Research Article

Evaluation of *in vitro* Anti-Inflammatory Activity of Leaves of *Terminalia chebula* Retz. (Combretaceae)

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ABSTRACT

Aim: The present study focused to establish the *in vitro* anti-inflammatory activity of leaf extracts of *Terminalia chebula* prepared using different solvents. **Materials and Methods:** The *in vitro* anti-inflammatory potentiality of leaves of *Terminalia chebula* were tested using the protein denaturation inhibition assay (bovine serum albumin inhibition assay). **Results:** Leaves of *Terminalia chebula* were extracted by ultrasonic assisted extraction using different solvents in increasing order of polarity. The extracts so obtained were screened for anti-inflammatory potential. Leaf extracts of different concentrations ranging from 6.25-100 µg/mL were incubated with bovine serum albumin and its ability to inhibit protein denaturation was measured. The percentage of inhibition of standard diclofenac sodium was determined and its IC_{50} value was found to be 49.57 µg/mL. The ethanol, ethanol: distilled water extracts (4:1, 3:2, 2:3, 1:4) and distilled water extracts exhibited greater inhibition of protein denaturation and their IC_{50} values ranged from 52.03 µg/mL to 77.75 µg/mL. **Conclusion:** The current study proved that leaves of *Terminalia chebula* exhibited considerable amount of anti-inflammatory activity and could be used as anti-inflammatory drug but only after proper scientific validation (further *in vitro* and *in vivo* studies).

Keywords: *Terminalia chebula*, *in vitro* study, Anti-inflammatory study, Protein denaturation assay.

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INTRODUCTION

Inflammation is a symptom commonly seen as a part of many chronic diseases induced either by pathogenic microbes or may be due to other harmful stimuli such as toxic compounds, damaged tissue or injured tissue. It is an immune response stimulated in the body of an organism against foreign substances or matters.[1-3] This immune response is characterized by oedema, pain, heat, redness and impaired function of tissues. The process of inflammatory response starts by increasing the permeability of blood vessels thereby increasing the flow of blood to the affected area causing the redness and heat in the affected area. Leukocytes are recruited to the affected area and inflammatory mediators are released which takes control of the inflammatory process.[4-7]

In all living organisms inflammatory response involves a signaling cascade whereby a number of immune cells and inflammatory mediators interact with each other to produce an effective healing. The signaling cascade in turn involves mediators from damaged cells and those mediators which are recruited from blood.[7-10] Different types of receptors such as pattern recognition receptors are present in the cells which get stimulated when come in contact with noxious microbes or cytokines such as interleukins, tumour necrosis factor alpha etc. When the receptors (PRRs) get activated, many signalling pathways are initiated such as MAPK pathway, NF-kappa B pathway and JAK-STAT pathway.[8,11-13] Once the noxious compounds or the harmful stimuli are eliminated, the leukocyte recruitment eventually stops and the release of inflammatory mediators gets reduced. Thus the process of inflammation ceases. This occurs

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only in the case of acute inflammation. Sometimes dysregulation of the inflammatory process leads prolonged inflammation which eventually results in many diseases like cardio vascular diseases, cancer and arthritis.[2,5,13]

Anti-inflammatory drugs are used to get rid of ache, soreness and chronic inflammation. However, anti-inflammatory drugs pose many side effects. Recently plants have been explored widely in medical field because of its medicinal potential and lesser side effects, which in turn paved the way to use herbal medicines to cure inflammatory diseases.[14,15] But proper scientific study and validation are required to produce fruitful result. In the current study leaves of *Terminalia chebula*, a medicinally valuable tree coming under Combretaceae was studied. *Terminalia chebula* commonly called as Haritaki is well known in Ayurveda as a detoxifier and cleanser.[16] Many studies conducted in the fruits of *Terminalia chebula* revealed its antioxidant,[17] anti-inflammatory,[18] antidiabetic,[19] and immunomodulatory effects.[20] The major secondary metabolites present in the plant were found to be responsible for its pharmacological actions. So in the present work leaves of *Terminalia chebula* were evaluated for its anti-inflammatory action.

