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Tecoma stans: A noxious weed put to beneficial use

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Abstract
Tecoma stans belongs to family *Bignoniaceae* native to America is an ornamental tree, commonly known as yellow bells. In the Global Invasive Species Database (GISD, 2008), it has been listed as a noxious weed in South Africa, Australia and America (prohibited plants that needs to be controlled). This type of weeds serves no economic purpose and possesses characteristics that are harmful to animal, environment and even human beings. A close literature survey revealed that *Tecoma stans* is not a toxic plant as it is used as a remedy for diabetes and moreover for feeding cattle and goats in Mexico and are extensively employed in the Mexican traditional medicine. There are number of bioactive compounds which have medicinal value from almost all parts *viz* leaves, fruits, flowers. The present investigation is just a first step to compare the bactericidal activity of plant leaves against standard human pathogenic strains along with phytochemical assay which are key route to the efficacy of plants in antimicrobial aspects. Zinc nanoparticle were synthesized and evaluated for increased or decreased efficacy of antimicrobial activity against four bacterial pathogens UV visible spectroscopy resulted in a peak at 396nm which confirmed the synthesis of zinc nanoparticles.

Keywords: *T. stans*, antimicrobial, zone of inhibition, phytochemical, noxious, weed

Introduction
Tecoma stans belongs to family *Bignoniacae* is an ornamental plant and official flower of United States, Virgin Islands and National flower of the Bahamas. It has cosmopolitan distribution throughout India. The showy flowers occur in clusters at the ends of the branches and are trumpet shaped with 5 rounded lobes, 6 cm long, pale to bright yellow in color. Traditional uses of plant leaves throughout Mexico and Central America for diabetes and urinary disorder control have been reported [1]. Costantino and his coworkers [2] reported the flower infusion can be taken orally for diabetes and stomach pains. There are many bioactive components extracted from fruits and flowers, which has antioxidant and antiproliferative effects against cancer cell lines [3]. The plant is used in the treatment of diabetes due to the presence of anthranilic acid in the roots and presence of alkaloids such as tecomas tamine and tecomine from leaves having potential hypoglycemic effects [4]. Plant has benefits of anti-allergic, anti fungal, anti inflammatory, anti oxidant and cytotoxic assays. Beside all these effects on human biological system, it has anti microbial activity against various human pathogenic microbes, which can be proved beneficial in future for new drugs against those pathogenic microbes which developed resistance against widely used drugs. So the noxious weed can be put to beneficial aspects by considering important bio constituents from plants to be extracted and analysed for newer drug developments.

Materials and Methods
Plant and culture collection
The seedling of *Tecoma stans* used in the present study for antimicrobial and phytochemical analysis was procured from Tau Devi Lal Herbal Park near Khizrabad highway in Churpur district, Yamunanagar, Haryana, India. The seedling was cultured following all agronomic practices. The leaves of plant were taken for investigating bacteriocidal activity. The human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC) Institute of Microbial Technology (IMTECH), Chandigarh; which included Gram-negative bacteria *E. coli* (MTCC 443), *P. aeruginosa* (MTCC-424) and Gram-positive bacteria *S. aureus* (MTCC- 96) and *B. subtilis* ((MTCC- 441) and *S. mutans* (MTCC- 1943).

Preparation of *Tecoma stans* leaves extract
Leaves of *Tecoma stans* were shade dried and powdered to fine powder. The 25 g powder was

soaked in 100 ml of different solvents for 72 hours and filtered. The filtrate was evaporated at 45-50 °C in water bath. The residual powder after solvent extraction was dissolved in DMSO and stored at 4 °C.

The callus was taken out in sterilized petri plates aseptically. The fresh callus was pressed in filter paper to remove the excess moisture from callus and dried in hot air oven at 40 °C for 24 hours. The dried callus was grinded into fine powder. The 5g powdered callus was soaked in 20 ml of solvent for 72 hours and filtered. The solvent present in filtrate was evaporated at 45-50 °C in water bath. The residual powder after solvent evaporation was dissolved in 1 ml DMSO and stored at 4 °C.

