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In Vitro Anti – Inflammatory Activity Of *Psoralea corylifolia* Seeds Ethanolic Extract

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ABSTRACT

Psoralea corylifolia (Family: Fabaceae) seeds, commonly known as Psoralea or Babchi seeds. Seeds are used as therapy for various skin problems and they also produce pigment for skin disorders as leukoderma and vitiligo. It is also having anti- bacterial, anti-fungal, anti-oxidant, anti-cancer, anti-larvicidal, anti-diabetic, and anti-inflammatory properties. The present study was to evaluate the preliminary Phytochemicals and the inhibitory activity of the cyclooxygenase and the lipoxygenase enzymes. The cyclooxygenases and lipoxygenase enzymes inhibitory activities were evaluated to determine the promising mechanism of the anti-inflammatory activity of *Psoralea corylifolia* seed. This study has revealed the presence of phytochemicals as Carbohydrates, Alkaloids, Phytosterols. Oils, Saponins, Proteins, Amino acids and Flavonoids.

KEYWORDS: *Psoralea corylifolia* seeds, Anti-inflammatory, Lipoxygenase, Cyclooxygenas

INTRODUCTION:

Inflammation is a dynamic process that is generated by mechanical injuries, burns, microbial infections, and other noxious stimuli that may damage the host's health (Cotran et al.,1999). Due to increased blood flow, increased vascular permeability, tissue destruction via activation and migration of leucocytes with the synthesis of reactive oxygen derivatives (oxidative burst), and the synthesis of local inflammatory mediators such as prostaglandins (PGs), leukotrienes, and platelet-activating factors induced by phospholipase A2, cyclooxygenases (COXs), and lipoxygenases (LOXs). Arachidonic acid is an important biochemical precursor that is transformed into several of the eicosanoids with various biological functions(Paul, W.E, 6thEdition). The COX pathway, which generates both PGs and thromboxanes, and the 5 - lipoxygenase pathway, which generates leukotrienes and 5S-hydroxy-6E, 8Z,

11Z, 14Z-eicosatetraenoic acid, are the two major processes of arachidonic acid metabolism (5- HETE)(Medzhitov R, 2008).

Psoralea corylifolia L., a therapeutic herb, is on the verge of extinction. It is widely spread throughout the Himalayan regions of India, China, and Pakistan (Khuranna et al., 2020; Wang et al., 2009). Psoralea is a genus of plants native to North America (Maisch, 1889). P. corylifolia is also known as 'Kusthanashini,' or leprosy killer, due to its capacity to treat leprosy. Because it is a fundamental component of both allopathic and conventional medical systems in various regions of the world, it has a wide range of applications. It is used to treat psoriasis, leucoderma, and vitiligo in traditional Chinese medicine and Indian healthcare systems such as Ayurveda, Siddha, and Unani (Khusboo et al., 2010).

The focus of this research was to explore preliminary Phytochemical analysis of ethanolic extracts of *Psoralea corylifolia* L. seeds, evaluate the LOX and COX inhibitory activity, and determine exactly whether they might perform in the treatment of inflammatory disorders.

MATERIALS AND METHODS:

COLLECTION AND PREPARATION OF EXTRACTS

The seed of *Psoralea corylifolia* was collected in the local folk medicine market in Cuddalore District, Tamil Nadu. After the collection of seeds, it was ground coarsely by using Morter and Pestle. The ground seed samples are sieved and stored for further processes. 10g of seeds powder of *Psoralea corylifolia* was mixed with 100 ml of Ethanol and incubated for 3 days. After incubation, the extract was filtered through Whatman No.1 filter paper. The filtered extract was used for further analysis.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical analyses were carried out for Ethanolic extracts of *Psoralea corylifolia* seed. The crude extracts are then subjected to preliminary phytochemical screening as per the standard methods of the procedure (Harborne, 1973), tested for the presence or absence of primary or secondary metabolites.

