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In vitro anti-inflammatory activity of betel leaf (*Piper betle* L.)

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Abstract-
Piper betle L. also known as 'Paan' in India, is an important species of the Piperaceae family. The betel leaves are mainly used as a mouth freshener and have long been in use in the Indian indigenous system of medicine for the relief of pain.
The present study was conducted to determine the *In vitro* anti-inflammatory activity of *Piper betle* L. leaf extracts. Ethanolic and aqueous extracts were prepared from dried betel leaves. *In vitro* anti-inflammatory activity of these extracts was investigated by inhibition of protein denaturation assay and results were compared with standard drug Diclofenac sodium. Both extracts exhibited concentration-dependent inhibition of protein denaturation. The IC50 value of both extracts was calculated and compared with standard drug.

Keywords- *Piper betle* L., *In vitro* anti-inflammatory activity, inhibition of protein denaturation

Introduction

Piper betle L. also known as 'Paan' in India, an important species of the Piperaceae family, is an evergreen and perennial creeper, with glossy heart-shaped leaves. The leaves of *Piper betle* L. have long been in use in the Indian indigenous system of medicine for the relief of pain. The betel leaf has long been used as a household remedy for the inflammation of the oral cavity. is The betel leaves are mainly used as a mouth freshener and is also well known for curing many communicable and non-communicable diseases like cold, cough, bronchial asthma, rheumatism, stomachalgia and used to treat other diseases like bad breath, boils and abscesses, conjunctivitis, constipation, swelling of gums, cuts and injuries (Gundala et al., 2014). *Piper betle* L. is a famous aromatic herb. Various studies on biological effects of betel leaves indicated the presence of antibacterial, anti-fungal, antioxidant and anti-inflammatory activities (Sengupta and Banik, 2013). Betel leaves have wound healing properties. In addition, the leaves can also be applied locally to cure inflammatory swelling such as orchitis, arthritis and mastitis. (Salehi et al., 2019). There have been a number of studies on the analysis of phytochemical components of the *Piper betle* L. as well as the extraction and isolation of interesting compounds from betel plants. (Hussain et al.,2014;

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Nguyen et al., 2020). Betelvine is a commercially important crop cultivated in Kelwa village of Palghar district, Maharashtra, India.

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation has been identified as the cause of inflammation. Indications are that when living tissues are injured, inflammation results. This is characterized by redness, swelling, pain, and heat in the affected area. Disruption of the electrostatic, hydrogen, hydrophobic and disulphide bonds in the protein structure occurs, causing the protein to lose its molecular conformation and functions or become denatured. It is therefore deduced that compounds which are able to prevent these changes and inhibit thermally or heat induced protein denaturation, have potential therapeutic value as anti-inflammatory agents. (Bailey-Shaw et al.,2017).

Currently, there is growing interest, both in the industry and in scientific research, for medicinal plants because of their anti-inflammatory properties. These properties are due to many active phytochemicals. Scientific research on the betel leaves reveals that it possesses many beneficial bioactivities and its extract has a great potential in developing anti-inflammatory drugs.

Materials and Methods

Collection of sample-

Fresh and healthy leaves of *Piper betle* L. were collected from the betelvine farm in Kelwa village of Palghar district, Maharashtra, India.

Preparation of extracts-

Betel leaves were washed under distilled water, shade dried and ground to get fine dry powder. Ethanolic and aqueous extracts were prepared from 15g powder of dried betel leaves and 300ml solvents, through Soxhlet extraction method. Then collected extracts were evaporated to dryness and stored in the refrigerator at 4ºC for further analysis. (Banik et al., 2020)

Determination of *In vitro* anti-inflammatory activity

Anti-inflammatory activity of betel leaf extracts was measured *in vitro* by Protein Denaturation Inhibition Assay (De et. al., 2017). The 5ml of assay mixture consisted of 0.5 ml of egg albumin (100mg/ml) as a protein source, 2.5 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract (200µg/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg/ml ethanolic and aqueous extracts of betel leaf). Similar volume of double-distilled water was used as control. All the assay mixtures were incubated at (37ºC±2)ºC in an incubator for 15 min and then heated at 70ºC for 5 min. After cooling, their absorbance was measured at 660 nm by vis-spectrophotometer. Diclofenac sodium at the final concentration of (200µg/ml, 400µg/ml, 600µg/ml, 800µg/ml, 1000µg/ml) was used as a reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

Percentage inhibition (%) = $[(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100$

The plant extract or drug concentration for 50% inhibition (IC50) was determined by plotting

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percentage inhibition with respect to control against concentration of plant extract or drug.

