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A Comparative Study on Anti-inflammatory Activity of Hydroethanolic of Leaf, Stem and Root Extracts of *Tecoma stans*

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Authors' contributions

This work was carried out in collaboration among all authors. Author Prateek managed Literature search, data collection analysis and wrote the first draft of the manuscript. Author GD Data Verification, Manuscript draft. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: *Tecoma stans* is an important medicinal plant. The major bioactive compounds like alkaloids, phenols, terpenoids, glycosides, flavonoids and saponins had been isolated from this plant. The leaves bark and roots contain biologically active chemicals, and extracts from those tissues are in use as traditional folk medicines. Inflammation is a biological response of the immune system that can be triggered by a variety of factors, including pathogens, damaged cells and toxic compounds. The aim of the study was to analyze the anti-inflammatory activity of hydroethanolic leaf, stem and root extracts of *Tecoma stans.*
Materials and methods: All chemicals and reagents were bought by sigma Chemicals Company. Anti inflammatory activity of the three different parts (leaf, stem and root) the plant were analysed by an *in-vitro* protein denaturation inhibition assays and inhibition calculation on protein inhibition percentage was done by the formula % inhibition=100−(A1 -A2)/A0)*100). Statistical analysis was done by ANOVA and Duncan's multiple range test.

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Results: After comparing the anti-inflammatory activity of the leaf root stem of *Tecoma stans*, the results were plotted in graphs through statistical analysis using ANOVA and Duncan's multiple range test. The results showed that the stem of the plant has maximum anti-inflammatory activity compared to leaf and root.
Conclusion: A comparative study of the extract or stem, leaf and root shows that the stem portion of the plant contains maximum anti-inflammatory activity.

Keywords: Tecoma stans; anti inflammation; phytochemicals; plant extract; innovative techniques.

1. INTRODUCTION

Plants have been used as an important source of phytochemicals that are used to make drugs for pharmacological use. Plant derived medicines have made large contributions to human health and wellbeing since ancient times. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing countries [1]. Plants are used medicinally in different ways varying from country to country and are a source of many potent and powerful drugs. Medicinal plants provide good remedies for human diseases and play a vital role in our everyday life [2]. Indian systems of medicines are all mostly based on the knowledge of drugs from plants. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinically used drugs are naturally produced and natural products play an important role in drug development programs in the pharmacological effects industry [3]. In India, people have been using plants and natural products for the treatment of various diseases since ancient times. In the last two decades of the century the scientists are sincerely trying to evaluate many plant drugs used in the traditional system of medicine. The pharmacognostical study is one of the major criteria for identification of plant drugs [4]. Active Bignoniaceae family plants are also widely used in traditional medicinal systems of a number of countries, where folk and tribal medicinal practitioners use a number of species as a preventive and curative measure for diverse ailments. *Tecoma stans* are commonly planted as an ornamental in warmer climates throughout the world because of its showy yellow flowers and pinnate foliage. *Tecoma stans* is an important medicinal plant. The major bioactive compounds like alkaloids, phenols, terpenoids, glycosides, flavonoids, and saponins had been isolated from this plant [5]. The leaves bark and roots contain biologically active chemicals, and extracts from those tissues are in use as traditional folk medicines. The presence of phytoconstituents like phytosterol, triterpene, glycosides, phenols, flavonoids, saponins, and tannins either individually or combined together may exhibit the synergistic effect towards healing of wounds.5 Researchers revealed that it is having antidiabetic, anticancer, antioxidant, antispasmodic, antimicrobial, and antifungal properties and extensively used in the treatment of diabetes [6].

Inflammation is a biological response of the immune system that can be triggered by a variety of factors, including pathogens, damaged cells and toxic compounds. These factors may induce acute or chronic inflammatory responses or both together, in the heart, pancreas, liver, kidney, lung, brain, intestinal tract and reproductive system, potentially leading to tissue damage or disease. Both infectious and non-infectious agents (stimuli) and cell damage activate inflammatory cells and trigger inflammatory signaling pathways, Inflammation if not treated will lead to chronic inflammation and further will lead to chronic inflammatory disease [6,7]. The level of inflammation relies on the magnitude of the stimuli. A wide range of anti-inflammatory drugs are already in use to control inflammation in the body. However, they have side effects and may not be as effective in some cases . Compounds that are present in certain herbal remedies also have the potential to be anti-inflammatory drugs [8]. Phytochemical constituents that are present in herbs are considered as an effective remedy as an anti-inflammatory agent. Our team has extensive knowledge and research experience that has translate into high quality publications [9–11,12–17,18,19,20,21, 22,23,24–28]. The aim of the study is to comparatively evaluate the anti- inflammatory effect of leaf, root and shoot of *Tecoma stans.*

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA.

