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PHYTOCHEMICAL AND IMMUNOMODULATORY SCREENING OF TECOMINE, ISOLATED FROM METHANOLIC EXTRACT OF *TECOMA STANS* LEAVES

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ABSTRACT

Immunology is one of the most rapidly developing areas of medical biotechnology research. Immunomodulators are becoming a viable adjunct to establish modalities offering a novel approach for the treatment of infectious diseases in coming decades. In the present study Phytochemical screening included extraction, isolation and identification bioactive compounds of methanolic extract of *Tecoma stans* leaves by different analytical methods. The isolated compound Tecomine was screened for immunomodulatory activity. The literature survey revealed that there is no evaluation of immunomodulatory activity of the isolated compound Tecomine. So, the present study was aimed to evaluate the immunomodulatory activity of Tecomine isolated from *Tecoma stans*. From research studies in the past and present investigation data, it can be assumed that the Tecomine is responsible for immunostimulant properties.

KEYWORDS: Phytochemical, Tecoma Stans, Tecomine, Immunomodulatory Activity.

INTRODUCTION

Immunology is one of the most rapidly developing areas of medical biotechnology research. All drugs which modify immune response generally categorized as immunomodulators[1]. These can either function as immunostimulants or immunosuppressants. Some of these can have both the properties depending on which component of immune response they affect.

Immunomodulators are becoming a viable adjunct to establish modalities offering a novel approach for the treatment of infectious diseases in coming decades.

Tecoma stans is a perennial shrub belonging to the family Bignoniaceae, commonly known as yellow trumpet bush. It has sharply toothed, lance-shaped green leaves. The chemical constituents reported were monoterpene alkaloids.[1-4]

A new phenyl ethanoid, 2-(3, 4-dihydroxy phenyl) ethyl-2-O- [6-deoxy-alpha-L-manno pyranosyl- 4-(3, 4 dihydroxy phenyl) -2-propenoate]-beta-D-glucopyranoside, and a novel monoterpene alkaloid, 5-hydroxy-skytanthine hydrochloride, along with eleven known compounds in the fruits and flowers was established in *Tecoma stans.*[5]

The past reported pharmacological activities were anti-diabetic, antioxidant, antifungal, antimicrobial.[6-8]

The literature survey revealed that there is no detailed study of chemical constituents using analytical methods such as HPLC, I.R., and NMR. So, the present work was aimed to study the detailed chemistry of active principle present in the methanolic extract of *Tecoma stans* leaves and screening of isolated component for immunomodulatory activity.

MATERIALS AND METHODS

Collection of Plant Material: The leaves of *Tecoma stans* were collected from the surroundings of University College of Pharmaceutical Sciences, Satavahana University, Karimnagar, Telangana, India. The plant parts were authenticated and deposited at the herbarium of University College of Pharmaceutical Sciences, Satavahana University, Karimnagar, Telangana, India.

Preparation of the extract: The leaves of *Tecoma stans* (2.0kg) were kept for maceration with methanol for seven days. The extracts were concentrated in desiccators.[9]

Chemicals: All the chemicals used for the investigation were of analytical grade.

Drugs: In the present study Levamisole was used as an immunostimulating agent.[10]

Antigenic Material: All groups of mice were antigenically challenged with SRBC (0.5x109cells/ml/100 g) on the 5th day intraperitoneally.[10]

Detection of phytoconstituents

The extract was tested for phytoconstituents by preliminary tests, isolated the compound by Column chromatography and identified pure component through HPLC, identified functional groups by I.R. and structure with the help of NMR data.

Screening of immunomodulatory activity

Methods

- ✓ Carbon clearance test
- ✓ Humoral antibody titre
- ✓ Delayed type hypersensitivity

METHOD OF EVALUATION

Detection of Phytoconstituents: The extract was tested for the presence of Carbohydrates, Tannins, Flavonoids, Alkaloids, Anthocyanin and Betacyanin, Glycosides, Proteins, Steroids and Phytosterols, Phenols.

Chromatography

a. Thin-layer chromatography (TLC): Thin layer chromatography (TLC) is a chromatography method which is employed to separate mixtures.[11] The analytes rise in the TLC plate at different rates, finally the mixture is separated.[12]

b. Column chromatography: Every compound in a mixture will have a specific solubility in the solvent and a specific affinity to be adsorbed by the solid adsorbent. No two compounds typically behave precisely alike in these respects. This principle is used in column chromatography.[13,14]

Preparation of Column

Materials Used

Column of size 90 cm X 4.0 cm

Silica gel 100-200 mesh as the adsorbent

Silica gel 100-200 mesh was poured into the column by tapping to avoid air space between the particles (dry column method). The bottom of the column was plugged with little cotton to prevent the adsorbent pass out. The methanolic extract of *Tecoma stans* was subjected to column chromatography over silica gel (100-200 mesh).

