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EVALUATION OF *IN- VITRO* ANTI-INFLAMMATORY ACTIVITY OF *EMBLICA OFFICINALIS* SEEDS

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ABSTRACT
Emblica officinalis (Indian gooseberry, Amla), a medicinal plant rich in tannins and vitamin C, has long been used in Ayurveda for its antioxidant and therapeutic properties. The present study aimed to evaluate the **in-vitro anti-inflammatory activity** of ethanolic seeds extract of Emblica officinalis. Phytochemical screening confirmed the presence of carbohydrates, alkaloids, saponins, tannins, phenolic compounds, and proteins. Anti-inflammatory potential was assessed through **albumin denaturation inhibition** and **anti-lipoxygenase assays**. Results revealed that the extract exhibited concentration-dependent activity, showing significant inhibition comparable to diclofenac sodium and indomethacin. These findings suggest that Emblica officinalis seeds possess promising anti-inflammatory potential and could serve as a natural alternative in managing inflammatory conditions. However, further research is required to isolate the bioactive compounds and elucidate their mechanisms of action.

KEYWORDS: *Emblica officinalis*, Amla, Anti-inflammatory activity, Protein denaturation inhibition, Lipoxygenase inhibition.

INTRODUCTION
Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, and is often associated with pain, protein denaturation, increased vascular permeability, and membrane alterations. The classical signs of inflammation include pain, redness, heat, and swelling. For example, following injury, dilation of arterioles increases blood circulation to the affected tissue, producing redness and localized heat.[1]

Inflammation is broadly classified into acute and chronic. Acute inflammation is a rapid, adaptive response to tissue injury or infection, whereas chronic inflammation is prolonged, often persisting for months or years. Chronic inflammation is a major global health concern and has been linked to more than 50% of deaths worldwide. Current therapeutic strategies rely heavily on steroidal and non-steroidal anti-inflammatory drugs (NSAIDs). These agents, including diclofenac, aspirin, and indomethacin, act primarily by inhibiting cyclooxygenase (COX) enzymes, thereby reducing prostaglandin biosynthesis and exerting anti-inflammatory, analgesic, and antipyretic effects.[3]

However, long-term use of NSAIDs is associated with serious side effects, including gastrointestinal irritation leading to ulcers, cardiovascular risks, and endocrine disturbances.[5] This has led to increased interest in **medicinal plants** as safer alternatives for managing inflammatory conditions. Plant-based therapies often provide effective anti-inflammatory activity with fewer adverse effects, and in-vitro and in-vivo studies have been widely employed to elucidate their mechanisms.[4]

One such plant is *Emblica officinalis* (syn. *Phyllanthus emblica*, commonly known as Amla or Indian gooseberry), a member of the family Euphorbiaceae. Revered in ancient Indian mythology as the first tree created in the universe, Amla holds a prominent place in Ayurveda and Unani medicine. Classical Ayurvedic texts describe Amla as one of the most potent rejuvenating herbs (*Amalaki Vayasthapnanam Sreshtham*). Modern phytochemical studies have shown that Amla contains hydrolysable tannins, such as Emblicanin A and B, which possess strong antioxidant properties.[5] Owing to these bioactive constituents, the present study was designed to examine the in-vitro anti-inflammatory activity of ethanolic seeds extract of *Emblica officinalis.*

MATERIALS AND METHODS

Collection of plant material

For this study, the seeds of *Emblica officinalis* are purchased from local store.

Preparation of ethanolic extract of Emblica officinalis seeds powder

The dried seeds of *emblica officinalis* were coarsely ground subjected to maceration with ethanol (95%) for 7 days with frequent shaking. Thereafter, the extract was concentrated by evaporating the solvent at 45^{o} C. The preliminary phytochemical screening of ethanolic extract of seeds of *emblica officinalis* revealed the presence of Carbohydrates, Tannins, saponins and proteins.

Evaluation of In-vitro Anti-inflammatory activity

1. Inhibition of egg albumin denaturation[6-7]

Preparation of 1% of egg albumin solution

Fresh Hen's egg was used to make a 1% egg albumin solution. Fresh Hen's egg was carefully cracked and transferred 1 mL of the translucent portion (clear component of the egg) to 100 mL distilled water, and stirring thoroughly. Cold water was used for making the solution.

Inhibition of Egg Albumin Denaturation Assay.

The anti-inflammatory activity of crude extract was determined in vitro for inhibition of the denaturation of egg albumin (protein). 0.2 mL of 1% egg albumin solution, 2 mL of sample extract or standard (Diclofenac sodium) at varying concentrations, and 2.8 mL of phosphate buffered saline (pH 7.4) were mixed to form a reaction mixture of a total volume of 5 mL. A total volume of 5 mL of the control was created by combining 2 mL of distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline. The reaction mixtures were then incubated at 37±2°C for 30 min and will be heated in a water bath at 70±2°C for 15 16 min. After cooling, the absorbance was measured at 280 nm by a suitable UV/Visible spectrophotometer using distilled water as the blank. The following equation was used to determine the % inhibition of protein denaturation.