2.2 Collection of Plant Material

Tecoma stans were collected from Chennai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, and Chennai, India. The bark leaves and flower parts of the plant were shade-dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with ethanol.

2.3 Preparation of Plant Extracts

1kg of dry powders from leaves from both plants were taken in individual aspirator bottles; 3 liters of ethanol was used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extracts were filtered before drying using whatman filter paper no 2 on a Buchner funnel and the solvent was removed by vacuum distillation in a rotary evaporator at 40˚C; the extracts were placed in pre-weighed flasks before drying.

2.4 Assessment of *in-vitro* Anti-inflammatory Activity

2.4.1 Inhibition of albumin denaturation

The anti-inflammatory activity of the plant extract was studied by the inhibition of albumin denaturation technique which was studied according to the methods of Oyedepo and Femur Was (1965) and Sakat et al. (2010) followed with minor modifications. The reaction mixture consisted of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl. The plant extract with increase in concentration (100 to 500 µg/ml) were incubated at 37^0C for 20 min and then heated to 51^0C for 20 min, after cooling the samples the turbidity was measured at 660nm.(UVVisible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate. In this study, Aspirin was used as a standard anti-inflammatory drug.

100 µL of bovine serum albumin was added to 100µl of plant sample with increase in concentrations (100-500µg/ml). This was incubated at room temperature for 5 minutes. Reaction was inhibited by the addition of 250 µl of trypsin followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Acetyl salicylic acid was used as a positive control.

Calculation:

% Inhibition=100−((A1 -A2)/A0)*100)

2.5 Statistical Analysis

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of $p<0.05$.

3. RESULTS AND DISCUSSION

Fig. 1 represents the protein denatured inhibition of stem extract of *Tecoma stans.* Fig. 2 represents the protein denatured inhibition of root extract of *Tecoma stans and* Fig. 3 represents the protein denatured inhibition of leaf extract of *Tecoma stans.* In this study the standard drug used is aspirin. It is detected that the concentration of aspirin increases from 100 micro g/ml to 500 micro g/ml in each of the graphs obtained, the concentration of leaf extract also increases.

Protein denaturation is the main common cause of prolonged inflammation. Therefore, the inhibition of this denaturation can have a favourable clinical effect on inflammation [29] have shown that these phytochemicals have anti-inflammatory effects when they inhibit protein denaturation [30]. Also carried out a similar study with slight modification with the technique to screen the anti-inflammatory effect. In this study it was observed that Tecoma stans has good anti-inflammatory activity similarly in another study conducted it was observed that the flavonoids in these plants act as protective agent against inflammatory disorders [31]. It was also reported that they reduce edema formation and

inhibit the synthesis of prostaglandin and thromboxane [32]. Hence this study was evaluated with these similar citations.

These phytochemicals bind to the specific receptors on cells that their dimensional confirmation to result in a specific pharmacological action. In the current study, as concentration of extract increased there was a significant rise in the anti- inflammatory effect. The stem,seed and root extract of *Tecoma stans* showed the same anti-inflammatory activity. In relevance to dose dependent protein protective activity [33].

The relative activity of bark, leaves and flowers could also be observed. The dose dependent increase was not uniformly present among the three groups that were being tested. That means, the seed, stem and root extracts showed almost the same activity, even at 200 µg/ml. They reached high values of activity at relatively low concentrations, but nevertheless remained constant thereafter. That implies that this drug will show some saturation effect at higher concentrations. Although, the values of percentage inhibition have reached high percentages at high concentrations.

The limitation of the study has only used aspirin as standard to evaluate the % inhibition extracts. In clinical scenarios, there are much more proteins that are involved in inflammation and repair. While indication is provided for potential protein protective activity, in vivo testing is essential to prove the clinical utility of the extracted phytochemicals.

