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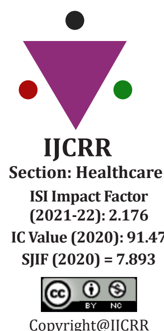

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# Antioxidant, Cytotoxic, Antidiabetic and Phytochemical Investigation of Root Extracts of *Tecoma stans* (*Bignoniaceae*)

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## ABSTRACT

**Introduction:** The plants are an alternative source for the management of a number of human disorders due to the presence of biologically active constituents

**Aims:** Current study aims to investigate underexplored therapeutic potentials of roots of Tecoma stans through phytochemical and biological assays.

**Methods:** The dried roots were extracted successively with methanol and dichloromethane by simple maceration and labeled with codes TSM and TSD respectively.

**Results:** The phytochemical investigation revealed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins, andphenols in the extracts. TSM fraction revealed the highest total phenolic, flavonoid and alkaloid contents159.79 mg GAE/g, 65.38to 13.04 mg QE/g and95.2 to 56.61 mg AT/g of extract respectively. As compared to TSD extract, TSM extract showed significant antioxidant activity using two models DPPH (79.8 %) and ABTS (71.06 %). Cytotoxic activity of methanol extracts against PC3 and HeLa cancer cell line exhibited 61.23% (IC50 22.89 µg/mL) and 74.03 % (17.78 µg/mL) inhibition of cancer cells respectively at concentration of 30 µg/mL. Extracts revealed antidiabetic activity through α-amylase and α-glucosidase inhibition with maximum inhibition 63.26% and 75.66% respectively was detected at the concentration of 1000 mg / mL by TSM extract.

**Conclusion:** Methanolic extracts of roots of Tecoma stanscontainshigher amount plant secondary metabolites and revealed antioxidant, cytotoxic and α-amylase as well as α- glucosidase inhibition activities.

**Key Words:** Secondary metabolites, Antioxidant activity, MTT assay, Tecoma stans, Antidiabetic, Brine shrimp lethality assay

## INTRODUCTION

Plants are an essential part of the earth. Since ancient time, human beings have been using plants as medicine. These medicinal properties of plants are due to the presence of biologically active constituents. It has been scientifically reported that these constituents obtained from plant extracts have various biological activities. The main purpose of these constituents in plants is to protect them from various harms but studies indicate that many of them can also be used against various disorders and diseases in human. The most important of these biologically active constituents are secondary metabolites for example flavonoids, glycosides, steroids alkaloids, terpenes, and tannins. These secondary metabolites can be extracted by using different solvents and used in the preparation of useful drugs. The importance of chemical as well as pharmacological evaluation of plant-derived bioactive compounds used to treat many human illnesses has been increasingly recognized in the past few decades, but still there are numerous useful medicinal plants and herbs waiting to be explored and evaluated for their effective medicinal application.[1,2,3,4]

*Tecoma stans* is distributed worldwide mostly growing in the tropical and subtropical countries. It belongs to the family Bignoniaceae and commonly known as yellow bell. Phytochemical studies on the plant have shown the presence of secondary and primary plant metabolites such as alkaloids, phenolic, sugars, sterols and triterpenoids. Almost each part of the plant is of therapeutic value for example flowers showed antidiabetic, anticancer, anti-inflammatory activity and antioxidant activity.[5,6,7,8] Survey indicates that very few

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reports are available on its roots part so there was a need to explore the root regarding its phytochemical and pharmacological analysis. Therefore, the present work aimed to study the qualitative photochemical screening and quantitative analysis of total phenolic, flavonoid and alkaloid contents of methanol and dichloromethane extracts of *Tecoma stans* roots as it is indicated that alkaloids, flavonoids and other polyphenols of *Tecoma stans* are main compounds thought to be responsible for positive results of many therapeutic activities. Plant extracts were also evaluated for antioxidant activity, alpha amylase, alpha glycosidase inhibitory. Roots were also investigated for their cytotoxic potential using lethality assay as well as MTT assay against two important cancer cell lines (PC3 and HeLa).

## MATERIAL AND METHODS

### Plant Material

Whole *Tecoma stans* plant was collected. The plant was identified by plant taxonomist Dr. Zafrulla Ullah Zafar (Associate Professor), institute of pure and applied biology, Bahauddin Zakariya University, Multan, Pakistan and a voucher specimen holding no. Kew – 318412 was deposited in the same institute.