Results and Discussion

Percentage inhibition of protein denaturation by betel leaf extracts was determined to study *In vitro* anti-inflammatory activity and the results are presented in Table 1 and Table 2. IC50 value was calculated by plotting a graph (Figure 1, Figure 2 and Figure 3) of percentage inhibition with respect to control against plant extract or drug concentration.

Conc. of extract/drug (µg/ml)	% inhibition of protein denaturation		
	Ethanolic extract of betel leaf	Aqueous extract of betel leaf	Diclofenac sodium
200	31.25	26.25	37.50
400	43.75	39.58	47.90
600	58.33	54.16	61.78
800	68.75	62.50	70.83
1000	80.41	71.56	83.33

Table 1 - Percentage inhibition of protein denaturation by betel leaf extracts and standard drug.

Test Extract/Drug	IC50 value (µg/ml)
Ethanolic extract of betel leaf	494.29
Aqueous extract of betel leaf	585.42
Diclofenac sodium	420.75

Table 2 - IC50 values of betel leaf extracts and standard drug against protein denaturation.

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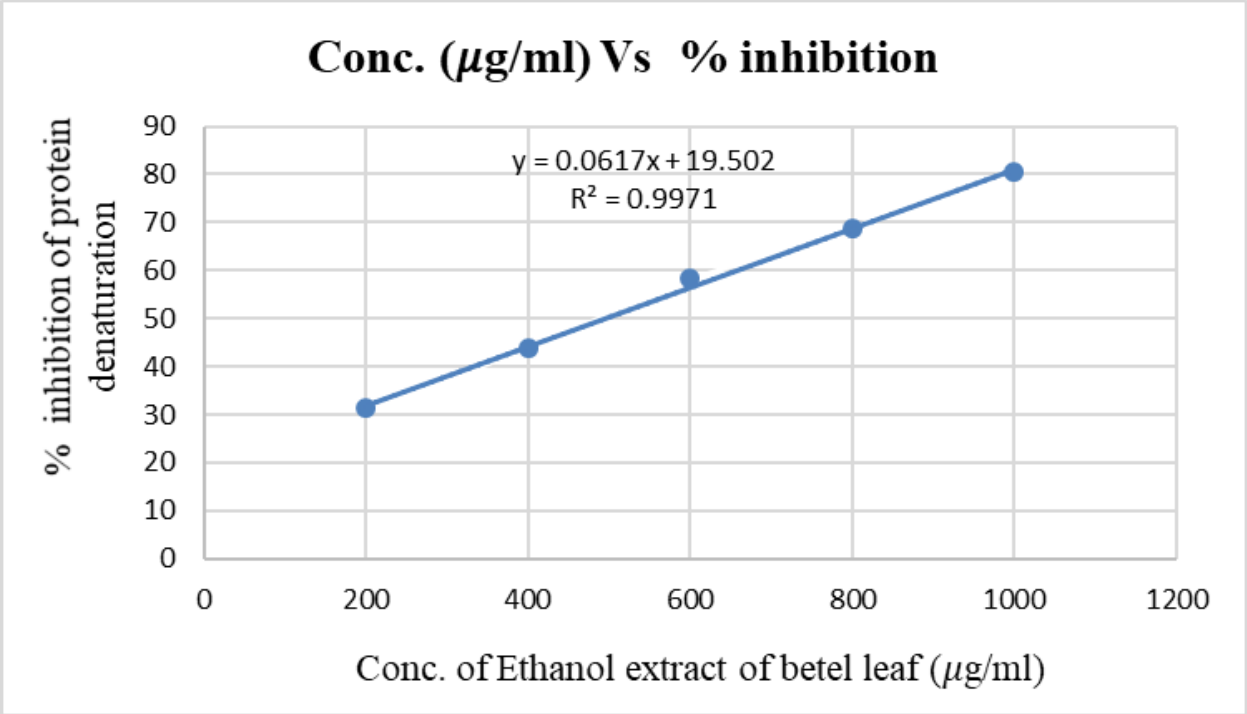

Figure 1- Percentage inhibition of protein denaturation by ethanolic extract of betel leaf.

