Section: Research Paper
ISSN 2063-5346

Phytochemical Screening and Evaluation of Anti-Inflammatory Activity of *Piper Betle*

Dipali Gupta1, Rajat Yadav[2], Dr. Nipun Agrawal[3], Shubhi Saxena[4], Rishita Goel[5] and Shivang Saxena[6]

[1]Associate Professor at Shri Ram Murti Smarak College of Engineering and Technology (Pharmacy), Bareilly
[2]Assistant Professor at Shri Ram Murti Smarak College of Engineering and Technology (Pharmacy), Bareilly
[3]Associate Professor at Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly (corresponding author)
[4]Research Scholar at Shri Ram Murti Smarak College of Engineering and Technology (Pharmacy), Bareilly
[5]Research Scholar at Shri Ram Murti Smarak College of Engineering and Technology (Pharmacy), Bareilly
[6]Research Scholar at Shri Ram Murti Smarak College of Engineering and Technology (Pharmacy), Bareilly

Corresponding Author:
Dr. Nipun Agrawal
Email:- nipun.agrawal@gmail.com
Postal address:- SRMS CET, Nanital Road Bhojipura,Uttar Pradesh Bareilly.
Phone number:- 9412409090

1.ABSTRACT

Background: A member of the Piperaceae family, which also contains pepper and kava, the betel (*Piper betle*) is a vine. Most Asian immigrants use betel leaf throughout Asia and abroad in the world as betel quid or paan, along with areca nut and/or tobacco. A preliminary phytochemical screening was performed on the leaves.

Objective: The anti-inflammatory qualities of *Piper betle* leaf extract were investigated in this work. The purpose of this study was to evaluate the physical characteristics and anti-inflammatory activity of the betel leaf extract at 1%, 2% and 4% concentration.

Methods: Using the Soxhlet apparatus, an alcoholic extract of leaves of *Piper betle* was obtained, and its phytochemical screening was examined. The carrageenan induced paw edema model was used to test the anti-inflammatory activity of these dosage forms.

Result: The anti-inflammatory activity of *Piper betle* is maximum at 4% concentration. The phytochemical screening of betle leaf shows the presence of the constituent i.e. hydroxychavicol (polyphenolic component), quercetin (flavonoid), β-caryophyllene (volatile component) and others like alkaloids, saponins, coumarin and glycosides.

Conclusion: In conclusion, it has been demonstrated that *Piper betle* extract has anti-inflammatory properties through a number of methods, such as the suppression of pro-inflammatory cytokines and enzymes.

2. KEYWORDS: Betle leaf, Carrageenan, Betle Extract, Indomethacin, Paw Edema.

3. INTRODUCTION

Betle leaf, often referred to as "paan ka patta," is frequently used as a mouth refresher and has a powerful, pungent flavor. Evergreen perennial dioecious creeper *Piper betle* L. (Piperaceae) [1]. The *Piper betle* leaf belongs to the Plantae kingdom with the Piperaceae family, Magnoliopsida as class and Piper as genus. The leaves are a fantastic source of calcium and are packed with vitamins like vitamin C, thiamine, niacin, riboflavin, and carotene. It is frequently referred to as "Paan" or "Nagavalli." In many Asian nations, betel has been chewed for centuries [2,3] along with areca nut, slaked lime, cardamom, and clove. In the Indian subcontinent, offering guests a betel morsel (Pan-Supari) is considered polite. In Bangladesh, India, Sri Lanka, Malaysia, Thailand, Taiwan, and other Southeast Asian nations, this plant is widely cultivated [4]. The nodes of the plant's branches are enlarged [5].

The literature has evidence of various beneficial uses of *Piper betle L*. such as treating bronchitis, difficulty in breathing and cough, inflammation and infections of the respiratory tract viz. cough, dyspnoea, indigestion, diphtheria, and hysteria, general and sexual debility[6]. Redness, swelling, heat, and pain are common manifestations of inflammation, which is the body's reaction to microorganisms that have invaded the area[7]. Many studies have shown that inflammation has a role in the development of a number of diseases, including aging[8], cardiovascular dysfunction[9], cancer[10] and other serious and disabling conditions. Free radical overproduction, complicated enzyme activity, and the release of a number of inflammatory and pro-inflammatory mediators are all components of acute inflammation. A well-known acute model of inflammation that is frequently used to screen new anti-inflammatory drugs is the carrageenan-induced paw edema. A biphasic edema was brought on by injecting carrageenan into the subplantar surface of rat paws[7]. Hydrophilic and hydrophobic chemicals found in betel leaf extract can be combined to create emulsion gels that can deliver multiple, controlled releases [11].

Figure No. 1: *Piper betle* leaves

In this review we determine the anti-inflammatory properties of the Piper betle leaf by using carrageenan induced paw edema model. Carrageenans are anionic polysaccharides because they possess 15–40% ester-sulfate concentration. The carrageenan-induced paw edema is a well-established model of acute inflammation that is characterized by the involvement of a wide range of inflammatory mediators in its development and has been extensively used to assess the anti-edematous activity of natural products [12].

