Research Article
ISSN: 2456-8473
International Journal of Chemistry, Pharmacy & Technology
Vol. 3, No.1, pp-7-10, 2018

IN VITRO CYTOTOXICITY AND ANTI-INFLAMMATORY ACTIVITY OF YELLOW FLOWERS OF *TECOMA STANS* L.

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Received 11-01-18; Revised & Accepted: 27-01-18

ABSTRACT

Tecoma stans (L.) Juss. (Yellow bells plants) of the Bignoniaceae family, is an important medicinal plant. It is ornamental surub with 2 to 4 meters hight. It is an important medicinal herb found as a weed throughout India. All parts like seeds, roots and bark are used medicinally. This plants has more medicinal compound constituents are phytosterols, alkaloids, quinines, Also used to cure anti-diabetic, diuretic, anti- spamodic, antimicrobial, anti-fungal and anticancer. The extract was prepared by different process like aqueous, decoction, microwave oven and methanolic maceration and activity was performed by cytotoxicity activity by using brine shrimp lethality assay and anti-inflammatory activity by Egg albumin method. The extracts of yellow flower showed moderate cytotoxicity against brine shrimp (LC50 285.71 µg/mL). The results showed that Methanol Extract of *Tecoma stans* (L.) Juss are at a concentration of 500 µg/ml significantly protects the heat induced protein denaturation as compared with other forms of extracts.

These findings provide scientific evidence to support traditional medicinal uses of *T. stans* and indicate a promising potential for the development of an Anti-inflammatroy and cytotoxicity agent from *T. stans* trees.

Keywords: Anti-inflammatroy, Cytotoxicity, brine shrimp lethality assay, Egg albumin method

INTRODUCTION

Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae) trees are grown in North America and East Asia and wildly distributed in tropical and sub-tropical countries. It is a flowering perennial shrub or small tree; 5 to 7.6 m in height and grows to 25 ft. *T. stans* is not a toxic plant because it is used in Latin America as a remedy for diabetes and moreover for feeding cattle and goats in Mexico and are extensively employed in the Mexican traditional medicine.[1] The primary applications of *T. stans* have been found in treating diabetes and digestive problems.[2]

The literature survey reveals that the genus *T. stans* possesses various bioactive compounds such as saponins, flavonoids, alkaloids, phenols, steroids, anthraquinones, tannins, terpenes, phytosterols, triterpenes, hydrocarbons, resins, volatile oil and glycosides.[3,4] Recent studies found that *Tecoma* genus possess various bioactive compounds that are reported to exhibit various pharmacological activities such as antioxidant, antimicrobial and antifungal activities.[5,6] A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). The technique is easily mastered, costs little, and

utilizes small amount of test material. The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays once the active compounds have been isolated[7].

A survey of literature indicated no systemic approach has been made to evaluate the cytotoxicity and anti- inflammatory potential of *Tecoma stans* (L.) Juss. ex Kunth are by in vitro method. The present study involves determination of anti-inflammatory activity of Tecoma *stans* (L.) Juss. ex Kunth are by Inhibition of albumin denaturation, and brine shrimp lethality bioassay.

MATERIALS AND METHODS

Plant material

Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae) flowers were collected from Maharashtra, India in Oct. 2017. The plant was identified by Wadmare Sir, Department of Botany, and voucher specimens have been deposited at Smt. Kasturba Walchand College, Sangli. The plant part flowers were air-dried under the shade and ground using a kitchen blender.

Preparation of the crude extracts and fractions

The shade-dried powdered plant samples (20 g) were extracted with methanol in soxhlet apparatus for 12 h. The methanol extracts were concentrated.

Toxicity testing against the brine shrimp

Hatching the brine shrimp

Brine shrimp eggs (*Artemia salina*, Sanders) were hatched in artificial sea water prepared from commercial sea salt (Aqua Marine, Thailand) 40 g/l and supplemented with 6 mg/l dried yeast. The two unequal compartments plastic chamber with several holes on the divider was used for hatching.

The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 hours incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipette from the illuminated side whereas their shells were left in another side.

Bioassay

(*Artemia salina*) eggs were hatched in artificial sea water (40 g sea salts/L) at room temperature (22-29 ºC). After two days these shrimps were transferred to vials (10 shrimps per vial) containing artificial sea water (5 mL) with 500, 50 and 10 µg/mL final concentrations of each compound taken from their stock solutions of 20 mg/mL in DMSO. After 24 hours number of surviving shrimps was counted[8].

Lethal concentration (lc_{50}) determination

The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC_{50}) was determined from the 24 h counts and the dose-response data were transformed into a straight line by means of a trendline fit linear regression analysis (MS Excel version 7); the LC_{50} was derived from the best-fit line obtained.

In vitro anti-inflammatory activity by Protein denaturation method[9]

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL of different forms of extracts

decoction , micro oven and methanol (500 µg/mL). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37^0c ±2) in a incubator for 15 min and then heated at 70^oc for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the 500 µg/mL) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% inhibition = 100 x (Vt / Vc - 1)

Where,
Vt = absorbance of test sample,
Vc = absorbance of control

RESULTS AND DISCUSSION

From Table, yellow flower extract showed potent cytotoxic activities against Brine shrimp (*Artemia salina*) lethality assay. Extract exhibited the higher activity in the brine shrimp assay for overall toxicity profile. In addition Table show that the cytotoxic activities increase as the doses increase, therefore the 500 µg/mL doses induced more cell death than the 10 µg/mL doses.

The methanol extracts of Tecoma *stans* (L.) Juss. ex. showed significant percentage inhibition of protein denaturation with 61.37, respectively, whereas the positive control, Diclofenac sodium, showed a percentage inhibition is 90.21 µg/mL. It can be concluded that the proposed cytotoxicity and Anti-inflammatory method has been successfully employed for the direct monitoring of flowers of *Tecoma stans L.* The cytotoxicity activity by brine shrimp lethality assay and anti-inflammatory activity was found to be significantly. The developed test method provides reliable results with, without the need for complicated instrumentation.

Table 1 Brine shrimp toxicity of flowers of Tecoma *stans* (L.) Juss. ex.

Compound	Conc. (µg/mL)	Shrimp Survivied			Total number of shrimp alive	% inhibition	LC50 (µg/mL)
Methanol extract of Tecoma *stans* (L.) Juss. ex	500	2	1	1	04	82.60	285.71
	50	3	2	5	10	56.52	
	10	6	5	6	17	26.08	

Table 2 In vitro anti-inflammatory activity of flowers of Tecoma *stans* (L.) Juss. ex.

In vitro anti-inflammatory activity 500µg/ml	
Entry	**% inhibition**
Control	--
Standard (Diclofenac sodium)	90.21
Decoction extracts	36.69
Micro oven extract	49.54
Methanol extract	61.37

CONCLUSION:

In the present study, results indicate that the methanol extracts of Tecoma *stans L* are possess anti-inflammatory as well as cytotoxic properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins. This study gives on idea that the compound of the plant Tecoma *stans L* are can be used as lead compound for designing a potent cytotoxic agent and anti-inflammatory drug which can be used for treatment of various diseases such as cancer, inflammation.

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