Bio-Beneficial Spectrum of *Tecoma stans* Flower Extract *in vitro* for Fighting Prostate and Ovarian Cancers with its Anti-diabetic and Antioxidant Activities

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People have long used plants and plant-derived products to treat a wide range of illnesses. In the present work, *Tecoma stans* flower was extracted using 90% ethanol. Flavonoids and total phenolic constituents of *T. stans* flower extract were screened, and polyphenolic compounds were assessed using high-performance liquid chromatography (HPLC). Anti-diabetic *via* α-amylase and α-glucosidase assays, antioxidant *via* 2,2-diphenyl-1-picryl-hydrazyl-hydrate, ferric reducing antioxidant power, and total antioxidant capacity of *T. stans* flower extract were assessed. The cytotoxic action for *T. stans* flower extract was assessed *versus* WI-38 (human fetal lung fibroblast cells), PC3 (prostate cancer cell line), and SK-OV3 (ovarian cancer cell line). The *T. stans* extract showed promising *in vitro* anti-diabetic effect with IC_{50} = 12.08 ± 0.2 µg/mL and 22.83 ± 0.3 µg/mL for α-amylase and α-glucosidase, respectively. Moreover, *T. stans* showed good *in vitro* antioxidant action with IC_{50}= 5.36 ± 0.2 µg/mL for DPPH testing, and the best antitumor impact *versus* PC3 cells with IC_{50} = 113.27 ± 1.59 µg/mL. Flow cytometric analysis confirmed the role of *T. stans* in acceleration in apoptosis of PC3 cells through regulation of oxidative enzymes. These results indicate that the derived materials from *T. stans* flower have multiple medicinal applications.

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INTRODUCTION

Cancer is a diverse illness characterized by compromised cellular function that spreads outside of normal limits (Gutschner and Diederichs 2012). It is a major contributor to global mortality rates and places a significant strain on health systems (McGuire 2015; Examinati *et al*. 2018; Qanash *et al.* 2022). Cancer of the prostate is one of the most common kinds of cancer affecting men. Despite the worldwide prevalence of prostate cancer, there is still an important knowledge gap concerning its management and manifestation (Sayan *et al.* 2024). In many nations, prostate cancer is a significant public health issue. Data from the United States and Europe showed the highest death and age-standardized incidence rates (ASIRs). Comparable reports from the Arab World are a great deal lower. Data on death rates are not available, but the ASIR rate ranged from 39.2% in Lebanon to 5.5% in Saudi Arabia (Wong *et al*. 2016; Arafa and Rabah 2017). Contrastingly, ovarian cancer is a deadly cancer of the female reproductive system that is often detected late in the disease's clinical course because there are no early warning indicators or screening programs. The incidence varies throughout the world. It is the

seventh most prevalent cancer diagnosis in females. It is listed as the fourth leading cause of death for women. There is geographical and ethnic variation in the epidemiology of ovarian cancer (Momenimovahed 2019; AlDakhil *et al*. 2022).

Free radicals are produced by either regular metabolic processes in cells or by being exposed to outside stimuli such as chemical substances in industries, UV rays, and air pollutants. They are very erratic, fleeting, and sensitive. Because they can quickly harm any type of macromolecule, notably proteins, genetic material, and lipids, a high concentration of free radicals or reactive oxygen species (ROS), has negative effects. Antioxidants, however, have the ability to neutralize or remove them. Through stabilizing free radicals prior to their causing physiological harm, an antioxidant molecule can neutralize them. Enzymes found in cells are the main antioxidants (Krobthong *et al*. 2022). Additionally, Diabetes mellitus is now regarded as a "lifestyle" illness, and over the centuries, many plant species have been recognized as a primary source of effective hypoglycemic agents (Chaddha *et al*. 2013). Particularly, therapeutic substances are used in many countries to treat diabetes to alleviate the cost that traditional drugs place on the populace (Sharma and Prajapati 2014). Because of the possibility for use in therapy, plants and their byproducts have been an important supplier of pharmaceuticals for many years. Medicinal plant agents have been proposed in recent years to treat diseases, such as diabetes, because these plants contain a variety of phytoconstituents with hypoglycemic activity (Anand and Basavaraju 2021).

The curative efficacies of a variety of medicinal plants in the cure of diabetes and the adverse effects accompanying diabetes have been confirmed by many investigations conducted over the past several years (Gupta and Behl 2021). Among these plants, *Tecoma stans*, which is regarded as the preferred medication in Mexico for the treatment of diabetes, also yield encouraging results. Due to the existence of a variety of beneficial phytoconstituents, *T. stans* has an enormous opportunity for managing a wide range of diseases, as evidenced by scientific research. The research has made it clear that *T. stans* has a wide range of pharmaceutical characteristics, including antimicrobial, cardioprotective, wound-healing, and neuroprotective, qualities. These characteristics are linked, both directly and indirectly, to the development of different metabolic illnesses (Gupta and Behl 2021).

T. stans (L.) is an ornamental tree in the Bignoniaceae family with beautiful yellow bell-like flowers. It is considered an invasive tree, similar to those found in South Africa and Namibia, due to its rapid growth and rate of development (Röder *et al*. 2016). Relevant antidiabetic qualities in the aqueous extract are consistent with its conventional applications (Kumar and Boopathi 2018; Mohammed *et al*. 2019). *Tecoma stans* have yielded over 130 molecules with a variety of compositions through phytochemical analysis. They include volatile ingredients, flavonoids, terpenoids, glycosides, and monoterpene alkaloids. Although every part of the plant has undergone chemical analysis, little is known about the chemistry of the flowers. Previous research on floral extracts has identified four glycosides and two carotenoids (Raju *et al*. 2011; Taher *et al*. 2016).