### Plant Extract Preparation

The collected roots of the *Tecoma stans* were first freed from soil and then shade dried. The dried roots were grounded to coarse powder and subjected to simple maceration process. The weighed amount of coarse powder in an extraction bottle was soaked with known volume of dichloromethane (DCM) for 24 hrs. with intermittent shaking. Then the filtration was performed after 24 hrs. of addition of solvent (DCM). This process was repeated in triplicates using the same solvent. Then extraction of the marc was completed by using methanol in the same way. Both extracts were concentrated in a rotary evaporator at 35C to obtain crude extracts. Dichloromethan and methanol extracts were labeled as TSD and TSM respectively.

### Qualitative estimation of Phytochemical Constituents

Preliminary phytochemical examination for extracts was completed as described by.[9]

### Quantitative estimation of phytochemical constituents

#### *Total Phenolic contents*

Folin–Ciocalteu colorimetric method with slight modification was adopted for the purpose of quantification of total phenol content in the methanolic and dichloromethane root extracts of *Tecoma stans*. Accurately weighed 10 mg of gallic acid was dissolved in 10ml methanol to make the concentration of the solution 1 mg/ml and from this different concentrations of gallic acid (10-200 µg/ml) were prepared. Aliquots of 0.5 ml of plant extracts (10mg/10ml in methanol) and each of the standard gallic acid solution were taken in tubes and mixed with Folin–Ciocalteu reagent five ml (1:10 in deionized $H_2O$). After few minutes, 4 ml of saturated solution of sodium carbonate (7.8% w/v) was added in each solution given in tubes and covered with silver foils and subjected to incubation for sixty minutes (at room temp.) with shaking. Now the absorbance of each solution measured at 765 nm wavelength. Blank was also used which was methanol only. All the samples were analyzed triplicates. Gallic acid (GA) was used as reference. Results obtained were expressed as gallic acid equivalent (GAE) per gram of the sample. Calibration curve was plotted from pure phenolic standard which was gallic acid to quantify phenolic contents in both extracts.[10]

#### *Total Flavonoid Contents*

$AlCl_3$ colorimetric assay with modification was adopted to quantify the methanol and dichloromethane root plant extracts of *Tecoma stans* regarding flavonoids. A standard was also used which was rutin and the results were expressed as Rutin equivalent (RUE) per gram of the sample. Dissolved 10 mg of rutin in 10ml methanol and from this varying concentration (10-120mg) solutions were prepared. Now 0.18 ml 5% w/v of sodium nitrite solution and 2 ml of distilled water were added in each solution of rutin. Similarly 0.8 ml of extracts (10mg/10ml) mixed with 0.18 ml 5% w/v of sodium nitrite solution and 2 ml of distilled water. After 10 minutes, 2ml 10% $AlCl_3$ w/v solution was also added in both extracts as well as in standard rutin solutions. All solution were allowed to stay for 10 minutes and then 1 ml 4% sodium hydroxide was added to each mixture and made volume up to 5 ml using distilled $H_2O$ and mixtures were allowed to stay for 15 minutes. The absorbance of each mixture and blank was determined at a wavelength of 510nm. With the help of standard curve of standard, amount of flavonoid was calculated as mg rutin equivalent per gram of extract. All readings performed in triplicates.[10]

#### *Total Alkaloid contents*

Total alkaloid contents in both extracts of *Tecoma stans* were calculated by spectrophotometrically.[11] Atropine solution was used as reference standard and its solution was prepared by dissolving 10mg of atropine in 10ml distilled water. The plant extracts with concentration1 mg/ml were dissolved in hydrochloric acid which was 2 normal and then filtered. 1.5 ml of filtered sol. was shifted to a separating funnel. In this separating funnel 6 ml of phosphate buffer of neutral pH and 4 ml of bromocresol green solutions were added and followed by vigorous shaking using 4ml chloroform for extraction purpose.

The extracted solution was further diluted with chloroform up to 10ml and absorbance of this diluted solution was measured at 470 nm wavelength. The calculations were performed in triplicates. Atropine calibration curve plotted by preparing varying dilutions of (50, 75, 100, 125, 150, 175, 200, 225 and 250 µg/ml) atropine solution and performed same procedure as mentioned above. Absorbance of each dilution noted at 470nm against blank solution (devoid of atropine).