Figure No. 2: Dried and crushed *Piper betle*

3.1 Indigenous (Vernacular) Names of Piper betle leaf **[13]:**
English: Betle-vine, Betle pepper, Betle
Sanskrit: Mukhbhushan, Tambool, Varnalata
Hindi: Paan ka patta
Gujarati: Tambola, Paan, Nagarbael
Bengali: Parnakari leaf, Paan, Tambulaballi plant, Paana
Indonesia: Daun sirih, Séwéh, Bakik serasa, Seureuh, Sirih
Chinese: Ch'ing Chu, Wei ye, Tu wei teng, Ju jiang, Da geng teng, Tu bi ba, Wei zi etc.

3.2 Physical Characteristics **[14,15]:**
A tiny climber or ground cover with green leaves that has growth characteristics quite similar to pepper. The betel leaf plant is a branching vine that can reach heights of 10 to 15 feet, however it often grows as a ground cover in the understory. Generally speaking, it is too delicate to grow outside of the tropics. Although it can handle some drought, the ideal circumstances for plant growth are warm and humid. The betel leaf is utilized in a variety of conventional treatments for infections, stomach disorders, and as a general tonic. It is frequently chewed alongside betel nuts (Areca catechu) as a stimulant. There is some evidence that betel leaves have both immune-boosting and anti-cancer qualities.

3.3 Nutritional Properties **[16]:**
The imminent analysis of the *Piper betle* leaves showed that it contained macro and micro nutrients as well as phytochemicals such as phosphorus (0.05-0.6%), vitamin A (1.9-2.9

mg/100g), nitrogen (2.0-7.0%), calcium (0.2-0.5%), tanin (0.1-1.3%), iodine (3.4 µg/100g), riboflavin (1.9-30 µg/100g), vitamin C (0.005-0.01%), thaimine (13-70 µg/100g), potassium (1.1-4.6%), fat (0.4-1.0%), iron (0.005-0.007%), essential oils (0.08 - 0.2%), nicotinic acid (0.63-0.89 mg/100g), minerals (2.3-3.3%), carbohydrate (0.5-6.10%), fibre (2.30%), energy (44 kcal/100g), chlorophyll (0.01-0.25%), water (85-90%) and protein (3-3.5%).

3.4 Chemical Constituents of the Piper betle leaf **[17,18]:**

Betel oil (volatile oil), chavibetol (betel phenol) (53.1%) and chavicol (volatile oil) are the primary constituents of the betel leaf. Others include; caryophyllene (3.71%), eugenol (0.32%), menthone, quercetin etc. The main constituents which are responsible for anti-inflammatory activity are; *hydroxychavicol* (polyphenolic component), *quercetin* (flavonoid) and *β-caryophyllene* (volatile component). Due to their inhibitory effects on the enzymes involved in the formation of the chemical mediator of inflammation, flavonoids and saponins are well known for their capacity to reduce both the sense of pain and their anti-inflammatory capabilities. Due to its high flavonoid concentration, piper betel has anti-inflammatory effects. Polyphenolic constituents potently inhibits the nitric oxide production and it's synthase activity which helps in reducing inflammation induced by carrageenan.

By acting as an effective, selective, and non-psychoactive full agonist for the CB2 receptor in vivo, β-caryophyllene has anti-inflammatory properties while by significantly inhibiting the action of the proinflammatory cytokine TNF-α, hydroxychavicol exhibits strong anti-inflammatory effects.

3.5 Modern Medicinal Use of Piper betle leaf **[19,20,21,22,23]**

1. Betle leaves are helpful in treating lung infections in children and the elderly. To treat coughs and difficult breathing, heated leaves mixed with mustard oil are applied to the chest.
2. A small amount of the leaves can effectively cause sore throats. The berries or fruits that have been flattened should be combined with honey and used to soothe annoying coughs.
3. Betle leaves are useful for treating debility, nervous weariness, and pain associated with the nervous system. A few betle leaves extracted with honey makes a healthy tonic.
4. When used locally, betle leaves are beneficial for treating swelling caused by rheumatoid arthritis and orchitis, or inflammation of the testicles.
5. Betle leaves also possess cooling and analgesic qualities.
6. Also, it works well on boils. A leaf is slightly warmed until it becomes soft, and then castor oil is applied on top of the leaf. The oiled leaf is applied to the sore.
7. A hot compress made of the leaves or their extract with a neutral oil, such as refined coconut oil, can be administered to the loins to treat lumbago.
8. Wounds can also be treated with the leaves. It is best to extract the leaf juice and apply it locally to the wounds.
9. When applied to the breast during lactation, leaves that have been coated with oil are reported to stimulate milk secretion.
10. The Unani system states that these leaves have a strong flavor and pleasant aroma that stimulates appetite.
11. Moreover, it is utilized as a liver, heart, and brain tonic.
12. Moreover, it supports strong teeth and skin.