The column was eluted with solvents of increasing polarity. They are.

1. The solvent system used-starting with Toluene 100%, then Toluene 90% & Ethyl acetate 10% up to Toluene 50% & Ethyl acetate 50% but no spots were observed for these fractions on TLC.[15]
2. Finally, Toluene: Ethyl acetate: Diethyl amine were used in the ratio of 7:2:1, two spots were observed on TLC (Fig 1).
3. Again same solvent system in the ratio of 7:2:2 was used, single spot was observed (Fig 2)

All chromatograms were visualized under ultra violet light.

Sprayed by using Dragendroff's reagent got the orange bands for alkaloids.

Chemical Test

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added (Potassium bismuth iodide solution). A prominent orange-red precipitate indicated the presence of alkaloids.

c. Preparative high performance liquid chromatography

This technique was used to identify the specific constituent present in the sample, which was isolated by column chromatography.[16]

HPLC conditions

Column: Hypersil BDS-C18 (150X4.6mm, 5µ)

Mobilephase: A: 0.1% TFA in Water (50%)

B: 0.1% TFA in ACN (50%)

Flowrate: 1.0 ml/min

Column temp: 35°C

Run time: 40min

Programme (Isocratic)

Diluent: MeOH

Sample Preparation: 1.0 mg/mL in diluent

Vail: 96

Injection Volume: 10 uL

Characterization of Isolated Plant Constituent

Isolated Compound

Spectroscopic Methods: The chemical constituents present in the drug possess characteristic features because of which its characterization becomes possible. At every stage of structure

determination from isolation and purification of constituents to its final comparison with an authentic sample, the spectral data facilitates the description of structure. Interpretation of molecular spectra is generally based on empirical correlations of spectral data with reasonable assurance to a particular group or arrangement of atoms in the molecule.

Infra red (IR) spectrum is generally complicated and out of many peaks relatively a few can be interpreted with assurance. Proton ('HNMR) spectra provide information about the number, nature and environment of the protons and carbon skeleton in the molecule, respectively.[17- 18]

Infrared Spectrum: The constantly vibrating molecules stretch and bend their bonds with respect to one another, by absorbing infrared light. IR spectrum is highly characteristic to establish the identity of compounds. The region 1430 – 910 cm-1 is called 'fingerprint' region where many more bending vibrations of the molecules are found. The identities of two samples that have identical spectra in the finger print region give conclusive identification of compounds. The crystals obtained from methanolic extract of *Tecoma stans* was subjected to Infrared Spectroscopy and the spectrum is shown in Fig 4.[19]

FT-IR conditions used

Model Name FT/IR-4100typeA

Light source- Standard

Detector- TGS

Accumulation- 16

Resolution- 4cm^{-1}

Apodization- Cosine

Scanning speed- auto (2mm/sec)

Filter- auto(30000 Hz)

Nuclear Magnetic ResonanceSpectroscopy (NMR): Nuclear Magnetic Resonance Spectroscopy deals with the study of spin changes in the presence of magnetic field, at the nuclear level when radio frequency energy is absorbed. As we are analyzing organic compounds for the nature, type, number and environment of protons (Hydrogen), the solvent used in the NMR spectroscopy should not contain hydrogen atoms. Hence we usesolvents Carbon tetrachloride (CCl4), Deuterated chloroform (CDCl3), Deuterated Water (D2O), Deuterated Methanol (CD3OD), Deuterated acetic acid (CD3COOD), Deuterated dimethyl

sulphoxide (DMSO). The crystals obtained from methanolic extract of *Tecoma stans* was subjected to NMR and the spectrum is shown in Fig 4.[19]

Screening of immunomodulatory activity of isolated component, Tecomine

Carbon clearance test

Swiss albino mice were divided into five groups which were administered drug for 5 days orally. On the last day, mice were injected with 0.1mlIndian ink via the tail vein. Blood samples were withdrawn at 0min and 15min. A 50µL blood sample was mixed with 4ml, 0.1% Sodium carbonate solution and the absorbance of this solution was determined at 660nm. The phagocytic index K was calculated using the following equation.

K= (Log OD1-Log OD2/15 where OD1 and OD2 are the optical densities at 0 and 15min respectively.

Delayed type hypersensitivity: Cell-mediated immunity (CMI) involves effector mechanisms carried out by T-lymphocytes and their products (lymphokines). The cell mediated immune response was assessed by DTH reaction, i.e. Footpad reaction.