## Antioxidant activity

### DPPH Assay

Antioxidant potential of dichloromethan as well as methanol root extracts of *Tecoma stans* was measured by using DPPH assay.[10] 1mg/ml solution of DPPH was prepared in methanol. 10 mg extract were carefully dissolved in10 ml methanol to prepare 1mg/ml solution. From this stock solution 60, 50, 40, 30, 20 and 10 µg/ml dilution prepared. In each of these dilutions, 75 µl 1, 1- diphenyl-2-picrylhydrzl solution was added. All test tubes were placed in dark for 35 minutes and then the absorption was noted at 517 nm. IC50 was also determined from % DPPH radical scavenging activity. Ascorbic acid was used as a reference. The stock solution of reference was prepared with the procedure same for each plant extract. The whole experiment was performed in triplicates. % inhibition of the 1, 1- diphenyl-2-picrylhydrzl free radical was measured by the formula given below:

$$Percentage\ Inhibition of\ DPPH\ radical\ activity = \frac{AB - AS}{AB} \times 100$$

AB is absorption of blank sample containing DPPH. Whereas, AS is the absorption of DPPH solution having tested plant extract solution/ Standard.

### ABTS Assay

ABTS assay is based on ABTS+• radical cation decolorization when it is reduced to 2, 2 – azino -bis (3-ethylbenzthiazolin 6 sulphonic acid). Radical cation was produced by reacting 1:1 volumes of seven mM ABTS solution in $H_2O$ with 2.25 mM $K_2O_8S_2$ and staying this mixture in dark at 30°C for 15 hours because time needed to get unchanging absorbance at 734 nm wavelength. After this, solution was diluted using methanol until absorbance value 0.69 ± .05 at 734 nm was achieved. Ascorbic acid was taken as reference standard. All stock solution and their dilutions were prepared as provided in DPPH assay. 2 ml of prepared solution of radial cation was reacted with 200µl of each of dilutions of plant extracts and ascorbic acid.Vortexed the solutions and after 30 minutes absorbance was measured at 734 nm. The amount of sample required to decrease the absorbance of ABTS by 50% (IC50) was also calculated. All calculations were repetitive at least three times. Following formula was employed to calculate percent radical scavenging activity.[12]

$$\%\ ABTS\ radical\ scavenging\ activity = \frac{AB - AS}{aB} \times 100$$

Where AB is the absorbance of ABTS radical and AS is the absorbance of ABTS+.plus plant extracts / standard.

## Cytotoxic activity

### The brine shrimp lethality test

The artificial seawater was placed in a chamber consisting of small plastic container having partition for light for attracting attract the hatched shrimp and dark areas (having shrimp eggs). Two days were given for the shrimp to hatch and mature as larva. Stock solution of both methanolic and dichloromethan root extracts (30mg/3ml) were prepared. From this stock solution 10, 100, 1000 µg/ml dilution were prepared. Each dilution was prepared in three replicates. A control was also prepared having 6ml only sea water. Both plant extracts with different dilution were added to different test tube and evaporating solvents, 6 ml of artificial seawater added to every tube with moderate shaking. Now 10 shrimps were added into each tube. Therefore, there were total of 30 shrimps in each dilution. After adding 6ml artificial seawater to each test tube, they were exposed to the light (lamp). After 24 hours, the no. of living (surviving) and dead shrimps were counted. The percentage mortality (%Mortality) was calculated by formula given below. Lethality concentration (LC50) was also calculated, whereas LC50 value of greater than 1000 µg/ml is non-toxic (inactive) while LC50 value less than 1000 µg/ml is toxic (active).[13,14]

$$\%\ Mortality = \frac{No.\ dead\ of\ nauplii}{Total\ no.\ nauplii\ used} \times 100$$

## Cytotoxicity against HeLa and 3T3 cell lines

Anticancer activity of both plant extracts was calculated in 96-well micro plates using MTT assay. In this study, two human cancer cell line namely prostate cancer cell line (PC3 cell line) and cervical cancer cell line (HeLa cell lines) were used to validate the plant cytotoxic potential. Both cell line were first cultured in Dulbecco's Modified Eagle's Medium having 100 µg/ml streptomycin, 100U/ml of penicillin and ten percent of fetal bovine serum and kept in 6.5% $CO_2$ incubator at 37 $^oC$ . Culture of the cells having concentration of 1 x $10^6$ cells/ml was prepared and placed into 96 well plates. Now cells were incubated (6.5% $CO_2$ incubator) with various concentrations (10-30 ug/ml in 0.5% DMSO) of both plant extracts and standard drug Doxorubicin for 48hrs at 37 $^0C$. After 48 hours, sample solutions were washed using phosphate-buffered saline having 7.4 pH. Into each well, 200µl 0.5% MTT phosphate buffer saline solution was added and subjected to incubation (6.5% $CO_2$ incubator) againfor more 4 hrs. Now absorbance at wavelength 570nm was noted. Similarly absorbance of wells without sample was also measured at

