Volume 2, Issue 3, 2023, Page 282-288

International Journal of Pharma Research and Technology

Formulation and Evaluation of *In-Vitro* Anti-Inflammatory Potential of Hydroalcoholic Leaves Extract of *Cinnamomum Tamala*

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Abstract

All the secondary metabolites discovered from the medicinal plants play a major role in treating various diseases as a complementary medicine. Microbial infection is nothing but the invasion and multiplication of germs in the body, which can take the form of bacteria, fungus, or other microbes. Inflammation may happen as a result of tissue injury, cell death, malignancy, ischemia, or degeneration and has the capacity to spread throughout the body, reside in specific tissues, or circulate in the circulation. The current study provides scientific support for the strong usage of Indian bay leaf (*Cinnamomum tamala*) as an anti-inflammatory medication. The goal of this study was to invent an anti-inflammatory ointment that prevents the inflammation. The in vitro anti-inflammatory effect has been investigated using the HRBC membrane stabilisation technique. The outcome demonstrated the concentration-dependent stabilising potential of membranes.

Keywords- *Cinnamomum tamala*, anti-inflammatory, HRBC Stabilization.

Introduction

Our bodies' natural defence against harmful stimuli like tissue damage or allergies is inflammation. It frequently includes actions like vascular permeability that is raised, protein denaturation that is increased, and membrane alteration, and it is connected to pain frequently. These events frequently follow processes like tissue degeneration, cell death, cancer, ischemia, and degeneration, as well as when infectious microbes like bacteria, viruses, or fungi enter the body or circulate in the blood. The primary indicators and symptoms of inflammation include pain, redness, warmth, and swelling. When there has been an injury, the blood flow to the injured tissue increases, causing redness. An inflammatory crisis can be controlled and suppressed using a variety of drugs. Steroids, NSAIDs, and immune-suppressants are a few of them. Despite the fact that these have a number of negative side effects, it is necessary to use natural anti-inflammatory drugs to boost pharmacological response and reduce unpleasant side effects. Herbal remedies are marketed as complementary or alternative treatments to allopathic ones, with fewer or no side effects.In the current investigation, *Cinnamomum tamala* leaf extract was used to examine the in vitro anti-inflammatory effects.[1]

Cinnamomum tamala is a perennial plant with several uses. It is sometimes referred to as

Indian bay leaf, Indian cassia, or Tezpat/Tezapatta. The perennial shrub known as bay leaf, which is a member of the Lauraceae family, is grown across Asia, Europe, and other tropical and subtropical regions. In India about 20 species occur.[2] There are eight different kinds of *Cinnamomum tamala* in the Himalayan area, and recent studies have found that they have considerable digestion-improving and appetite-stimulating qualities. The plant has thicker, ovate-shaped leaves with a pointy or acuminate form. Its progeny/young leaves are reddish pink in colour and transform to a dark green tint and a glossy look as they age. It is 12–20 cm long and 5-8 cm broad (at the middle)

Figure No.1: Dried Bay leaves

The plant's leaves are made up of three nerve-like lines that go from the base to the top. Leaves have a pungent aroma and a clove-like flavor.3 Alkaloids, steroids, sugar, flavonoids, tannins, saponins, amino acids, phenolic compounds, glycosides, phytosterol, proteins, oils, and lipids may all be found in the leaves of *Cinnamomum tamala*. It gives essential oil by steam distillation known to be cinnamon oil. Numerous qualities of cinnamon tamala include immunosuppressive effects, anti-diabetic effects, anti-hyperlipidemic effects, antibacterial effects, anti-fungal effects, anti-inflammatory effects, and anticancer effects. Anti-tyrosinase, antioxidant, and possible Alzheimer's disease prevention properties. It is frequently used in cuisine for its aromatic qualities, and certain sectors of the perfumery industry utilise it for scent[4,5].

Material and methods

Collection of Plant Materials

The dried bay leaves were procured from the Butibori market Nagpur in Maharashtra. The dried bay leaves were identified and verified by Botanist Dr. Dongarwar, Department of Botany R.T.M. Nagpur University Nagpur, Maharashtra. All of the parts were cleaned and air-dried before the extraction procedure began.

Preparation of extract

A hydro alcoholic extraction (Soxhlet Extraction) was carried out on a 100g leaf. To achieve a semi-solid consistency, it was filtered and evaporated using a rotating vacuum evaporator. The aqueous extract was redissolved in water at a ratio of 1 mg/ml and used to test the in-vitro anti-inflammatory activity.[6]

Preparation of Herbal gel

- Two formulations with different Carbopol and leaf extract concentrations were made.