Humoral antibody titre: The animals were immunized by injecting 0.1ml of SRBCs suspension, containing 1×10^8 cells, intraperitoneal on day 0. Blood samples were collected in micro centrifuge tubes from individual animal by retro orbital puncture on day 7. Briefly, equal volumes of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25µl volume of normal saline, in U-bottomed micro titration plates were added 25µl of freshly prepared 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37ºc for 2h and examined visually for agglutination. The reciprocal of the highest dilution of the test serum causing visible haemagglutination was taken as the antibody titre.[20]

RESULTS AND DISCUSSIONS

TLC results of alkaloids for methanolic extract of leaves *Tecoma stans*

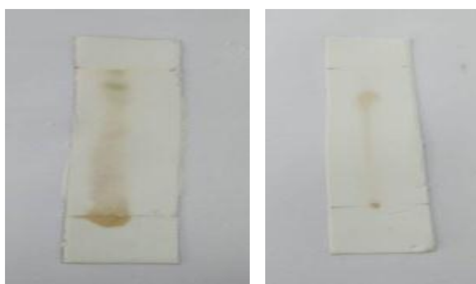

Fig 1 Fig 2.

HPLC fingerprints of methanolic extract of leaves of *Tecoma stans*

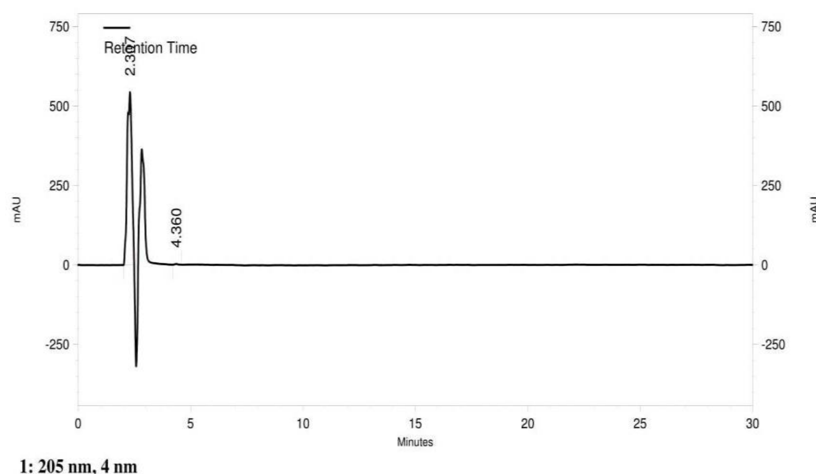

Fig. 3.

Fourier Transform Infrared Spectroscopy (FTIR) methanolic extract of leaves of *Tecoma stans*

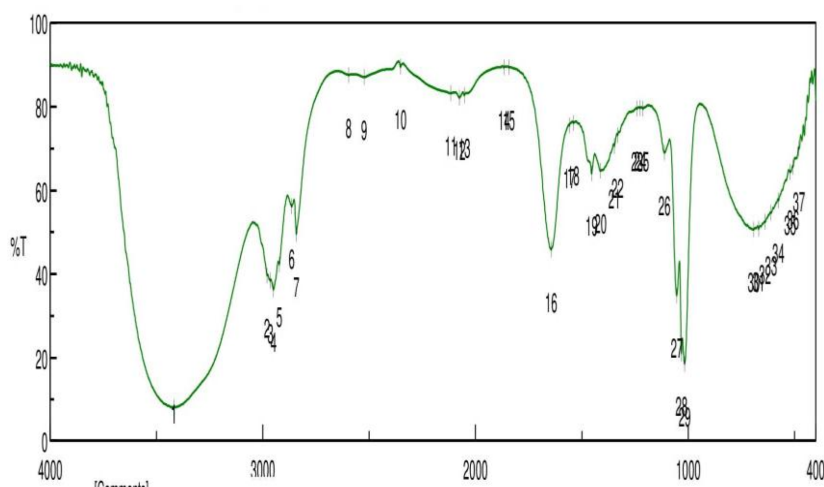

Fig. 4.

FTIR spectral data interpretation

Table. 1: The FTIR spectrum of methanolic extract of *Tecoma stans* leaves are given in Fig 4.

