

Antibacterial and Antioxidant Potential of Ethanol Extract of *Tecoma Stans* (L.) Juss. Ex Kunth Leaves

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ABSTRACT

The aim of this research is to evaluate the total phenolic content, antibacterial activity and antioxidant activity of *Tecoma stans*. leaves were collected from the College of Veterinary Medicine, University of Baghdad, and the ethanolic extract was prepared using a magnetic stirring. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, phenols, alkaloids, tannins, saponins, and glycosides. Antioxidant activity was evaluated using the DPPH assay, along with Total Phenolic Content (TPC) analysis. The extract exhibited strong antioxidant activity that increased with concentration. High-Performance Liquid Chromatography (HPLC) analysis identified six major phenolic compounds: quercetin, gallic acid, caffeic acid, apigenin, ferulic acid, and rutin. The extract also demonstrated antibacterial activity against multidrug-resistant *Pseudomonas aeruginosa* at concentrations of 128 and 256 mg/ml, with inhibition zones ranging from (12.33 ± 0.42) mm to (21.67 ± 0.42) mm, respectively, indicating its potential as a promising natural antibacterial agent.

Keyword: alkaloids, bignoniaceae, free radicals



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INTRODUCTION

Tecoma stans (L.) Juss ex Kunth (Bignoniaceae) is native to Mexico (the tropical dry ecosystems of North America) and the temperate high altitudes of South America and is colloquially called kusi urakame, koyawari, Palo amarillo, tronadora, yellow-elder, yellow trumpet bush, trumpet-flower, yellow bells, trumpet bush, ginger-thomas, esperanza, and timboco. (Anand and Basavaraju 2021). The plant species *Tecoma stans* has previously been classified under several nomenclature authorities; however, the currently accepted scientific name is *Tecoma stans* (L.) Juss. ex Kunth. (Singh *et al* 2024). It shows antibacterial, antidiabetic, antiproliferative, anti-inflammatory, antioxidant activity and this plant is naturalized in tropical and subtropical regions of Asia, Oceania, and Africa (Bakr 2019, Verma *et al* 2016). The pharmacological activities of the flowers and leaves of *T. stans* are most commonly used for

the treatment in managing cancer whereas the leaves have been known to possess antifungal activity against yeast (Gonçalves *et al* 2022). Other studies researched the antioxidant and the anticancer properties of the plant. *T. stans* contains a variety of bioactive compounds including saponins, flavonoids, alkaloids, phenols, steroids, anthraquinones, tannins, terpenes, phytosterols, triterpenes, hydrocarbons, resins, essential oils and glycosides (Balasubramanian *et al* 2024). Flavonoids have various functions and roles in plants, including protection against UV radiation and oxidation, and antifungal, antimicrobial, and insecticidal effects (Dias *et al* 2021). Flavonoids increase the action of vitamin C and role as antioxidant (Al-Quhli *et al* 2024). *Tecoma's* leaf and flower has also been used for synthesizing silver nanoparticles, and it is already promising for biomedicine, food packaging, and wound healing (Anand and Basavaraju 2021). In

addition to its use as an ornamental plant, *Tecoma stans* is characterized by properties, it has some health benefits being high in Fiber, in addition to fast growth and good durability which enables *Tecoma* in landscaping both hot and dry areas (Selvan *et al* 2024). A glossy green leaves, bright yellow, trumpet flowers, bring in pollinator sweeps of bees and butterflies (Singh *et al* 2024). It doesn't need many requirements, as it thrives in well-drained soil and full sunlight (Selvan *et al* 2024). Application of antioxidants has been studied in many sciences such as medicine, nutrition and cosmetics. Antioxidants are significant defence mechanisms and also important key inhibitors in protection (Jin, 2009). Because of the alterations in skin physiology, bacterial infections are the most frequent cause of complications and fatalities among burn victims, the individuals are susceptible to burn wound sepsis and infection (Darweesh *et al* 2024). Burn patients often have *pseudomonas* infection (Hilal, 2023). Since the skin's environmental barrier function has been compromised, these patients are more vulnerable to microbial invasion and colonization by harmful bacteria like *P. aeruginosa* (AL-Sabagh *et al* 2023). A common Gram-negative bacterium that may thrive in a variety of circumstances, *Pseudomonas aeruginosa* is a member of the Pseudomonadaceae family. Plants, animals and most importantly humans are among the many organisms that can become infected by the opportunistic pathogen *Pseudomonas aeruginosa*, especially those with cystic fibrosis burn patients and immunocompromised patients (Pachori, *et al* 2019). *P. aeruginosa* presents a significant clinical problem owing to its Multi Drug Resistance (MDR) phenotype, antimicrobial resistance is caused by a variety of methods, including enzyme synthesis, protein loss from the outer membrane, target alterations, and multidrug efflux systems, clinically treating MDR in *P. aeruginosa* is crucial (Avakh *etal* 2023). The efflux pump is one resistance mechanism that is intimately linked to the formation of MDR and XDR (Ali, *et al* 2024). *P. aeruginosa* readily acquires resistance to individual agents via

chromosomal mutations and lateral gene transfer (Morita, *et al* 2014). This research aims to explore the bioactive potential of *Tecoma stans* leaf extract by assessing its total phenolic content and antioxidant properties. Additionally, it investigates the extract's antibacterial activity against multidrug-resistant *Pseudomonas aeruginosa*, with the goal of identifying promising natural alternatives to conventional antibiotics.

