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Research Article

Comparative Evaluation of Anti-Inflammatory Potential of Ethanolic Extract of Leaf, Bark and Flower of *Tecoma stans* with Ibuprofen- An *In vitro* Analysis

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ABSTRACT

Background: Endodontics has always been indebted to pharmaceutical sciences to provide it with necessary analgesics and anti-inflammatory agents. More specifically, there has always been a need for anti-inflammatory phytotherapeutic agents as the commercially available synthetic anti inflammatory drugs have their own limitations due to undesirable side effects. Hence, novel potent analgesic and anti-inflammatory drugs without considerable side effects from the natural sources are under evaluation. *Tecoma stans* (Bignoniaceae) is an ornamental plant found throughout India. It has been shown to have variety of medicinal properties. In the present study, we have shown that potential anti inflammatory activity of different parts of *Tecoma stans (T.stants)* and compared with standard drug. **Aim:** To evaluate the *in vitro* anti inflammatory potential of different parts of *T.stans* ethanolic extract and to compare the anti inflammatory activity with standard drug ibuprofen. **Methodology:** The ethanolic extraction of *T.stans's bark,* leaves and floweres was done as per the standard method. Different concentrations (100, 200, 300, 400 and 500 µg/ml) of the extracts were used for anti-inflammatory activity by inhibition of albumin denaturation. All samples were analyzed in triplicate. The results were statistically analyzed. **Results:** All the three parts of the plant extract have shown to have anti inflammatory activity in a dose-dependent manner. However, the leaf and flower extracts of *T.stans* were found to have 100 percent anti-inflammatory pontential than standard drug ibuprofen. **Conclusion:** It is concluded from the present findings that *T.stants* possess anti-inflammatory properties which could be due to presence of active constitutents presen in the plant extracts. Hence, *T.stans* may serve as one of the anti inflammatory herbal durgs for Endontic infection-induced inflammation and related to dental diseases. Further studies on the identification of the active principles present in the leaf and flower extract are warranted to assertatin its potentials.

Key words: *Tecoma stans,* Anti-inflammatory effect, Leaf, Bark, Flower.

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INTRODUCTION

Endodontics has always been indebted to pharmaceutical sciences to provide it with necessary analgesics and anti-inflammatory agents. More specifically, there has always been a need for anti-inflammatory agents. This is because of wide range of action of these drugs, resulting in undesirable side effects. As the sciences grew, more biologically specific molecules were produced and were tried to be delivered locally. Nevertheless, many factors work in unison to orchestrate the inflammatory process.

In this context, since antiquity, man had been searching various plants for cure of illnesses. Many such endeavors have been successful and have proved to be potent precursors for modern drugs. Currently, more herbs are being sought and studied from traditional wisdom all over the world.[1] From the endodontic perspective, it is essential to have a drug that can both systemically and topically be delivered to reduce the inflammation, without causing side effects.[2]

The search has led to exploration of a perennial shrub called *Tecoma stans*. It is easily available in India and has been used for this purpose since antiquity. Numerous reports have been published recently showing that its extracts have antibacterial, antifungal, anti-inflammatory and gastroprotective activity.[3-6]

Phytochemicals are known to work synergistically to produce the pharmacological action. Also, the content of every phytochemical will vary with the part from which the extract was prepared. Certain phytochemicals are abundant in bark rather than in greener parts. Metabolism of plants produce such variations and it has to be tackled by isolation of specific bioactive molecule. Moreover, phytochemical isolation is largely dependent upon solvent used to extract them too. Hence, inorder to effectively separate the bioactive molecule, it is necessary to identify the plant part that shows the best activity. While there can be several potential activities for any type of extract, it is necessary to individually study all the effects to result in clinical translation of the discovery.

Though there had been a lot of reports on beneficial effects of various *Tecoma Stans* extracts, till date, comparative evaluation of ethanolic extract of

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Tecoma stans from bark, leaf and flowers have not been compared for *in vitro* anti-inflammatory activity, in comparison with known standard drug. Hence, this study throws light on *in-vitro* anti-inflammatory efficacy of ethanolic extract of *T.stans* compared to ibuprofen.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in the present study were of molecular and analytical grade; and they were purchased from Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom; and Sisco Research Laboratories, Mumbai, India.

Collection of plant material

Tecoma stans bark, leaves and flower 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, 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.

