 inhibitory activity compared to the standard Ibuprofen.

Inflammation is a very complex and multifactorial dynamic phenomenon, with a series of events in the process. Actually the inflammatory process occurs in three distinct phases as the acute exudative phase, a sub acute phase and a chronic proliferative phase. In this study the carageenan, formaldehyde and cotton pellet granuloma methods represent the acute, sub acute and chronic models of inflammation. The chronic proliferative phase is usally characterized by tissue degeneration, fibrosis formation and eventually responsible for the release of prostaglandins like substances. The carrageenan induced edema model is a prototype of acute or exudative phase of inflammation characterized by increased vascular permeability and also responsible for the release of histamine, kinins & serotonins. The formaldehyde induced edema model represents a subacute phase characterized by increased migration of leucocytes and phagocytes in the area of inflammation.

It is already reported that reactive oxygen species (ROS) like hydroxyl and superoxide radicals participate in the development of inflammatory disorders and the role of lipid peroxidation in the involvement of inflammation is also well established. The cell membrane is primarily composed of poly unsaturated fattyacids which are highly susceptible to attack of oxygen free radicals. This may lead to a decrease in membrane fluidity, destabilization of membrane receptors and distortion of DNA synthesis.

Conclusion

The results of the present work clearly indicated that the drug extract (PFiL & PFiR) inhibit the oxygen free radicals formation effectively compared to the control and this may be one of the possible mechanisms of anti-inflammatory activity of these plant drugs. Further studies are in progress in the laboratory on the isolation of active constituents responsible for the anti-inflammatory activity.

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