MATERIALS AND METHODS

Collection of Plant Material: *Tecoma stans* leaves were collected from the internal departmental garden of the College of Veterinary Medicine, University of, after being determined as a type (by the specialist in the Plant Science branch, Biology Department, College of Science, University of Baghdad). The leaves were rinsed with water, dried at room temperature, ground in a mill and stored at 4 °C for further analysis.

Plant extraction procedure: After air-drying in the shade, 100 g of fresh leaves of *Tecoma stans* were pulverized using a coffee grinder. The generated powder was thereafter deposited in a volumetric cylindrical flask and completed to 1000 ml with 70% ethyl liquor to produce 1/10 (W/V). After 24 h of mixing the solution with a magnetic stirrer device, the liquid was passed through four folds of medical gauze followed by through Whatman (No.1) filter paper. The filtered mixture was then concentrated for 72 h at 40 °C in an incubator to obtain crude extract. The extract (10 g) were maintained at 4 °C in the dark, in a sterile screw bottle until use (Jin, *et al*, 2009).

Phytochemical screening: All *Tecoma stans* powder determinations were achieved by chemical tests through using Standard protocol for recognition of the constituents as given below:

Test of Phenols: Based on the method of Harborne (Harbone *et al*, 1984), 2ml of 1% ferric chloride solution was added to 3 ml of plant extract, and bluish green color was indicated the presence of phenols. To verify this observation, a volume of 3 ml of the test samples were placed in separative tubes, followed by adding the aqueous basic lead acetate which led to the appearance of a reddish-brown precipitate.

Test of Flavonoids: One gram of ethanolic *Tecoma stans* extract was dissolved in ten milliliters of 95% ethanol and filtered to create Solution (A). Ten milliliters of 50% ethyl alcohol and ten milliliters of 50% potassium hydroxide were combined to create solution (B). The presence of flavonoids was shown by the yellow coloration that resulted from mixing equal parts of solutions (A) and (B) (Jaffer *et al*, 1988).

Test of Alkaloids: By dissolving in 5 ml distilled water acidified with 4% hydrochloric acid then taking 0.5 ml of this mixture in a watch glass, the following reagents were then used with 1 gram of the various *Tecoma stans* leaves extract:

- Mayer reagent: white precipitate appears (Harborne, 1998).
- Wagner reactant: this was thought positive if there was brown precipitate (Sailaja *et al*, 2016).

Tests of Glycosides: To the test tube, 1 ml of methanolic and aqueous *Tecoma* leaves extract solution were added and incubated with 2 drops of alpha naphthol alcohol. Next, 0.5 ml sulfuric acid was added slowly. When the test

is positive, a violet ring is seen (AACC). 2001, Al-Khafaji *et al*, 2022).

Test of Tannins: One gram of the ethanolic extract of *Tecoma stans* leaves was dissolved in 10 ml of 70% methanol to obtain a homogeneous solution. The solution was then equally divided into two portions. A few drops of 1% lead acetate solution were added to one portion, while the other portion was left untreated as a negative control. The appearance of a white gelatinous precipitate in the treated portion indicated the presence of tannins in the extract (Sailaja, *et al*, 2016).

Test of saponins: The presence of saponins was detected by vigorously shaking five ml of the ethanolic extract of *Tecoma stans*, which produced a stable persistent foam (Harborne, 1998).

High-Performance Liquid Chromatography (HPLC): Identification of the ethanolic extract of *Tecoma stans* preparation was carried out based on HPLC technique as described by Mradu *et al* (Mradu, *et al*, 2012) and separated under the following conditions Table(1):

Table 1. Conditions of HPLC analysis

Pump model	S 2100 Quaternary Gradient Pump
The mobile phase (A)	Methanol
The mobile phase (B)	1% formic acid in water (v/v)
Column	C18-ODS
Column dimension	25 cm × 4.6 mm
Detector UV	280 nm
Flow rate	1.1ml/min
Sample volume	100 µl
Temperature	30°C

Determination of Total Phenolic Content (TPC): Folin-Ciocalteu method, described by Jayaprakasha *et al* 2001. The total phenolic content of *Tecoma stans* ethanolic extracts was spectrophotometrically determined using Folin-Ciocalteu method. 0.4 ml of each sample were mixed with 2.0 ml of the 10 fold diluted Folin-Ciocalteu reagent and 1.6 ml of 7.5% sodium carbonate solution. The top

volume was brought to the mark with distilled water up to 5 ml. After wrapping the tubes in parafilm and allowing them to stand at room temperature for 30 minutes, the absorbance at 760 nm was measured. They were reported as milligrams of gallic acid equivalent (GAE mgg-1 dry weight) based on calibration standard curve of gallic acid Figure (1).