Preparation of plant extracts

1kg of dry powders from bark, leaf and flower were taken in individual aspirator bottle; 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.[7]

Assessment of *in vitro* anti-inflammatory activity by *T.stans* leaf, bark and flower ethanolic extracts

Inhibition of albumin denaturation assay

The anti-inflammatory activity of *Tecoma stans* leaf, bark and flower ethanolic extracts was studied by inhibition of albumin denaturation method which was studied according to Mizushima *et al.* (1968) and Sakat *et al.* (2010) followed with minor modifications.[8,9] The reaction mixture was consisted of test extracts and 1% aqueous solution of bovine albumin fraction, then the reaction mixture was adjusted to pH 6.3 with 1N HCl. The sample extracts of leaf, bark and flower at different concentrations (100, 200, 300, 400 and 500 µg/ml) were incubated at 37ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples the absorbance was measured at 660 nm using UV Visible Spectrophotometer Model 371, Elico India Ltd. The experiment was performed in triplicate. In the present study, the anti-inflammatory standard synthetic drug, Ibuprofen was used to compare the efficacy. The Percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control.

RESULTS

Effect of *T.stans* bark, leaves and flower ethanolic extract on inhibition of albumin denaturation

Figure 1 and Table 1 represent the effect of different concentration of *T.stans* ethanolic extract on inhibition of albumin denaturation. Protein denaturation is a well established cause of inflammation. As a part of the investigation on anti-inflammatory activity, ability of different concentration of *T.stans* ethanolic extracts showed differential inhibitory activity (Table 1 and Figures 1-3). Leaf extract showed the inhibitory activity of 23,53,85,100 and 100% respectively while Bark extract showed a inhibitory activity of 23,70,85,89, and 85 % respectively. The flower part of the plant showed the inhibitory activity of 36,71,79,100 and 100 % respectively which could comparable with the commercially available synthetic anti inflammatory drug Ibuprofen. Among the different parts of the plant exgtracts used, the leaf and flower were found to be more effective than bark.

DISCUSSION

Prolonged inflammation is the major reason for failure in endodontics. In order to test such activities, laboratory animal models are necessary. From traditional wisdom, numerous drugs are being developed and hence it is a fertile area for future research in pharmaceutical sciences. However, scanning these phytochemicals for all possible biological activity is a huge task and hence should be subjected to initial screening before subjecting them to expensive testing models.

Protein denaturation is the most common cause of prolonged inflammation. Therefore, inhibition of such denaturation can have a clinically favorable effect on inflammation. Moreover, stabilizing the regenerative molecules can yield better clinical results. Mizushima and Kobayashi (1968) have shown that when these phytochemicals inhibit protein denaturation and they have anti-inflammatory activity.[8] Sakat *et al.*, (2010) have also slightly modified the technique to screen the

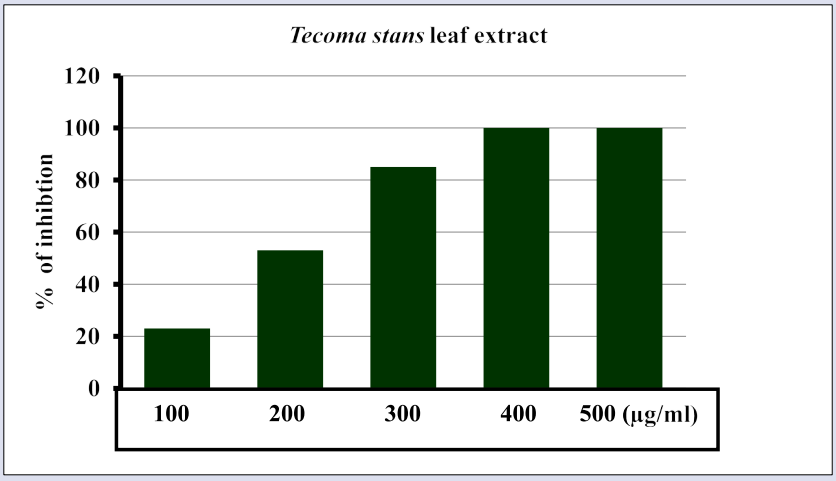

Figure 1: *Tecoma stans* leaf extract vs % of inhibition.

Table1: Effect of *Tecoma stans* leaf, bark and flower extracts on heat-induced protein denaturation.