MATERIALS AND METHODS

Plant sample collection

The leaves of *Terminalia chebula* were collected from Thiruvananthapuram district, Kerala. The plant sample was identified and the voucher specimen (collection number 95980) deposited at JNTBGRI, Palode, Thiruvananthapuram, Kerala.

Chemicals and Instruments

Diclofenac sodium (DFS) and bovine serum albumin were collected from Sigma Aldrich. All the solvents (used for extraction purposes) were of analytical grade. Extraction was carried out using Ultra autosonic probe-type ultrasonicator and anti-inflammatory activity was determined using a UV spectrophotometer.

Preparation of plant sample

As mentioned in standard procedures sample preparation involved thorough cleaning, shade drying and blending of leaves of *Terminalia chebula*.[21] The blended leaf powder was then subjected to ultrasonic-assisted extraction using different organic solvents viz. hexane, chloroform, ethyl acetate, acetone, ethanol, ethanol: distilled water (in different ratios such as 4:1, 3:2, 2:3, 1:4) and distilled water.[22]

Determining anti-inflammatory potential

Protein denaturation inhibition assay[23,24]

Leaf extracts [ethyl acetate, acetone, ethanol, and ethanol: distilled water (4:1, 3:2, 2:3, 1:4) and distilled water] of *Terminalia chebula* were taken in different concentrations (6.25, 12.5, 25, 50 and 100 µg/mL respectively). Analysis was performed according to standard procedures mentioned by Mizushima and Kobayashi, 1968 and Sakat *et al*, 2010. The spectrophotometric reading was done at 660 nm. The per cent inhibition of protein denaturation was estimated as

$$\text{Percent inhibition} = \left[\left(\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}}\right)\right] \times 100$$

Where 'Abs' stands for absorbance.

Statistical analysis

The results (all the triplicate values) were statistically analyzed using Microsoft excel and the values obtained were represented as standard error of the mean.

RESULTS

In the present work *in vitro* anti-inflammatory activity of the sample leaves were done by protein denaturation assay. The percent inhibitions of denaturation of albumin (protein) for the extracts were calculated. All the extracts exhibited a dose-dependent inhibition of protein denaturation. Initially, at lower concentrations, percentage inhibition was lower for all extracts. However, as the concentration of the extract increased from 6.25-100 µg/mL percentage of inhibition of protein denaturation also increased. Ethanol, ethanol: distilled water extracts namely (4:1), (3:2), (2:3), (1:4) and distilled water extracts showed greater percentage inhibition against protein denaturation ranging from 60.53±0.03% to 77.44±0.04%. Comparatively lower percentage inhibition against protein denaturation was seen in ethyl acetate and acetone extract (37.46±0.06% and 37.75±0.05% respectively (Table 1, Figure 1).

IC_{50} values were also calculated for all the extracts and the standard drug, diclofenac sodium. In the current context, the IC_{50} value represents half the maximal concentration of either the extract or the standard required to inhibit the protein denaturation. IC_{50} value of the standard drug was found to be 49.57 µg/mL (Table 2, Figure 2). Ethanol, ethanol: distilled water extracts [(4:1), (3:2), (2:3), (1:4)] and distilled water extract showed IC_{50} values close to diclofenac sodium

Table 1: Percentage of inhibition of protein denaturation of leaf extracts of *Terminalia chebula*.