570nm as blanks. All calculations were performed in triplicates. The% cell inhibition was calculated by using formula given below.[15]

$$Percentage\,Cell\,Inhibition = 1 - \frac{Absorbance\,of\,Sample}{Absorbance\,of\,Blank} \times 100$$

## Antidiabetic Activity

### α Amylase Inhibition Activity

250 μL of both extracts of *Tecoma stans* having dilutions 150, 250, 500 and 1000 mg/mL was added in tubes, mixed with 0.020 molar 250 μL of sodium phosphate buffer having pH 6.9 which was already containing 0.50 mg/ ml of enzyme solution (α-amylase). This solution was preincubated at 25 C for 15 min followed by mixing of 250 μL of starch solution (1%) in 0.020 molar buffer 6.9 pH (sodium phosphate) at timed intervals and incubated again at 30 C for 15 minutes. The reaction was ended by adding 500 μL of DNS (dinitrosalicylic acid reagent). The tubes were ungergone for incubation for 6 minutes and subjected to cooling to room temperature. Solution (The reaction mixture) in the tube was diluted with 8 mL of distilled $H_2O$. After dilution, absorption of this mixture was determined at 540 nm using spectrophotometer. Control was prepared by the same method but here the extracts was repalced with distilled water. All reactions were performed in triplicates. The inhibitory activity of α-amylase was calculated as percentage inhibition using formula giving below.[16]

%Inhibition = [A conl – A ext/A conl] × 100

Whereas, Aconl is absorbance of control and Aext is the absorbane shown by the extract. IC50 (50% inhibition of enzyme activity) were also calculated.

### α Glucosidase Inhibition Activity

The substrate solution for assay consisting of p-nitrophenyl glucopyranoside abbriviated as pNPG was prepared in 20mM phosphate buffer having 6.90 pH. 100μL of alpha glucosidase (1.0U/mL) was preincubated using 50μL of the varing dilutions of both methanolic and dichloromethan root extracts (150,250,500 and 1000ug/ml). Then 50μL of substrate solution in the form of p-nitrophenyl glucopyranoside 3.0mM mixed in 20mM phosphate buffer with 6.90 pH was added to initiate the reaction. This reaction mixture was then incubated at 37C for 25 min. After this, reaction was stopped by mixing 2mL 0.1Molar sodium carbonate solution (Na-2CO3). The enzyme inhibitory potential was calculated by quantifying the yellow colored para nitro phenol released from substrate at 405nm.[17]All the reactions were performed in triplicates. Percentage inhibition is calculated as

%Inhibition =[ A conl – A ext/ Aconl ] × 100

Whereas A conl is absorbance of control and A ext is the absorbane shown by the extract. IC50 (50% inhibition of enzyme activity) were also calculated.

## Statistical analysis

To calculate the IC50, a logistic linear regression model was fit to the data using Microsoft Excel 2010. The obtained values were expressed as 'Mean ± SD'. A value of $p<0.05$ was considered as significant.

# RESULTS

## Extraction

Metahnol and dichlorometha solvents were used for extration purpose. Results indicated that amount of methanol extarct was higher 60g than that of dichloromethan 26.7g extract.

## Preliminary Phytochemical analysis

Preliminary phytochemical screening or qualitative phytochemical analysis of methanolic and dichloromethane root extracts of *Tecoma stans* was performed in order to verify either the absence or presence of plant sec. metabolites. The data shown in table 1 summarizes plant secondary metabolites in both root extracts. According to results it is clear that dichloromethan extract displayed the presence of flavonoids, alkaloids, glycosides, terpenoids, tannins, phenols except saponins, proteins and carbohydrates. The methanol root extract of *Tecoma stans* extract exhibited the availability of alkaloids, glycosides, flavonoids, phenols saponin, terpenoids, tannins, carbohydrates and proteins.