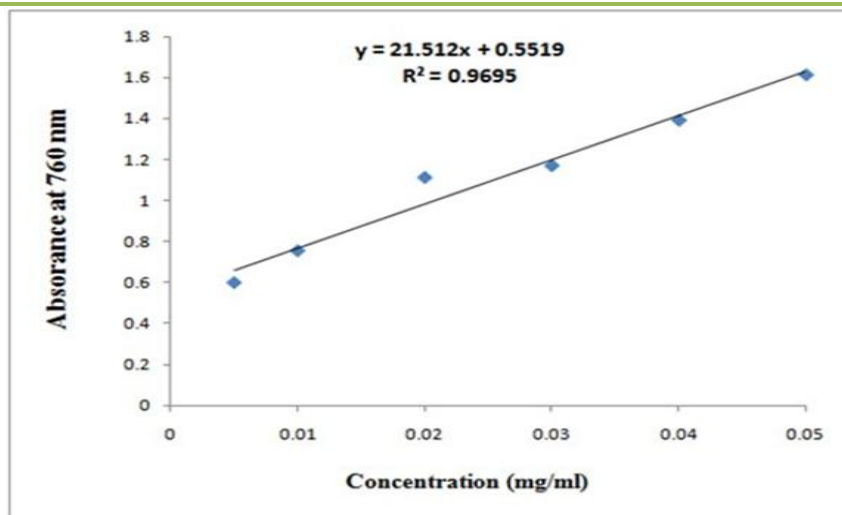


Figure 1. Standard curve of Gallic acid (1)

Evaluation of the antioxidant activity

(DPPH assay) : The antioxidant activity of the prepared ethanolic leaf extract of *Tecoma stans* was evaluated according to the method described in (Ogunmoyole *et al* 2013). A volume of 5 ml of freshly prepared 0.004% 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol was mixed with 50 µl of different concentrations (0.625, 1.25, 2.5, 5 and 10) mg/ml in distilled water (0.010g extract). To prepare a working solution of 10 mg/ml, the volume was subsequently brought up to 10 ml volume. Ten-fold serial dilutions of *Tecoma stans* extract were performed to reach the concentrations 10-0.625 mg/ml. After 30 minutes, each dilution's absorbance was taken at 517 nm. Positive controls were Butylated Hydroxy Toluene (BHT) and vitamin C. Three independent tests were performed for all tests. The percentage of DPPH scavenging (or the percentage of DPPH reduction ability) was calculated according to the following formula:

Where the % Reduction = $(\text{Abs DPPH} - \text{Abs Dil.}) / \text{Abs DPPH} \times 100$

Whereby: **Abs DPPH** = DPPH solution average absorption, **Abs Dil.** Absorption = mean of the three absorption were each dilution

A graphic was carried out using the values obtained from the above using Microsoft Excel. Graphic values of effective concentration of extract or compound (EC₅₀) needed to reduce 50% of DPPH of each extract.

Collection and identification of *pseudomonas aeruginosa*:

Samples were obtained from patients with wounds and burns. Isolates were cultured using selective media blood agar, MacConkey agar, and cetrimide agar colony morphology was observed, and biochemical tests were performed using the VITEK-2 system.

Disc diffusion method: The standard approach by Razmavar *et al* (33, 1). To determine whether the *Tecoma stans* extracts had antibacterial properties. Muller Hinton agar plates were uniformly inoculated with the bacterial culture (adjusted to 0.5 McFarland standard) using a sterile swab. After 15 minutes of drying the plates were used for the sensitivity test. A stock solution of plant extract was created by dissolving 0–512 g of the extracts in 1 ml of their respective solvents (distilled water for one extract and Di Methyl Sulf Oxide (DMSO) for another extract) in order to reach a final concentration of 512 mg/ml. After that, extract concentrations of 128 and 256 mg/ml were obtained by diluting the stock solution. Twenty microliters of each dilution were impregnated into sterile blank discs measuring six millimeters in diameter. The negative controls were distilled water and DMSO discs. Each disc was dried completely before being placed on the Mueller Hinton agar surface. The plates were incubated for 24 hours at 37°C. To evaluate the antibacterial activity after incubation the diameter of the inhibition zone encircling the discs was measured. Three runs of the test were performed to ensure reliability.

Statistical analysis: The Statistical Analysis System (SAS) (2018) program was utilized to identify the effect of various factors on study parameters. A difference of least significant-LSD test was used to significant compare between means (Cary, 2012).