Treatment (s) Concentration	Concentration (µg/ml) Tecoma stans	% Inhibition leaf extract	% Inhibition bark extract	% Inhibition flower extract
Tecoma stans	100	23	23	36
Tecoma stans	200	53	70	71
Tecoma stans	300	85	85	79
Tecoma stans	400	100	89	100
Tecoma stans	500	100	85	100
Ibuprofen (Standard drug)	100 mg/ml		90	

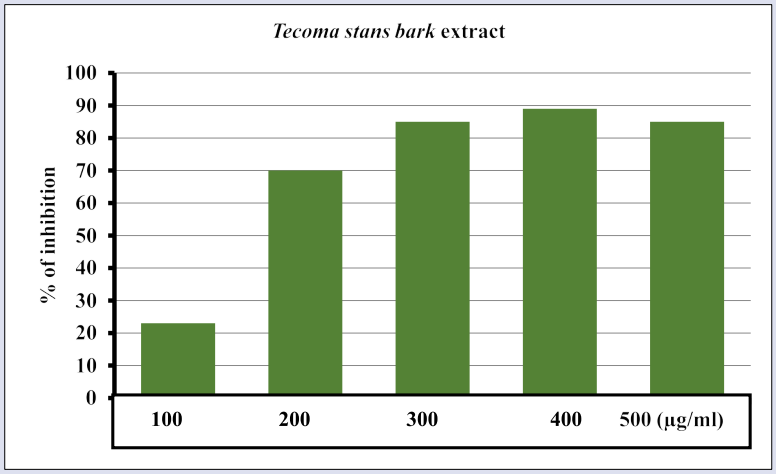

Figure 2: *Tecoma stans* bark extract vs % of inhibition.

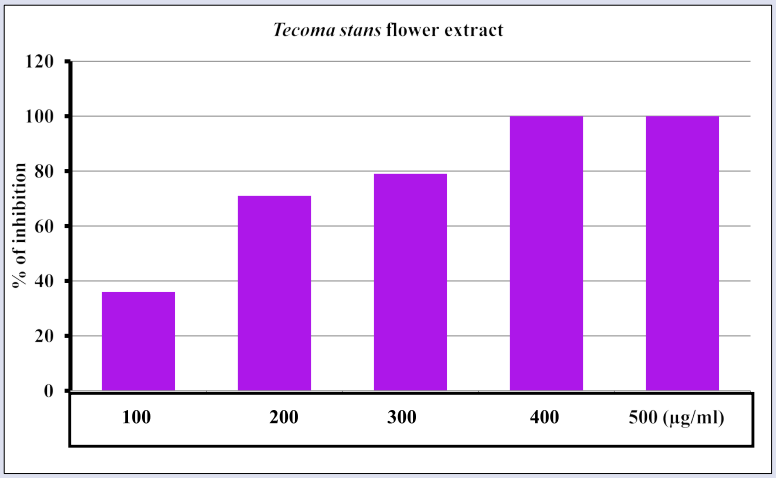

Figure 3: *Tecoma stans* flower extract vs % of inhibition.

anti-inflammatory activity *in vitro*.[9] Recently, methanolic extract of *E.axillare* has been studied successfully.[10] The relation between *in vitro* protein denaturation and *in vivo* anti-inflammtory action has been seen in *Mikania scandens*.[11] Hence, this study utilized these methods to evaluate *in vitro* anti-inflammtory activity.

Major alkaloid found in *Tecoma stans* is Tecomine. It is known to produce antidiabetic effect in rats.[12,13] Other biologically active alkaloids include 5b-Hydroxyskitanthine and Boschniakine. While it is a known fact that every phytochemical can exhibit more than one kind of biological activity, its mechanism of action should be thoroughly studied. These phytochemicals bind to specific receptors on cells that match their three dimensional conformation to result in specific pharmacological action. As these molecules are not specifically synthesized to bind to these receptors, their affinity and activity may vary largely. It is up to us to properly characterize the binding of these molecules to receptors that we intend to target.

In current study, it was seen that as the concentration of extract increased there was a steady rise in anti-inflammatory activity in all the groups. Also, flower and leaves showed higher activity compared to bark. This may be due to variation in bioactive molecule concentration in various parts of the plant. Unlike animal metabolism, plants show high variation with climatic conditions. It is usual to find abundance of phytochemicals in flowers than in bark. So far, this study has seen the potential protein protecting effect in phytochemicals obtained from *Tecoma stans*.