13. It aids in the treatment of several eye ailments, skin conditions, and disorders of bodily physiology.
14. Betle leaf has diuretic qualities as well. Giving leaf juice mixed with milk or honey can make it easier to urinate.
15. Betel leaf is employed as an aphrodisiac, or an agent that piques one's desire for sexual activity.
16. The leaves' essential oils have qualities that are antibacterial, antiprotozoal, and antifungal. As a result, the oil prevents the growth of egregious bacteria that cause typhoid, cholera, tuberculosis, etc. and aids in correct assessment and utilisation.
17. Six leaves plus a tiny amount of slaked lime are reported to be equivalent to around 300 ml of cow milk since the leaves are nutrient-rich and contain a significant amount of vitamins and minerals.
18. The leaves also contain significant amounts of all the essential amino acids, with the exception of lysine, histidine, and arginine, which are only present in minute amounts.

4. METHODOLOGY

*4.1 Materials Required***:** Betel leaves, ethanol, distilled water, 0.9% NaCl solution, chloroform and betle extract. A water bath (Memmert®), porcelain saucer, Petri dish, autoclave (Shenan®), incubator, inoculation loop, micropipette (Socorex®), analytical scales, and glassware (Iwaki Pyrex®) were among the research tools used.

*4.2 Method used***:**

*4.2.1 Dried Piper betle leaves preparation***:** The dried betel leaves were prepared by gathering, sorting, drying, and cutting them into smaller pieces. These dried specimens underwent testing for drying losses, followed by macro- and microscopical identification of the blotches [24].

*4.2.2 Preparation of Extract***:**

4.2.2.1 Betel Extract: Five hundred grams of the dried betel leaves were macerated with 5L of ethanol 95% (solvent) with a ratio of 1:10 for 24 hours[25] by using Soxhlet apparatus. The macerated mixture was collected and put through a rotary evaporator at 50 degrees Celsius until concentrated extracts with a solid green hue and a strange betel leaf odor were produced [26]. The extraction of leaves has enormous possibilities for creating industrial products like cream, emulsion gel and nanoemulsion [27,28]. The benefits of both nanoemulsion and emulsion gel have led to high understanding of acceptability for nanoemulgel [29].

4.2.2.2 Detection test of ethanol: This test was performed to make sure that the extract was free of ethanol-related substances. In a test tube, the extract was first dissolved in H_2SO_4 before being mixed with acetic acid. Cotton was used to seal the tube, which was then heated until it boiled. Esters in the cotton's aroma indicate ethanol presence [30].

*4.3 Phytochemical Screening***:**

In order to identify several phytoconstituents such as flavonoids, phenolic component volatile components etc. a freshly made alcoholic extract of *Piper betle* leaves was exposed to phytochemical screening. The test has been done as follows;

*4.3.1 Test for alkaloids***:** Add a few drops of strong hydrochloric acid and a few drops of Dragendroff's reagent to 1 ml of the extract. Alkaloids are present when a reddish brown tint is present[31].
*4.3.2 Test for flavonoids***:** To 1 ml of the extract, a few drops of pure sulfuric acid were added. The presence of flavonoids is indicated by the presence of a persistent yellowish orange colour [32].

*4.3.3 Test for saponins***:** In a test tube, vigorously stir 5 ml of distilled water and 1 ml of the extract. The presence of saponins is indicated by lather development [32].

*4.3.4 Test for terpenoids***:** Add 2 ml of chloroform and 3 ml of concentrated sulphuric acid to 5 ml of the extract to create a layer. Terpenoids are present because a reddish brown precipitate forms at the contact [33].

*4.3.5 Test for tannins***:**
*4.3.5.1 Ferric chloride test***:** Equal quantities of newly produced 10% ferric chloride should be added to 1 ml of the extract. The presence of tannins is indicated by the occurrence of a greenish black color [32].

*4.3.5.2 Lead Acetate test***:** Add 2 ml of 10% Lead Acetate to 1 ml of the extract. The appearance of tannins is indicated by the formation of a white precipitate [32].

*4.3.6 Test for phenols***:** Add 2 ml of 10% Lead Acetate to 1 ml of the extract. The presence of phenols is indicated by the precipitation of a white substance.[32].

4.3.7 Test for proteins (Biuret test): A copper sulphate and sodium hydroxide solution is added to the sample as part of the biuret test. The sample's colour shifts from blue to violet when proteins are present [34].

*4.3.8 Test for Reducing Sugars (Fehling's test)***:** Mix equal parts of Fehling's A (copper(II) sulphate) and Fehling's B (alkaline tartrate solution) to make Fehling's reagent. The Fehling's reagent should be combined with a tiny amount of Piper betel extract, and the two should be heated over a hot plate or in a water bath. Depending on how much reducing sugar is present, reducing sugars in the extract will react with the copper ions in Fehling's reagent to change the colour of the solution from blue to green, yellow, orange, or brick-red. The amount of reducing sugar in the extract can be calculated based on how strongly the colour changes [35].

4.3.9 Test for phytosterols (Liebermann-Burchard test): A test tube should contain a little amount of Piper betel extract. To the test tube, add a few drops of acetic anhydride, and stir thoroughly. To prevent mixing the concentrated sulfuric acid with the acetic anhydride, add 2-3 drops of the acid gently and carefully along the side of the test tube. Check out the mixture's colour. Depending on the type of phytosterol present, the extract will colour green, blue, or violet if phytosterols are present [36].

