

Original Article

Anti-Inflammatory Activity of Phytosterols and their derivatives Isolated from the Leaves of *Tridax procumbens* Linn.

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Abstract

Currently, inflammatory diseases are being treated with drugs most of which have side effects. There is a need to develop newer analogs especially from phytochemical sources that have lesser or no side effects. Hence, we thought it worthwhile to isolate phytosterols from leaves of *Tridax procumbens* plant, prepare their semisynthetic derivatives and evaluate them for anti-inflammatory activity. Column chromatography and preparative TLC were employed to isolate β -sitosterol and stigmasterol. Their acetylated derivatives were prepared using conventional organic reactions. The compounds were evaluated for *in vivo* anti-inflammatory activity using carrageenan-induced paw oedema method in rats. Results revealed that both β -sitosterol and stigmasterol have significant anti-inflammatory activity viz., 65% and 67%; respectively with standard diclofenac exhibiting 68% inhibition. Derivatives betasitosteryl acetate and stigmasteryl acetate showed 61% and 62% inhibition; respectively. Thus, phytosterols and derivatives of *Tridax procumbens* leaves can be used as leads to make future drug candidates to treat inflammatory conditions.

Keywords: Anti-inflammatory activity, *Tridax procumbens* Linn, Phytosterols.

1. Introduction

Inflammation is a diseased condition in which body tissues are affected by heat, redness, swelling and pain¹. There is a plethora of reviews and textbooks outlining the pathology of inflammation, including the sequence of events, network of mediators, such as prostaglandins, leukotrienes and cytokines along with complex molecular mechanisms that are involved². Inflammation is a key feature of a number of diseases and the clinical features of these diseases are described extensively in literature. Although there are many non-steroidal anti-inflammatory drugs in the market, many of these suffer from various side effects and related metabolic toxicities. Hence, there is a need to search new potent anti-inflammatory agents with no toxicities and minimal or no side effects.

Tridax procumbens has been reported to act against inflammatory conditions and hence, it was thought worthwhile to isolate its active principles and evaluate them for anti-inflammatory activity. *Tridax procumbens* Linn (family-asteraceae) is a green perennial plant and is available in all seasons in many parts of India³. It is listed as a weed and a pest plant, it has been known by several names including coat buttons in English, Jayanti veda in Sanskrit, ghamra in Hindi, dagadi pala in Marathi, herbe caille in French and thata poodu in Tamil. It habitats in waste places, road sides and hedges throughout India⁴. Some of the reported chemical constituents present in the aerial parts of the plant are phytosterols; beta-sitosterol, stigmasterol, campesterol⁵ and a characteristic triterpene; beta-amyrin. The leaves of this plant including other aerial parts except flowering tops have been claimed to be useful in the treatment of inflammatory conditions. It is also known for

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several other potential therapeutic activities like antiviral, anti-oxidant, anti-bacterial, wound healing, insecticidal and activities. In the Indian traditional medicine, it has been used as anticoagulant, hair tonic, antifungal and insect repellent, in bronchial catarrh, diarrhea, dysentery, and wound healing ⁶.

Taxonomical Classification⁷

Kingdom: Plantae

Sub kingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Asterales

Family: Asteraceae

Subfamily: Caesalpinaceae

Genus: *Tridax L*

Species: *Tridax procumbens L*

Amongst the various reported activities of this plant *Tridax procumbens* Linn, anti-inflammatory activity was considered for evaluation after isolation of its phytosterols viz., β -sitosterol and stigmasterol.

2. Materials and Methods

2.1. Phytochemical investigations

2.1.1 Plant material

The plant material of *Tridax procumbens* were collected from the local areas of Nashik and authenticated from the Department of Botany, N.D.M.V.P Samajs, K.T.H.M College Nashik-2, Maharashtra, India.

2.1.2 Preparation of aqueous extracts

The freshly collected leaves plant were dried and coarsely powdered. The powdered leaves (750g) were extracted with petroleum ether (60-80°C) in Soxhlet extractor. This extract was saponified with alcoholic potassium hydroxide and unsaponified matter was then separated as per procedure given in Indian Pharmacopoeia⁸. Thin layer chromatography (TLC) of this unsaponified portion showed the presence of phytosterols which were subjected to column chromatography.

2.1.3 Isolation of phytosterols and preparation of its derivatives

It was achieved by employing column chromatography of unsaponified portions. The

column was set several times till reproducible yields of phytosterols were attained. The phytosterol fractions were combined together to get an overall yield of 1.79gm. Among the reported phytosterols, β -sitosterol and stigmasterol could be isolated. These were detected by the presence of bluish fluorescence under ultra-violet light. These were separated from the other pigments and unidentified constituents by recolumn chromatography. Finally, separation of β -sitosterol and stigmasterol from each other was done by preparative TLC. To achieve reproducible yields of these phytosterols, recolumn chromatography and preparative TLC were repeated several times. The phytosterols were acetylated by treating them independently with acetic anhydride in presence of pyridine to afford β -sitosteryl acetate and stigmasteryl acetate. The structures of the isolated phytosterols were confirmed by infrared and NMR spectroscopic techniques.