According to previous reports, water is the least expensive and safest green solvent of polar active compounds, while organic solvents are required to extract less polar constituents. For instance, polyphenols might have better solubility in organic solvents but poorly soluble in water (Giacobbo *et al.* 2015). Moreover, low-viscosity solvents such as acidified water or organic solvents such as methanol or ethanol may be chosen for formulating plant extracts because of the probability of promoting mass transfer (Gil-Martín *et al.* 2022). Strugała *et al.* (2017) indicated that flavonoid glycosides are more

soluble in water than aglycones; furthermore, both classes may be extracted with dual mixtures of water-alcohol and pure alcohols. Therefore, from the mentioned literature investigations, the goal of this study was to screen the polyphenolic and flavonoid contents in *T. stans* flower, and their potential antidiabetic, antioxidant, and anticancer effects were assessed.

EXPERIMENTAL

Chemicals and Flower

The chemicals used for the study were purchased from Sigma Co., Ltd. (Heidelberg, Germany). Fresh *T. stans* flowers were gathered from South Sinai, Egypt. The collected flowers were shade dried for 3 days.

Extraction of *Tecoma stans*

About 50 g of ground dry flower taken in a stoppered glass container was homogenized, mixed with 0.5 L of 90% ethanol, and macerated for three days at room temperature. For the conventional extraction process, the extract was placed in a disruptor set to 40 °C for 60 min. Crude extract was then produced by filtering and centrifuging the extract at 40 °C under pressure using a rotatory evaporator (Marinova *et al*. 2005).

Phytochemical Testing

Assessment of the total phenolic level

A mixture of 2.5 mL Folin-Ciocalteu reagent and 2.5 mL (75 g/L) $CaCO_3$ was mixed with 500 µL aliquots of extracts. After being vortexed for 10 s, the tubes were kept at 30 °C for 2 h. A Biosystem 310 spectrophotometer (Cole-Parmer Ltd., Eaton Socon, England) was used to measure absorbance at 760 nm (Marinova *et al*. 2005; Sembiring *et al*. 2018).

Assessment of total flavonoid content

A 10-mL volumetric flask was filled with 1 mL of extract and 2 mL of methanol. In a 25-mL flask, solutions of 6% $NaNO_3$, 6% NaOH, and 7% $AlCl_3$ were prepared using water. In a sealed glass vial, 200 µL of extract was mixed with 70 µL of 5% $NaNO_3$ and allowed to react at room temperature for 10 min. The vial was then filled with 1.30 mL of $AlCl_3$ and 0.5 mL of NaOH. Disruption was then performed, and the vials were allowed to react at the surrounding temperature for 10 min. Using a Bio-system 310 spectrophotometer, the absorbance of all working solutions and the standard solution was evaluated at 510 nm in relation to methanol blank after incubation (Marinova *et al*. 2005; Sembiring *et al*. 2018).

Investigation of Polyphenols and Flavonoids by High Performance Liquid Chromatography

Ten microliters of the *T. stans* extract were injected into the HPLC (Agilent Technologies, Santa Clara, CA, USA) and analyzed using a C18 column (10 µm and 4.6 mm × 250 mm) at 45 °C. Acetic acid (0.05%) in acetonitrile (B) and water (A) at a flow rate of 0.9 mL/min made up the mobile phase. The following was the sequence in which the mobile phase was programmed: 0 min (82% A); 0 to 1 min (85% A); 1 to 11 min (70% A); 11 to 18 min (65% A); 18 to 22 min (80% A); and 22 to 24 min (80% A). Analysis was

done at 280 nm *via* a multi-wavelength detector. For every sample solution, a 6.0 µL injection volume was used. There was no deviation from the 40 °C column temperature. A single wavelength ultraviolet (UV) detector at 280 nm was used (Icon Scientific Inc., North Potomac, MD, USA) (Gupta *et al*. 2023).

Anti-diabetic Testing

Amylase inhibition testing

Through applying the 3,5-dinitrosalicylic acid (DSNA), the experiment was conducted. After dissolving the extract in a 10% dimethyl sulfoxide, it was mixed with buffer (0.02 M NaH_2PO_4/NaH_2PO_4 and 0.009 M NaCl at pH 7.2) to yield levels ranging from 1.9 to 1000 µg/mL. After mixing 200 µL of the extract with 2.0 units/mL of α-amylase solution, the mixture was kept for 10 min at 30 °C. Following that, 200 µL of the 1% starch in water (w/v) solution was added to each tube, and it was then incubated for 3 min. Approximately 200 µL of DNSA reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M sodium hydroxide and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) was added to end the reaction, and it was then heated for 10 min at 80 °C in a water bath. After bringing the mixture down to room temperature, it was diluted with 5 mL of deionized water, and the absorbance at 560 nm was determined in a UV-Visible Biosystem 310 spectrophotometer (Alsolami *et al*. 2023).

Alpha-glucosidase Testing

To measure the specimens' α-glucosidase activity, 50 µL specimens with different concentrations (1.97 to 1000 µg/mL) were kept for 25 min at 35 °C after mixing them with 10.0 µL of the α-glucosidase enzyme solution (1 U/mL) and 125.0 µL of 0.11 M phosphate buffer (pH 7.2). After 20 min, the reaction was initiated by incorporating 20 µL of 1 M pNPG (substrate), and the resulting mixture was then kept for 35 min. The reaction was ended by adding 50 µL of 0.1 N Na_2CO_3, and the absorbance at 405 nm was evaluated (Taher *et al*. 2016).

Antioxidant Testing

DPPH testing

To evaluate the sample's *in vitro* antioxidant impact, a 0.1 mM DPPH ethanol solution was employed. This solution (1.0 mL) was divided into three and combined with samples in varying concentrations of ethanol (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL). After giving the mixture a good shake, it was left to remain at ambient temperature for 30 min. The absorbance at 517 nm was then evaluated using a Biosystem 310 spectrophotometer (Al-Rajhi *et al*. 2023).

Total Antioxidant Capacity (TAC) Testing

A total of 3 mL of the reagent solution (0.72 M H_2SO_4, 28 mM sodium phosphate, and 4.0 mM ammonium molybdate) were combined with 1.0 mL of *T. stans* extract (0.5 mg/mL) using the phosphor-molybdenum method. There was only 4.1 mL of reagent solution in the blank solution. For 150 min, the mixtures were kept at 100 °C. Utilizing a microtiter plate reader (Thermofisher Scientific ELX700; Waltham, MA, USA), the absorbance was measured at 650 nm after the mixture had cooled to surrounding temperature. Ascorbic acid equivalent (AAE) µg/mg of extracts was employed to convey the outcomes (Lahmass *et al*. 2018).