4.3.10 Test for anthraquinones (Bornträger's test): A test tube should contain a little amount of Piper betel extract. Shake well after adding 2 to 3 mL of chloroform to the test tube. To the test

tube, add a few drops of the 10% ammonia solution, and then shake it one more. Look at the chloroform layer's colour. The chloroform layer will turn pink, crimson, or violet if the extract contains anthraquinones [35]

*4.3.11 Test for Anthocyanosides (Vanillin-HCl test)***:** A test tube should contain a little amount of Piper betel extract. Mix well before adding 1-2 mL of 95% ethanol to the test tube. Mix well after adding a few drops of the 1% hydrochloric acid in ethanol to the test tube. Add a few crystals of vanillin to the test tube and stir well. Heat the mixture in a water bath for a few minutes. Check out the mixture's colour. The combination turns pink, red, or violet if the extract contains anthocyanins [37].

4.3.12 Test for Phlobatannins (ferric chloride test): A test tube should contain a little amount of Piper betel extract. The test tube should now contain a few drops of 5% ferric chloride solution. Mix thoroughly. Check out the mixture's colour. If the extract contains phlobatannins, the mixture will turn blue or green [38].

4.3.13 Test for Volatile Compounds (gas chromatography-mass spectrometry (GC-MS)): You can make Piper betel extract by soaking the leaves in an ethanol or methanol-based solvent. To get rid of any solid components, filter the extract. Put the extract inside the GC-MS device. Determine the volatile chemicals included in the extract by analysing the findings [39].

4.3.14 Test for Coumarin (thin-layer chromatography (TLC) test): Place the extract on a TLC plate, then let it dry. A solvent system like ethyl acetate-methanol-water (7:2:1) or chloroform-methanol-acetic acid (90:10:1) can be used to develop the plate. Look at the plate under UV light with a 254 nm wavelength.Look for any spots that match the retention factor of coumarin, which is normally between 0.5 and 0.6 [40].

4.3.15 Test for Acids (acid-base titration test): A tiny quantity of the extract should be weighed before being dissolved in a known volume of water or another suitable solvent. A few drops of phenolphthalein indicator should be added to the mixture. When the solution turns pink or crimson, titrate it with a standardised sodium hydroxide (NaOH) solution. The titration's endpoint is indicated by the pink or red colour. Note the quantity of NaOH solution needed to reach the destination. Using the formula acid content (mg/g) = (volume of NaOH solution x concentration of NaOH solution x equivalent weight of acid) / weight of extract, you may determine how much acid is present in the extract [41].

4.3.16 Test for Glycosides (Keller-Killiani test): In a test tube, put a small amount of the Piper betel extract. A few drops of hydrochloric acid (HCl) in a 5% solution should be added to the test tube. Fill the test tube with a little amount of concentrated sulfuric acid. For 5 to 10 minutes, warm the mixture in a water bath. After the mixture has cooled, gently add a few drops of potassium hydroxide (KOH) 10% solution to the test tube. Look for red or pink colouring in the combination, which denotes the presence of glycosides [42].

4.4 Animal Species:

In total, 12 albino rats weighing 150-200 grams (6±2 months) were used which were procured as well as housed for 20 days prior to study in SRMS CET (Pharmacy) animal house. Animal housing was kept in good shape under generally accepted hygienic standards, including a humidity level of 60±10%, a 12-hour day/night cycle, and unlimited access to food and water.

These rats were divided into 2 groups viz. control group (group 1) and treatment group (group 2) containing 6 animals each. 2 ml saline/day was given to the control group and betel extract at 1%, 2% and 4% concentrations is given to the treatment group which are monitored at 30, 60, 120, 180, 240 and 300 minutes.

4.5 Procedure for anti-inflammatory activity assessment:

The assessment was carried out by using carrageenan induced paw edema model and it involves following procedure:

1. Animals should be weighed and recorded.
2. Put a mark on the Albino rat's two rear paws.
3. Configure the digital plethysmometer.
4. Take note of each rat's initial paw volume using the mercury displacement method.
5. As a control and treatment group, divide the animals into two groups (6 rats each)
6. Administer saline solution into the control group and betel extract via the oral route into the treatment group.
7. After 30 minutes, inject (i.v. route) 0.1 ml of 1% (w/w) carrageenan into both groups' left paw's plantar area (the right paw of each group will serve as reference non-inflamed paw).
8. Take note of the leg and group paw volumes at 30, 60, 120, 180, 240 and 300 minutes.
9. Compare the % difference in each rat's two paws' volume (both groups).
10. Comparing the two groups of rats' average paw volume changes.
11. Express to what extent did the anti-inflammatory betel extract prevent edema.