## Total flavonoid, phenolic and alkaloid contents

The present work has been carried out for quantification of the total flavonoid, alkaloid and phenolic contents of dichloromethane and methanolic extracts of roots of *Tecoma stans*. The results of total flavonoid, phenolic and alkaloid contents of both extracts are summarized in table 2. Quantity of phenolic compounds in TSD and TSM expressed in gallic acid equivalent and determined by regression equation of calibration curve ($y=0.0024x+0.0494$, $R^2=0.9974$) figure.1. Among the extracts, highest amount of gallic acid equivalent phenolic content of 159.79±5.27 mgGAE/g of extract was observed in TSM extract followed by TSD extract 32.41±2.50 mgGAE/g of extract. The content of the flavonoid in both methanol and dichloromethane root extract quantified from regression equation of standard curve ($y=0.0017x+0.0634$, $R^2=0.9942$) figure 2 and were expressed in rutin equivalent. The results revealed that extract TSM possess high flavonoid contents 65.38±3.19 mgRU/g of extract as compared to TSD 13.56±1.67 mgRU/g of extract. Similarly quantity of alkaloid contents of both extract that in TSM and TSD root extract were also quantified and expressed in atropine equivalent which were 95.20±3.72 and 56.61±2.21 mgAT/g of extract respectively. Regression equation of calibration curve was ($y=0.0005x+0.106$, $R^2=0.9922$) figure 3 used as well in this regard. According to results it is clearly indicated that TSM root extract of *Tecoma stans* contains high

amount of flavonoids, phenolic compounds and alkaloid as compared to TSD.

## Antioxidant activity

### *DPPH assay and ABTS assay*

Antioxidant activity of *Tecoma stans* was measured by DPPH method using ascorbic acid. All concentrations tested showed a dose dependent escalation in the % antioxidant activity. At concentrations of 10µg/mL and 60 µg/ml, ascorbic acid showed a percentage inhibition of 44.07% and 95.85% respectively as given in the table 3. The IC50 value of ascorbic acid was also measured which was 12.72 µg/ml. Similarly at maximum concentration 60µg/mL, TSD and TSM extracts displayed 56 % and 79 % inhibition of radical respectively. According to results, TSM extract reveal significant scavenging activity against DPPT radical cation with IC50 value 26.02 µg/ ml.

The dichloromethane and methanol extracts of root of *Tecoma stans* were also analyzed against the ABTS radical. All tested exhibited a dose dependent increase in the percentage antioxidant activity. At 60 µg/ml, reference (ascorbic acid) showed percentage inhibition 90.09% as given in the table 3 with IC50 value 20.26 µg/ml. Similarly at 60 µg/ml, TMS and TSD showed scavenging activity 71% and 31% respectively which is the same trend as DPPH antioxidant activities. According to results, TSM extract revealed significant radical scavenging activity with IC50 value 37.78 µg/ml. Scavenging of DPPH radical by the plant *Tecoma stans* was found to be slight higher than that of ABTS radical. According to studies some compounds exhibiting ABTS +radical scavenging activity did not reveal DPPH scavenging activity but this is not the case in this study.[18]

## Cytotoxic activity

### *Brine shrimp lithality assay*

Methanolic extract of root of *Tecoma stans* (TSM) exhibited potent brine shrimp larvicidal activity with lethality concentration (LC50) 384.31 µg/mL as given in the table 4. Whereas, 96.66 % mortality was seen at a concentration of 1000 µg/ml in methanolic extract (TSM). Dichloromethane extract (TSD) exhibited 20 % mortality at 1000 µg/ml. Based on results, TSM extract, exhibited a dose dependent increase in brine shrimp lethality. The detected lethality of TSM to the brine shrimps showed the availability of potent cytotoxic and possibly anticancer constituents.

### *Cytotoxicity against HeLa and PC3 cell lines*

In the present study, *in- vitro* cytotoxic effects of both root extracts of *Tecoma stans* were evaluated against the human cancerous cell lines namely PC3 (prostate cancer cell line) as well as HeLa (cervical cancer cell line). The cytotoxic potential of both plant extracts with IC50 are given in table 5. The results showed that with the increase of concentrations of both extracts, the percent inhibition of the cancerous cell was also increased. Among the two extracts, TSM extract at 30 µg/ml exhibited higher cytotoxicity against PC3 (61%) and HeLa (74%) cell lines with IC50 17.78 and22.89µg/ml respectively using Doxorubicin as standard with IC50 1.44 µg/ml.

### *Enzymatic activity*

Alpha amylase inhibitory activity of both extracts was evaluated and results are give in table 6. Results were compared with standard acarbose. Results showed that TSD extract showed miled enzyme inhibitory activity 35.22% while TSM reveald prominent and dose dependant inhibitory activity against alpha amylase. At 1000ug/ml concentration, inhibitory potential of standard acarbose and TSM were 83.65% (IC50 390.96 µg/ml) and 63.68% (IC50 638.68µg/ml) repectively. Similarly, plant extracts were also evaluated against alpha glucosidase enzyme while acarbose used as standard and results are give in table 6. Results indicated that like alpha amylase inhibitory activity TSD extract was very less active against this enzyme and at 1000ug/ml it showed less inhibitory activity 29.49% against enzyme as compared to methanolic extract which was potent inhibitor of alpha glucosidase enzyme. At 150mg/ml dose, TSM did not showed significant inhibitory activity (27.07%) but at 1000mg/ml this methanolic extract showed significant inhibitory effects 75.66% on alpha glucosidase enzyme when compared with standard (84.04%). IC50 values of methanolic and acarbose were calculated which were 457.03and 355.23µg/ml respectively.