Ferric Reducing Antioxidant Power (FRAP) Testing

The FRAP testing was created by combining 20.0 mM $FeCl_3.6H_2O$ solution, 10.1 mM TPTZ solution in 40.1 mM HCl, and 300.0 mM acetate buffer (pH 4.0) in a 10:1:1 ratio (Raju *et al*. 2011). The standard solution was ascorbyl glucoside solution (3.9 to 1000 μg/mL), and *T. stans* flower extract solutions (0 to 1000 μg/mL) were made. In 96-well plates, samples (20 μL) were left to react with 180 μL of FRAP solution at 37 °C for 30 min in the dark. At 595 nm, the absorbance of the standard and extract ferrous tripyridyltriazine complex was measured. The extract's FRAP content concentration was expressed in equivalent (AAE) μg/mg of sample. Using a ferrous sulphate solution ranging from 9.8 to 5000 μM, the standard curve was constructed to determine the FRAP content (Fernandes *et al*. 2016).

Cytotoxicity Assay

The cell lines PC3, SK-OV3, and WI-38 were obtained from Prof. Dr. Tarek M. Abdelghany, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, 71524, Egypt. The cytotoxic effect of *T. stans* was determined by extraction on PC3, SK-OV3, and WI-38 cells by MTT analysis, which followed its dissolution with DMSO. The outcome showed a blue color, for which the concentration is directly correlated to the number of living cells using standard curve dilutions. Using an automated microplate reader (Thermofisher Scientific ELX700; Waltham, MA, USA), the absorbance was determined at 570 nm. Following 24 h of adhesion until merge, samples of the extract ranging in level from 500 to 15.63 μg/mL were added, and the cells were then kept for another 24 h at 36 °C. Following the addition of the new medium, 100 μL of MTT solution (5.0 mg/mL) was added and kept for 4 h at 36 °C. The cells were examined by microscope (Nikon, 1106, Tokyo, Japan) connected with a Charge-coupled device camera (Al-Rajhi and Ghany 2023a).

Annexin V/PI Apoptosis Detection Assay

Using the Annexin V-FITC apoptosis determination package (Sigma-Aldrich, San Francisco, CA, USA) following the supplier's leaflet directions, cell death mechanisms were investigated. The PC3 cells were first seeded at a density of 1.5×10^6 cells/well in a tissue culture plastic plate, and they were incubated for 24 h. After that, they were treated for an additional 24 h, and the IC_{50} of *T. stans* extract was determined. After treatment, trypsinization was performed on both the treated and untreated (negative control) cells, and three PBS washes were then performed. Then, the treated and untreated cells were resuspended in a PBS. After adding 6.0 μL of Annexin V-FITC and 10.0 μL of PI solution to each suspension, the cells were labelled and kept for 15 min at ambient temperature without light. Using (BD FACSCaliber software, USA), a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was utilized to measure cell apoptosis in less than 60 min (Chittasupho *et al*. 2023).

Cell Cycle Arrest Assay

The effects of *T. stans* extract on the cell cycle pattern of PC3 cells were examined using flow cytometry. The PC3 cells were first cultured in a tissue culture plastic plate for 24 h at a level 1.5×10^6 cells/mL, and then they were exposed to the extract's IC_{50}. After trypsinization, the cells were fixed at -20 °C with ice-cold 70% ethanol after being rinsed with PBS buffer. The cells were then incubated at 4 °C for 30 min with PI solution (BD Biosciences, USA). The cell cycle phases were examined through flow cytometry and (BD

FACSCaliber software, USA) (Cháirez-Ramírez *et al*. 2021).

Oxidative Enzymes Detection

The activities of the major antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), in addition to the level of malondialdehyde (MDA), nitric oxide (NO), and hydrogen peroxide (H_2O_2), were assessed to evaluate the role of *T. stans* extract on certain indicators of oxidative stress. The cells were grown at 36 °C in a humid place with 6% CO_2 binder incubator in DMEM enriched with 10% fetal bovine serum and 1% penicillin-streptomycin for these assessments. Following the acquisition of the cell monolayer, the cells were exposed to IC_{50} values of *T. stans* extract for a duration of 6 h. A positive control procedure involved treating the sample with 100 µM H_2O_2 for 15 min and then it was washed twice with a cold PBS solution to obtain the cell lysates, which were trypsinized concurrently with other treated groups. The samples were placed in a cold 0.2 M potassium phosphate buffer (pH 7.5), 1.15% KCl, and ultrasonically sonicated eight times for 1 min at a power of 35% in an 8 × 10 cycle. After that, the specimens were centrifuged for 15 min at 3000 rpm, and the supernatant was used to identify biomarkers of oxidative stress. The steps in the published work were used to determine the target enzymes (Weydert and Cullen 2010).

Statistical Analysis

At the 0.05 level of probability, statistical designs were performed utilizing the computer programs Microsoft Excel version 365 (Microsoft Corp., Redmond, WA, USA) and SPSS v.25 (statistical package for the social science version 25.0) (SPSS Inc., Chicago, IL, USA).

RESULTS

Assessment of Various Phytochemicals in *T. stans* Extract

Both flavonoids and total phenolic molecules in *T. stans* flower extract were quantitatively assessed. Where, the total flavonoids were 21.3 ± 0.9 mg (QuE)/mL. The total phenolic content was 53.83 ± 0.026 mg(GAE)/mL (Data not tabulated).

HPLC Analysis of *T. stans* Ethanol Extract

The levels of the various flavonoids and polyphenolic molecules in the ethanolic flower extract of *T. stans* were qualitatively measured using HPLC analysis. About 18 different compounds could be seen in the extract, as shown (Fig. 1, Table 1). Ellagic acid was the most predominant compound. While gallic acid, rosmarinic acid, rutin, vanillin, chlorogenic acid, caffeic acid, coumaric acid, catechin, naringenin, and querectin were present at moderate levels. Furthermore, kaempferol, cinnamic acid, daidzein, ferulic acid, hesperetin, methyl gallate, and syringic acid were present in minimal levels.