Extract prepared in	Peak number	Peak value	Functional group
Methanol	1	3400	RR’N-H (Tertiary amines)
	4	2950	Aliphatic –CH
	16	1650	C=O (Keto group)

NMR spectral data of *Tecoma stans* extract

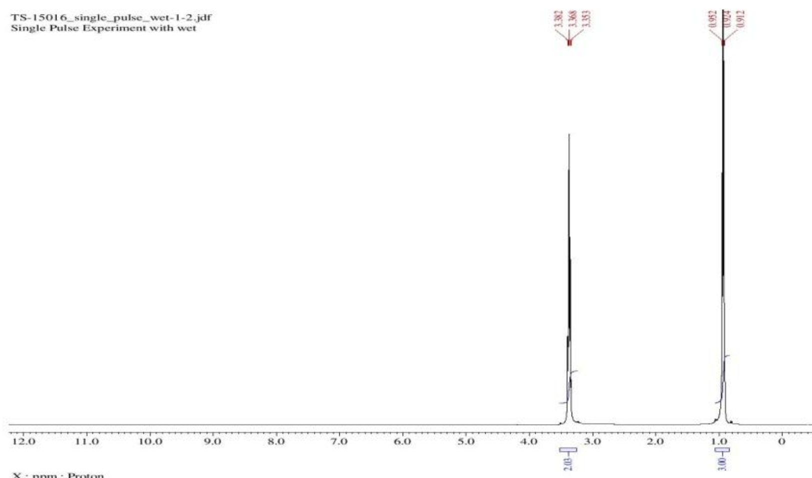

Fig 5.

Table. 2: Effect of methanolic extract of *Tecoma stans* leaves by Carbon Clearance test, Humoral Antibody (HA) Titre and Delayed Type Hypersensitivity (DTH) response.

Treatment dose	Carbon Clearance test	DTH response	HA Titre
Control	0.020± 0.2	12.00± 0.013	5.12±0.50
Std (Levamisole)	0.025± 0.2	12.84± 0.020	5.53±0.54
TME (200mg)	0.023±0.1	12.09± 0.011	5.08±0.32
TME (400mg)	0.024±0.4	12.88± 0.021	5.28±0.40
TME (600mg)	0.029±0.4	14.86± 0.036	6.90±0.42

Values are mean ± SEM; n=6 in each group; P<0.05 in comparison with control

Phytochemical screening

TLC analysis: TLC of methanolic extracts from the T. stans leaves used in this study revealed the presence of alkaloids by using Dragendroff's reagent to reveal characteristic orange bands of alkaloids (Fig 1 & 2).

HPLC analysis: Results of HPLC analysis (Fig 3) of *Tecoma stans* methanolic extract of leaves, at 205nm, shows presence of a constituent as evidenced by the chromatogram obtained at retention time 2.307.

IR Report: FTIR spectrum analysis of *Tecoma stans leaves* was found the presence of primary amines, alkanes, carboxylic acids, alkenes, aliphatic alkyl halides responsible for potential medicinal properties (Fig 4) FTIR spectrum analysis of *Tecoma stans leaves* was found the presence of tertiary amine, aliphatic alkyl and a keto group (Table 1).

NMR spectral data: The 1H NMR spectrum of 1 displayed signals for three methyl groups at δ 0.912, 0.924, 0.952 and three characteristic signals of a –CH2– group at δ 3.353, 3.368, 3.382 (Fig 5)

Screening of immunomodulatory activity: The isolated compound Tecomine was screened in animals using the haemagglutinating antibody titre to assess humoral immune response and Carbon clearance test to assess scavenging activity. The animals were also evaluated for delayed type hypersensitivity by the difference between the pre and post challenge footpad thickness. They have shown significant immunostimulant properties i.e., immunodulatory activity for all the methods used. The data were analyzed using statistical methods and compared to that of the standard drug, obtained values at a dose of 200mg/kg body weight (Table 2).

CONCLUSION

Phytochemistry: In the present study, the reports of HPLC reveal the presence of the compound with retention time 2.307; I.R. reveals the presence of tertiary amine, aliphatic alkyl and a keto group. The 1H NMR spectrum displayed signals for three methyl groups at δ 0.912, 0.924, 0.952 and three characteristic signals of a –CH2– group at δ 3.353, 3.368, 3.382. Therefore, compound was identified as Tecomine, in comparison with published data.

Immunomodulatory activity: The isolated compound Tecomine was screened in animals using the haemagglutinating antibody titre to assess humoral immune response and carbon clearance test to assess scavenging activity. The animals were also evaluated for delayed type hypersensitivity by the difference between the pre and post challenge footpad thickness. They have shown significant immunostimulant properties i.e., immunodulatory activity for all the methods used. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's test to analyze the data and compared to that of the standard drug, obtained values at a dose of 200mg/kg body weight. The significance in difference was accepted at $P<0.05$.

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