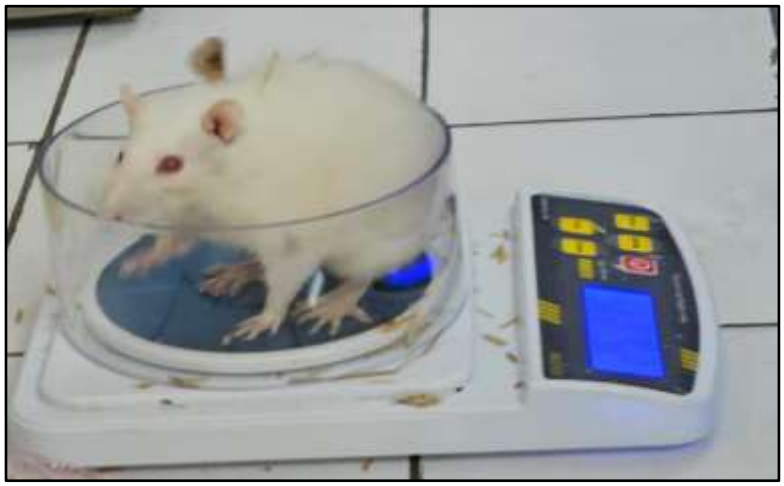


Figure No. 3: Weighing animals

Figure No. 4: Marking of animal

Figure No. 5: Carrageenan induced paw edema through i.v. route

Figure No. 6: Administration of betel extract through cannula

4.6 Evaluation of Physical Characteristics:
An organoleptic test, pH readings, adhesion, spreadability, and viscosity measurements were all part of the study of the physical qualities.

4.6.1 Organoleptic Test: The concoctions' form (shape), flavor, and appearance were examined during the organoleptic examination [43].

4.6.2 pH Testing: In this experiment, a pH meter that had been calibrated with acetate and phosphate buffers at pH 4.0 and 7.0, respectively, was employed. 10 mL of distilled water were used to dissolve one gramme of the extract. The solution's pH was measured using an electrode dipped in it to determine the base pH of the betel extract [44].

4.6.3 Test for Spreadability: The distribution diameter is measured every minute after adding 150 g of ballast to a half gramme of extract that has been placed on a round glass scale and covered with another round glass for 1 minute [45].

4.6.4 Test for Adhesive Capacity: The extract was tested by sandwiching two pieces of glass with a 0.25g sample. During five minutes, a 1 kilogramme weight was placed on top of the glass. The load was raised, and an 80g weight was used to separate the pieces of glass from one another. It was timed how long it took the glass objects to separate [46].

4.6.5 Test for Viscosity: A Rheosys Merlin VR II viscometer with 25mm concentric cylinders or spindles was utilized for this test. It processed 10 points at a temperature of 25 °C, a delay duration of 20 seconds, with rotating speeds ranging from 0.1 to 100 rpm.

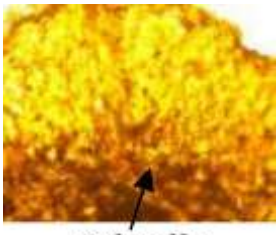
5. RESULT

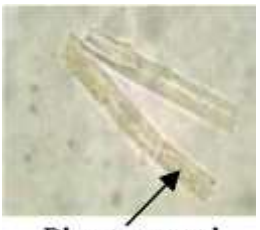
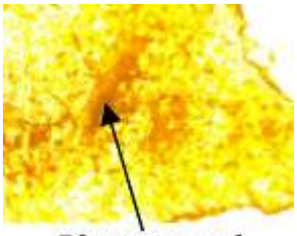
*5.1 Microscopic and Macroscopic Results***:** The tests, both macroscopic and microscopic, proved that the dried samples were betel leaves (Piper betle L.). Table II and III, respectively, provide the findings of the betel leaf's macroscopic and microscopic tests [47]. The extract had a solid green hue, a thick viscosity, and the typical betel leaf aroma based on the organoleptic examination. A 10% w/w extract made from 500 grams of leaves of dried betel weighs 50 grams (yield). The extract had a solid green hue, a thick viscosity, and the typical betel leaf aroma based on the organoleptic examination. After the extract was heated with H_2SO_4 and acetic acid for the ethanol test, there was no ester smell detected, indicating that the extract does not contain ethanol [30].

Table No. I: The results of betel leaf macroscopic identification

Parameters	Standard Features	Results
Length	5-18 cm	11 cm
Width	3-12 cm	8 cm
Shape	Egg-shaped with a rounded tip	Egg-shaped with a rounded tip
Odor	Distinctive	Distinctive
Color	Brownish Green	Dark Green
Taste	Spicy	Spicy and bitter
Lower surface	Rough and Softer in tone	Rough and Softer in tone

Table No. II: The outcomes of betel leaf identification under a microscope [47]

Parameters	Standard Features	Results
Upper Dermis		
Lower dermis		

Plant vessels thickened in a scalariform pattern		

5.2 Result of anti-inflammatory activity:- The study's findings demonstrated that carrageenan significantly reduced the rat hind paw edema caused by intraplantar injection, suggesting significant anti-acute, effective inflammation. The anti-inflammatory effect of Indomethacin was further assessed in the current research to clarify the underlying mechanisms operating in this animal model. We showed that the impact may be caused by inhibition of inflammatory enzyme expression, pro-inflammatory cytokine release, and the production of their byproducts. Furthermore, we demonstrated that anti-oxidative processes could stop liver damage brought on by carrageenan.