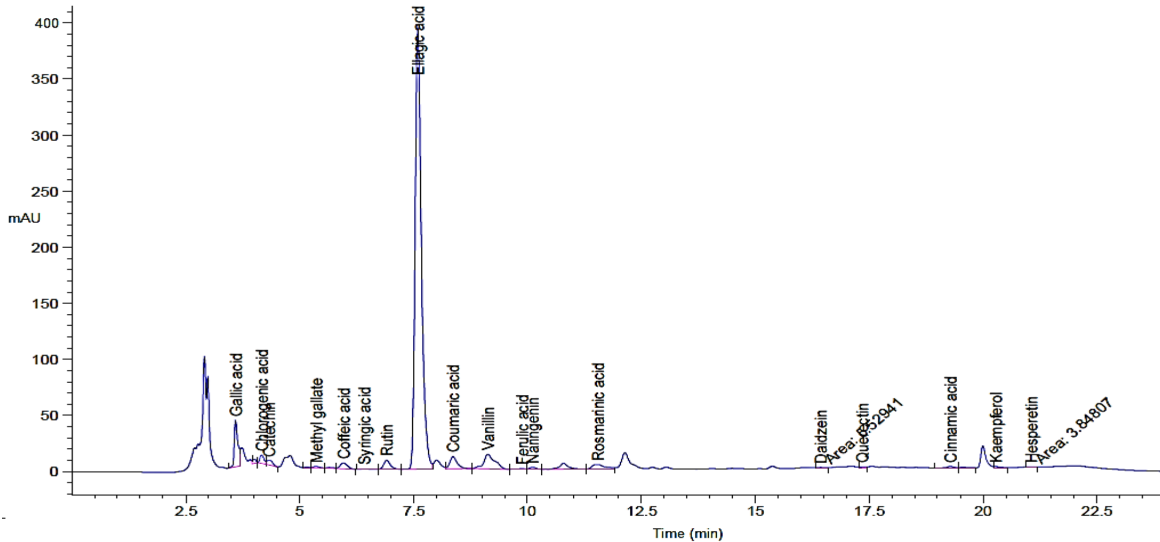


Fig. 1. Various peaks of polyphenolic molecules and flavonoids in ethanol of *T. stans* extract by HPLC

Table 1. Various Polyphenolic Molecules and Flavonoids in Ethanol Flower Extract of *T. stans* (dilution 1:10) Analyzed by HPLC

Compound Name	Area	Conc. (µg/mL)	Conc.(µg/g)
Gallic acid	225.94	19.99	999.25
Chlorogenic acid	45.77	5.94	296.97
Catechin	38.35	8.27	413.66
Methyl gallate	13.95	0.70	35.14
Caffeic acid	58.08	4.49	224.72
Syringic acid	4.39	0.32	16.06
Rutin	82.44	12.16	608.03
Ellagic acid	3767.47	376.33	18816.71
Coumaric acid	122.08	4.34	217.23
Vanillin	222.25	8.26	412.98
Ferulic acid	7.57	0.44	21.98
Naringenin	17.44	1.59	79.69
Rosmarinic acid	83.79	8.98	449.17
Daidzein	5.53	0.31	15.51
Querectin	8.82	1.19	59.50
Cinnamic acid	16.19	0.29	14.50
Kaempferol	10.09	0.64	31.81
Hesperetin	3.85	0.19	9.46

Antidiabetic Testing

α-amylase and α-glucosidase inhibition

Tecoma stans flower extract was tested for α-amylase inhibition and showed a promising IC_{50} = 12.08 ± 0.2 µg/mL. The acarbose standard showed an inhibition value with IC_{50} = 4.32 ± 0.1 µg/mL. Moreover, *Tecoma stans* flower extract was tested for α-glucosidase inhibition and depicted a good inhibition with IC_{50} = 22.83 ± 0.3 µg/mL. The acarbose standard showed an inhibition value with IC_{50} = 2.64 ± 0.6 µg/mL (Table 2).

Table 2. α- Amylase and α- Glucosidase Inhibition by *T. stans* Flower Extract and Acarbose (standard)

Concentration (µg/mL)	α- Amylase Inhibition		α- Glucosidase Inhibition	
	Extract	Acarbose	Extract	Acarbose
1000	88.0±3.32	95.0±3.33	82.7±1.00	94.6±2.33
500	82.2±1.00	89.0±2.50	76.9±0.56	92.7±2.45
250	77.3±2.50	83.0±1.40	71.0±0.5	89.1±0.5
125	71.7±2.2	77.3±1.5	65.0±0.5	83.2±0.45
62.5	65.6±0.55	71.8±1.00	59.3±1.02	76.7±0.5
31.25	58.9±1.0	66.0±0.5	53.5±1.56	70.5±1.66
15.62	53.0±0.66	60.2±0.55	45.2±0.2	64.4±1.00
7.81	46.1±1.33	55.1±2.2	39.4±1.00	57.8±0.5
3.9	39.6±0.57	49.9±0.33	33.1±0.66	53.2±0.11
1.95	32.8±0.25	43.2±1.0	31.1±0.5	46.6±0.21
Control 100%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
IC_{50} (µg/mL)	12.08 ± 0.2	4.32 ± 0.1	22.83 ± 0.3	2.64 ± 0.6

Detection of Antioxidant Efficiency

DPPH assay

Tecoma stans flower extract was tested for DPPH scavenging percentage and exhibited a notable antioxidant capacity with IC_{50} = 5.36 ± 0.2 µg/mL. The ascorbic acid standard showed antioxidant value with IC_{50} = 2.85 ± 0.1µg/mL, as shown in Fig. 2.

