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# Anti protein denaturation activity and bioactive compound screening of *Piper betel* aqueous and alcoholic leaf extract

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**Abstract**
Nature provides several herbal phyto-chemicals for the beneficial of human since the ancient periods. *Piper betel* is one of the common and valuable herbs in traditional medicine. In the current study, qualitative screening of different bioactive compounds and Anti protein de-naturation property was checked in aqueous and alcoholic extract of *Piper betel*. Three different concentration 50, 75, 100 microgram/ml was taken for protein de-naturation method using same amount sodium diclofenec as a reference drug. The study revealed that *P. bete* have several bioactive compounds and significant Anti protein de-naturation property. Presence of several phyto-chemicals may help to scavenge the highly heterogeneous conformational isomers derived by denatured proteins in human. So, in future it could be possible to develop a new phyto derived drug for membrane destabilization related disease in human.

**Keywords:** *Piper betel*, anti protein de-naturation, bioactive compound, plant extract

## Introduction
Betel leaf is a member of the family Piperaceae and widely consumed in India. About 15-20 million peoples consumed betel leaf in this country [1] with areca nut, slaked lime, catechu with or without tobacco. In other part of South-Asian and Southeast-Asian country like Bangladesh, Myanmar, Indonesia, Vietnam and Sri Lanka, chewing of Betel Quid is very much popular and often taken as a traditional mouth refreshing habit [2]. Other than that, betel leaf has tremendous use in social, cultural and religious ceremonies like marriage occasions, puja festival etc [3]. In Indian society, it is also used as a symbol of respect and offered to the guest as an honor. Betel leaf is also used in many folk medicines to reduce bad breath, boils and abscesses, conjunctivitis, constipation, headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, ringworm, otorrhoea, swelling of gum, abrasion, rheumatism, cuts and injuries etc [1, 4, 5]. Since the Vedic age, betel leaf was also used in ayurveda, unani and siddha medicine [6]. This literature review prompted us to investigate the presence of bioactive compounds and *in vitro* anti-inflammatory activity of betel leaf extract through anti-Protein de-naturation method. Protein de-naturation results in the disorganization and unfolding of the protein secondary and tertiary structure without breaking or hydrolysis of peptide bonds. De-naturation may under ideal condition, be reversible, and its original native structure will retain by refolding when the de-naturating agent is removed. However, most protein, once de-natured, remains permanently disordered. Denatured proteins are often insoluble and therefore precipitate which increases the activity of macrophase in the protein de-naturation site within the tissue leading some neurodegenerative disease and inflammatory disease [7, 8].
The management of protein de-naturation related diseases is a big challenge to the medical practitioner as there are huge side effects for the long term consumption of conventional drug [9, 10]. For the remedy of this serious problem, clinician tries to believe in some alternative or herbal medicine. Nature provides huge medical agents for thousands of years and a significant numbers of modern human drug are isolated from natural resources. So, in future, development of new plant based drug with better bioactive potential and without or less side effects is the principal objective to the researcher.

## Materials and Methods
### Collection and Preparation of Extract
Fresh leaves of Paan or *Piper betel* were collected from a local market during the month of March. Fresh leaves were washed twice through running tap water then followed by distilled water and air dried. After proper drying, leaves were blended to make a fine powder. The shade dried powder of leaves was stored in room temperature for future use.

One gram of the dried powdered leaves was taken in two different pre-labeled conical flask and 40 ml of double distilled de-ionized water and ethanol was added in each. The mixtures were kept in the BOD shaker incubator at 30 ºC temperature in 120 rpm for overnight. Next day both the mixture was filtered through Whatman filter no- 1. During the anti protein de-naturation assay every time freshly prepared aqueous and alcoholic extract were used.

**Phyto-chemical Screening**

Freshly prepared extract of *Piper betel* was screened for the presence of bioactive compounds like alkaloids, flavonoids, tannin, carbohydrate, amio acids and proteins, terpenoids, saponin, sterols etc. The qualitative analysis was done by the standard method of Harbone [11].