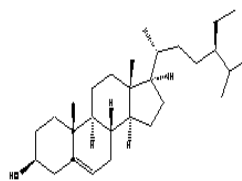


Figure 1: β -sitosterol

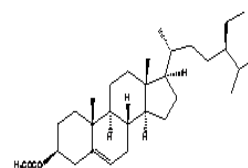


Figure 2: β -sitosteryl acetate

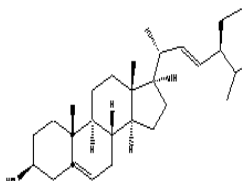


Figure 3: Stigmasterol

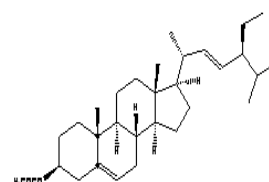


Figure 4: Stigmasteryl acetate

2.2. Pharmacological Screening

2.2.1 Animals

Male albino rats (wistar strain) weighing 150-200gm, procured from National toxicological Centre, Pune, Maharashtra were used for the study.

2.2.2 Housing of the Animals

The animals were kept for one week to acclimatize to laboratory conditions before starting the experiment. Animals were housed

in groups of five rats in standard polypropylene cage, on 12 h light /12 h dark cycle. They were given free access to water and standard rat feed. 12 hrs prior to an experiment, the rats were deprived of food but not water.

2.2.3 Acute toxicity studies

The rats were starved to overnight and were divided into groups (n=5 per group) and were orally fed with increasing doses (10, 20, 40 and 100 mg/kg body weight) of the selected compound. These did not produce any evident sign of toxicity and any mortality in rats when observed upto 4 days after administration. Out of these doses, the dose of 40 mg/kg b.w was found to be an effective dose (ED50). This dose was selected on the basis of the dose-dependent variation in responses in animals for the said activity.

2.2.4 Preparation of dosage

Diclofenac sodium, phytosterols and their derivatives were administered as suspension prepared 30 mins before experiment by triturating with water and 1 % Tween 80.

2.2.5 Anti-inflammatory activity testing

The isolated phytosterols and its acetyl derivatives (1 to 4) were tested for their anti-inflammatory activity using carrageenan-induced rat hind paw oedema method in rats⁹. For the study, male wistar albino rats weighing between 150 and 200 g were randomly selected. The animals were divided into control, standard and test groups and the experiments were performed in the morning according to the guidelines for the care of laboratory animals¹⁰ and carried out with strict compliance of Institutional Animal Ethics Committee regulations. In all groups, acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in the right hind paw of the rats.

The paw volume was measured plethysmometrically from 0 to 180 min after carrageenan injection. The compounds (25 mg/kg body weight) were administered orally, standard group was treated with diclofenac sodium (10 mg/kg body weight) orally 1 h before carrageenan injection and control group received only vehicle. The difference in volume gave the amount of oedema that had been developed. The mean of this difference in paw volume was measured and percentage inhibition was calculated by using formula

% inhibition of oedema = $[(V_c - V_t) / V_c] \times 100$;
where, V_t and V_c are the mean paw volumes of test group and control group; respectively.

2.2.6 Statistical analysis

The mean paw volume was expressed in terms of mean \pm SEM and checked for statistical significance^{11,12} by using ANOVA technique and employing Dunnett's test thereafter, with the readings showing statistical significance at $P < 0.01$.

Results and Discussion

In the present study, the isolated phytosterols and their acetylated derivatives were studied for anti-inflammatory activity by carrageenan induced oedema method. The results of this study are summarized in Table 1. It was found that both β -sitosterol and stigmasterol have significant carrageenan inhibitory activity viz., 65% and 67%; respectively with the standard drug having 68% inhibition. The derivatives betasitosteryl acetate and stigmasteryl acetate showed 61% and 62% inhibition; respectively which is comparatively lesser than that of parent sterols. Thus, the phytosterols and the derivatives of leaves of *Tridax procumbens* can be used as leads for making future drug candidates to be used against inflammatory diseases.

Table 1: Inhibition of paw oedema shown by the isolated phytosterols and the derivatives of the leaves of *Tridax procumbens*.

Time (min)	Carrageenn (Control)	Diclofenac sodium	B-sitosterol	Betasitosteyl acetate	Stigmasterol	Stigmasteryl acetate
Dose	0.1 ml (1 %)	25 (mg/kg b.w)	40 (mg/kg b.w)	40 (mg/kg b.w)	40 (mg/kg b.w)	40 (mg/kg b.w)
0	0.45±0.031	0.23±0.012	0.24±0.018	0.29±0.01	0.23±0.03	0.29±0.018
15	0.48±0.018	0.25±0.015	0.27±0.012	0.3±0.027	0.25±0.02	0.3±0.015
30	0.59±0.024	0.27±0.012	0.24±0.018	0.33±0.02	0.23±0.02	0.32±0.02
45	0.67±0.012	0.27±0.02	0.32±0.012	0.33±0.02	0.3±0.015	0.31±0.01
60	0.79±0.018	0.24±0.016	0.28±0.012	0.31±0.029	0.28±0.012	0.3±0.015
90	0.79±0.018	0.24±0.016	0.26±0.01	0.3±0.022	0.24±0.018	0.29±0.018
120	0.85±0.015	0.2±0.015	0.24±0.01	0.29±0.01	0.23±0.012	0.23±0.012
150	0.9±0.016	0.2±0.015	0.23±0.025	0.21±0.018	0.22±0.012	0.21±0.018
180	0.92±0.025	0.17±0.012	0.22±0.02	0.17±0.012	0.2±0.015	0.2±0.022
Vt	0.71 / Vc	0.23	0.25	0.28	0.24	0.27
% Inhibition	--	68	65	61	67	62

All the readings are statistically significant by ANOVA technique and Dunnett's test at P<0.01.

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