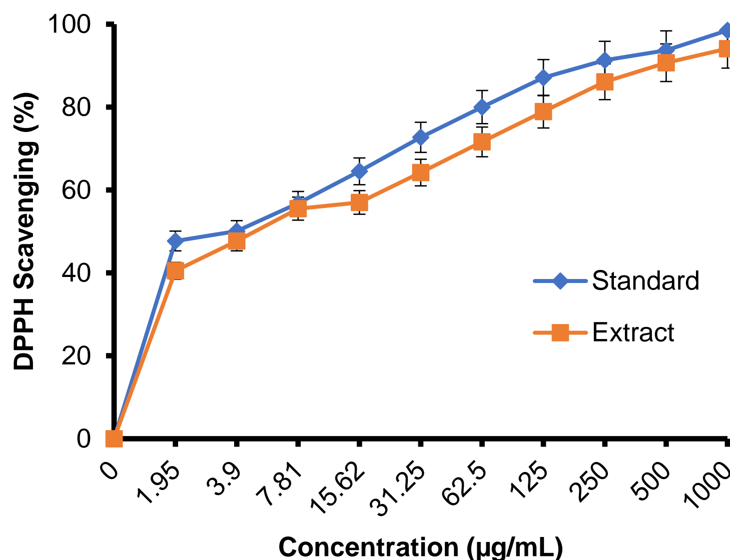


Fig. 2. Antioxidant impact of *T. stans* flower extract *versus* ascorbic acid standard (Data are depicted as means ± SD.)

TAC and FRAP Assays

Antioxidant impact of *T. stans* flower extract was further confirmed using total antioxidant capacity analysis, which resulted in a value of 715.8 ± 0.1 (equivalent (AAE) µg/mg of sample). Additionally, ferric reducing antioxidant power (FRAP) testing indicated an antioxidant role of *T. stans* flower extract, with a value of 626.64 ± 0.4 (equivalent (AAE) µg/mg of sample) (Data not tabulated).

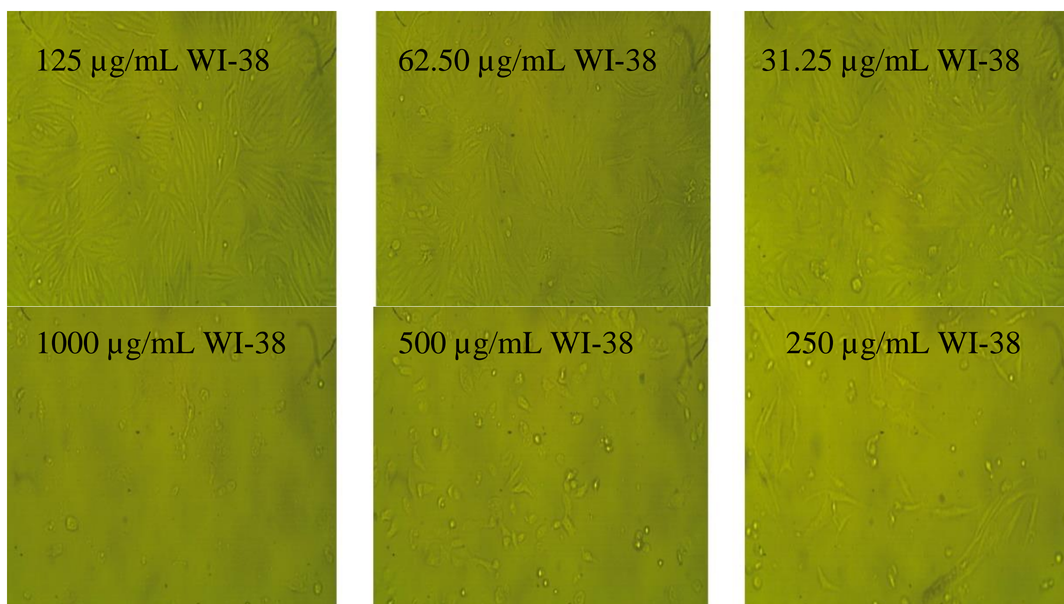
Antitumor Impact of *T. stans* Flower Extract

The cells WI-38, PC3, and SK-OV3 were exposed to six levels of *T. stans* flower extract (1000 µg/mL to 31.25 µg/mL) (Table 3), where a destruction of cells could be seen in a descending level in values of 1000, 500, 250, and 125 µg/mL. A gradient formation of monolayer cells increased gradually upon decreasing concentrations of treatment using *T. stans* flower extract, where a complete sheet and classical structure of cells could be seen upon treatment using 125 µg/mL of *T. stans* flower extract with IC_{50} = 240.37 ± 3.48 µg/mL for WI-38 cells. Furthermore, a gradient formation of monolayer cells increased gradually upon decreasing concentrations of treatment, such that a complete sheet and classical structure of cells could be seen upon treatment using 62.5 µg/mL of *T. stans* flower extract with IC_{50} = 113.27 ± 1.59 µg/mL for PC3 cells. Moreover, a gradient formation of monolayer cells increased gradually upon decreasing concentrations of extract, where a complete sheet and regular shape of cells could be seen upon treatment using 125 µg/mL of *T. stans* flower extract with IC_{50} = 158.34 ± 1.76 µg/mL for WI-38 cells, as shown in Fig. 3.

Table 3. Cytotoxicity of *T. stans* Flower Extract Toward WI-38, PC3, and SK-OV3

Concentration (µg/mL)	WI-38		PC3		SK-OV3	
	Viability (%)	Toxicity (%)	Viability (%)	Toxicity (%)	Viability (%)	Toxicity (%)
0.0	100	0.0±0.0	100	0.0±0.0	100	0.0±0.0
31.25	99.85	0.15±0.01	99.74	0.26±0.21	99.81	0.19±0.01
62.5	99.00	1.00±0.21	96.01	3.99±0.25	99.58	0.42±0.11
125	99.90	0.10±0.01	40.96	59.04±2.25	50.09	49.91±1.66
250	46.91	53.09±0.55	15.82	84.18±1.33	17.37	82.63±1.0
500	19.06	80.94±1.33	3.12	96.88±1.78	2.62	97.38±2.50
1000	2.99	97.01±2.57	2.47	97.53±2.10	2.53	97.47±2.66
IC_{50}± SD	240.37 ± 3.48 µg/mL		113.27 ± 1.59 µg/mL		158.34 ± 1.76 µg/mL	