RESULTS AND DISCUSSION

Phytochemical screening: Qualitative phytochemical analysis of the ethanol extract of *Tecoma stans* leaves showed that the extracts included different kinds of active phytochemical constituents Table (2). This result has been ensured by Singh *et al.* (2024) when they observed phenols, terpenoids and flavonoids in ethanolic leaf extract of *Tecoma stans*. Equally, (Salem *et al* 2013) revealed that ethanolic extract of leaves has significant amounts of flavonoids and phenolic compounds. Nevertheless, variations in the phytoconstituents of *Tecoma stans* leaf extracts have been reported in some studies. For instance, Ngoupayo *et al.* 2024 noticed flavonoids, polyphenols, cardiac glycosides, coumarins and tannins in a hydroethanolic extract of the leaves, while no alkaloids nor saponins were detected. Differences in the polarities of the solvents used for extraction, extraction procedures, geographic location and

environmental circumstances may be responsible for these discrepancies as they can influence the biosynthesis and accumulation of phytochemicals in plants

Table 2. Phytochemical screening of *Tecoma stans* leaves extracts

Active ingredient	Results
Phenols	Present
Flavonoids	Present
Alkaloids	Present
Glycosids	Present
Tannins	Present
Saponins	Present

Hydrostatic High Performance Liquid Chromatography: HPLC method followed for individual phenolic compositions of *Tecoma stans* (Mradu *et al* 2012). Figure (2) shows the identification of 6 phenolic compounds in ethanolic extract (Quercetine, Gallic acid, Ferulic acid, Caffeic acid, Rutin and Apigenin) based on comparison with standard Compounds (Figures 3, 4, 5, 6, 7, and 8). So, the composition of phenolic compounds in the extracts is so much dependent on the plant raw material kind, region of origin, and the selection of solvent Ethanol is an ideal solvent due to its high content of bioactive compounds (Durgadevi *et al* 2024).

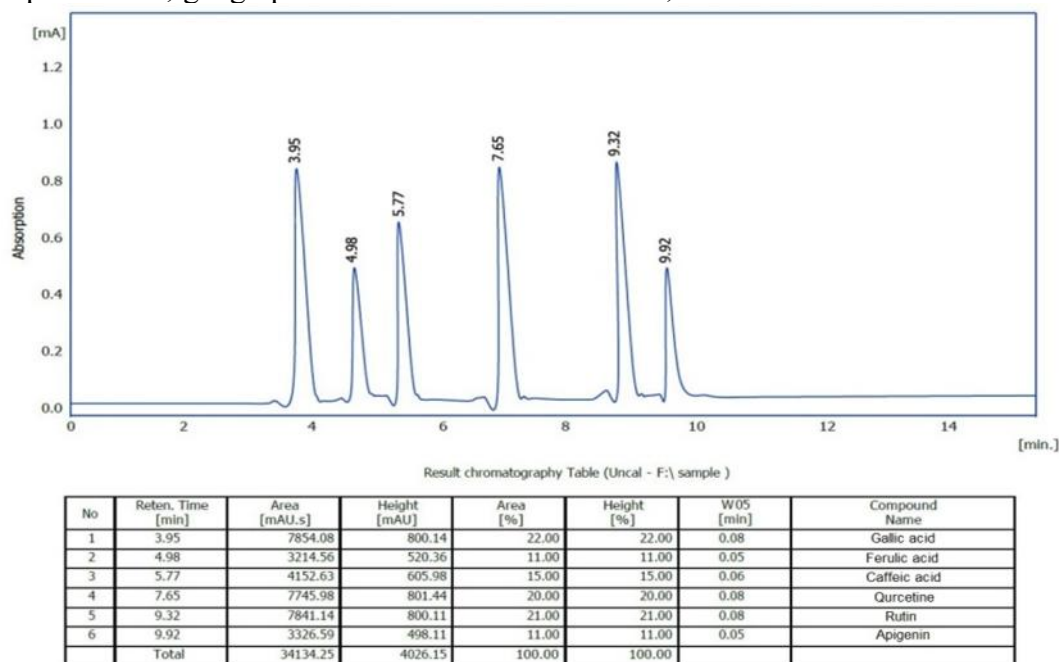


Figure 2. HPLC chromatogram of phenolic compounds in ethanolic *T. stans* leaves extract