The carrageenan-induced paw edema is a well-established model of acute inflammation that is characterized by the involvement of numerous inflammatory mediators in its development and has been extensively used to assess the anti-edematous impact of natural products [48]. In the current research, we demonstrated that dose-dependent anti-inflammatory effects of Indomethacin were produced in carrageenan-induced rat paw edema. Our data supported earlier research showing that Indomethacin has a pronounced anti-inflammatory effect in animal models. Neutrophil infiltration, on the other hand, is well known to contribute a significant part in the inflammation brought on by carrageenan in the hind leg. A histopathological analysis of our findings showed the significant reduction of the neutrophil infiltration into the carrageenan-treated paws.

The class of analgesics known as nonsteroidal anti-inflammatory drugs (NSAIDs) contains both conventional nonselective and selective cyclooxygenase-2 inhibitors, which prevent prostaglandins and thromboxane from being synthesized [49]. Since the middle of the 1960s, indomethacin, a nonsteroidal anti-inflammatory drug, has been used successfully to treat mild to moderate pain. It has strong antipyretic, analgesic, and anti-inflammatory action. Although it has shown efficacy in the treatment of a number of other painful conditions, it is frequently recommended for the relief of acute gouty arthritis pain. The clinical usefulness of indomethacin in comparison to other commonly used analgesics has been supported by numerous comparative studies [50].

An increasing body of research has shown that triterpenoids, phenolic acids, and flavonoids have anti-inflammatory and antinociceptive properties in animal models. According to studies, flavonoids like luteolin, quercetin, and rutin generated significant antinociceptive and anti-inflammatory effects. As a result, it was proposed that Indomethacin content might be responsible for its antioxidant and anti-inflammatory properties [51]. One key mediator of inflammation has been identified as the expression the inducible isoform. However, generation by is a mediator of inflammatory diseases and causes cell injury by producing reactive radicals, despite physiological production having positive antimicrobial and anti-tumour effects.

Studies conducted in vitro and in vivo have shown a cross-talk between the production for the control of inflammation. According to data, Indomethacin inhibits the synthesis of prostaglandins produced primarily by cyclooxygenase enzymes has a significant impact on COX-2's catalytic action. The mechanisms underlying this impact, however, remain unclear. One explanation is to lengthen the half-life of COX-2 by producing free radicals and preventing COX-2 auto-inactivation [52]. Another explanation is that arachidonic acid is released from the cell membrane as a result of lipid peroxidation caused by peroxynitrite. One of the most potent inflammatory mediators in the inflammatory reaction is PGE2 [53,54], which is a COX-2 product.

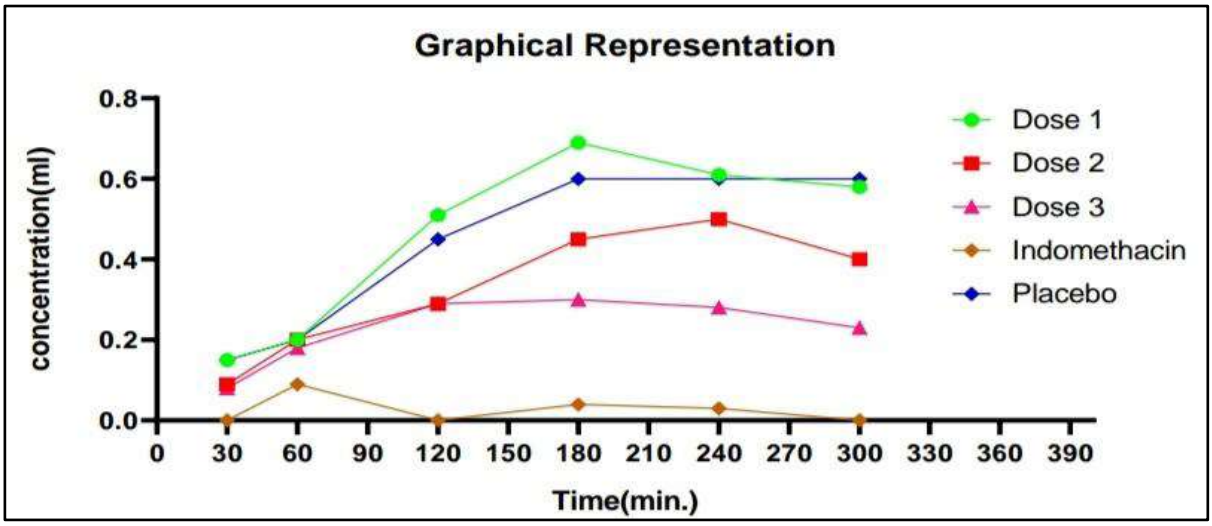
The effect of the dose of the betel extract on the concentration of edema (induced by carrageenan injections) is shown in Table III.

Table No. III: Effect of Dose of betel extract at concentration 1%, 2% and 4% on the concentration of edema using Plethysmometer equipment at different time durations.