Data are represented as means ± SD



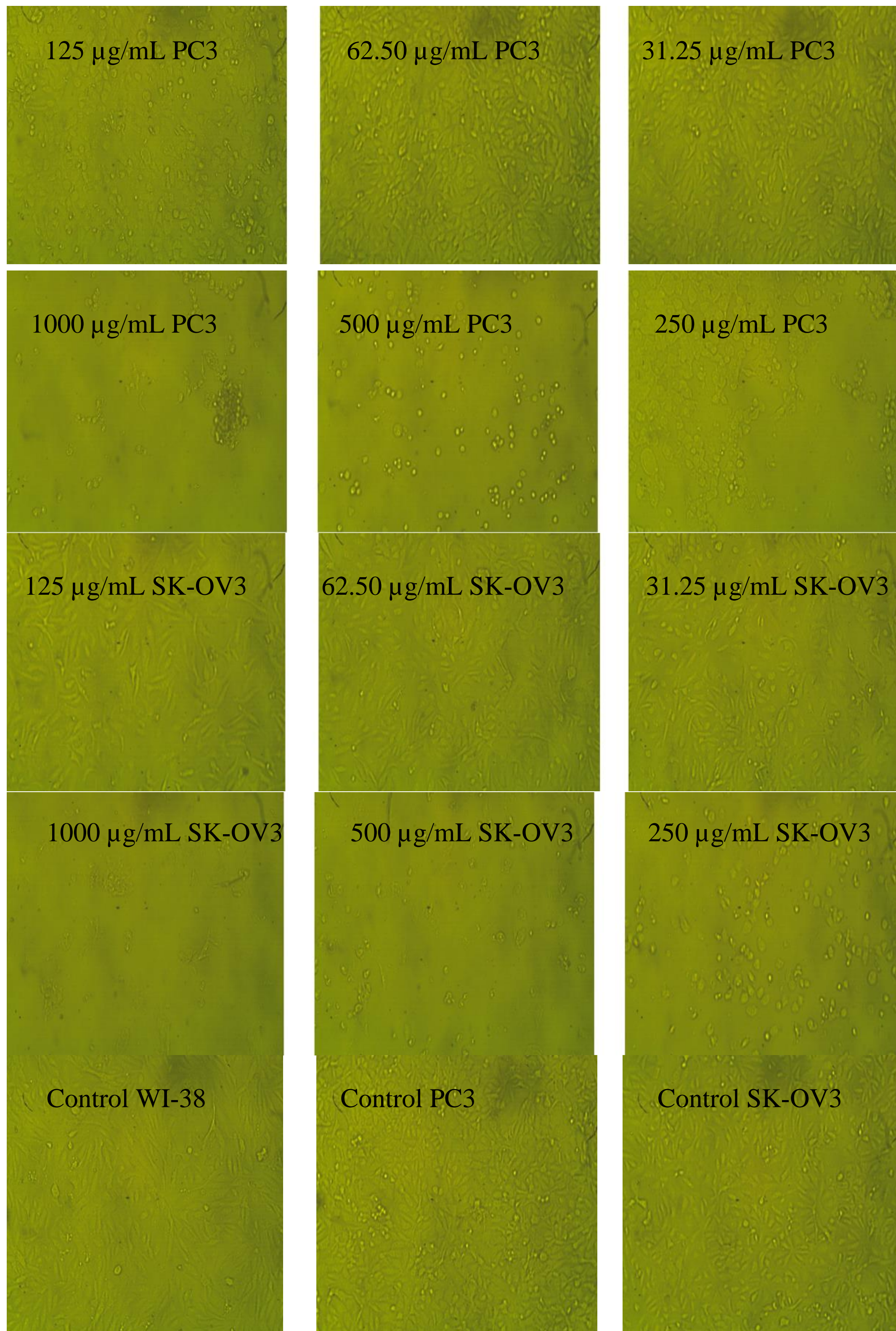


Fig. 3. Antitumor action of ethanolic extract of *T. stans versus* (A) WI-38, (B) PC3, and (C) SK-OV3 cells. Photos were collected at various levels in a range of 31.25 and 1000 µg/mL, prior to 24 h incubation by inverted microscope (Magnification 40X)

Annexin-V FTIC Apoptotic Assay

Ethanolic extract of *T. stans* flower had the best antitumor impact on PC3. Examining the effects on PC3 through flow cytometry and the Annexin V-FTIC staining experiment allows one to determine whether the cells were killed by apoptosis or non-specific necrosis. Upon comparison with the control, it was seen that *T. stans* extract meaningfully increased the proportion of PC3 cells that were Annexin V-FITC positive during both the early and late stages of apoptosis by roughly three times ($p < 0.05$). Additionally, as demonstrated in a dramatic elevation in number of necrotic cells as depicted in (Fig. 4).

Cell Cycle Outcome

Tecoma stans flower extract placed on PC3 cells at its IC_{50} resulted in separate cells at different phases of the cell cycle. When contrasting untreated PC3 cells to treated PC3 control cells, *T. stans* flower extract showed a notable increase in the number of cells at the G2/M phase. Furthermore, the results show that the extract inhibited PC3 cells from proliferating in the G0/G1 phase for it to have its harmful effect as shown in (Fig. 5).

