

## Research Article

## In-Vitro Evaluation on Anti-Inflammatory Activity of Methanol Extract of *Piper betle* Leaf

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### ABSTRACT

To investigate the in vitro anti-inflammatory effect of methanol extract of piper betle leaf against the denaturation of protein. The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the piper betle leaf. The effect of diclofenac sodium was found to be less when compared with the test extract. From the present study it can be concluded that piper betle leaf possessed marked in vitro anti-inflammatory effect against the denaturation of protein. The effect was plausibly due to the most important dietary flavonoids is quercetin (group of flavonols) contents of piper betle leaf.

**Keywords:** Antiinflammation, piper betle, Diclofenac sodium and methanol.

### INTRODUCTION

In the modern world, usage of drugs has become indispensable to lead a normal life for the changed dietary habits. As the life goes on; a day comes where an average human body becomes stuffed with synthetic drugs and such a usage is bound to produce adverse side effects. These side effects need to be taken care of by introducing new drugs into the body and the vicious circle continues. The best alternative to keep a check on this situation is by searching for the alternative sources of drugs that have no side effects. Since long, it is well known that the plants are the richest sources of bioactive substances that have a wide variety of applications in the field of therapeutics. The world health organization (WHO) estimates that 80% of the world populations presently use herbal medicine for some aspects of their primary health care<sup>1</sup>.

Inflammation is a dynamic and multifactorial process involving with many systems in the body. Rheumatic diseases are common inflammatory diseases in the world. There is a great disadvantage of present anti-inflammatory synthetic drugs like Steroidal and non steroidal drugs lie in their toxicity and reappearance of symptoms after discontinuation of treatment. Plant based traditional medicine system play a vital role in healthcare and various plant

extracts and their isolated compounds are excellent anti-inflammatory agents<sup>5,9</sup>.

The *Piper betle* (Tamil: vetrilai) is a glabrous climbing vine belonging to the family *Piperaceae*. It is abundantly distributed in many Asian countries. In India it is found in Bihar, Bengal, Orissa, Tamilnadu, Andhra Pradesh and Karnataka. In Tamilnadu, three varieties of Piper betel leaves, Sirugamani, Karpoori and vellaikodi are available mostly<sup>1</sup>. The chief constituent of the leaves is a volatile oil varying in chemical composition from different countries and known as betel oil. The active ingredient of *piper betle* oil which is obtained from the leaves are primary a class of allyl benzene compounds, chavibetol (betelphenol; 3-hydroxy-4-methoxyallylbenzene), Chavicol (p-allylphenol; 4-allyl-phenol), Estragole (p-allylanisole; 4-methoxy-allylbenzene), Eugenol (allylguaicol; 4-hydroxy-3-methoxyallylbenzene; 2-methoxy-4-allylphenol), methyl Eugenol (Eugenol methyl ether; 3,-dimethoxy-allylbenzene) and hydroxycatechol (2,4-dihydroxy-allylbenzene)<sup>2</sup>. Previous studies on the betel leaves, roots and whole extract (mixture of volatile and non-volatile) of the green variety showed a very strong antiinflammatory and microbial activity. In the South East Asia region, *piper betle* L, is among the plants that have been associated with the control of caries and periodontal

diseases and to the control of bad breath in traditional practice<sup>3</sup>. Piper betel L. leaves is widely used as a mouth freshener after meal. This plant is extensively grown in Bangladesh, India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Its common name is betel in English, paan in India and Bangladesh. Indian system of medicine and health has adopted the use of betel leaves in various ways. In Indian folkloric medicine, betel leaf is popular as an antiseptic and is commonly applied on wounds and lesions for its healing effects. Essential oil extracted from betel leaf may be used as an industrial raw material for manufacturing medicines, perfumes, mouth fresheners, tonics, food additives. Mouthwashes and tablets containing pulverized betel nuts were used for the treatment of dental and periodontal diseases<sup>4</sup>.

Inflammation is a bodily response to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair<sup>5</sup>. It is triggered by the release of chemical mediators from injured tissue and migrating cells. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities<sup>9</sup>. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost. In the current investigation, the chemical composition, physico-chemical parameters and antiinflammatory potential of leaf extract of *Piper betle* from vellaikodi variety has been evaluated against antiinflammatory.

#### MATERIALS

Plant material dried piper betle leaves were ground mechanically into a coarse powder and kept into an air-tight container for use in the study.

#### Drugs and chemicals Diclofenac

sodium was procured from Organic Chemical Industries Pvt. Ltd., Kolkata 70001, West Bengal, India. All the other chemicals were of analytical grade obtained commercially. Double distilled water from all-glass still was used throughout the study.

#### Preparation of extract

The powder plant material (50 g) was extracted with 400 mL distilled water by boiling for 45 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (methanol extract of piper betel leaf, yield: 27.28%). The dry extract was kept in a vacuum desiccator until use.

#### METHODS

The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug.