S. No.	Duration (minutes)	Placebo concentration of edema (in ml)	Indomethacin concentration of edema (in ml)	1% dose concentration of edema (in ml)	2% dose concentration of edema (in ml)	4% dose concentration of edema (in ml)
1.	30	0.15	0	0.15	0.09	0.08
2.	60	0.2	0.09	0.2	0.20	0.18
3.	120	0.45	0	0.51	0.29	0.29
4.	180	0.6	0.04	0.69	0.45	0.30
5.	240	0.6	0.03	0.61	0.50	0.28
6.	300	0.6	0	0.58	0.40	0.23

The current study's findings concluded that Piper betel extract had similar anti-inflammatory effects to indomethacin in rat paw edema caused by carrageenan. Indomethacin anti-inflammatory properties may be linked to a decrease in TNF- and IL-1, which may inhibit the expression of iNOS and COX-2 and the production of NO and PGE2. Furthermore, Piper betel anti-oxidative properties may contribute to its protective impact against liver damage [55]. Our research offers fresh views on the therapeutic application of Piper betel in the treatment of inflammatory diseases.

The graph for the Table III is represented below;



Graph No. 1: Concentration of edema vs Time duration

The y-axis represents the concentration of edema in ml and the x-axis represents the time duration. The most appropriate dose for anti-inflammatory activity is 4% dose.

5.3 Result of Evaluation of Physical Characteristics: The emulsion gels' physical properties, including pH, spreadability, adhesion, and viscosity, were evaluated in order to identify their organoleptic features. Table V provides a summary of the assessment's findings.

Table No. IV: The result of evaluation of physical characteristics

Physical Characteristics	1% concentration	2% concentration	4% concentration
Organoleptic	Semisolid Emulsion gel with typical green color	Semisolid Emulsion gel with typical green color	Semisolid Emulsion gel with typical green color
pH	6.40 ± 0.12	6.20 ± 0.13	5.68 ± 0.12
Spreadability ($g.cm.s^{-1}$)	1.85 ± 0.45	1.83 ± 0.05	1.78 ± 0.09
Adhesive Capacity (s)	112 ± 10.80	127.3 ± 8.50	143.7 ± 13.50
Viscosity (cps)	1993.95	2164.12	2645.35

The organoleptic test of emulsion gel of betel leaf was identified as being green, semisolid, and smelling like betel leaf extract. The emulsion gel's whitish semi solid base, meanwhile, had an HPMC-like odor. The emulsion gels with 1, 2, and 4% betel leaf extract had pH values of 6.40±0.12, 6.20±0.13, and 5.68±0.12, respectively. The purpose of the pH measurement is to confirm that the preparations fall within the safe pH range for cutaneous application, which is 4.5

to 6.5 [56]. The alkaline gels might result in scaly skin, while the acidic gels can irritate the skin [57]. The results of the pH analysis revealed that the pH was lower in the emulsion gels containing more betel leaf extract. The pH in this study is within the permitted range of a safe pH for topical application.

Spreadability, which is correlated to ease of application to the skin, customer acceptance and removal from containers, is a crucial component of topical medicines [27]. The spreadability test reveals information regarding a preparation's capacity to cover a surface. The greater the dispersion of the preparation on the skin, the greater the absorption of its therapeutic components [57]. The emulsion gels containing 1, 2, and 4% of the betel extract had the spreadability of 1.85±0.45, 1.83±0.05, and 1.78±0.09, respectively. The findings demonstrated that a lesser spreadability was associated with a higher level of extract in the emulsion gel. It may be said that it has really good spreadability, simple to apply to skin.

Adhesive capacity, the preparation's ability to adhere to the skin can be evaluated using their adhesive capacity. The length of adherence has an impact on the drug's level of absorption. The rate at which medicines are absorbed via the skin increases with contact time [26]. Earlier studies revealed that topical treatments must adhere for at least 4 seconds in order to be considered appropriate [58]. The emulsion gels containing 1, 2, and 4% of the betel leaf extract had adhesive capabilities of 112±10.80, 127.3±8.50, and 143.7±13.50 seconds, respectively. The emulsion gels have satisfied the criteria for topical dosage forms based on their capacity. Moreover, raising the extract's concentration seems to make adherence stronger.

The viscosity test was designed to measure the thickness of the preparations. The viscosity increases with increasing thickness. Spreadability and adhesive capability are inversely correlated with viscosity, meaning that the stronger the preparation sticks to the skin, the higher the viscosity. The viscosities of the emulsion gels containing 1, 2, and 4% of the betel extract were 1993.95, 2164.12, and 2645.35 centipoises (cps), respectively, according to the viscometer's measurement at 100 revolutions per minute. 2000–4000 cps is the acceptable viscosity range for semisolid preparations [28]. The findings demonstrated that emulsion gels increase in viscosity as extract content increases.

5.4 Phytochemical Screening Analysis: Essential oils, phenyl propane, chavicol, flavonoids, tannins, terpenoids, and other phytochemicals are among the phytochemical components found in betel leaves. The primary chemicals recovered from the betel leaf extract for the anti-inflammatory effect of the betel leaf are hydroxychavicol (polyphenolic component), quercetin (flavonoid), and β-caryophyllene (volatile component). Moreover, it has the power to alter the composition of cell membranes, which would affect membrane permeability.