Zinc nanoparticles were synthesized using leaves as plant part and the aqueous plant extract was prepared by dissolving 10gram of leaf material in 100ml of double distilled water and heated for 7 minutes on hot plate. The extract was filtered and stored at room temperature. The extracts were filtered using Whatman filter paper No.-1. After filtration the extract was stored at 4 °C till further use and was used within a week for analysis.

Synthesis and Characterization of zinc nanoparticles

Zinc nitrate solution was prepared by dissolving 10 gram powder in 100ml de-ionized water and incubated in water bath for 20 minutes. Solution was used immediately after preparation. 50ml of plant extract was incubated in water bath with zinc nitrate solution for 20 minutes and were mixed in a ratio of 1:2 (12.5ml PE: 25ml Zinc nitrate) in fully sterilized condition under laminar air flow by adding drop wise one solution into another. The solution prepared was again incubated for one hour in water bath at 75ºC. The cream colored appearance of solution and UV visible spectroscopy confirmed the zinc nanoparticle synthesis resulting in a peak at 396nm.

Antimicrobial activity of callus, different solvent extracts and zinc nanoparticle (Leaves)

The antimicrobial activities of plant leaves, callus and zinc nanoparticle synthesized using leaves aqueous extract were evaluated by agar well diffusion assay [5]. In each well 50 µl of plant extract was poured. DMSO was used as a negative control whereas ciprofloxacin was used as positive control. The anti microbial activity of extract was determined by inhibition zone diameters. The zones were measured by high media zone scale. The experiment was repeated twice and the average values were recorded for anti bacterial activity. The solvent showing best activity in case of leaves was selected for callus extract preparation and comparison in activities of leaves, callus and nanoparticles was done. For zinc nanoparticles three different concentrations of 50, 75 and 100µl were subjected to antimicrobial assay to cross verify the results of different solvent extracts in comparision to nanoparticles efficacy.

Phytochemical screening

The crude extract of plant leaves were subjected to qualitative phytochemical screening for identification of active chemical constituents using the methods described by Trease and Evans [6].

Results and Discussion

Comparative analysis of antimicrobial activity

The different solvent extracts were found to possess significant antimicrobial activity against both gram positive and gram negative bacteria. The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zones against the tested pathogens. DMSO served as negative control in which no inhibition zone was not observed, while ciprofloxacin (0.1 mg/ml) served as positive control for bacterial cultures. Ciprofloxacin showed inhibition zones of 20 mm for *B. subtilis*, 22 mm for *E. coli*, 25 mm for *S. aureus*, 19mm for *P. aeruginos*, 18 mm for *S. mutans*. The inhibition zone of plant extracts were compare to the antibiotic used as positive control.

The inhibition zone diameters (in mm) of leaf extract of various solvents against pathogenic strains is shown in Table-1. The antimicrobial activity of various extracts of *Tecoma stans* leaves exhibited significant activity. The best activity was observed in methanolic and chloroform extracts. The methanolic extracts of leaves showed maximum inhibition zone diameters of 29 mm for *B. subtilis*, 27 mm for *E. coli*, 35 mm for *S. aureus*, 22 mm for *P. aeruginos*, 25 mm for *S. mutans*. Senthil Kumar and his coassociates reported *in vitro* antibacterial activity of crude leaf extracts of *Tecoma stans* using disc diffusion method against 5 human pathogenic bacteria and observed high susceptibility of crude extract of leaves against all pathogens [7]. Our results are in well corroboration with Senthilkumar except that the zones acquired larger area in size. Chloroform extracts of leaves showed inhibition zones of 20 mm for *B. subtilis*, 18 mm for *E. coli*, 23 mm for *S. aureus*, 14mm for *P. aeruginosa*, 12 mm for *S. mutan*. The antimicrobial activity of *Tecoma stans* revealed that methanolic extracts of leaves showed better inhibition zones which proves that methanol is more effective solvent as compared to others. Ghosh and his coworkers reported similar findings in five different medicinal plants *viz; T. bellerica, T. chebula, E. officinalis, Punica granatum and Lawsonia inermis* [8]. Govindappa alongwith his associates evaluated the antimicrobial potentials of ethanolic, methanolic and aqueous extracts of *Tecoma stans* against 7 seven bacterial pathogens [9]. The antimicrobial analysis revealed significant zones of inhibitions against all pathogens selected. The methanolic and ethanolic extracts resulted in more potent activity. The phytochemical and antimicrobial analysis of crude flower extracts of *Tecoma stans* were tested against selective gram positive and gram negative bacterias *in vitro*. The extracts resulted in broad spectrum antimicrobial activities against nine human pathogens using agar well diffusion assay [10].