## DISCUSSION

Plant secondary metabolites have played a vital role in curing and preventing various human ailments. Therefore, a detailed investigation of all medicinal plants is required before their use as medicine, since the therapeutic activity completely depends on the quality of plant material. The study of any plant crude sample is valuable only if it contains the active constituents which have to be recognized to validate its real value. Moreover, information about different plant constituents is a very essential and beneficial as it is much valuable in screening of their biological activities and the production of complex chemical compounds. Therefore, to screen these constituents and their therapeutic activities we selected roots of *Tecoma stans*. Our results revealed that roots of *Tecoma stans* contains important secondary metabolites like alkaloids flavonoids, phenol, saponins etc.Similarly in this study, low and high polarity solvents are also tested to study their effects on extraction of phyto constituents. The resultant extracts were also screened for quantitative phytochemical analysis as alkaloids, flavonoids and other phenols

are responsible for many pharmacological properties.[20,21] Result indicated that both extracts TSM and TSD contained almost equal secondary metabolites but their amounts regarding alkaloids, phenols and flavonoids are higher in methanolic extract (TSM). It was also observed that a high amount of extract was obtained when methanol was used as solvent for extraction purpose. The above-mentioned preliminary phytochemical screening and quantitative phytochemical analysis has provided some basis to perform some pharmacological studies of roots of *Tecoma stans*

Radical scavenging property plays a significant role in preventing illnesses for example cancer, inflammation, CVS diseases etc. The qualitative phytochemical study of *Tecoma stans* shown that the it has alkaloids, flavonoids, steroids, phenols, glycosides. Many aerial parts of *Tecoma stans* have been evaluated regarding anti-oxidant activity.[19,20,21,22] In this study, antioxidant activity performed by DPPH assay and ABTS assay. It was also indicated that TSM contains potent scavenging activity that could be due to the high amount of polyphenol and alkaloid contents in this extract.

The antioxidant assay findings further, directed the study towards cytotoxic assay. Flowers, leaves, fruits of *Tecoma stans* having antioxidant activity have been identified and their anticancer activity was also evaluated against different cancer cell line.[23,24] TSM contains greater amount of secondary metabolites which helps to explain its higher cytotoxicity than dichloromethane root extract and TSM is toxic (active) as its LC50 value is less than 1000 µg/ml.[14] In present study this brine shrimp lethality was verified by applying MTT assay against PC3 and HeLa human cancer cell lines. *Tecoma stans* was evaluated first time against these cell lines and the results revealed that only methanol plant extract exhibited potent inhibitory activity against both cell lines. The choice of HeLa and PC3 cell line was based on the fact that according to American Institute for Cancer Research, in men prostate cancer is the 2nd most commonly occurring cancer and the 4th most commonly occurring cancer overall. Whereas, cervical cancer is the 4th most commonly diagnosed cancer in women worldwide. Thus require new therapies for their treatments

Alpha glucosidase inhibitors in the small intestine delay the carbohydrate breaking and reduce the postprandial blood glucose excursion in diabetics.[25] Inhibition of carbohydrate digesting enzymes such as $\alpha$-glucosidase and $\alpha$-amylase in the gastrointestinal glucose absorption is one of the strategies used to treat diabetes mellitus.[26] In present study, the effect of *Tecoma stans* roots extracts (TSM and TSD) on the inhibition of alpha glucosidase and alpha amylase was assessed. The results of the inhibitory assays (alpha glucosidase and alpha amylase) revealed that the extract TSM of the roots has greater alpha glucosidase inhibitory activity as compared to alpha amylase. According to the previous reports that plant phytochemicals are strong inhibitors of alpha-glucosidase and mild inhibitors of alpha-amylase.[27] It is a quality that have advantage over synthetic drugs (acarbose) use by diabetics in postprandial blood glucose management. These drugs are strong inhibitors of alpha-amylase. Mild inhibition of alpha amylase and stronge inhibition of alpha glucosidase activity of the vegetable extracts could address the major side effetcs of currently used alpha amylase and alpha-glucosidase inhibitor drugs having side effects (flatulence, diarrhea, meteorism and abdominal distention.[28] It has been suggested that excessive pancreatic alpha-amylase inhibition causes abnormal bacterial fermentation of undigested carbohydrates in the colon might be the cause of such adverse effects.[27]