Table No. V: The result of phytochemical constituents of the extract

Phytochemical Constituents	Result of extract
Alkaloids	+
Flavonoids	+

Saponins	+
Phenol	+
Proteins	-
Reducing Sugars	-
Tannins	-
Phytosterols	-
Anthraquinone	-
Anthocyanosides	-
Terpenoids	-
Phlobatannins	-
Volatile Compounds	+
Coumarin	+
Acids	-
Glycosides	+

+ Represents the presence of the constituent i.e. hydroxychavicol (polyphenolic component), quercetin (flavonoid), β-caryophyllene (volatile component) and others like alkaloids, saponins, coumarin and glycosides.
- Represents the absence of the constituent

6. DISCUSSION

Paan, also known as P. betle, is a well-known medicinal plant in India [59], with its leaf extracts being used in situations where inflammation is a key component. To determine some of the underlying mechanisms causing *P. betle* leaves anti-inflammatory effect, we examined an ethanolic extract of the plant's leaves for anti-inflammatory activity. The study showed that the ethanolic extract of *P. betle* leaves had anti-inflammatory properties, which were partially mediated by NO production reduction, preventing the release of inflammatory mediators.

A test for anti-inflammatory drugs that work by blocking the mediators of acute inflammation is the carrageenan-induced rat paw edema [60]. The biphasic event is the carrageenan-induced oedema development in the rat paw [61]. Carrageenan-induced edema, which is thought to be biphasic, has frequently been utilized as an experimental animal model for acute inflammation. The early stage of the carrageenan model (1-2 h) is mostly mediated by histamine, serotonin, and enhanced prostaglandin synthesis in the vicinity of the injured tissue. Prostaglandin release and mediators such as bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins made by tissue macrophages support the late phase [62,63,64]. Since the extract significantly reduced

the second-phase paw edema caused by carrageenan, this result raises the possibility that the extract inhibits the production of cyclooxygenase. This effect is comparable to that produced by non-steroidal anti-inflammatory drugs like indomethacin, whose mechanism of action involves inhibiting the cyclooxygenase enzyme.

Due to their inhibition of enzymes involved in the formation of the chemical mediator of inflammation, flavonoids and saponins are well known for both their capacity to reduce the sense of pain and for having anti-inflammatory activities [65]. This hypothesis is strongly supported by the previous study, which has shown that P. betle possess anti-inflammatory activity due to the presence of high flavonoid content [66,67]. Current findings [68,69] imply that NO synthase activity and NO generation are both strongly inhibited by polyphenols. The findings of the trials imply that *P. betle* might be utilized as a complementary or alternative herbal therapy for the treatment of inflammation and pain.

The anti-inflammatory activity of 1% concentration of the betel extract was very low and that of 2% was slightly better than 1% concentration. The maximum anti-inflammatory activity was shown at 4% concentration. The phytochemical screening shows the presence of essential components required for anti-inflammatory activity which includes hydroxychavicol (polyphenolic component), quercetin (flavonoid) and β-caryophyllene (volatile component).

The phytochemical screening revealed the presence of various bioactive compounds, including alkaloids, flavonoids, terpenoids, and tannins. These compounds have been reported to possess various pharmacological activities, including anti-inflammatory properties. It has been reported that hydroxychavicol discovered by phytochemical screening exhibits antioxidant, anti-mutagenic, anti-carcinogenic, anti-microbial, and antifungal effects [70,71,72,73]. In addition to being isolated from plants like clove, nutmeg, cinnamon bark and leaves, pepper, and ginger, eugenol is another active component of *Piper betle* that is frequently employed as a food or flavoring additive [74,75].

The anti-inflammatory activity of the extract was evaluated in vivo using a rat model of inflammation. The results showed that the extract significantly reduced paw edema, which is a hallmark of inflammation. The extract was found to inhibit the production of inflammatory mediators such as prostaglandin E2 and nitric oxide in lipopolysaccharide-stimulated RAW 264.7 macrophage cells. The extract was also found to inhibit the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1 beta [76].

7.CONCLUSION

In studies using carrageenan-induced rat paw edema, the extract of betel leaf has demonstrated anti-inflammatory effects comparable to indomethacin. In addition, this betel extract possesses the necessary physical qualities for, such as viscosity, spreadability, adhesion, and organoleptic characteristics. Several investigations have shown that *Piper betle* extracts significantly reduce inflammation by preventing the synthesis of cytokines and enzymes that contribute to inflammation, such as TNF-, IL-1, COX-2, and iNOS. Overall, the results point to *Piper betle* as a possible source of organic anti-inflammatory compounds.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENT

I would like to express our sincere thanks to the SRMS Trust that supported this research. I would also like to thank our guides, Mrs. Dipali Gupta and Mr. Rajat Yadav, for their invaluable assistance and support. Lastly, we are grateful to the technicians who generously gave their time and effort to make this study possible, as well as to our family and friends for their unwavering support throughout the research process.

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