As the nanoparticle synthesis from medicinal plants have been gaining momentum in the past fifteen years. Considering this aspect present investigation proposed a simple economical and ecofriendly method for zinc nanoparticle synthesis from aqueous leaf extracts of this noxious weed plant. Zinc oxide nanoparticles are witnessed in many marketable products and could be further exploited as safe agents in food industries [11]. Although the characterization part has limitation of preparing sample on a large scale for further analysis but the visual change in color and UV visible spectra showing a peak at 396nm confirmed the zinc nanoparticle synthesis. Zinc nanoparticle synthesized using aqueous leaf extracts were tested against four pathogens at three different concentrations of 50, 75 and 100 µl and resulted in effective zones of inhibition. Although the zones obtained using different solvent extracts at a higher concentration were almost equivalent in size as compared to zinc nanoparticles bur still some contradiction does exist. Significant zones of inhibition were reported against *B. subtilis* a gram positive bacteria showing a zone of 25mm which showed linear relationship

with increasing concentration while a zone 31mm was observed at 100ul concentration. In comparision to *B. subtilis* a comparable zone of 17-18mm was observed at three different concentrations against *S. aureus*, while different sizes zones were reported in different solvent extracts. Similarly comparable zone of 17-18mm was observed against *E. coli* which was quiet equivalent to different solvent extracts except methanol where a bigger zone of inhibition (27mm) was observed. Linear relationship was observed in another gram negative bacteria *P. aeruginosa* where the zone size increase from 18mm(50µl) to 20mm(75µl) and then to 22mm at 100µl as shown in Table-2.

Callus was produced by using leaf explant on MS medium supplemented with 2, 4-D and BAP following all recommended tissue culture practices. The antimicrobial activity of callus (*in vitro*) was compared to leaves (*in vivo*) of *Tecoma stans.* Methanol and chloroform were selected as extractant because of literature survey proving them to be the best extractant, moreover the meager callus amount was the other constraint. *Tecoma stans* methanolic extracts of callus showed inhibition zones diameters of 20 mm for *B. subtilis*, 22 mm for *E. coli*, 19 mm for *S. aureus*, 16 mm for *P. aeruginosa*, 18 mm for *S. mutans*. Chloroform extracts of callus showed inhibition zones diameters of 15 mm for *B. subtilis*, 14 mm for *E. coli*, 19 mm for *S. aureus*, 14 mm for *P. aeruginosa* and 12 mm for *S. mutans* (Table-3). Methanolic extracts of callus showed better inhibition zones than chloroform. Hence methanol was used as solvent for extraction of bioactive metabolite for antimicrobial assay for callus. *Tecoma stans* callus was less effective against used pathogenic cultures when compared to intact plant leaves. It was clear that callus has less anti microbial potential than leaves of intact plant when compared. Muthu and his associates in 2012 evaluated the antimicrobial activity of various extracts from heartwood of *Tecoma stans* [12]. The strong antimicrobial activities were observed in the methanolic and chloroform extracts of *T. stans* than that of other solvents. Mohammed in 2013 with his coworkers reported antibacterial efficacy of different extracts of leaves and branches of *Tecoma stans* [13]. Salem with his associates in 2013 reported the different extracts of the leaves and branches of *T. stans* have antibacterial potential against the growth of some human bacterial strains [14]. The plant resulted in effective and significant zones of inhibitions against slected bacterial pathogens. Our findings provide scientific proof to support medicinal aspects of *T. stans* and a promising potential for development of antimicrobial and antioxidant agents from *T. stans* which is otherwise considered to be a noxious weed.