## CONCLUSION

The current study concludes that roots of *Tecoma stans* are potential source of phytochemicals and suggests that the plant samples are found to have suitable antioxidant activity that may be credited to the availability of significant phenolic and flavonoid contents. It is shown that among the plant extracts tested, the TSM extract revealed highest antioxidant activity using DPPH inhibition and ABTS assay. Extract of *Tecoma stans* (TSM) exposed notable cytotoxic activity when assessed against PC3 and HeLa cancer cell line. The results of *in vitro* anti-diabetic activity reveals that pant possess a potent inhibitory activity against both alpha amylase and glucosidase enzymes and can helpful for management of postprandial glucose level in diabetes. The obtained results claim further pharmacological characterization and bioactivity guided isolation of the compounds responsible for the observed activities.

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We had no funding sources for this study.

**Conflict of Interest**

The authors declare that there are no conflicts of interest.

**Authors' Contribution**

The authors Atif Shahzad[1], Samina Afzal[1] and Imran Ahmad[1] have made substantial contribution to design and perform the

experiments. They were involved in planning, implementation and analysis of the research study and its presentation in the form of final manuscript

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**Table 1: Preliminary Phytochemical Investigation of Dichloromethane (TSD) and Methanolic Extracts (TSM)**

| Test | TSD | TSM |
|---|---|---|
| Alkaloids | + | + |
| Glycosides | + | + |
| Flavonoids | + | + |
| Terpenoids | + | + |
| Tannins | + | + |
| Saponins | - | + |
| Phenols | + | + |
| Carbohydrates | - | + |
| Proteins and amino acids | - | + |

(+) present, (-) absent.

**Table 2: Total Phenolic, Flavonoid and Alkaloid contents of roots of *Tecoma stans*.**

| Sr. No | Extract | Total Phenol contents (mgGAE/g) ±SD | Total Flavonoid contents (mgRU/g) ±SD | Total Alkaloid contents (mgAT/g) ±SD |
|---|---|---|---|---|
| 1 | TSM | 159.79±5.27 | 65.38±3.19 | 95.20±3.72 |
| 2 | TSD | 32.41±2.50 | 13.04±1.67 | 56.61±2.21 |

RU: Rutin equivalen, GAE: Gallic acid equivalen, AT: Atropine equivalent.Each value is Mean ± SD (Standard deviation).

**Table 3: Percent inhibition of DPPH and ABTS radical using TSD and TSM extract. Ascoric acid as standard.**

| Concentration (µg/ml) | % Inhibition of DPPH radical | | | % Inhibition of ABTS radical | | |
|---|---|---|---|---|---|---|
| | TSD | TSM | Ascorbic Acid | TSD | TSM | Ascorbic Acid |
| 10 | 9.81±2.49 | 36.79±3.53 | 44.07±3.34 | 8.55±0.99 | 17.34±1.84 | 36.33±3.54 |
| 20 | 13.67±2.30 | 45.63±4.67 | 61.35±2.23 | 12.44±2.07 | 32.33±2.51 | 52.73±2.46 |
| 30 | 17.19±1.82 | 53.35±1.70 | 68.10±1.44 | 16.89±1.34 | 41.73±3.24 | 64.70±1.10 |
| 40 | 21.75±3.21 | 61.27±2.14 | 73.85±1.46 | 20.83±1.65 | 56.14±0.72 | 75.93±2.69 |
| 50 | 23.54±1.14 | 67.57±2.25 | 83.17±3.47 | 23.89±1.69 | 61.50±1.88 | 80.96±1.65 |
| 60 | 25.56±1.61 | 79.88±3.02 | 79.88±3.02 | 31.79±3.12 | 71.06±2.17 | 90.09±1.33 |

Each value is Mean ± SD (Standard deviation).Differences were considered significant atp < 0.05 level.