Test for Carbohydrates

In 2 mL of distilled water, a small amount of extract was combined. A few drops of Molisch's reagent (which contains -Naphthol) and a tiny amount of concentrated sulphuric acid was added to the thoroughly mixed solution, as well as a small amount of concentrated sulphuric acid along the sidewall of the test tube. A purple ring appeared at the interface between the acid and test layers, signaling the presence of carbohydrates.

Test for Alkaloids

A small portion of the alcoholic extract was separated and filtered after being agitated with a few drops of dilute hydrochloric acid. Mayer's and Dragendorff's tests were performed on the filtrate.

Dragendorff's Test

A small amount of filtrate was treated with Dragendorff's reagent and the appearance of orange red

precipitate indicated the presence of alkaloids.

Mayer's Test

The filtrate was treated with Mayer's reagent and the appearance of creamy precipitate indicated the presence of alkaloids.

Test for Phytosterols

Salkowski test was done for the detection of phytosterols. The extract was dissolved in 2 ml of chloroform in a test tube. 1 ml of concentrated sulphuric acid was added along the sides of the tube and allowed to stand for 5 minutes. A reddish-brown color at the interface indicated the presence of phytosterols.

Test for Fixed Oil

Spot test was done for the detection of fixed oil. In this test, a small quantity of alcoholic extract was pressed between two filter papers. The appearance of oil strain on the paper indicated the presence of fixed oil.

Test for Saponins

A Froth test was done for the detection of saponins. Each extract was diluted with 20 ml distilled water and agitated in a graduated cylinder for 15 minutes. The formation of a 1cm layer 'honey comb' froth indicated the presence of saponins.

Test for Proteins and Free Amino

acids Biuret Test

The plant extract was diluted with distilled water and treated with a Biuret reagent. Appearances of pink or purple color indicated the presence of proteins and free amino acids.

Ninhydrin Test

The diluted extracted was treated with ninhydrin reagent and observed for a characteristic purple color which indicated the presence of free amino acids.

Test for Flavonoids

An alkaline reagent test was done for the detection of flavonoids. The extract was treated with a few drops of sodium hydroxide to give a deep yellow color. The disappearance of yellow color after the addition of dilute hydrochloric acid indicated the presence of flavonoids.

In-vitro Anti-inflammatory

screening Cell lines:

RAW 264.7 cell line was purchased from NCCS, Pune and was maintained in Dulbecco's modified eagle's media (Himedia, India) supplemented with 10% fetal bovine serum (Himedia, India) and grown to the confluence at 37℃ at 5% CO_2 in a CO_2incubator.

Preprocessing

RAW 264.7 cells were cultured to 60% confluence before being activated with 1 µL of lipopolysaccharide (1 g/mL). RAW cells that had been activated with LPS were subjected to varied concentrations of sample solution. Diclofenac sodium, a common anti-inflammatory medicine, was also added in varying concentrations to match the sample and incubated for 24 hours. The anti-inflammatory experiments were done using the cell lysate after incubation.

Lipoxygenase (LOX) activity:

The activity of 5-LOX was measured according to Axelrod et al (1981). The reaction mixture (2 mL final volume) contains Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and 200 µL of sodium linoleate (10 mg/ml). The synthesis of 5-hydroxyeicosatetraenoic acid from linoleate was measured as a difference in absorbance at 234 nm, which reflects LOX activity (Axelrod et al., 1981)

Percentage inhibition of the enzyme will be calculated using the formula:

$$\% \text{ inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}} \times 100$$

Cyclooxygenase (COX) activity:

The cell lysate in Tris-HCl buffer (pH 8) was incubated with glutathione 5 mM/L, and hemoglobin 20 µg/L

for 1 minute at 25°C. The reaction was started with the addition of 200 mM/L arachidonic acids and

ended with the addition of 10% trichloroacetic acid in 1 N hydrochloric acid after 20 minutes of incubation at 37°C. COX activity was evaluated by measuring absorbance at 632 nm after centrifugal separation and the addition of 1% thiobarbiturate (Walker and Gierse, 2010). Percentage inhibition of the enzyme was calculated as,

$$\% \text{ inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening:

The ethanolic extract of *Psoralea corylifolia* seeds was screened for the presence of preliminary Phytochemicals by using Harborne (1973) procedures. This study has revealed the presence of phytochemicals as Carbohydrates, Alkaloids, Phytosterols. Oils, Saponins, Proteins, Amino acids and Flavonoids (Table 1). The presence of these bioactive metabolites might be responsible for medicinal attributes and also a reason for the Anti-inflammatory activity of the seed.