**Protein Denaturation Assay**

In this experiment 0.2 ml of egg albumin (from fresh hen's egg) act as a protein source, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extract (50µg/ml, 75µg/ml, 100µg/ml alcoholic and aqueous extract of *Piper betel* leaves) were mixed to prepare assay mixture. Similar volume of double-distilled water served as control. The mixtures were incubated at 37±2ºC in a BOD incubator for 15 minutes and then heated at 70ºC for 5 minutes in water-bath. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentration of (50µg/ml, 75µg/ml, 100µg/ml) was used as reference drug and treated similarly for determination of absorbance. [12, 13]

**Calculations**

The percentage inhibition of protein de-naturation was calculated by using the following formula

$$\% \text{ inhibition} = 100 \times [\, Vt / Vc - 1]$$

Where, Vt = absorbance of test sample, Vc = absorbance of control

**Result**

In the current study, presence of different bioactive compound in the extract of *Piper betel* was depicted in Table-1 and the anti protein denaturation property of aqueous and ethanolic extract was depicted in Table-3. Concentration of reference drug and the experimental samples were also mentioned in the same table.

**Table 1:** Bioactive compound study in the extract of *Piper betel*

| Particulars | Observation |
|---|---|
| Alkaloids | +++ |
| Flavonoids | +++ |
| PolyPhenols | +++ |
| Tannin | +++ |
| Carbohydrate | +++ |
| Saponin | ___ |
| Carbonyls | +++ |
| Terpenoids | ___ |
| Proteins | +++ |
| Sterols | +++ |
| Triterpensess | +++ |
| Anthraquinone | ___ |

+++ =Positive, --- =Negative

**Table 2:** *In vitro* anti protein denaturation activity of two different vehicles

| Type of Vehicle | Optical Density at 660 nm | Inhibition |
|---|---|---|
| 1. Water | 0.419 | Minimum |
| 2. Ethyle Alcohol | 0.241 | Moderate |

**Table 3:** *In vitro* anti protein denaturation activity of aqueous and ethanolic extract of *Piper betel*

| Treatment | Concentration(µg/ml) | % Inhibition |
|---|---|---|
| Control | ---------- | ---------- |
| Ethyle Alcohol Extract | 50 | 39.05 |
| | 75 | 43.71 |
| | 100 | 49.01 |
| Aqueous Extract | 50 | 30.28 |
| | 75 | 31.70 |
| | 100 | 35.04 |
| Sodium Diclofenac | 50 | 48.99 |
| | 75 | 68.74 |
| | 100 | 84.73 |

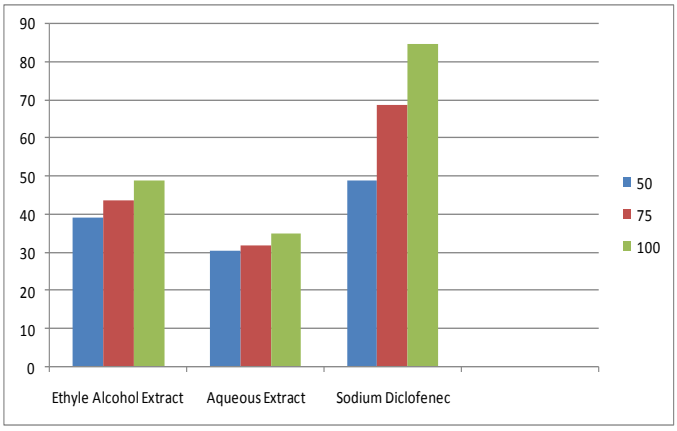


**Fig 1:** Comparative analysis of inhibition percentage of protein denaturation in different extract of Betel leaf

**Discussion**

*Piper betel* is very common mouth freshener throughout the world. The leaves of *Piper betel* are full of nutrients, anti-oxidants and different bioactive molecules like phyto-chemicals and many nutraceuticals. But many few people know about the beneficiary effect of betel leaf as there was several products manufactures from betel leaves on industrial scale like Tooth-pastes Skin emollients Tooth-powders Paan masala De-odourants Mouth freshners Facial creams Anti-septic lotions Cold drinks Chocolates Appetizers Digestive agents Tonics and medicines Beauty and cosmetics products [14].