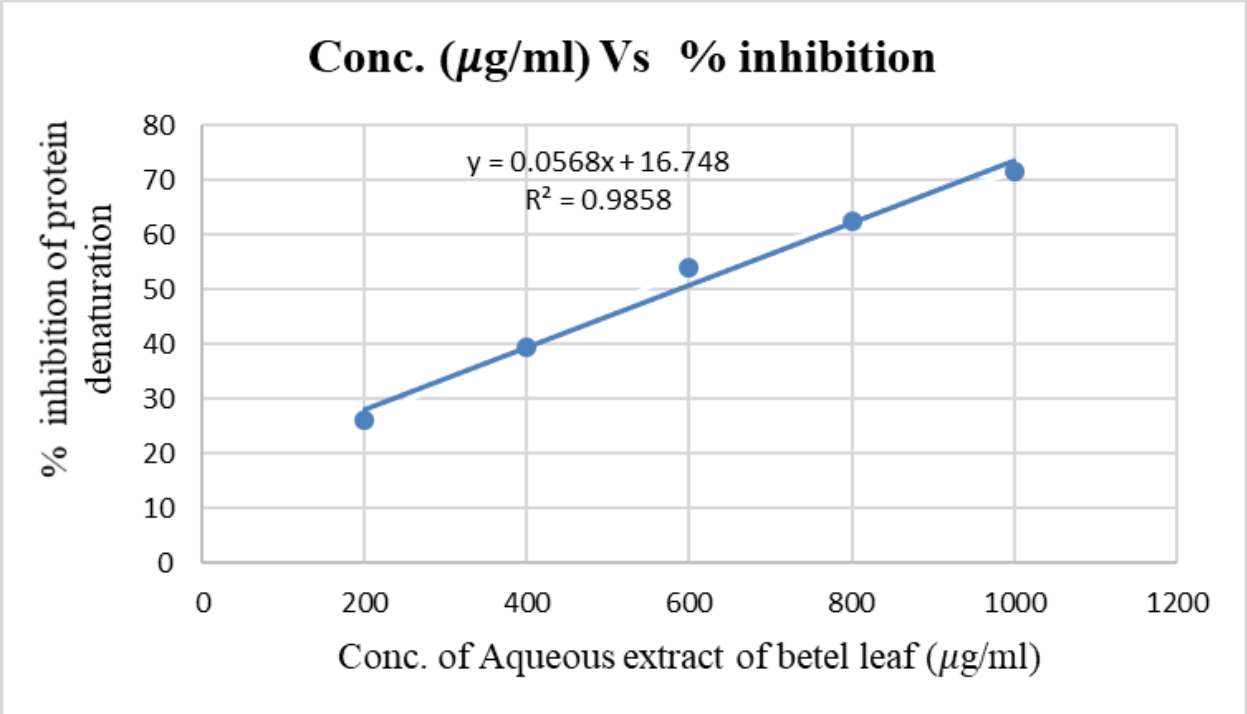

Figure 2- Percentage inhibition of protein denaturation by aqueous extract of betel leaf.

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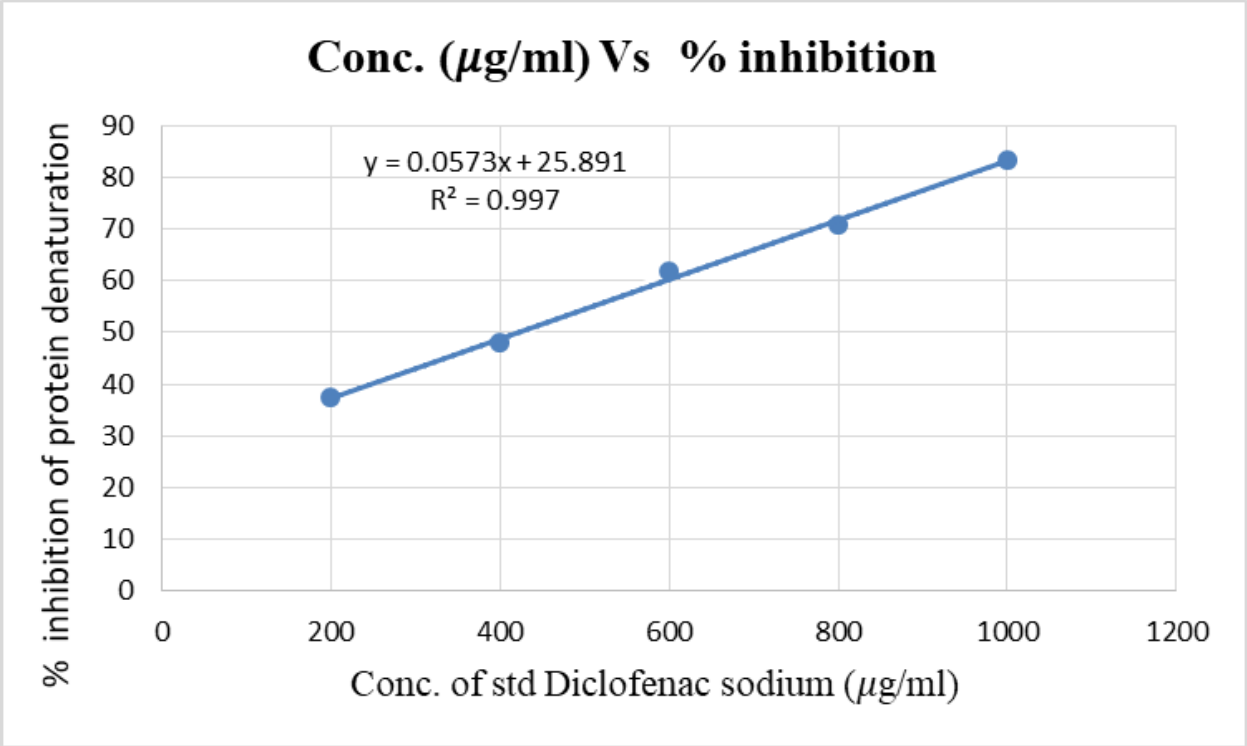