Table 1: Phytochemical screening of seed extracts of *Psoralea corylifolia*

S.No	Test	Ethanol
1	**Carbohydrates**	+
2	**Alkaloids**	
	Dragendorff's	+
	Mayer's	+
3	**Phytosterols**	
4	**Fixed oil**	+
5	**Saponins**	+
6	**Protein and Free amino acids**	+
7	**Flavonoids**	+

Lipoxygenase (LOX) Activity

The anti-inflammatory effects of ethanolic extracts of *Psoralea corylifolia* seeds on the leukotrienes production were analyzed by inhibition of lipoxygenase activity. LOX assay of ethanolic extracts of seeds is analyzed with the Standard Anti-Inflammatory drug Diclofenac on various concentrations as 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml. The results of the LOX inhibition assay showed good inhibitory activities (Table 2). In the LOX inhibition assay, the percentage inhibition was found to be 2.62%, 12.5%, 22.99%, 29.94%, and 46.45%. This present study revealed the seed extracts having inhibitory properties of the lipoxygenase enzymes were dose-dependent.

Table 2: Results of Lipoxygenase inhibition were depicted.

Diclofenac concentration (µg/ml) (Standard anti-inflammatory drug)	**Absorbance at 234 nm (Triplicate readings)**	**Absorbance (Average)**	**Percentage inhibition**
Control	0.660 0.670 0.665	0.665	-
6.25	0.612 0.624 0.636	0.624	6.17
12.5	0.588 0.579 0.570	0.579	12.93
25	0.494 0.483 0.472	0.483	27.37
50	0.310 0.322 0.316	0.316	52.48
100	0.257	0.257	61.35

	0.264 0.250		
Ethanol Seed Extract			
Sample MRL concentration (µg/ml)			
Control (Without LPS)	0.038 0.031 0.026	0.031	-
Control	0.652 0.643 0.649	0.648	-
6.25	0.638 0.629 0.620	0.631	2.62
12.5	0.574 0.560 0.567	0.567	12.5
25	0.491 0.507 0.498	0.499	22.99
50	0.466 0.454 0.450	0.455	29.94
100	0.345 0.353 0.341	0.347	46.45