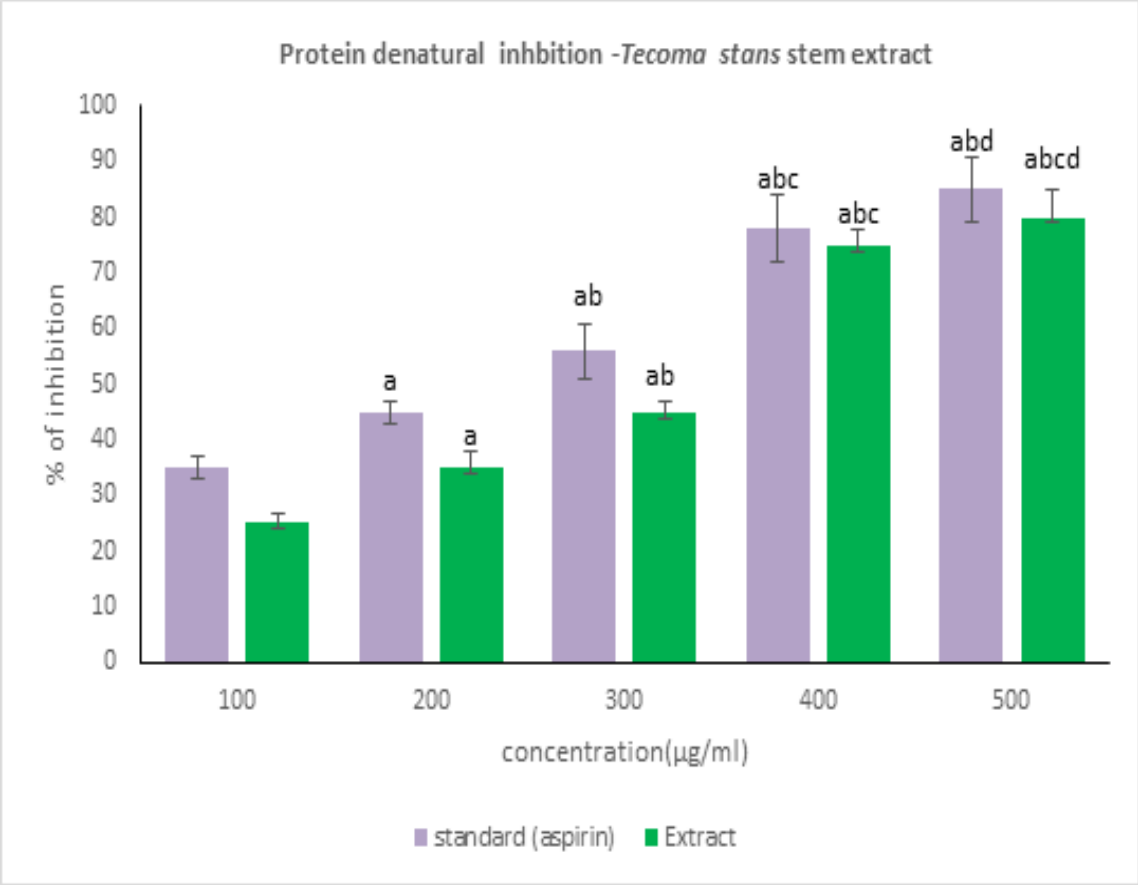

Fig. 1. Protein denaturation inhibitory activity of stem extract of *Tecoma stans* . Each bar represents the mean ± SD of 6 observations. Significance at the levels of P < 0.05.a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.; d-compared with 400µg