- 1 g of Carbopol 934, accurately weighed, was dispersed in 50 ml of distilled water, and the beaker was left to stand for 24 hours while being vigorously swirled to form a gel.
- Use a water bath to dissolve the required amounts of methyl paraben (0.1 ml) and propyl paraben (0.2 ml) in 5 ml of distilled water.
- After the solution had cooled, 5 cc of propylene glycol was added. A further 1 ml of C. tamala leaf extract was added to the previously described combination, and the remaining distilled water was then added to produce the final amount 100 ml.
- Finally, with steady swirling, all of the mixed components were fully incorporated into the Carbopol 934 gel. The pH (6.8-7) of the skin was then balanced using triethanolamine, which was then gradually added to the mixture to give the gel the correct consistency.[7]

Formulation of herbal gel:

The herbal gel containing hydroalcoholic leaves extract of *Cinnamomum tamala* was prepared by following formula. Two batches were prepared.

Table No.1: Formulation of herbal gel containing hydroalcoholic leaves extract of *Cinnamomum tamala*

Sr. No	Ingredients	Additive property	Composition %	
			F1	F2
1	Carbopol 934	Gelling or thickening agent	1 gm	1.5
2	C. Tamala extract	Anti-microbial property	1ml	1.5ml
3	Methyl paraben	Preservative	0.2g	0.2g
4	Propyl paraben	Preservative	0.1g	0.1g
5	Propylene glycol	Humectants	5 ml	5ml
6	Triethanolamine	Emulsifying agent	3-4 drops	2-3 drops
7	Distilled water	To-make formulation stable	q.s to 50 ml	q.s to 50ml
8	Carbopol 934	Gelling or thickening agent	1 gm	1.5
9	*Cinnamomum tamala* extract	Anti-microbial property	1ml	1.5ml

Figure No.2: Herbal gel containing hydroalcoholic leaves extract of Cinnamomum tamala

Evaluation parameter of Herbal gel

The herbal gel was evaluated by means of physic-chemical properties. The anti-inflammatory potential of formulation was determined through in-vitro assay method.

Physical evaluation

Physical characteristics including colour, style, and consistency were examined.[8]

pH

The pH of the gel compositions that were formed was measured using a digital pH metre. 1 g of gel was combined with 100 ml of distilled water, and the combination was allowed to settle for two hours. The pH of each formulation was tested three times, and the average values were calculated.[8]

Homogeneity

After the gel was placed in the container and spread out on the slide, it was visually inspected to see whether any lumps, flocculates, or aggregates were present.[8]

Skin Irritation

On human participants, the skin irritation experiment was conducted. Two volunteers were chosen to test the prepared gel, and 1.0g of the gel was spread over a two-square-inch area to the back of the volunteers' hands. The volunteers' skin reactions and irritation were noted.[8]

Viscosity

The viscosity of the gel was measured using a Brookfield viscometer. At 100 rpm spindle number 7, the viscosity was discovered to be 8900 centipoises.

Spreadability

The equipment, which consists of a wooden block with a pulley at one end, was used to gauge spreadability. By using this technique, spreadability was assessed based on the gels' properties of slip and drag. On this ground slide, extra gel (approximately 2 gm) was used for the experiment. Spreadability was calculated using the following formula: S= M × L/ T Where, S= Spreadability, M= weight in the pan (tied to upper slide), L= Length moved by the slide, T= Time (in sec.)[9]

In-vitro anti-inflammatory activity

HRBC Membrane Stabilization

As previously mentioned, a suspension of human red blood cells (HRBC) was generated. Healthy human participants who had not taken any NSAIDs for at least two weeks previous to the experiment's start had their blood drawn. The blood sample was then transferred to the centrifuge tubes, which were spun for 1-2 minutes at 2000 rpm. Three equal washes of normal saline were applied to the sample. 2 ml of regular saline were added to the centrifuged tubes after the supernatant was removed to create the HRBC blood suspension. For the membrane stabilisation test, haemolysis brought on by hypo tonicity was utilised. The reaction mixture (4.5 ml) contained 0.5 ml of 10% human RBC in normal saline, 1 ml of phosphate buffer saline, 1 ml of extract (200, 400, 600, 800, and 1000 g/ml), and 2 ml of hypotonic saline (0.25% NaCl). In the blank, red blood cells were absent, and the control included 1 ml of isotonic saline in place of the extract. For 30 minutes, the mixes were incubated at 370C. After chilling the tubes under running water for 20 minutes, they were centrifuged at 2000 rpm for 2–3 minutes. At 261 nm, the absorbance of the supernatant was measured.

The following formula was used to compute membrane stabilisation:

(Abs of blank – Abs of extract) / Abs of control × 100. The control represents 100%

Because drugs and membranes interact, the stabilisation of the erythrocyte membrane was chosen as the study's paradigm. Erythrocytes are stabilised by NSAIDs against stress haemolysis. Additionally, they inhibit the release of haemoglobin due to their function to stabilise membranes. The human red blood cells (HRBC) model is chosen to evaluate *Cinnamomum tamala*'s anti-inflammatory properties. The HRBC membrane stabilisation activity of 05 various concentrations of *Cinnamomum tamala* leaves extract was

examined in this study. The HRBC membrane was shown to be stabilised by an extract of the leaves at a high concentration (1000 g/ml), which is equivalent to the action of the common NSAID diclofenac (%). In all dilutions of the extract, the membrane stabilisation action was seen in a dose-dependent manner.10

Figure No.3: The suspension of human red blood cells (HRBC)

Results and Discussion

The formulations were evaluated for their Organoleptic as well as physico-chemical properties determination as given below.