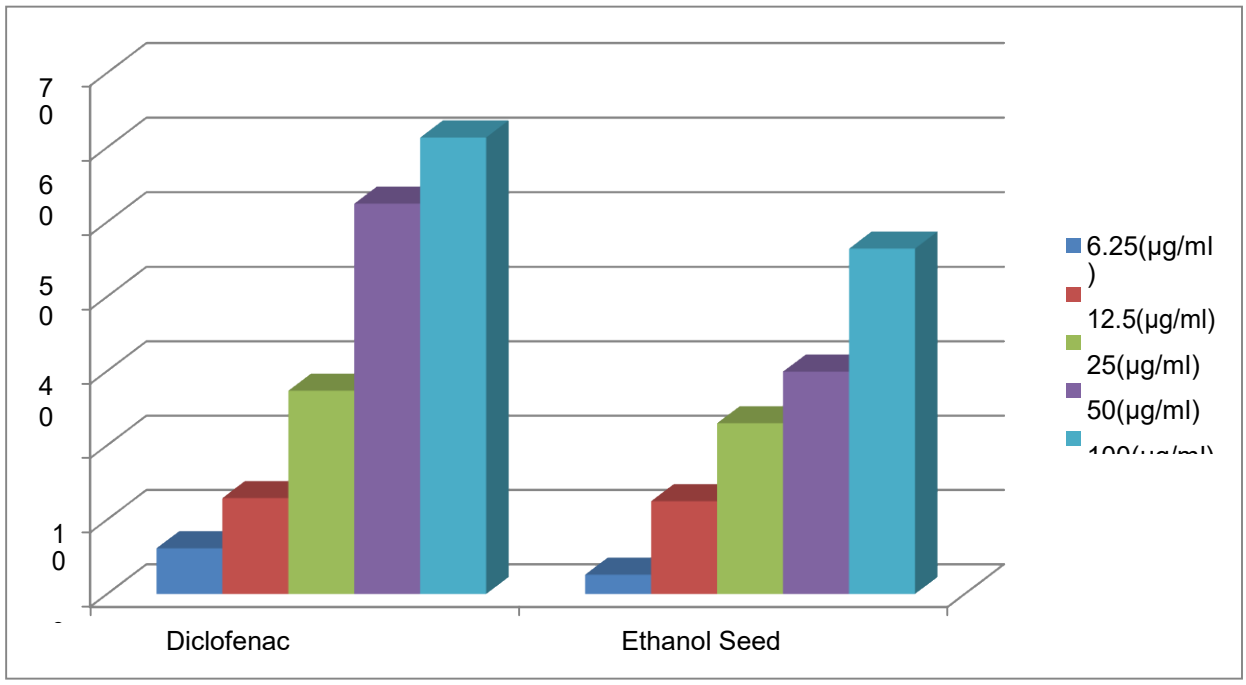

Fig 1: Graphical representation of *Psoralea corylifolia*Seed extract with Standard drug.

Cyclooxygenase (COX) activity:

The anti-inflammatory effects of ethanolic extracts of *Psoralea corylifolia* seeds on prostaglandins production were analyzed by inhibition of cyclooxygenase activity. COX assay of ethanolic extracts of seeds is analyzed with the Standard Anti-Inflammatory drug Diclofenac on various concentrations as

6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml. In the LOX inhibition assay, the percentage inhibition was found to be 4.40%, 11%, 21.09%, 26.26%, and 40.49%. The results of the COX inhibition assay showed good inhibitory activities (Table 3). This present study revealed the seed extracts having inhibitory properties of the cyclooxygenase enzymes were dose-dependent.

Table 3: Results of cyclooxygenase inhibition were depicted

Diclofenac concentration (µg/ml) (Standard anti-inflammatory drug)	**Absorbance at 632 nm (Triplicate readings)**	**Absorbance at 632 nm (Average)**	**Percentage inhibition**
Control	0.766 0.760	0.760	-

	0.754		
6.25	0.680 0.670 0.675	0.675	11.18
12.5	0.602 0.612 0.622	0.612	19.47
25	0.564 0.534 0.549	0.549	27.76
50	0.486 0.492 0.480	0.486	36.05
100	0.381 0.389 0.385	0.385	49.34
Ethanol Seed Extract			
Sample MRL concentration (µg/ml)			
Control (Without LPS)	0.050 0.044 0.059	0.051	-
Control	0.762 0.774 0.782	0.773	-
6.25	0.732 0.749 0.735	0.739	4.40
12.5	0.681 0.688 0.694	0.688	11

25	0.609 0.623 0.598	0.610	21.09
50	0.564 0.569 0.577	0.570	26.26
100	0.452 0.473 0.455	0.460	40.49