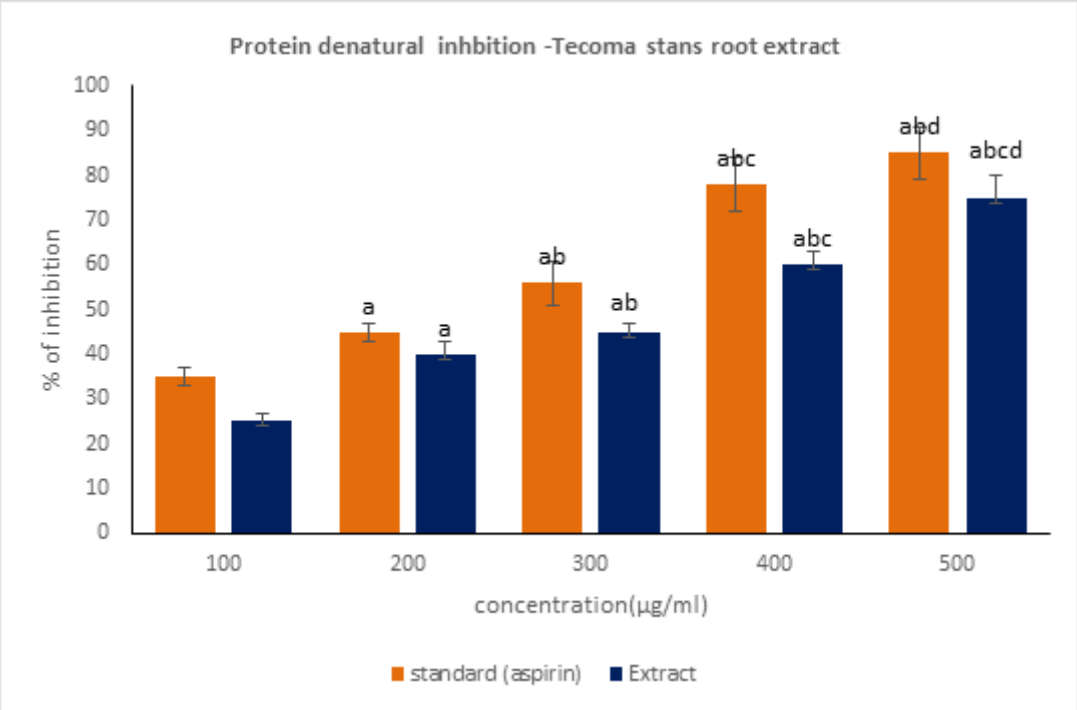

Fig. 2. Protein denaturation inhibitory activity of root extract of *Tecoma stans.* Each bar represents the mean ± SD of 6 observations. Significance at the levels of P<0.05.a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.; d-compared with 400µg

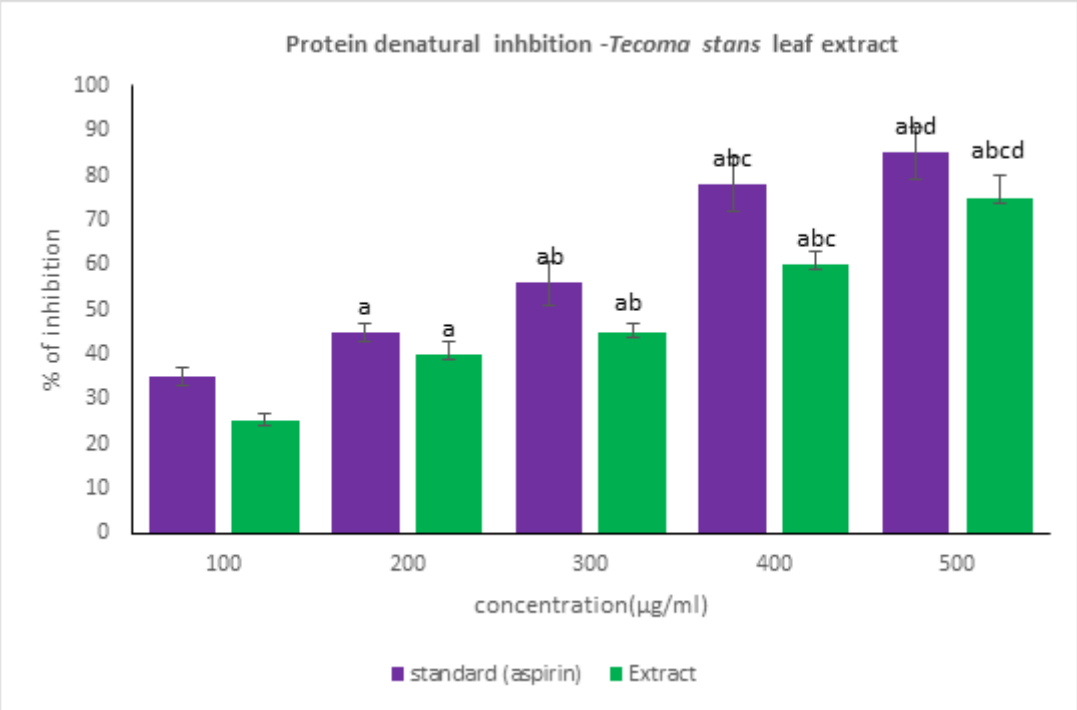

Fig. 3. Protein denaturation inhibitory activity of leaf extract of *Tecoma stans*. Each bar represents the mean ± SD of 6 observations. Significance at the levels of P < 0.05.a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.; d-compared with 400µg

4. CONCLUSION

The *Tecoma stans* is widely used by traditional medical practitioners for the treatment of various diseases. Studies show a diversity of pharmacological activities in Tecoma Stans. investigations of this plant, Tecoma stans, showed that the crude extracts exhibited antidiabetic, antimicrobial, free radical scavenging, anti-inflammatory, wound healing, cytotoxic and anticancer properties. Phytosterols, alkaloids, quinones, amino acids, monoterpenes, triterpene, glycosides, phenols, flavonoids, saponins, and tannins are well known for their biological properties and although a suite of compounds belonging to this class of phytochemicals have been isolated, very few have been subjected to pharmacological assays. Different plant parts possess varying amounts of phytochemicals beneficial to the medical industry. Hence through this study it can be concluded that the plant stem / bark part consists of maximum anti-inflammatory activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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