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In vitro antipsoriatic effect of extract of *Strobilianthes ciliatus* using Hacat cell lines and its cyclooxygenase inhibition using RAW 264.7 cell lines

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Abstract
Inflammation is the beneficial host response to challenges by foreign bodies or to tissue injury. There is an ongoing research to develop safer and more effective drugs for the therapy of inflammation and psoriasis. Psoriasis can be considered as a T-cell mediated disease, with a complex role for a variety of cytokines interaction between keratinocytes and T- lymphocytes. Epidermal hyper proliferation, abnormal keratinocyte differentiation, angiogenesis and blood vessel dilatation can be observed in this disease condition. *Strobilianthes ciliatus* of Acanthaceae family is highly potential medicinal plant in Ayurveda in the treatment of inflammation and skin diseases. Petroleum ether and ethanolic extract of leaf and ethanolic extract of stem of *Strobilianthes ciliatus* were screened for anti-psoriatic and anti-inflammatory activities by *in-vitro* models. Psoriatic activity was studied by MTT assay on HACAT cell lines. Cyclooxygenase inhibition of the extracts were analyzed using RAW 264.7 cell lines. Petroleum ether extract of leaf showed high anti-psoriatic activity and cyclooxygenase inhibition than the other extracts. Significant activity shown by the extract suggests bioactivity guided isolation to identify the promising constituent present in the extract.

Keywords: *Strobilianthes ciliatus*, cyclooxygenase, psoriasis, HACAT cell line, RAW264.7cell line

1. Introduction
Psoriasis is described as the hyper proliferation and aberrant differentiation of keratinocytes, which leads to inflammation in dermis, epidermis and leukocyte infiltration. It is a genetically determined chronic inflammatory skin disease characterized by red, scaly and raised patches that affect 2.3% of the population world wide [1].

It usually appears first between the ages of 15-30 years. The lesions are characterized by brownish red papules and plaques which are sharply demarcated and are covered with, fine silvery white scales. As the scales are removed by gentle scrapping, fine bleeding points appear. Commonly involved sites are scalp, upper back, sacral region and extreme surfaces of the extremities especially knees and elbows [2].

Inflammation is a protective response that is initiated either after injury, physical and chemical damage, or infection by microorganism, but persistent inflammation may cause chronic diseases. It is a complex biological response to harmful stimuli and is classified as acute or chronic. Inflammation is caused by the body release of hormone-like substances called prostaglandins (PGs) and leukotrienes (LTs). These inflammatory agents are produced from arachidonic acid (AA) by the enzymes cyclooxygenase (COX) and lipoxygenase (LOX). COX is the first enzyme in the pathway for producing PG and thromboxane (Tx) from arachidonic acid, and can occur as three isoforms, COX-1, COX-2 and COX-3 [3].

Strobilianthes ciliatus of Acanthaceae family is a highly potential medicinal plant in Ayurveda in the treatment of inflammatory disease. The entire plant was recognized as variable drug and frequently used by many of the ancient traditional medical system. The leaves, stem, seed and root of the plant possess a number of therapeutic effects such as diuretic, diaphoretic, anti-inflammatory, lumbago, sciatica, chest congestion, urogenital infection, leprosy, anti-diabetic, fever, skin disease, treatment of rheumatism etc [4]. Numerous secondary metabolites such as saponin glycosides, sterols, tannins, flavanoids were reported from the plant. Lupeol, stigmasterol and betulin has been isolated [5] from the plant. The acetone and ethanolic extract of *S. ciliatus* have been evaluated for the antimicrobial, antioxidant and cytotoxic properties [6].

2. Methodology

2.1 Extraction

The shade dried powdered leaf was extracted with petroleum ether (60-80 ^{0}C) followed by ethanol. The air dried stem powder was extracted with ethanol to get the total ethanolic extract. All the extracts were concentrated and calculated the percentage yield.

2.2 Preliminary phytochemical screening

Preliminary phytochemical screening was carried out on all the extracts [7, 8].