Percentage inhibition = [{Abs control- Abs sample}/Abs control] x 100

2. Anti-Lipoxygenase Activity[8]

Anti-Lipoxygenase activity of sample extract was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25ml of 2M borate buffer pH 9.0 and added 0.25ml of lipoxidase enzyme solution (20,000U/ml) and incubated for 5 min at 250C. After which, 1.0ml of lenoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as reference standard.

The percent inhibition was calculated from the following equation,

Percentage inhibition= [{Abs control- Abs sample}/Abs control] x 100

Statistical analysis Statistical analysis was done using one-way analysis using ANOVA where $p< 0.05$ was considered as statistically significant. Values are expressed as ± SD (n=3).

RESULTS

Inhibition of Egg Albumin Denaturation

In the present investigation, the in vitro anti-inflammatory effect of the extract was evaluated against denaturation of egg albumin and compared with the standard drug diclofenac sodium. The results are summarized in Table No. 1

Table 1: Inhibition of Egg Albumin Denaturation by of seeds extract of *Emblica officinalis and* diclofenac sodium.

Sl. No.	Concentration (µg/mL)	% Inhibition of egg albumin denaturation	
		Seeds extract of *Emblica officinalis*	Diclofenac sodium
1.	100	20.2 ±0.6	22.6±0.6
2.	200	28.6±0.5	35.3±0.3
3.	300	46.3±0.2	56.4±0.4
4.	400	79.5±0.7	88.4±0.4
5.	500	89.2±0.6	97.4±0.7

The present findings exhibited a concentration dependent inhibition of egg albumin (protein) denaturation by ethanolic extract of emblica officinalis and diclofenac sodium throughout the concentration range of 100 to 500 µg/mL.

Anti-Lipoxygenase activity

Anti- lipooxygenase activity of extract was evaluated with varying concentrations, 100, 200, 300, 400 and 500µg/ml and compared with the standard drug indomethacin. The results are summarized in Table 2.

Table 2: Anti-Lipoxygenase activity of seeds extract of *emblica officinalis* and indomethacin.

Sl. No.	Concentration (µg/mL)	Anti-Lipoxygenase activity (% Inhibition)	
		Seeds extract of *Emblica officinalis*	Indomethacin
1	100	18.4 ±0.4	21.5±0.2
2	200	23.6±0.8	30.1±0.6
3	300	42.4±0.4	58.7±0.4

4	400	74.2±0.3	85.4±0.8
5	500	86.2±0.6	94.5±0.4

The present findings exhibited a concentration dependent Anti-lipooxygenase activity by ethanolic extract of *emblica officinalis* and indomethacin throughout the concentration range of 100 to 500 µg/mL.

DISCUSSION

The present study evaluated the in-vitro anti-inflammatory potential of ethanolic seeds extract of *Emblica officinalis* using two well-established models: inhibition of protein (egg albumin) denaturation and anti-lipoxygenase activity. Both assays demonstrated a concentration-dependent inhibitory effect, supporting the traditional use of *E. officinalis* in managing inflammatory conditions.

In the egg albumin denaturation assay, the extract significantly inhibited protein denaturation in a dose-dependent manner, with 89.2% inhibition at 500 µg/mL, which is comparable to diclofenac sodium (97.4%). This suggests that the extract can stabilize protein structure and prevent denaturation-associated inflammatory responses. Similar findings were reported in studies with other medicinal plants where protein stabilization was linked with anti-inflammatory properties.

The lipoxygenase inhibitory assay further confirmed the anti-inflammatory potential of the extract, showing 86.2% inhibition at 500µg/mL compared with 94.5% inhibition by the standard indomethacin. Lipoxygenase is an enzyme involved in arachidonic acid metabolism and leukotriene synthesis, both of which contribute to inflammation. Inhibiting lipoxygenase therefore represents a critical mechanism of anti-inflammatory action. The high inhibitory activity of *E. officinalis* seeds suggests that the extract may interfere with lipid peroxidation pathways and reduce the availability of hydroperoxides necessary for lipoxygenase activity.

Previous reports support the antioxidant, antimicrobial, and anti-inflammatory properties of *E. officinalis* fruit extracts, with studies showing activity in both in vitro and in vivo models.[9-12] The current findings align with these earlier observations but highlight the potential of seed extracts, which are less studied compared to the fruit. The comparable efficacy of seed extract to standard drugs (diclofenac and indomethacin) is significant, as it indicates possible therapeutic value with fewer side effects than synthetic NSAIDs.

CONCLUSION

Overall, the results suggest that ethanolic seed extract of *Emblica officinalis* possesses strong anti-inflammatory properties mediated through protein stabilization and lipoxygenase inhibition. However, further research is needed to isolate the active constituents, determine their molecular mechanisms, and evaluate efficacy and safety in vivo.

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