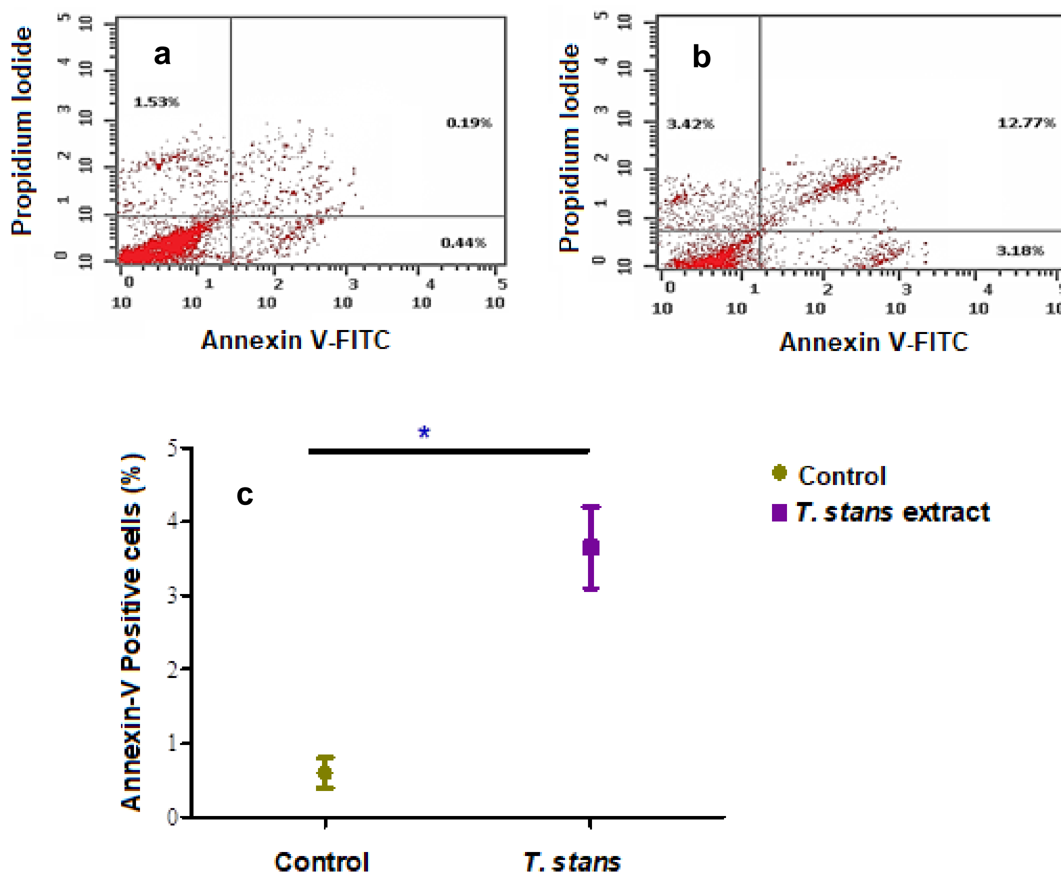


Fig. 4. The percentage of Annexin V-FITC dying in PC3 cells treated for 24 h with 113.27 ± 1.59 µg/mL of *T. stans* extract: (a) Control, (b) Treated cells with *T. stans* extract illustrating dramatic enhancement of apoptosis, (c) Statistical testing between control and treated cells ($P < 0.05$)

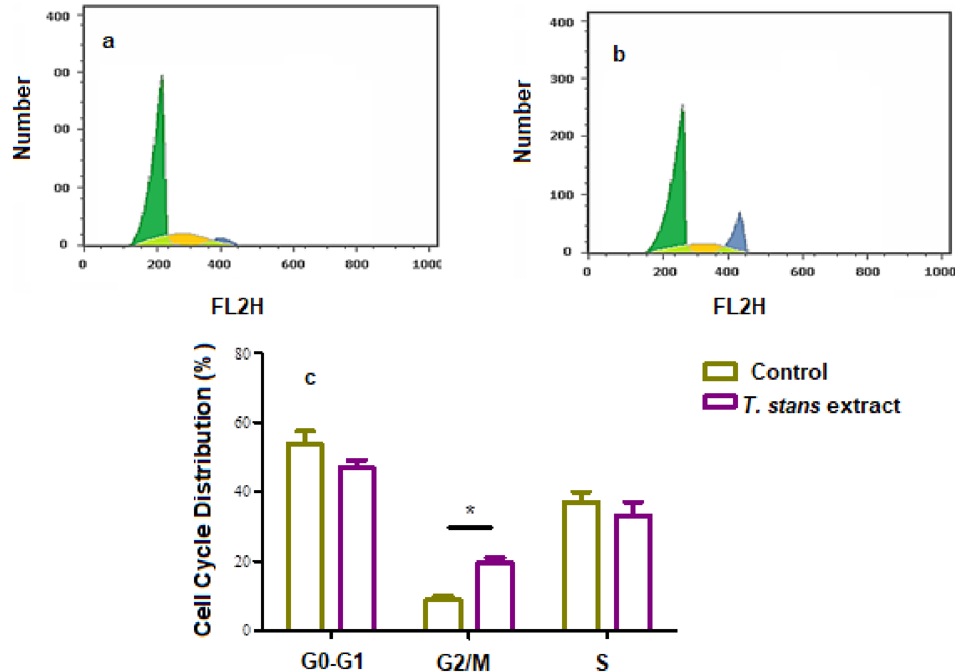


Fig. 5. The cell cycle of population of PC3 cells prior to treatment with 113.27 ± 1.59µg/mL of *T. stans* flower extract for 24 h illustrating different phases of the cell cycle (G1, S, and G2/M) repeated three times, and a representative outcome was drawn. (a) Flow cytometry histogram of non-treated PC3 cells; (b) flow cytometry histogram of PC3 cells treated with *T. stans*; (c) Statistical testing among various phases of cell cycle (Data are represented as means ± SD where $P \leq 0.05$ considered as significant).

Measurement of Oxidative Markers

Using *T. stans* flower extract, treated PC3 cells exhibited a dramatic ($P \leq 0.05$) decrease in the values of GSH and Catalase enzymes upon comparing to untreated PC3 cells. In contrast to untreated PC3 cells, treated PC3 cells with *T. stans* flower extract showed a detrimental increase ($P \leq 0.05$) in MDA and NO levels. Moreover, a significant increase in H_2O_2 levels ($P \leq 0.001$) was observed in PC3 cells treated with *T. stans* flower extract as illustrated in Table 4.