Extracts and standard drug	% inhibition of protein denaturation by leaf extracts in different concentrations				
	6.25 (µg/mL)	12.5 (µg/mL)	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)
Eth acet	14.66±0.06	18.72±0.06	24.88±0.02	28.52±0.04	37.46±0.06
Acet	16.95±0.02	18.01±0.03	21.69±0.04	24.12±0.02	37.75±0.05
Ethanol	10.95±0.07	24.63±0.04	27.16±0.02	34.97±0.01	65.10±0.04
Eth:dw (4:1)	22.15±0.03	27.50±0.04	30.61±0.03	45.73±0.02	70.08±0.05
Eth:dw (3:2)	22.53±0.02	22.70±0.03	32.34±0.06	51.37±0.05	77.44±0.04
Eth:dw (2:3)	10.81±0.05	23.60±0.03	24.95±0.02	39.59±0.09	60.53±0.03
Eth:dw (1:4)	10.68±0.06	20.46±0.05	23.06±0.02	31.78±0.03	63.20±0.05
Dw	18.07±0.04	27.61±0.03	31.70±0.01	43.88±0.05	71.47±0.05
Diclofenac	5.12±0.1	14.21±0.03	25.99±0.11	54.05±0.10	92.99±0.05

Values were represented as Mean±Standard Error.

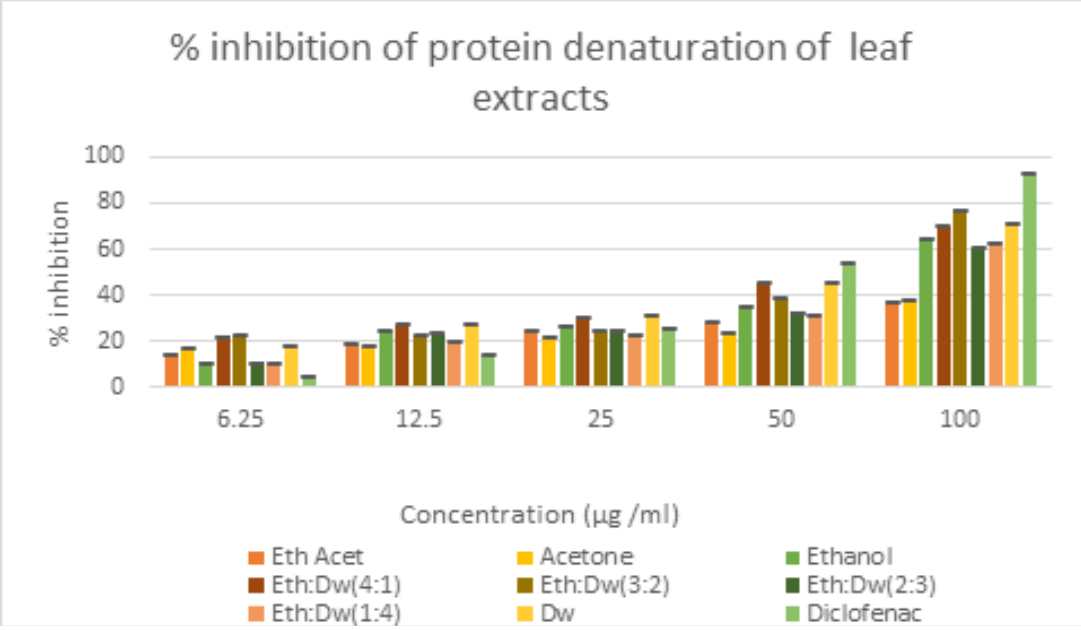

Figure 1: Graphical representation of % inhibition of protein denaturation in leaf extracts and diclofenac in different concentrations.

ranging from 52.03 µg/mL to 77.75 µg/mL. However, IC_{50} value was found to be greater for ethyl acetate and acetone extract [174.77 µg/mL and 157.86 µg/mL respectively]. Since the IC_{50} values of ethanol, ethanol: distilled water (4:1, 3:2, 2:3, 1:4) and distilled water extracts were lower compared to ethyl acetate and acetone extracts, we could infer that ethanol, ethanol: distilled water (4:1, 3:2, 2:3, 1:4) and distilled water extracts were more potent anti-inflammatory agents. (Table 2, Figure 2).

Table 2: IC_{50} values of leaf extracts and standard diclofenac.