Phytochemical Screening

The phytochemical screening revealed the presence of tannin, flavonoids, phenol, alkaloids, steroids and saponins in all solvent extracts of *Tecoma stans* as shown in Table-3. The phytochemicals were strongly present in the ethanolic and methanolic extracts. The water extract yielded less quantity of phytochemicals. The saponins, flavonoids, tanins and phenols were strongly present in the methanolic extracts while the steroids, diterepenes and carbohydrates were present in trace amounts and phytosterols were altogether absent. Flavonoids, tanins, phenols and diterepenes were present in high quantity in the ethanolic and ethyl acetate extracts. Phytosterol were present in minute quantities while saponins and carbohydatres were absent. Choloroform extracts of plant extract lacks saponins, flavonoids, tanins steroids and carbohydrates but contains traces of diterpenes and phytosterols. On the other hand aqueous extracts contains almost all the phytochemicals in very less quantity. The phytochemicals analysis of tecoma leaves have been reported earlier which showed the presence of tannins, flavonoids, alkaloids, phenols and traces of saponins and steroids [14]. Sowjanya and his coworkers have investigated the phytochemical and antimicrobial properties of crude flower extracts of *Tecoma stans* [10]. The extracts resulted in broad spectrum antimicrobial activities. Similar results have been reported in 2011, where phytochemical evaluation revealed the presence of tannins, flavonoids, phenols, alkaloids, steroids anthraquinones and saponins in all solvent extracts (methanol and ethanol) while the aqueous extracts resulted in less quantity of phytochemicals [9]. Similar observations have been made in *Tecoma* reported earlier also in the same plant species [12, 15]. Alonso-Castro in 2010 also observed similar observation showing higher percentile of phenolic, flavonoids, alkaloids than other species and further characterization resulted in presence of quercetin and atropine presence along with phenols and flavonoids [4]. The possible mechanism of action of alkaloids is attributed to the fact that they intercalate with DNA [15]. The flavonoids display a wide range of biochemical actions by acting as free radical scavengers and terminating the radical chain reaction that occur during oxidation of triglycerides in the food system [16].

Table 1: Inhibition zones diameters (in mm) of different solvent extract of *Tecoma stans*

Solvent	*B. subtils*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. mutans*
Methanol	29	35	27	22	25
Ethyl acetate	19	18	18	--	13
Butanol	14	7	17	--	--
Chloroform	20	23	18	14	12
n-Hexane	20	16	18	--	--
(-) control	--	--	--	--	--
(+) control	20	25	22	19	18

Table 2: Inhibition zone diameters (in mm) of *Tecoma stans* Zinc nanoparticles against various pathogenic strains.

Conc.(µl)	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*
50	25	17	17	18
75	24	17	18	20
100	31	18	17	22
Aq.(PE)	-	-	-	-

Table 3: Inhibition zone diameters (in mm) of *Tecoma stans* callus in methanolic and chloroform extracts against various pathogenic strains.

Solvent	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. mutans*
Methanol	20	22	19	16	18
Chloroform	15	14	19	14	12

Table 4: Phytochemical analysis for the different solvent extracts of *Tecoma stans.* (+++ strong; ++ medium; + poor; - absence. The measuring was repeated 3 times and the classification was based on the color intensity of the precipitates)

Extracts	Saponin	Flavonoids	Tannins	Phenols	Diterepenes	Steroids	Carbohydrates	Phytosterol
Methanol	+++	+++	+++	+++	+	+	+	-
Ethyl acetate	-	++	+++	+++	++	-	-	+
chloroform	-	-	-	-	++	-	-	++
Benzene	+	+	-	+	++	+	-	+
Ethanol	-	+++	+++	+++	+++	+	-	+
Water	-	+	+	+	+	+	+	-

Conclusion

In the present investigation using leaf extract from *Tecoma stans*, highest zone of inhibition was observed against *S. aureus* that reflects the bacterial sensitivity towards plant leaf extract. The *S. aureus* was susceptible to methanolic leaf extract obtained from *Tecoma stans*. Almost all plant parts of this plant need to be tested against different pathogens to design herbal drugs which may reveal potent bacteriocidal activity and could be explored for antibacterial activity against most of the resistant strains of *S. aureus* including other pathogenic strains. Instead of medicinal and edible fruit plants biotechnologist and microbiologist should focus on exploitation of weed plants of India to be taken under consideration for developing newer drugs for mankind.

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