**Table 4: Brine shrimp lethality assay of TSD and TSM extracts of *Tecoma stans*.**

| Name of extract | Concentration ug/ml | No. of shrimps | No. of survivors | Percent Mortality | LC50 ug/ml |
|---|---|---|---|---|---|
| TSD | 10 | 30 | 28 | 6.66 % | |
| | 100 | 30 | 28 | 6.66% | - |
| | 1000 | 30 | 24 | 20% | |
| TSM | 10 | 30 | 26 | 13.33% | |
| | 100 | 30 | 19 | 36.33% | 384.31 |
| | 1000 | 30 | 01 | 96.66% | |

**Table 5: *In vitro* cytotoxic activity of TSD and TSM extracts of *Tecoma stans* on PC3 and HeLa cell line**

| Concentration (µg/ml) | % Inhibition of PC3 cell line | | | % Inhibition of HeLa cell line | | |
|---|---|---|---|---|---|---|
| | TSD | TSM | Doxorubicin | TSD | TSM | Doxorubicin |
| 10 | 05±0.66 | 33.06±1.59 | 62.76±1.67 | 06±0.97 | 31.64±1.31 | 62.8±2.31 |
| 20 | 09±1.09 | 47.80±2.01 | 78.15±2.88 | 11±1.34 | 55.07±2.37 | 88.03±1.99 |
| 30 | 11±0.79 | 61.23±3.27 | 92.86±3.21 | 14±1.85 | 74.03±1.62 | 100±3.01 |
| $IC_{50}$ µg/ml | - | 22.89±0.42 | 1.44±0.63 | - | 17.78±2.24 | 1.92±0.93 |

Each value is Mean ± SD (Standard deviation).Differences were considered significant atp < 0.05 level

**Table 6: Inhibitory activity of TSM, TSDextracts of *Tecoma stans* and standard against α amylase and α glucosidase.**

| Concentration (µg/ml) | % Inhibition of α Amylase | | | % Inhibition of α Glucosidase | | |
|---|---|---|---|---|---|---|
| | TSD | TSM | Acarbose | TSD | TSM | Acarbose |
| 150 | 12.31±2.11 | 26.33±0.50 | 29.23±1.29 | 10.20±0.83 | 27.07±0.92 | 33.23±1.94 |
| 250 | 17.23±1.52 | 35.97±1.68 | 43.58±2.14 | 14.33±1.34 | 42.85±3.87 | 47.16±2.49 |
| 500 | 23.81±3.27 | 47.66±2.31 | 65.05±0.94 | 21.14±1.45 | 58.15±1.15 | 63.62±5.23 |
| 1000 | 35.22±4.10 | 63.26±4.90 | 83.65±3.61 | 29.49±2.37 | 75.66±2.08 | 84.04±3.57 |
| $IC_{50}$ µg/ml | - | 638.86± 3.6 | 390.96± 2.5 | - | 457.03±5.6 | 355.23±3.5 |

Each value is Mean ± SD(standard deviation). Difference were considered significant at $p<0.05$.

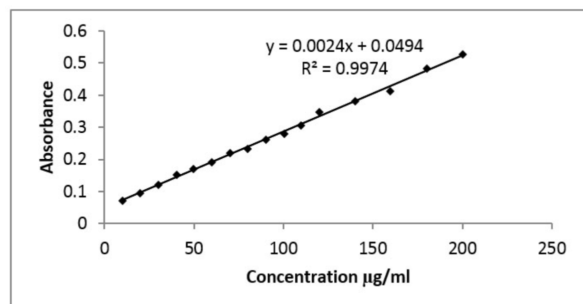


**Figure 1:** Standard calibration curve of gallic acid for total phenolic content.

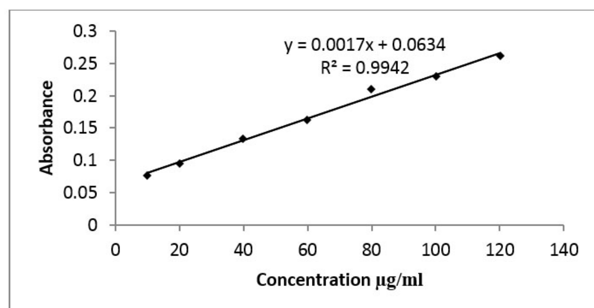


**Figure 2:** Standard calibration curve of Rutin for total flavonoid content.

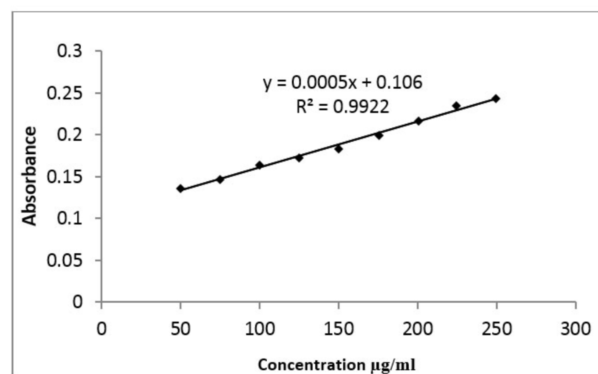


**Figure 3:** Standard calibration curve of Atropine for total alkaloid content.