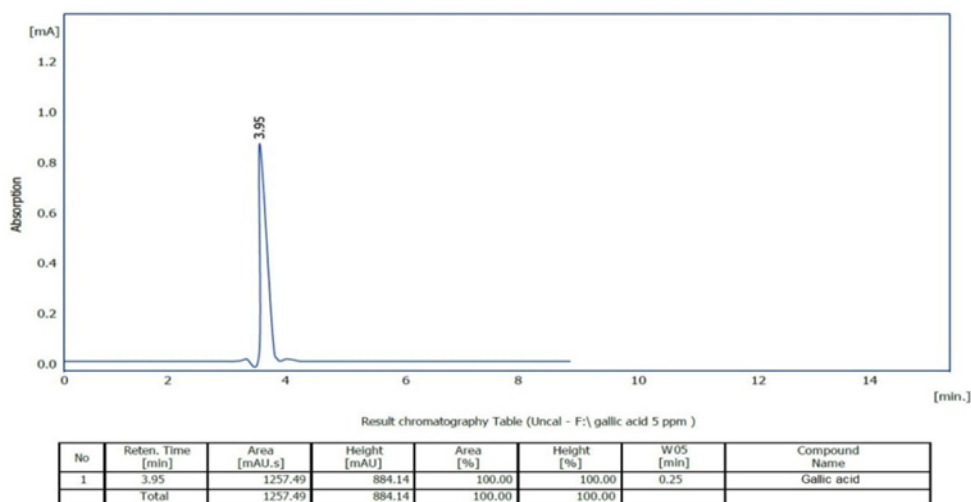


Figure 3. HPLC chromatogram of phenolic compounds standard gallic acid

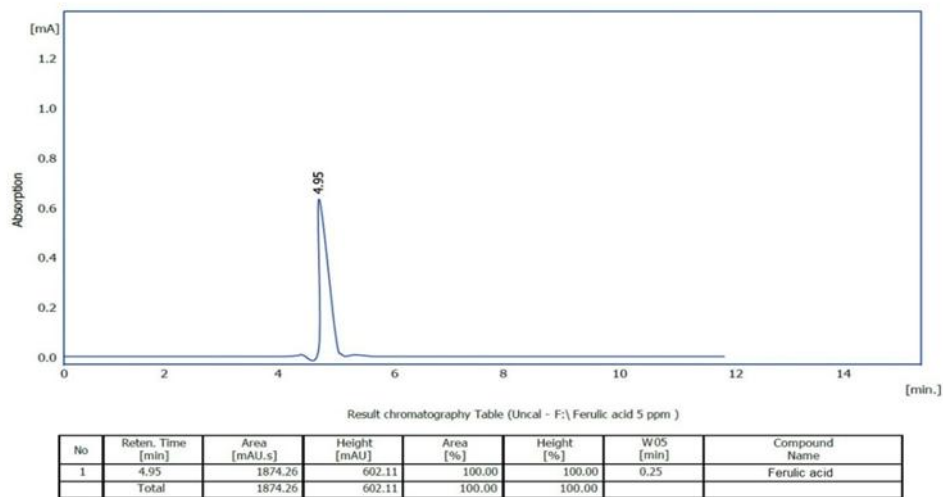


Figure 4. HPLC chromatogram of phenolic compounds standard ferulic acid

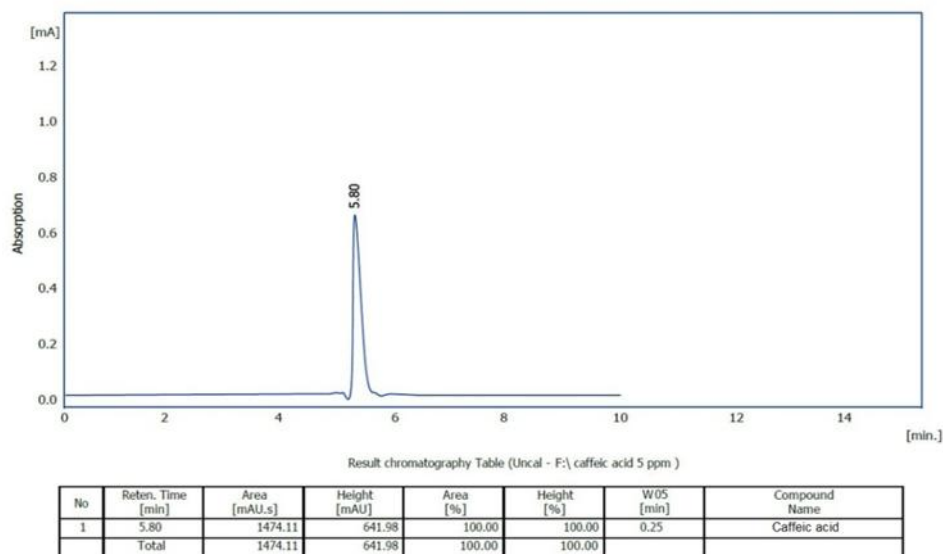


Figure 5. HPLC chromatogram of phenolic compounds standard caffeic acid

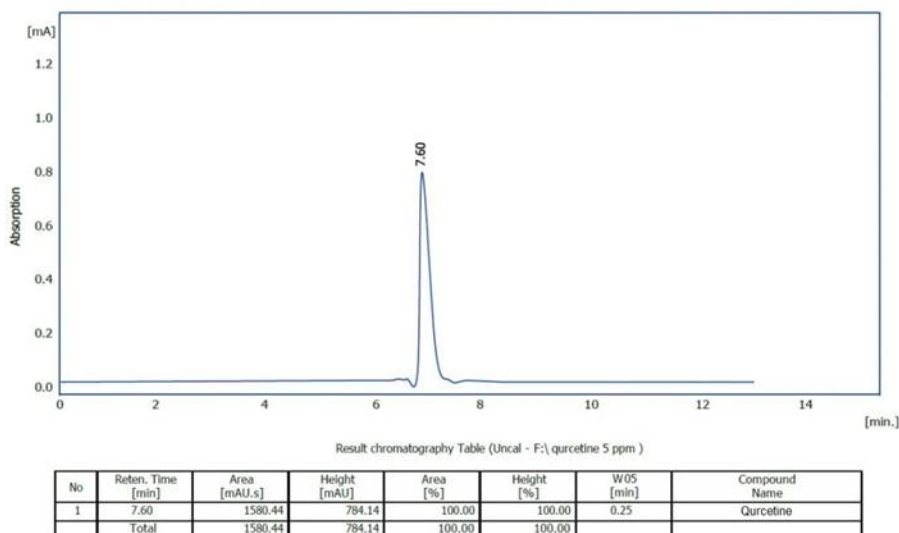


Figure 6. HPLC chromatogram of phenolic compounds standard quercetin

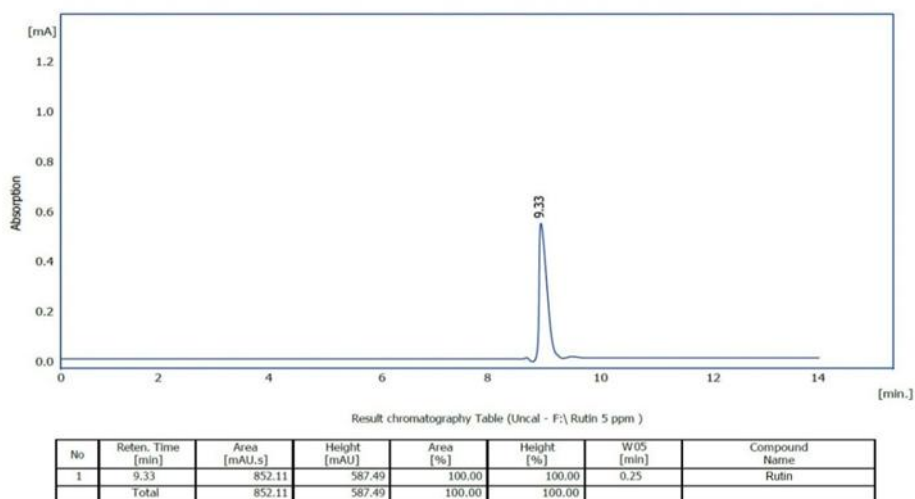


Figure 7. HPLC chromatogram of phenolic compounds standard rutin

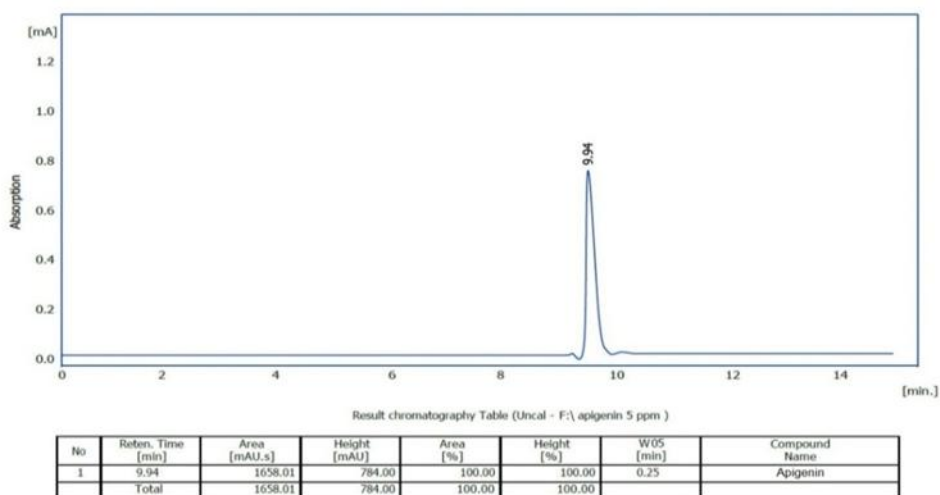


Figure 8. HPLC chromatogram of phenolic compounds standard apigenin

Total Phenolic Content

Results demonstrated a significant increase in total phenolic content of *Tecoma stans* extract

as the concentration increased ($P \leq 0.01$) in a stepwise manner. The highest values were in 10 mg/ml 83.25 ± 0.04 mg/g Table (3). The

phenolic compounds are critical plant components with redox properties that are responsible for antioxidant activity. Many phenolic compounds have been investigated for their biological activity and health significance in humans (Mohsen and Abas. 2024). Hence, the Folin-Ciocalteu reagent was used to determine the total phenolic content of the ethanolic extract.