From ancient periods betel leaf was traditionally used as medicinal purpose to cure several health problems like bad breath, conjunctivitis, , boils and abscesses constipation, hysteria, headache, itches, mastoiditis, mastitis, leucorrhoea, otorrhoea, ringworm, rheumatism, abrasion, swelling of gum, cuts and injuries etc and the root is known for its female contraceptive effects [4; 5]. The essential oil contained in the leaves possesses anti-fungal, anti-protozoan and anti-bacterial properties and its inhibitory action against tuberculosis, cholera and typhoid causing bacteria needs proper evaluation and exploitation [1]. Several literature studies revealed that betel leaves have full of nutrition and contain substantial amount of vitamins and minerals along with enzymes like

catalase and diastase. It also contains significant amount of essential amino acids without histidine, arginine and lysine [15; 16; 17]. According to Guha-2006, six leaves is almost equivalent to about 300 ml cow milk particularly for the vitamin and mineral quantity. In modern scientific research revealed that *Piper betel* leaves have anti carcinogenic properties. So, the cause of oral cancer is not for the betel leaves it may be due to some other carcinogen containing ingredients like tobacco [18].

Denatured proteins comprise highly heterogeneous conformational isomers and typically devoid of their intended biological activities. Due to the complexity of structure and the lack of biological function, structural and functional analysis of denatured proteins has been generally regarded as a daunting and futile effort. However, the importance of characterizing denatured protein is increasing in recent years as conformational change of proteins has proven to be the underlying cause of many neurodegenerative and inflammatory diseases. Any attempt to elucidate the mechanism of these diseases would have to entail meticulous characterization of diverse isomers of disease-associated proteins. In addition, conformational isomers of denatured proteins are conceivably one of the most opulent resources of bio-molecules that have remained untapped for their potential use in the disease diagnosis and treatment [19].

In the modern age of pharmaceutical research use of animal models associated with certain problems like ethical issues and different mechanism of body homeostasis during adverse condition. This problem leads us to look for alternative methods on the view point of basic mechanism [13]. Hence, in the present study the protein de-naturation assay methods are selected for assessment of *in vitro* anti- inflammatory property of *Piper betel*. Protein de-naturation is one of the key features of inflammatory tissue and it was a well-documented cause of inflammation related disease like arthritis. It is believed that agent that can help in anti-protein de- naturation could be used as a potent anti-inflammatory drug in future. As the agro-economy of this crop is not exploring mainly in post harvesting part thousand tons crop is wasted throughout India. So, production of anti-inflammatory drug and other nutrceutical from betel leaf will have an exploring industrial prospect.

In the present study the *in vitro* Protein denaturation activity of *Piper betel* was evaluated against heat induced protein denaturation. The present findings exhibit concentration dependant anti protein denaturation by the selected plant extract. The inflammatory response was generated by the release of denatured protein of lysosomal constituents which may activated neutrophil and proteases, leads more tissue inflammation by extra cellular release.

Qualitative analysis revealed presence of several phytochemicals like alkaloids, flavonoids, polyphenols, sterols, carbohydrate, and tannin in betel leaf extract. Among these bioactive compounds several have well known potential biological properties. The anti-protein denaturation property of Paan (*Piper betel*) may be due to the presence of these bioactive compounds. The effect may be synergistic rather than single one.

## Conclusion

It has been reported that several non-steroidal anti-inflammatory drugs have the ability to stop protein denaturation. Therefore, form the findings of the present preliminary experiment it can be concluded that the ethanolic and aqueous extract of *Piper betel* had marked anti protein denaturation effect *in vitro*. So, the anti-inflammatory effect of this plant should be further evaluated in pursuit of newer phytotherapeutics against inflammatory diseases.

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**Competing Interests Statement**

The authors declare that they have no competing interests.

**Contributor ship Statement**

Dr. M.J Mukhopadhyay helped with the concept and discussion. Mr. Puspal De and Mr. Subhradeep Sarkar performed all the experiments and analysis part in the laboratory. All authors read the manuscript and agreed for publication.

**Data Sharing Statement**

We cannot share any unpublished data with other laboratory or person.

**Ethics Approval**

Present study was approved by the institutional ethical committee.

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