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In Vitro Screening of Immunomodulatory Activity of Methanolic Leaves Extract of *Tecoma stans*

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Abstract*—Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. In the present study, methanolic leaves extract of Tecoma stans was screened for immunomodulatory activity using anti-oxidant activity by determining DPPH radical scavenging activity, Hydrogen peroxide scavenging activity, Ferric reducing antioxidant power assay, Immunomodulatory activity by Nitroblue tetrazolium test. The extract showed that the plant extracts have ability to act as free radical scavengers in DPPH radical scavenging activity, reducing ability in Ferric reducing antioxidant power assay, ability to prevent lipid peroxidation in Hydrogen peroxide scavenging activity, ability to stimulate the phagocytic activity in leucocytes in Nitroblue tetrazolium test. The literature survey revealed that these studies were not reported earlier. So, the present study was aimed to reveal the antioxidant and immunomodulatory activity of the plant extract. The study reports concluded that the extract possess immunomodulatory activity.*

Keywords— *Tecoma stans, Immunomodulatory activity, DPPH radical scavenging activity, Hydrogen peroxide scavenging activity, Ferric reducing antioxidant power assay.*

I. INTRODUCTION

Plants synthesise hundreds of chemical compounds for functions including defence against insects, fungi, diseases and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. Immunomodulators stimulate the immune system – immunostimulants **o**r are drugs which either suppress the immune system – immunosuppressants. They modify the immune system either on a positive or at a negative way.

Tecoma stans is a perennial shrub belonging to the family Bignoniaceae, commonly known as yellow trumpet bush, yellow bells, yellow elder. It has sharply toothed, lance-shaped green leaves and bears large, showy, bright golden yellow trumpet-shaped flowers. The chemical constituents reported are 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 are anti diabetic, antioxidant, antifungal, antimicrobial[6-8].

The literature survey revealed that these studies were not reported earlier. The present study was aimed to study the immunomodulatory activity of methanolic extract of *Tecoma stans* leaves.

II. MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Tecoma stans* were collected from the surroundings of lower manair dam, 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

Acids, bases, solvents and salts used for the investigation were of analytical grade and were obtained from Rankem Laboratories, Haryana, Merck Company and S.D. Fine chemicals Mumbai and Finar, Ahmadabad, India.

Drugs

DPPH, Potassium ferricyanide, Trichloro acetic acid, Ferric chloride, Ascorbic acid, Hydrogen peroxide.

III. METHODS OF EVALUATION

Screening of Immunomodulatory Activity

1. In vitro Methods for Antioxidant Activity

I. Determination of DPPH Radical Scavenging Activity[10, 11]

Accurately weighed amount of 6 ml DPPH taken in (20µg/ml) methanolic solution which was in turn added to 20µl of DMSO solution of each extract at room temperature. The mixture was shaken vigorously and absorbance was measured at about 515nm in a spectrophotometer.

% scavenging DPPH free radical = 100 × (1-AE/AD)

Where AE, is the absorbance of the solution after adding the extract and AD is the absorbance of the blank DPPH solution.

II. Determination of Hydrogen Peroxide Scavenging Activity[12]

The scavenging activity of extract towards hydrogen peroxide radicals was determined in this method. Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the

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absorbance at 560nm using UV spectrophotometer. 0.1mg/ml of the extract was added to hydrogen peroxide solution and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula:

Percentage scavenged [H_2O_2] = 1- Abs (standard)/Abs (control) x100

Where, Abs control was the absorbance of the control (without extract) at 560nm;

Abs sample was the absorbance in the presence of the extract at 560nm.

III. Ferric Reducing Antioxidant Power Assay[13-15]

The method relies on the reduction of the complex ferric ion-TPTZ (2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine) by the antioxidant ability of the drug/extract. 1 ml of different concentrations (10 to 40µg/ml) of the extract was mixed with potassium ferricyanide (2.5 ml, 1%) prepared in 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken and 2.5 ml water and 0.5 ml FeCl3 (0.1%) were added to it. The absorbance was measured at 700nm to test the amount of iron reduced.

2. In vitro Immunomodulatory Activity Using Nitroblue Tetrazolium Test (NBT)[16]

A suspension of 5 × 10^6/ml leukocyte was added with 0.2ml of Phosphate buffer solution (PBS). To this 0.2ml of freshly prepared 0.15% NBT solution was added. 0.1ml of Phosphate buffer solution was taken as negative control, 0.1 ml of endotoxin-activated plasma was added to the 0.15% NBT solution and leucocytes mixture which served as a positive control (standard). To all the remaining test tubes 0.2ml of freshly prepared 0.15% NBT solution and different concentrations of extract 10µg/ml, 20µg/ml, and 40µg/ml were added. Then, all the tubes were incubated at 37ºC for 20 min. Then centrifuged at 400g for 3-4 min and the supernatant were discarded. The cells were re-suspended in a few ml of PBS. From this suspension, a drop was taken on a slide and made a film, air-dried, heat fixed and stained with Carbol fuschin for 15sec and washed under tap water, dried and observed the slides under microscope using oil immersion objective. 200 neutrophils were counted and the % of NBT positive cells containing the blue spots.