Extracts and standard	IC_{50} (µg/mL)
Ethyl acetate	174.77
Acetone	157.86
Ethanol	72.93
Ethanol:distilled water (4:1)	60.62
Ethanol:distilled water (3:2)	52.03
Ethanol:distilled water (2:3)	76.14
Ethanol:distilled water (1:4)	77.75
Distilled water	60.18
Diclofenac sodium (standard)	49.57

DISCUSSION

Denaturation of protein is a common event occurring during inflammatory diseases. Hence protein

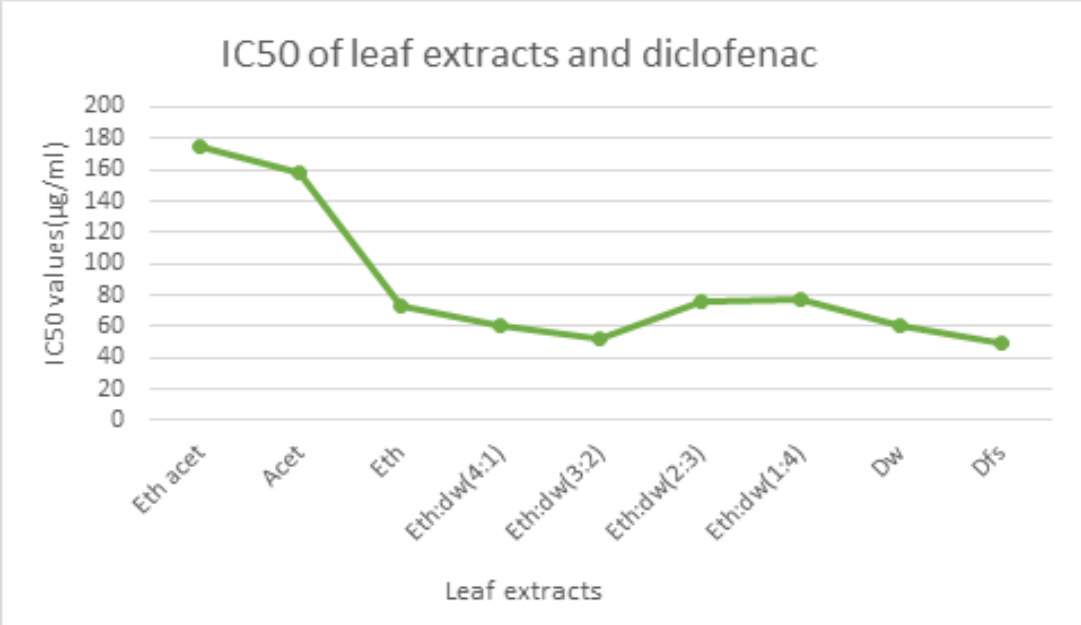

Figure 2: Graphical illustration of IC_{50} of leaf extracts and standard diclofenac.

denaturation inhibition paves way to anti-inflammatory activity.[25] In the current study, *in vitro* anti-inflammatory analysis of leaves of *Terminalia chebula* was evaluated by protein denaturation inhibition assay and the results obtained suggested positive anti-inflammatory action. Ethanol, Ethanol: distilled water (4:1, 3:2, 2:3, 1:4) and distilled water extracts exhibited good anti-inflammatory potential. IC_{50} values of ethanol, ethanol: distilled water and distilled water extracts were found in close proximity to standard drug diclofenac sodium.

Inflammation is a natural phenomenon occurring in the body to combat injury. Whenever the body is injured, cells in the affected area become stressed. As a result, several chemicals are released into the area such as histamines, and prostaglandins which cause increased blood flow, pain and inflammation.[1,2,6] Improper functioning of inflammatory pathways may lead to continuous release of inflammatory mediators which results in chronic inflammation.[5] In order to combat this problem anti-inflammatory drugs such as NSAIDs (Non-Steroidal Anti-inflammatory Drugs) are used. Although NSAIDs exhibit many positive effects such as antipyretic, anti-inflammatory and analgesic effects it has innumerable side effects such as damage to gastrointestinal tracts, indigestion, stomach ulcers, dizziness and headaches.[26,27] Here comes the importance of use of medicinal plants with anti-inflammatory potential.