In relevance to dose dependent protein protective activity, the change in concentration of extract showed proportional increase in activity.

This was almost linear and hence be attributed only to increase in phytochemical content. In certain cases, small change in dose can produce a large change in activity. At times, phytochemicals can also exhibit therapeutic window. In this study, such phenomena were not observed in the selected concentrations. This also means that the concentration almost achieved saturation as it reached higher levels. This suggests that at dose cannot be indefinitely increased and maximum protective activity will be seen at around 500 µg/ml concentrations. Though direct correlation of this value to *in vivo* studies cannot be found, it stands as a guideline for proper dosing.

Next question would be to observe the relative activity of bark, leaves and flowers. The dose dependent increase was not uniformly seen among the three groups tested. That means, bark and flower extract showed almost 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 100 percent in in leaf and flower extract at high concentrations, this study has only used bovine serum albumin to evaluate the extracts. In clinical scenario, there are much more number of proteins, that are involved in inflammation and repair. While indication is provided for potential protein protective activity, through *in vivo* testing is essential to prove the clinical utility of the extracted phytochemicals.

As said earlier, synergistic action of crude phytochemicals are worthy of investigation. But, due to wide variations, it is essential to isolate specific components by suitable means to test the anti-inflammatory activity. In economic perspective, isolation from flowers and leaves can offer good advantage over bark. While extraction from bark will require tree to be destroyed, extraction from leaves and flowers would result in constant and dependable production of raw materials. In future, the comparisons should be made among leaves and flowers isolated in various seasons around the year.

CONCLUSION

Our present study concules that *T.stants* possess anti-inflammatory properties which could be due to presence of active constitutents presen in the plant extracts. Hence, *T.stans* may serve as one of the anti inflammatory herbal durgs for Endontic infection-induced inflammation and related to dental diseases. Further studies on the identification of the active principles present in the leaf and flower extract are warranted to assertatin its potentials. In future, oral bioavailability and mechanism of action should be elaborated and the drug should be used in pre-clinical lab animal models.

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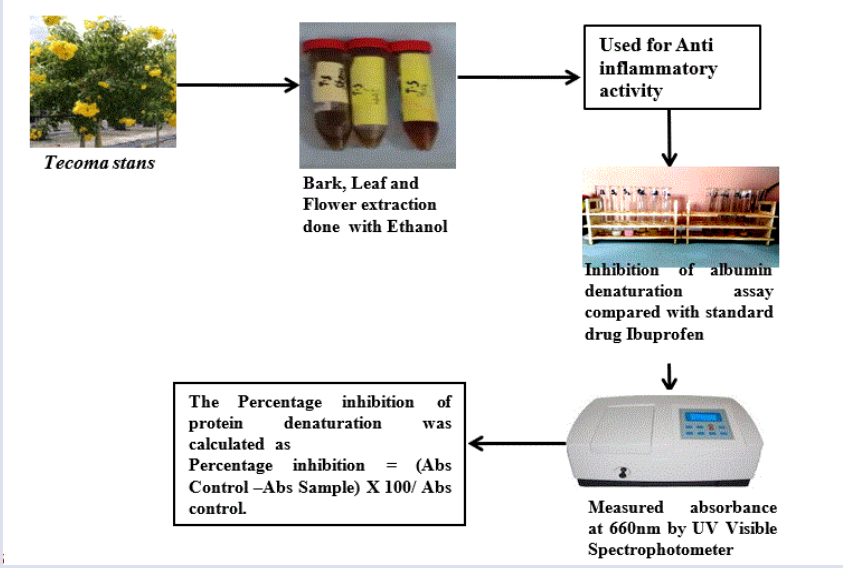
GRAPHICAL ABSTRACT



SUMMARY

- *Tecoma stans* Leaf, Bark and Flower extracts were prepared using ethanol extract as per the standard method.
- The 3 different parts of the plant extracts were subjected to albumin denaturation analysis to study the anti-inflammatory potentials of the plant extracts.
- Among three extracts used, the leaf and flower extracts showed 100% efficacy compared to bark extract than the standard drug ibuprofen.
- *T.stans* leaf and flower extracts may be used as one of the potential anti inflammatory herbal drugs for treatment of various diseases including Endodontic infection-induced inflammation and other dental related diseases.

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