#### Evaluation of in vitro anti-inflammatory activity

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of methanol extract of piper betel leaf so that final concentrations become 31.25, 62.5, 125, 250, 500, 1 000 µg/mL. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37°C±2) °C in a BOD incubator (Labline Technologies) for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2 500 µg/mL) was used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

Where,  $V_t$  = absorbance of test sample,  $V_c$  = absorbance of control. The extract/drug concentration for 50% inhibition (IC<sub>50</sub>) was determined by plotting percentage inhibition with respect to control against treatment concentration.

## RESULTS AND DISCUSSION

**Table 1: Effect of Methanol extract of *piper betel* leaf on protein denaturation**

Concentration (g/mL)	% of Inhibition	Viscosity (cp)
Control	--	1.45
31.25	20	0.71
62.50	120	0.75
125.00	400	0.79
250.00	1 320	0.84
500.00	2 800	0.88
1000.00	3 700	1.00

**Table 2: Effect of diclofenac sodium on protein denaturation**

Concentration (g/mL)	% of Inhibition	Viscosity (cp)
Control	--	1.45
78.125	12.5	1.45
156.25	12.5	0.80
312.5	25.0	0.86
625	50.0	1.00
1 250	212.5	1.13
2 500	812.5	1.15

In the present investigation, the in vitro anti-inflammatory effect of methanol extract of piper betel leaf was evaluated against denaturation of egg albumin. The results are summarized in Table 1. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by methanol extract of piper betel leaf throughout the concentration range of 31.25 to 1000 $\mu$  g/mL. Diclofenac sodium (at the concentration range of 78.125 to 2500 $\mu$  g/mL) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 2); however, the effect of diclofenac sodium was found to be less when compared with methanol extract of piper betel leaf. This was further confirmed by comparing their IC<sub>50</sub> values. methanol extract of piper betel leaf possessed IC<sub>50</sub> value 40  $\mu$ g/mL whereas that of diclofenac sodium was found to be 625  $\mu$ g/mL.

**DISCUSSION**

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of methanol piper betel leaf extract. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo. Agents that can prevent

protein denaturation therefore, would be worthwhile for anti-inflammatory drug development<sup>4</sup>.

The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by methanol piper betel leaf extract and reference drug diclofenac sodium. From the IC<sub>50</sub> values it becomes evident that methanol piper betel leaf extract was more active than diclofenac sodium, being effective in lower concentrations. This anti-denaturation effect was further supported by the change in viscosities<sup>6,7</sup>. It has been reported that the viscosities of protein solutions increase on denaturation. In the present study, the relatively high viscosity of control dispersion substantiated this fact. Presence of methanol piper betel leaf extract prevented this. The viscosities were found to decrease with concomitant decrease in concentration of test extract and reference drug as well. Although, the viscosities of the test samples (extract/drug), of all concentrations were always less than that of control. This decrease in viscosities may be due to decrease in concentration of test extract/drug in reaction mixture, which resulted in decreased viscosity; and/or other uncertain physico-chemical factors. Nevertheless, the viscosity data indicated inhibition of protein (albumin) denaturation. The effect of concentration of test agent on viscosity behaviour of denatured protein dispersion requires further studies. The major constituents of piper betel leaf bean are an alkaloid, polyphenolic compounds like

tannins and a phenolic acid namely chlorogenic acid. Polyphenols are well known natural products known to possess several notable biological properties. In the present study, the in vitro anti-inflammatory activity of methanol piper betel leaf extract can be attributed to its polyphenols content. The effect may be due to the synergistic effect rather than single constituent. 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 (pH: 6.2-6.5).

### CONCLUSION

Therefore, from the results of the present preliminary study it can be concluded that piper betel leaf possessed marked in vitro anti-inflammatory effect against the denaturation of protein. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions<sup>4</sup>.

### REFERENCES

1. Kirtikar RK and Basu DB. Indian Medicinal Plant, Vol.III. 2nd Ed, International Book Distributors, Uthanchal. 2005;2131-2133.
2. Ghosh K and Bhattacharya TK. Chemical Constituents of piper betle Linn. (Piperaceae) Roots, Molecules. 2005;10(7):798-802.
3. Jenie BSL. Antimicrobial activity of Piper betle Linn extract towards food borne pathogens and food spoilage microorganisms, FT Annual Meeting, New Orleans, Louisiana. 2001.
4. Ashutosh Kar. Pharmacognosy and Pharmacobiotechnology. 2nd Ed, New Age International Publishers, New Delhi. 2007;240-247.
5. Das M, Ram A and Ghosh B. Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice. Inflammation Research. 2003;52:101-106.
6. Dekkers BGJ, Maarsingh H, Meurs H and Gosens R. Airway structural components drive airway smooth muscle remodeling in asthma. Proceedings of the American Thoracic Society. 2009;6(8):683-692.
7. Govindan S, Viswanathan S, Vijayasekaran V and Alagappan R. pilot study on the clinical efficacy of Solanum xanthocarpum and Solanum trilobatum in bronchial asthma. J. Ethnopharmacol. 1999;66:10-205.
8. Fryer AD and MacLagan J. Muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig. Br J Pharmacol. 1984;83:973-978.
9. Fahy JV. Anti-IgE: Lessons learned from effects on airway inflammation and asthma exacerbation. The Journal of Allergy and Clinical Immunology. 2006;117(6):1230.