Numerous studies regarding anti-inflammatory potential of different plants had been reported. According to earlier reports, similar *in vitro* study conducted on *Terminalia bellerica* fruits suggested positive inhibition of protein denaturation and the activity was mainly contributed by ellagic acid.[28] Similarly 'Curcumin', a major constituent of Curcuma longa was reported to down-regulate several inflammatory mediators such as TNF-α, lipoxygenase and inducible nitric oxide synthase (iNOS) thereby controlling chronic inflammation.[29] Fruits of *Terminalia chebula* which were a storehouse of different tannins, also exhibited anti-inflammatory activity.[18] *Terminalia arjuna* barks were also reported to have anti-inflammatory activity and it was contributed mainly by the flavonoids. Flavonoids usually inhibit the production of prostaglandins, the end product of COX and lipoxygenase pathways.[30] Lucetti *et al.* reported that triterpenoids impose anti-inflammatory activity by reducing the cells that express inducible nitric oxide synthase (iNOS).[31] Some studies reported that alkaloids reduced carrageenan-induced oedema by inhibiting vascular permeability which was in turn induced by histamines.[32] All the data collected and cited suggested that the anti-inflammatory potential of many plants was mainly due to the presence of different phytochemicals such as hydrolysable tannins (ellagic acid), flavonoids, alkaloids, saponins and triterpenoids. Hence we could infer that the anti-inflammatory activity of leaves of *Terminalia chebula* may also be due to the presence of different types of pharmacologically active secondary metabolites.

From the present work we could infer that leaves of *Terminalia chebula* may form a potent source of anti-inflammatory drugs but only after proper scientific validation. The major advantage of the present work was that a natural anti-inflammatory agent was derived

without creating any ethical concerns. However, for widespread use of the drug *in vivo* studies are mandatory.

CONCLUSION

Inflammation is a defence mechanism of living organisms against foreign stimulants. Although the process of inflammation promotes faster healing and brings back homeostasis, prolonged inflammation is an adverse condition. NSAIDs commonly used to treat acute and chronic inflammation have many side effects. Therefore, health care providers and patients mostly prefer plant-based anti-inflammatory sources to treat inflammation. This shows the importance of medicinal plants in curing inflammatory diseases. The present study demonstrated that all the leaf extracts of *Terminalia chebula* especially ethanol, ethanol: distilled water extracts (4:1, 3:2, 2:3, 1:4) and distilled water extracts exhibited greater anti-inflammatory activity. Review of literature suggested that the anti-inflammatory property of many medicinal plants are due to the presence of many pharmacologically important secondary metabolites viz, hydrolysable tannins (ellagic acid), flavonoids, alkaloids, saponins and triterpenoids which in turn suggested that the presence of these phytochemicals in leaves of *Terminalia chebula* contributed to its anti-inflammatory property. However, further studies are needed for scientific elucidation and validation of the anti-inflammatory potential of leaves of *Terminalia chebula*.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

Both the authors contributed towards the analysis, evaluation, discussion and preparation of final draft of the work equally.

ABBREVIATIONS

Eth acet: Ethyl acetate; **Eth:** Ethanol; **DW:** Distilled water.

SUMMARY

In the current investigation leaves of *Terminalia chebula* Retz. were evaluated for its *in vitro* anti-inflammatory potential. It was found that the leaves exhibited considerable level of *in vitro* anti-inflammatory activity.

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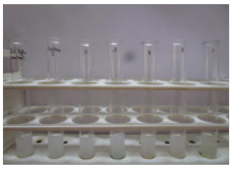
Illustration

FRESH LEAVES

SHADE DRIED, BLENDED

ULTRA SONICATOR

COLLECTION OF EXTRACT



ANTI-INFLAMMATORY STUDY (INHIBITION OF PROTEIN DENATURATION ASSAY)

POSITIVE ANTI-INFAMMATORY ACTIVITY

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