2.3. Determination of *in vitro* antipsoriatic effect of extract on cultured HACAT cell line

HACAT cell lines was purchased from NCCS Pune and maintained in Dulbecceo's modified eagles media and supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5% Co_2 in a humidified atmosphere in a Co_2 incubator. The cells were trypsinized for 2 minutes and passaged to T flask in complete aseptic conditions.

The two days old confluent monolayer of cell were trypsinized and the cells were suspended in 10% growth medium, 100 µl cell suspension was added in 96 well tissue culture plate and incubated at 37°C in humidified 5% CO_2 incubator. 1 mg of each plant extract or compound was added to 1 mL DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure sterility.

The freshly prepared plant extract in DMEM were five times serially diluted by two fold dilution and each one of 100 ml were added in triplicate to the respective wells and incubated at 37°C in humidified 5% CO_2 incubator.

2.4. Antipsoriatic assay by direct microscopic observation

Entire plate was observed at an interval of each 24 hours in an inverted phase contrast tissue culture microscope and microscopic observation were recorded as images. Any detectable change in the morphology of cell such as rounding or shrinking of cells granulation and vacuolization is the cytoplasm were considered as indicator of cytotoxicity.

15 mg of MTT was reconstituted in 3 mL PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in the wells were removed and 30 µl of reconstituted MTT solution was added to all test and control wells. The plate was gently shaken well, and then incubated at 37 ^{0}C is a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100 µl of MTT solubilization solution (DMSO) was added and wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm.

The percentage of growth Inhibition was calculated by using formula.

$$\% \text{ viability} = \frac{\text{Mean OD Sample x100}}{\text{Mean OD of cntrol grp}}$$

2.5 *In vitro* anti-inflammatory screening

RAW264.7 cell line was purchased from NCCS, Pune and were maintained in Dulbecco's modified eagle's media (Himedia, India) supplemented with 10% fetal bovine serum (Invitrogen. USA) and grown to confluence at 37° C at 5% CO_2 in a CO_2 incubator (Eppendorf, Germany).

RAW 264.7 cell were grown to 60% confluency followed by activation with 1 µl lipopolysacchariride (LPS, 1 µg/mL) LPS stimulated RAW cells were exposed with different concentration of sample and diclofenac sodium was added and incubated for 24 hrs. After incubation, cells were lysed using cell lysis solution, then anti-inflammatory assay were performed using cell lysate.

2.6 Cyclooxygenase (COX) activity

The COX activity was assayed by the method of Walker and Gierse [9]. The cell lysate was incubated in Tris-HCl buffer (pH8), glutathione 50mM/L and hemoglobin 5 M/L for 1 minute at 25° c. The reaction was initiated by the addition of arachidonic acid 200 M/L and terminated after 20 minute incubation at 37° c, by the addition of 10% trichloroacetic acid in hydrochloric acid. After the centrifugal separation and the addition of 1% thiobarbituric acid, COX activity was determined by reading absorbance at 632 nm.

Percentage inhibition of enzyme was calculated using the following equation.

$$\text{Percentage inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}} \text{ x100.}$$

3. Result and discussion

Percentage yield of the extracts were found to be 9.586 % w/w. The yield of ethanolic extract of leaf and stem was 7.2% w/w and 8.7%w/w respectively.

Preliminary phytochemical screening showed the presence of saponins, carbohydrates, tannins, flavonoid and sterols.

Anti-psoriatic activity was found to be better with ethanolic extract of leaf followed by petroleum ether extract of leaf and stem extract. Psoriasis is a chronic inflammatory proliferative skin disease characterized by pathological skin lesions due to various intrinsic and extrinsic factors [10]. Recently it has been suggested that increased reactive oxygen species (ROS) production and compromised function of anti-oxidant system may be involved in the pathogenesis of this disease [11].