Table 4. Concentrations of Oxidative Enzymes Secreted in Normal PC3 Cells and upon Treatment using *T. stans* Flower Extract

Treatment	GSH (mg/g protein)	SOD		MDA (nmol/g protein)	Catal-ase (U/g protein)	NO (umol/g protein)	H_2O_2 (nmol/g)
		Inhibi-tion (%)	Activity (U/g protein)				
Untreated cells	10.62±0.5	93.6±1.0	349.1±2.5	4.6±0.33	41.8±2.0	11.2±0.5	2.15±0.33
Treated cells	1.25±0.20	95.2	355.1±3.33	33.82±1.5	22.7±1.0	30.8±1.0	12.2±1.0

Data are represented as means ± SD

DISCUSSION

Many plants are abundant origins of natural substances that have good pharmacological properties and no negative side effects (Alawlaqi *et al*. 2023; Alsalamah *et al.* 2023; Qanash *et al*. 2023; Al Abboud *et al*. 2024). Antioxidants' ability to scavenge free radicals further qualifies them as molecules that promote health. Despite the presence of synthetic and manufactured antioxidants such as butylated hydroxytoluene and *t*-butyl-hydroquinone, their usage is restricted by their adverse reactions (Al-Rajhi *et al.* 2023a). As a result, there is a lot of interest in natural, safe, and environmentally friendly substitute antioxidants. Additionally, natural substances from herbal remedies including glycosides, flavonoids, alkaloids, and coumarins can work in harmony to generate positive therapeutic benefits for life (Al-Rajhi *et al.* 2023b).

In this study, using *T. stans* flower ethanolic extract was screened for its biomedical applications, through various *in vitro* investigations to test its antidiabetic, antioxidant, and antitumor impacts. Understanding the chemical composition of plants is crucial for both the production of novel medications and the identification of curative biologically active substances present in herbal remedies (Abdelghany *et al.* 2021; Bakri *et al.* 2024). Diverse plants' extracts can have beneficial interactions with one another due to the presence of medicinal substances, which can affect the extracts' ability to produce the anticipated or preferred pharmaceutical impact (Al-Rajhi and Ghany 2023b). In this study, notable levels of flavonoids and total phenolic molecules could be detected with 21.30 ± 0.9 mg and 53.83 ± 0.026 mg (GAE)/mL), respectively. Moreover, the present results revealed the presence of various compounds, such as ellagic acid, chlorogenic acid, caffeic acid, coumaric acid, catechin, naringenin, and quercetin, as well as hesperetin, and other compounds reported to be used in various traditional therapies (Garcia-Oliveira *et al.* 2022).

The current results revealed the promising antidiabetic role of *T. stans* flower ethanolic extract, which is likely due to the existence of flavonoids, especially naringenin, that occur spontaneously among the more significant flavanones, which are mostly present in various plants. Notably, three units of malonyl-CoA and *p*-coumaroyl-CoA are condensation products that yield naringenin. Furthermore, the precursor of naringenin production in dicotyledonous plants is *p*-coumaroyl-CoA, which is produced *via* PAL deamination from phenylalanine (Wilcox *et al.* 1999; De Souza Bido *et al.* 2010). The aforementioned enzyme is subsequently triggered by a CoA-dependent ligase and hydroxylated at C4 by a cinnamate-4-hydroxylase. Further, *T. stans* flower ethanolic extract showed a promising antioxidant impact, which is likely due to the presence of chlorogenic acid, a class of phenolic chemicals found in many different plant sources. Chlorogenic acids, which include caffeic acid, *p*-coumaric acid, and ferulic acid, are esters of quinic acid plus one *trans*-cinnamic acid component (Nakatani *et al.* 2000; Upadhyay *et al*. 2013). It is well known that chlorogenic acids function similarly to ascorbic acid in terms of eliminating free radicals (Liu *et al.* 2020). Moreover, chlorogenic acid can bind transitional metals, such Fe^{2+}, to neutralize free radicals and stop reactive chains (Lu *et al.* 2020).

In this work, *T. stans* flower ethanolic extract showed promising anticancer properties towards PC3 and SK-OV3 and minimal effect on WI-38. Polyphenol substances, including hesperidin, gallic acid, and ellagic acid, have antiproliferative, antiangiogenic, and antioxidant characteristics. These natural compounds target many signaling pathways and are less expensive and side effect-prone than other chemo preventive medicines. About 47% of cancer-fighting drugs on the market today are derived from natural substances or

their imitations (Mohammadinejad *et al.* 2022). Furthermore, quercetin is a polyphenolic flavonoid that directly induces tumor cells to undergo apoptosis, hence impeding the advancement of several malignancies in humans (Rauf *et al.* 2018). It has been reported earlier that ellagic acid and quercetin work in association with resveratrol to induce apoptosis and temporarily stop the cell cycle within human myeloma cells (Mertens-Talcott *et al.* 2005).

T. stans flower ethanolic extract was shown to contain catechin, which has essential function in promoting of apoptotic rate of tested breast cancer cells. It has been reported that catechin controlled the cell cycle arrest of A549 cells where p21 and p27 expressions were upregulated, and phosphorylated protein kinase B and cyclin functions were downregulated in cancer cells. These effects of catechin also assist in preventing the growth of cancer cells (Sun *et al.* 2020). Furthermore, the current report illustrated the role of *T. stans* flower ethanolic extract in regulation of enzymes responsible for lipid peroxidation and antioxidant enzymes. *T. stans* flower extract contained *p*-coumaric acid as a plant-derived metabolite, which is found in many edible plants with a variety of health-promoting advantages. Numerous research studies have shown how effective its antioxidant properties are in lowering oxidative damage and response to inflammation (Peng *et al*. 2018; Contardi *et al.* 2019).

CONCLUSIONS

1. *Tecoma stans* is a decorative foliage that is rich in flavonoids and polyphenols with unique biologic function.
2. *T. stans'* ethanolic floral extract showed a promising anti-diabetic, antioxidant, and cytotoxic effect on ovarian and prostate cancer cell lines.
3. The bioactive flavonoids and polyphenols essentially contribute to the flower's therapeutic potential to trigger cancer cell death and regulate enzymes that control oxidative activities in prostate cancer cell line.

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