Table 3. Total phenolic content of *Tecoma stans* (Mean $\pm$ SD)

Concentration (mg/ml)	Ethanolic extract (mg/g)
10	83.25 $\pm$ 0.04
5	54.92 $\pm$ 0.03
2.5	31.46 $\pm$ 0.06
LSD value	0.081**
** (P $\leq$ 0.01)	

DPPH assay for antioxidant activity

Antioxidants are substances that stop autoxidation by stopping the free radicals formation or stopping their spread (Wichayapreechar, *etal* 2024). Significant

variations (P $\leq$ 0. 01) were observed between concentrations of *Tecoma stans* extract which demonstrated concentration-dependent free radical scavenging activity. As the extract concentrations rose, the scavenging activity progressively increased. Each extracts capacity to scavenge radicals was evaluated at concentrations of 0.625, 1.250, 2.500, 5.000 and 10.000 mg/ml. Vitamin C and BHT served as controls. According to Table (4) the ethanolic extracts free radical scavenging activity was 95. 70 $\pm$ 0. 02 in a 10 mg/ml. This was compared to the natural antioxidant vitamin C which was 97. 32 $\pm$ 0. 02 and the artificial antioxidant BHT which was 92. 12 $\pm$ 0. 01. An increase in phenolic compound content was clearly associated with a higher scavenging effect of the extract according to numerous studies that examined the relationship between antioxidant activity and phenolic compound content (Dairi *et al* 2017).

Table 4. Radical scavenging activity of *Tecoma stans*

Concentration (mg/ml)	Mean $\pm$ SD			
	<i>Tecoma stans</i>	Vit. C	BHT	LSD value
10.000	95.70 $\pm$ 0.02	97.32 $\pm$ 0.02	92.12 $\pm$ 0.01	0.047**
5.000	91.87 $\pm$ 0.02	94.17 $\pm$ 0.03	87.56 $\pm$ 0.03	0.077**
2.500	76.35 $\pm$ 0.02	90.43 $\pm$ 0.02	69.60 $\pm$ 0.47	0.830**
1.250	68.59 $\pm$ 0.12	86.44 $\pm$ 0.05	57.45 $\pm$ 0.04	0.228**
0.625	45.11 $\pm$ 0.06	80.07 $\pm$ 0.06	34.75 $\pm$ 0.04	0.170**
LSD value	0.156**	0.101**	0.553**	----
** (P $\leq$ 0.01).				

In the present study, the ethanolic extract of *Tecoma stans* demonstrated a notable

antioxidant activity, as reflected by its EC50 value of 0.55 mg/ml Figure (9).

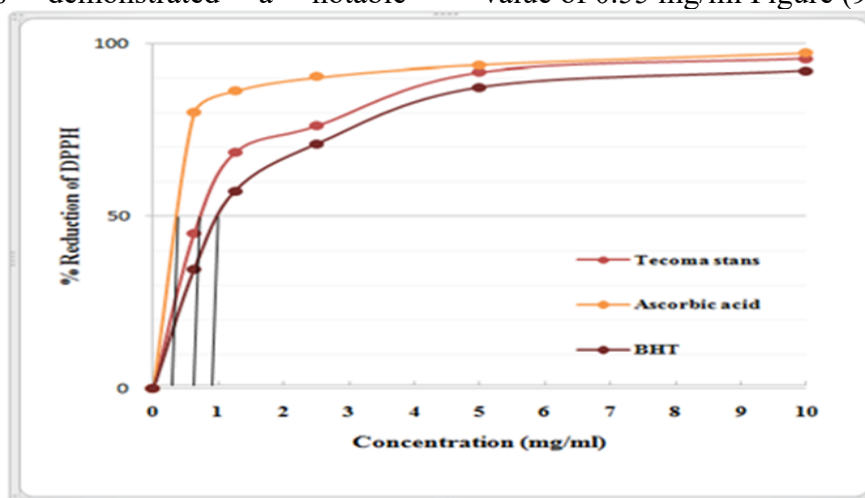


Figure 9. EC50 of *Tecoma stans*

The EC50, which represents the concentration required to achieve 50% of the maximal

scavenging effect, is a widely accepted indicator of antioxidant potency (Lee, 2020).

The inverse relationship between EC50 values and antioxidant capacity implies that lower EC50 values correspond to stronger antioxidant effects. These findings are consistent with previous research by (Salem *et al.* 2013), who reported that ethanolic extracts of the aerial parts of *T. stans* exhibited significantly higher antioxidant activity compared to methanolic and acetone extracts, using the DPPH assay as a radical scavenging indicator. This suggests that the ethanol-based extraction method may be more effective in

isolating bioactive compounds with antioxidant properties from *T. stans*.