Table 1: Antipsoriatic study of extracts of *S. ciliates*

Extract	Concn.(µg/mL)	Absorbance± SD	% viability
STD	Control	0.4550±0.0141	100
	6.25	0.3493±0.0071	76.769
	12.5	0.2749±0.0020	60.417
	25.0	0.2170±0.0141	47.692
	50.0	0.1965±0.0028	43.186
	100.0	0.1245±0.00149	27.362
LPE	6.25	0.8928±0.0124**	73.69
	12.5	0.8798±0.1499**	72.59
	25.0	0.7987±0.0631**	65.89
	50.0	0.6952±0.0196**	57.36

	100.0	0.5359±0.0120**	44.22
LAE	6.25	0.8743±0.0304**	72.14
	12.5	0.8385±0.0099**	69.19
	25.0	0.7873±0.0521**	64.96
	50.0	0.7275±0.0040**	60.03
	100.0	0.6131±0.0120**	50.59
SAE	6.25	0.9822±0.0143**	81.04
	12.5	0.7578±0.0151**	62.52
	25.0	0.7488±0.0067**	61.78
	50.0	0.6394±0.0075**	52.75
	100.0	0.5821±0.0095**	48.03

Values expressed are mean± SD, n=3
STD –Standard (Diclofenac sodium),
LPE- Petroleum ether extract of leaf,
LAE -Ethanolic extract of leaf,
SAE -Ethanolic extract of stem.
**p-value<0.001

IC_{50} value of the COX inhibition of the standard was found to be 2.84 µg /ml. Highest COX -2 inhibition was exhibited by petroleum ether extract of leaf of *S.ciliatus* followed ethanolic extract of stem and ethanolic extract of leaf.

Variety of phytochemicals like flavonoids, terpenoids, alkaloids and saponins has been described to possess significant anti-inflammatory activity. Several studies proved that naturally occurring flavonoids [12] act as dual inhibitors of cyclooxygenase and 5-lipoxygenase activities. Flavanoids inhibit biosynthesis of prostaglandins (the end products of the COX and lipoxygenase pathways) which act as secondary messengers. World-wide, there is an increasing concern in finding new anti-inflammatory remedies, not only having improved therapeutic index but also harmless [13].

Table 2: Cycloxygenase inhibition by extracts of *S.ciliatus*

Extract	Concentration	Absorbance± SD	% Inhibition	IC $_{50}$
STD	Control	0.1937±0.00004	-	-
	3.125	0.0993±0.0004	48.73	
	6.25	0.0613±0.0009	68.35	
	12.5	0.0577±0.0006	70.21	
	25	0.089±0.0055	72.021	
	50	0.063±0.0098	80.194	
	100	0.048±0.0026	84.910	
LPE	Control	0.1523±0.0019	13.85	89.76
	25	0.1312±0.0077*	35.19	
	50	0.0987±0.0058*	48.71	
	75	0.0781±0.0057*	55.67	
	100	0.0675±0.0052*	61.26	
	125	0.0590±0.0043*	78.92	
	150	0.0321±0.0061*		
LAE	25	0.1326±0.0025*	12.93	130.32
	50	0.1244±0.0040*	18.32	
	75	0.1102±0.0045*	27.64	
	100	0.0927±0.0033*	39.13	
	125	0.0811±0.0022*	46.74	
	150	0.0623±0.0011*	59.09	
SAE	25	0.1271±0.0025*	16.55	119.27
	50	0.1196±0.004*	21.47	
	75	0.0997±0.0014*	34.53	
	100	0.0884±0.0053*	41.95	
	125	0.0723±0.0025*	52.52	
	150	0.0582±0.0042*	61.78	

Values are expressed are mean ±SD, n=3
Standard (Diclofenac sodium),
LPE-Petroleum ether extract of leaf,
LAE-Ethanolic extract of leaf,
SAE-Ethanolic extract of stem.
**p-value<0.05

S. ciliatus was proved to be a good anti-oxidant [14] and that can be a factor behind the COX inhibition by the extracts.

A comprehensive and integrated anti-oxidant defense mechanism of skin is crucial in protecting the skin from ROS [15].

The above references substantiate the study that the anti-psoriatic activity of these extracts can be by the inhibition of COX enzyme with the support of anti-oxidant components present in the plant [16].

These findings provide scientific evidence to support traditional medicinal uses of *Strobiliathes ciliates* as anti-inflammatory agent and for skin diseases.

Conclusion

As per the above findings, the extracts of *S. ciliatus* were found to have anti-psoriatic activity which may be mainly, through cyclooxygenase inhibition. Bioactivity guided isolation of active constituents are in progress in our lab.

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