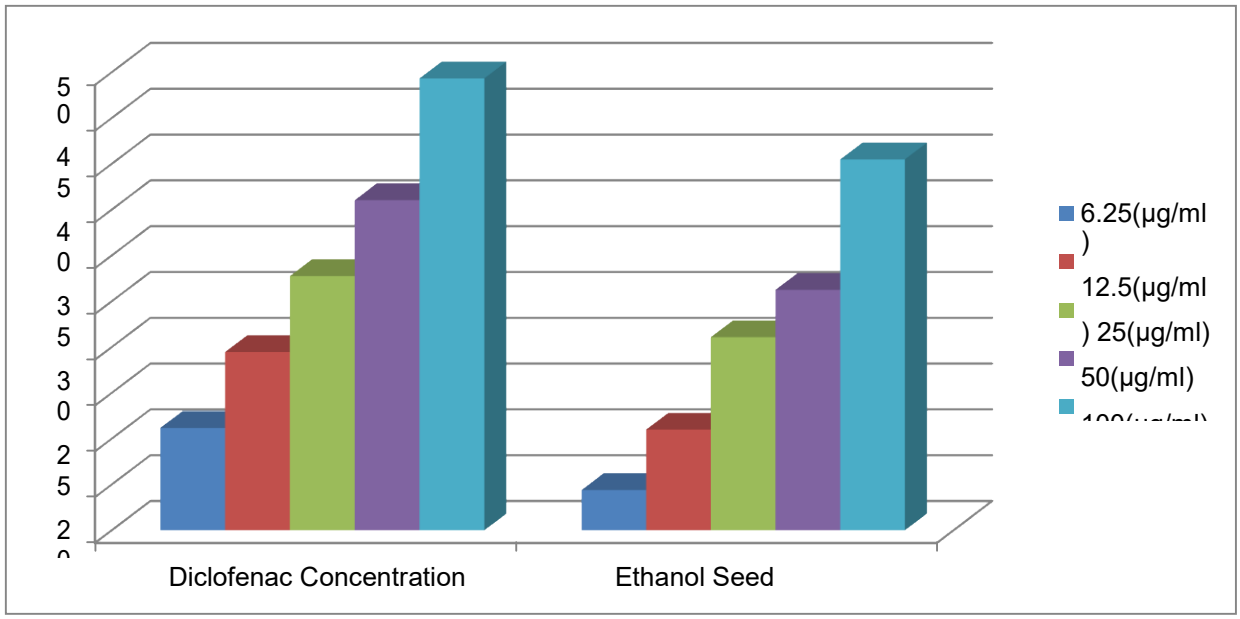

Fig 2: Graphical representation of *Psoralea corylifolia*Seed extract with Standard drug.

COMPARING COX AND LOX ACTIVITY:

Comparing both the enzymes cyclooxygenases and lipoxygenases, the inhibitory activity was evaluated that it was dose-dependent. The evaluation of anti-inflammatory activity by the inhibition of cyclooxygenase of 50 and 100 µg/ml – 26.26% and 40.49% and lipoxygenase of 50 and 100 µg/ml – 29.94% and 46.45%, which shows good anti-inflammatory activity.