Antibacterial activity: The results shown in Table (5) indicate that the ethanolic extract of *Tecoma stans* leaves, exhibited clear antibacterial activity against multidrug-resistant *Pseudomonas aeruginosa* isolates. All tested isolates showed inhibitory responses at concentrations of 256 and 128 mg/mL, with inhibition zones ranging from (21.67 ± 0.4235 mm) to (12.33 ± 0.4235 mm), and statistically significant differences at P<0.001.

Table 5. Antibacterial activity of *Tecoma stans* extract on *P. aeruginosa* isolates

Isolate	Concentration 256	Concentration 128	LSD Value	Significance
P1	19.330±0.4235	16.000±0.4235	0.5329	**
P2	16.670±0.4235	13.330±0.00	0.5329	**
P3	15.330±0.4235	12.330±0.4235	0.5329	**
P4	17.330±0.4235	14.670±0.4235	0.5329	**
P5	18.000±0.00	16.670±0.4235	0.5329	**
P6	20.670±0.4235	18.000±0.4235	0.5329	**
P7	17.330±0.4235	14.000±0.4235	0.5329	**
P8	19.000±0.4235	15.330±0.4235	0.5329	**
P9	17.000±0.4235	13.670±0.4235	0.5329	**
P10	21.670±0.4235	17.330±0.4235	0.5329	**
LSD_Value	1.258	1.258	---	**
**(P<0.001)				

This activity is likely due to the presence of bioactive phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids in the leaves of *Tecoma stans*. Ethanol is a suitable solvent for extracting both polar and non-polar compounds, thus enhancing the extract's antimicrobial potential. The findings of the present study are consistent with those reported by Bakr *et al.* 2019, who demonstrated the antimicrobial activity of the methanolic extract of *Tecoma stans*. Similarly, the results align with the study conducted by Javid *et al.* (2015) which showed the antibacterial potential of *Tecoma stans* extracts against *Pseudomonas aeruginosa*, and they noticed that different solvent different solvent fractions such as chloroform, butanol, and ethyl acetate exhibited notable inhibitory zones ranging from 15 to 20 mm. Although the solvents used differed from those employed in the current study, both investigations confirm the antibacterial efficacy of *Tecoma stans* against *P. aeruginosa*. This suggests that the plant harbors a wide range of bioactive compounds with antimicrobial properties that

can be extracted using solvents of varying polarity.

CONCLUSION

The present research shows the potential of *Tecoma stans* leaf extract as a natural source of phenolic compounds with strong antioxidant properties. The extract also demonstrated significant antibacterial activity against multidrug-resistant *Pseudomonas aeruginosa*, suggesting its promise as a natural therapeutic alternative. These results support further investigation into plant-based treatments as a sustainable approach to combat antibiotic-resistant pathogens.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original. Any Figures or images adopted from other sources were used

with the necessary permissions for re-publication.

All authors have approved the final version of the manuscript.

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AUTHOR'S CONTRIBUTION STATEMENT

All authors contributed to the study conception, data collection, laboratory analysis, interpretation of results, manuscript writing, and final approval of the submitted version.

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الإمكانات المضادة للبكتيريا ومضادات الأكسدة لمستخلص الإيثانول من أوراق نبات تيكوما (*Tecoma stans* (L.)

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المستخلص

يهدف البحث الى تقييم المحتوى الفينولي الكلي والنشاط المضاد للبكتيريا والنشاط المضاد للأكسدة لمستخلص اوراق *Tecoma stans* جمعت أوراق التيكوما من كلية الطب البيطري، جامعة بغداد، وحضر المستخلص الإيثانولي باستخدام التحريك المغناطيسي. كشفت الفحوصات الكيميائية النباتية عن وجود مركبات فعالة مثل الفلافونويدات، الفينولات، القلويدات، التانينات، الصابونينات، والجليكوسيدات. تم تقييم النشاط المضاد للأكسدة باستخدام اختبار DPPH، وقياس المحتوى الكلي من الفينولات، حيث أظهر المستخلص نشاطاً مرتفعاً يزداد بزيادة التركيز. حددت ستة مركبات فينولية رئيسية باستخدام HPLC، شملت كيرسيتين، حمض الغاليك، حمض الكافيين، الأبيجينين، حمض الفيروليك، والروتين. كما أظهر المستخلص فعالية مضادة لبكتيريا *Pseudomonas aeruginosa* المقاومة للأدوية المتعددة عند تركيزي 128 و 256 مغم/مل، حيث تراوحت مناطق التثبيط بين (0.42 ± 12.33) و (0.42 ± 21.67) ملم، مما يشير إلى إمكانية استخدامه كمضاد بكتيري طبيعي واعد.

الكلمات المفتاحية: القلويدات ، الفصيلة البجنونية ، الجذور الحرة.