IV. Results and Discussion

Antioxidant Activity

DPPH radical scavenging activity has been widely used to test the ability of compounds/plant extracts to act as free radical scavengers. In this present study, the DPPH radical scavenging activity of methanolic extract of unripe fruits of *Physalis minima* was determined and compared with the standard (Ascorbic acid).. The radical scavenging activity of extract was more significant than the standard, as the results shown in Table1.

TABLE 1. DPPH radical scavenging activity of methanolic extract of *Tecoma stans* leaves

Sample	Concentration (ug/ml)	Absorbance	% inhibition	IC50 (µg/ml)
Methanolic extract of leaves	10 20 40	2.8976 3.0456 3.5440	41.89 43.58 45.90	42.60
Ascorbic acid(Standard)	10 20 40	1.9630 1.9454 1.9275	38.56 40.25 41.32	38.04

Ascorbic acid was used as reference standard
Values were performed in triplicates and represented as mean ± SD
Mean values followed by different superscript in a column are significantly different ($p<0.05$)

In ferric reducing antioxidant power assay, the presence of radicals causes the conversion of the Fe 3+ / ferricyanide complex to the ferrous form indicated by a colour change from yellow to green-blue. The reducing capability of methanolic extract of unripe fruits of *Physalis minima* was determined and compared with standard (Ascorbic acid). The reducing antioxidant activity of the extract increased significantly with increase in concentration indicated in the Table 2.

TABLE 2. Reducing power activity of methanolic extract of *Tecoma stans* leaves

Sample	Concentration (ug/ml) 40	20	10
Methanolic extract of leaves (Absorbance at 700nm)	$0.95^a \pm 0.50$	$0.90^b \pm 0.22$	$0.88^c \pm 0.55$
Ascorbic acid (Absorbance at 700nm)	$0.64^a \pm 0.45$	$0.58^b \pm 0.34$	$0.53^c \pm 0.04$

Ascorbic acid was used as reference standard.
Values were performed in triplicates and represented as mean ± SD
Mean values followed by different superscript in a column are significantly different ($p<0.05$)

In Hydrogen peroxide (H_2O_2) scavenging activity, H_2O_2in turn generate hydroxyl radicals (•OH) resulting in initiation and propagation of lipid peroxidation. The hydrogen peroxide scavenging activity of methanolic extract of unripe fruits of *Physalis minima* were determined and compared with the standard (Ascorbic acid). The scavenging activities of extract were more than the standard which are illustrated in Table 3.

TABLE 3. Hydrogen peroxide (H_2O_2) scavenging activity of methanolic extract of *Tecoma stans* leaves

Sample	Concentration (ug/ml)	% inhibition (mean±SD)	IC_{50} (µg/ml)
Methanolic extract of leaves	10 20 40	48.95±0.35 70.00±0.51 79.54±0.32	74.12
Ascorbic acid(Standard)	10 20 40	28.65±2.25 45.25±1.89 65.32±1.45	51.62

Ascorbic acid was used as reference standard.
Values were performed in triplicates and represented as mean ± SD
Mean values followed by different superscript in a column are significantly different ($p<0.05$)

Immunomodulatory Activity

Nitroblue tetrazolium dye test is used to assess the Immunomodulatory activity of the test compound by determining its ability to stimulate the phagocytic activity in

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leucocytes. Once stimulated, the membrane permeable, water soluble, yellow-colored, nitroblue tetrazolium is reduced to blue NBT formazan crystals by the leucocytes. The methanolic extract of unripe fruits of *Physalis minima* stimulated phagocytic activity of the leucocytes in a concentration dependent manner as seen by the increased percentage of NBT positive cells, results shown in Table 4.

TABLE 4. Percentage of reduced neutrophils after treatment with the methanolic extract of *Tecoma stans* leaves, determined by Nitroblue tetrazolium test

Samples	**Concentration (ug/ml) 40**	**20**	**10**
Phosphate buffered saline	22.17±0.31	20.12±0.06	19.34±0.04
Endotoxin-activated plasma	67.15±0.09	57.97±0.95	51.49±0.25
Methanolic extract of leaves	70.95±0.22*	66.54±0.25*	58.20±0.24*

Phosphate buffered saline as Standard
Values were performed in triplicates and represented as mean ± SD
Mean values followed by different superscript in a column are significantly different ($p<0.05$)

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