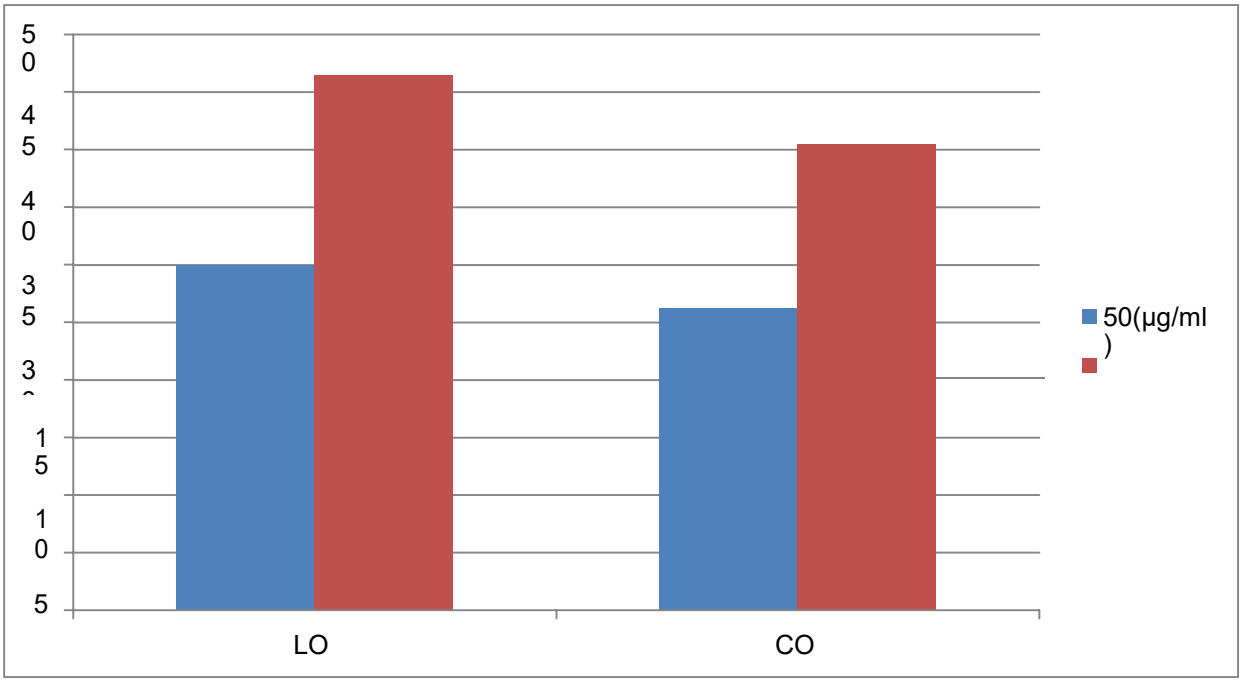

Fig 3: Graphical representation of both LOX and COX.

CONCLUSION:

The present study of In-Vitro analysis concluded the results showed that the extracts of the ethanolic seeds of *Psoralea corylifolia* inhibit the enzymes of lipoxygenases (LOX) and cyclooxygenases (COX) of Anti-Inflammatory activity and also proved that the *Psoralea corylifolia* seeds having Anti-Inflammatory and other medicinal properties.

CONFLICT OF

INTEREST: Nil

REFERENCES:

1. Axelrod B, Cheesebrough TM, Laakso S. Lipoxygenase from soyabean. Methods Enzymol .71,1981,441-5.
2. Cotran RS, V Kumar, T Collins and SL Robbins (Eds.) (1999)Robbins Pathologic Basis of Disease, 6th Edition (WB Saunders, Philadelphia, PA, USA), pp 1425.
3. Gierse Walker MC, Gierse JK. In vitro assays for cyclooxygenase activity and inhibitor characterization. Methods Mol Biol. 644, 2010, 131-44.
4. Harborne, J. B. (1973). Phenolic compounds. In Phytochemical methods (pp. 33-88). Springer, Dordrecht.

5. Khuranna, D., Sharma, S., Mir, S. R., Aqil, M., Ahmad, A., Rehman, M. U., & Mujeeb, M. (2020). Extraction, Quantification, and Cytokine Inhibitory Response of Bakuchiol in Psoralea coryfolia Linn. Separations, 7(3), 48.
6. Khushboo, P. S., Jadhav, V. M., Kadam, V. J., & Sathe, N. S. (2010). *Psoralea corylifolia* Linn.— "Kushtanashini". Pharmacognosy reviews, 4(7), 69.
7. Maisch, J. M. (1889). USEFUL PLANTS OF THE GENUS PSORALEA. American Journal of Pharmacy (1835-1907), 345.
8. Medzhitov, R. (2008). Origin and physiological roles of inflammation. Nature, 454(7203), 428- 435.
9. Paul, W.E. (2008) Fundamental Immunology. 6th Edition, Lippincott Williams & Wilkins, Philadelphia.
10. Wang, Y. F., Wu, B., Yang, J., Hu, L. M., Su, Y. F., & Gao, X. M. (2009). A rapid method for the analysis of ten compounds in *Psoralea corylifolia* L. by UPLC. Chromatographia, 70(1), 199-204.