Figure 3- Percentage inhibition of protein denaturation by Diclofenac sodium.

The results supported the traditional use of this plant in some painful and inflammatory conditions. De et. al. (2017) have reported higher percentage inhibition of protein denaturation in alcoholic extract than aqueous extract of *Piper betle* L. collected from Kolkata, West Bengal, India. Abhishek et. al. (2021) have also reported significant anti-inflammatory activity in extracts of *Piper betle* L. collected from different regions of Karnataka, India.

Based on IC50 value, aqueous and ethanolic extracts effectively inhibit protein denaturation (albumin) caused by heat at a concentration of 494.29 µg/ml and 585.42 µg/ml respectively. IC50 value of diclofenac sodium is 420.75 µg/ml.

Conclusion

It has been reported that several anti-inflammatory drugs have the ability to stop protein denaturation. Therefore, from the findings of the present study it can be concluded that the ethanolic and aqueous extracts of *Piper betel* L. had significant anti protein denaturation effect *in vitro*. The anti-protein denaturation property of betel leaf may be due to the presence of bioactive compounds present in this plant. So, the anti-inflammatory effect of *Piper betle* L. cultivar from Kelwa village of Palghar district should be further evaluated in pursuit of designing a potent anti-inflammatory drug which can be used for treatment of various diseases.

References

1. Bailey-Shaw Y.A., Williams L.A.D., Green C.E., Rodney S., Smith A.M. 2017. In- vitro evaluation of the anti-inflammatory potential of selected jamaican plant extracts using the

bovine serum albumin protein denaturation assay. Int. J. Pharm. Sci. Rev. Res., 47(1):145-153.

2. Banik A., Tamanna Z., Sultana R. 2020. Comparative Phytochemical and Antibacterial Properties of *Piper betle* Leave Extracts from Barguna and Moheshkhali, Bangladesh. Iranian Journal of Medical Microbiology, 14(2): 125-132.
3. De P., Sarkar S., Mukhophadhyay M. J. 2017. Anti protein denaturation activity and bioactive compound screening of Piper betel aqueous and alcoholic leaf extract. Journal of Pharmacognosy and Phytochemistry, 6(2): 52-55.
4. Gundala S.R., Yang C., Mukkavilli R., Paranjpe R., Brahmbhatt M., Pannu V., Cheng A., Reid M. and Aneja R. 2014. Hydroxychavicol, a betel leaf component, inhibits prostate cancer through ROS-driven DNA damage and apoptosis. Toxicology and Applied Pharmacology, 280(1):86–96.
5. Hussain R.M., Abdullah N.F. 2014. Isolation of allylpyrocatechol from *Piper betle* L. leaves by using high-performance liquid chromatography. Journal of Liquid Chromatography and Related Technologies, 38:289-293.
6. Nadig Abhishek P. R., Mandira T. G. and Kumar J. R. 2021. Comparative studies on the Antioxidant, Anti-Inflammatory and Phytochemical constituents of *Piper Betel* Leaves from the Western Ghats and Northern Region of Karnataka. International Journal of Pharmaceutical Sciences and Research, 12(6):1000-1011.
7. Nguyen L.T.T., Nguyen T.T., Nguyen H.A., Bui Q.T.P. 2020. Simultaneous determination of active compounds in Piper betle Linn. leaf extract and effect of extracting solvents on bioactivity. Engineering Reports, 2e12246:1-8.
8. Rai K. R., Trivedi R. V. and Umekar M. J. 2019. Review on Betel Leaf used in various ailments. International Journal of Pharmacognosy 6(8): 259-267.
9. Salehi, B., Zainul, A.Z., Rabin, G., Salam, A.I., et. al. (2019). Piper Species: A Comprehensive Review on Their Phytochemistry, Biological Activities, and Applications. Journal of the National Library of Medicine, 24(7):1364.
10. Sengupta R. and Banik J.K. (2013). A review on betel leaf (pan). Int. J. Pharm. Sci. Res., 4(12):4519-24.