Table No.2: Organoleptic evaluation of formulated herbal gel (F1 & F2)

Organoleptic evaluation	Formulation F1	Formulation F2
Colour	Yellow greenish colour	Greenish colour
Appearance	Jelly like	Smooth and transparent
Consistency	Thick and greasy	Smooth and consistent
odour	Pleasant	Aromatic

Table No.3: Physicochemical parameters of the formulated herbal gel (F1 & F2)

Physical parameters	Formulation F1	Formulation F2
pH	5.19	5.26
Viscosity	15200cps	18900cps
Skin irritation	No skin irritation	No skin irritation
Spreadability	25gm-cm/sec	30gm-cm/sec

Table No.4: HRBC membrane stabilization activity of the aqueous extract

Concentration (µg/ml)	Absorbance* (extract)	Stabilization (%)
100 µg/ml	3.591	10.72
200 µg/ml	2.982	25.96
400 µg/ml	2.524	36.95
800 µg/ml	2.387	40.33
1000 µg/ml	2.054	48.65
Diclofenac 1000 µg/ml	1.229	69.25

(*Reading from UV Spectrophotometer shimadzo 1800)

Conclusion

Bay leaf, a naturally occurring spice, may be used to make an anti-inflammatory gel. The current study looks at a significant in-vitro evaluation of the anti-inflammatory potential of *Cinnamomum tamala* leaves. According to the results of this investigation, *Cinnamomum tamala* may be a viable option for the development of a novel herbal anti-inflammatory medicine. The ability of *Cinnamomum tamala* hydroalcoholic extract to stabilise HRBC membranes has been tested at various concentrations. Additionally, it was shown to stabilise the HRBC membrane up to 48.65% (Table 4) at high concentrations (1000 g/ml), which is equivalent to the action of the common medication Diclofenac at 69.25%. The study's findings showed that the hydroalcoholic extract of *Cinnamomum tamala* has anti-inflammatory qualities. At large extract dosages, the percentage of inhibition is considerable and dose-dependent. It is reasonable to claim that bay leaf-containing gel exhibits synergistic effects with the extract tested for anti-inflammatory activity at the specified percentage of inhibition.

The anti-inflammatory activities of the extracts may also be due to other phytochemicals in the leaves. Tannins and other phytochemicals have been Shown in previous studies to have anti-inflammatory effects. To fully understand the process by which *Cinnamomum tamala* phytochemicals work, more study is required.

Acknowledgement: The author/s are thankful to Management and Principal of Kamla Nehru College of Pharmacy, Butibori for providing laboratory facilities for research.

References

1. *Sarveswaran R, Jayasuriya WJ, Suresh TS. In vitro assays to investigate the anti-inflammatory activity of herbal extracts a review.*
2. *Pravin B, Krishnkant L, Shreyas J, Ajay K, Priyanka G. Recent pharmacological review on Cinnamomum tamala. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013;4(4):916-7.*
3. *Tiwari, S., Talreja, S, Importance of Cinnamomum Tamala in the Treatment of Various Diseases. Pharmacognosy Journal- (2020).*
4. *Jeyasree P, Dasarathan P. Screening of phytochemicals and immuneomodulatory potential of amedicinal plant, Cinnamomum tamala. Int. J Pharma. Sci. Res. 2012 Apr 1;3: 1049-52.*

5. *Ahmed J, Sultana N, Dewan SM, Amin MN, Uddin SN. Determination of chemical groups and investigation of anthelmintic, cytotoxic, and antibacterial activities of leaves of Cinnamomum Tamala (Family: Lauraceae). Int J Pharmamedix India. 2013;1(2):222-32.*
6. *Ganer R, Kamble MA, Dhabarde DM, Ingole AR, Baheti JR. Evaluation of Analgesic and In-vitro Anti-Inflammatory potential of Fruit Flesh Extract of Terminalia catappa Linn. Research Journal of Pharmacognosy and Phytochemistry. 2017;9(4):228-30.*
7. *Dange V, Dinde S, Doiphode A, Dhavane S, Dudhal B, Shid S, Yadav A. Formulation and evaluation of herbal gel containing Lantana camara for management of Acne vulgaris. Journal of University of Shanghai for Science and Technology. 2020;22(11):799-809*
8. *Al-Hadi LM. The antibacterial activity of aqueous extract of peppermint and Bay leaf against Staphylococcus aureus. J. Bagh. Coll. Dent. 2011;23:146-50.*
9. *Harahap NI, Nainggolan M, Harahap U. Formulation and evaluation of herbal antibacterial gel containing ethanolic extract of mikania micrantha kunth leaves. Asian J Pharm Clin Res. 2018 Mar 1;11(3):429-31.*
10. *Yesmin S, Paul A, Naz T, Rahman AB, Akhter SF, Wahed MI, Emran TB, Siddiqui SA. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clinical Phytoscience. 2020 Dec;6(1):1-0.*