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Phytochemical Profile, Antioxidant and Antitumor Evaluation of *Tecoma stans* Leaves and Flower Extracts

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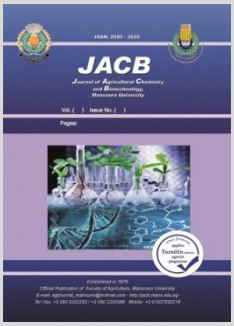

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ABSTRACT

This study examines the chemical profile, antioxidant, and antitumor activities of *Tecoma stans* extracts. The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent and ranged from 23.07±0.32 to 558.48±0.34 (mg GAE)/g dry weight. The total flavonoid content (TFC) ranged from 12.61±0.38 to 162.68±0.35 (mg QE)/g D.W. A total of 19 phenolic compounds, including 11 phenolic acids and 8 flavonoids, were identified and quantified using HPLC in *Tecoma stans* MeOH extract. Coumaric acid had the highest phenolic compound value of 8270.03 µg g^{-1} in *Tecoma stans* leaves and 1158.93 µg g-1 in the flower MeOH extract. Naringenin was the main flavonoid in Tecoma stans MeOH extract from leaves (2365.46 µg g-1) and flowers (928.35 µg g-1). A total of 18 phenolic compounds, including 11 phenolic acids and 7 flavonoids, were identified and quantified using high-performance liquid chromatography (HPLC) in Tecoma stans EtOAc extract. Vanillic acid had the highest phenolic compound value of 5379.38 µg g-1 in Tecoma stans leaves MeOH extract. Rutin was the highest flavonoid in Tecoma stans EtOAc extract, with values of 1235.70 µg g-1 for leaves and 1704.86 µg g-1 for flowers. EtoAc extract from *Tecoma stans* leaves and flowers exhibited superior antioxidant activity compared to its MeOH, MC, and Butanol fractions. EtOAc extract showed significant cytotoxic effects against (MCF7) and (HePG2) cell lines at concentrations ranging from 50 to 400 µg mL-1. These results indicate that *Tecoma stans* plants are promising natural sources of bioactive compounds with potential antioxidant and antitumor properties.

Keywords: *Tecoma stans* extracts, Polyphenols, antioxidant and antitumor activity

INTRODUCTION

Medicinal plants are essential for their various therapeutic properties and have been utilized for centuries for their medicinal benefits. These plants contain a wide range of chemical compounds that serve different functions, including protection against insects and fungi. They are also known for their antioxidant, antifungal, antitumor, and antidiabetic properties (Mohamed *et al.,* 2025). This study specifically examines the medicinal properties of *Tecoma stans* plant.

*T*ecoma stans, also known as yellow elder, is an erect shrub or small tree in the Bignoniaceae family. This plant has been traditionally used in herbal medicine for treating diabetes and digestive concerns. Extracts from *Tecoma stans* leaves have shown inhibitory effects on yeast infections. Research by Marzouk *et al.,* (2006) has investigated the plant's anticancer and antioxidant properties. Alanso-Castro *et al.,* (2010) reported that *Tecoma stans* extracts have antidiabetic activity. Compounds found in the fruits and flowers of *Tecoma stans* have demonstrated antioxidant and anti-proliferative effects on cancer cell lines (Marzouk *et al.,* 2006). Natural antioxidants are crucial in determining the pharmaceutical potential of plants and their efficacy in combating chronic diseases.

Most human disorders are caused by free radicals, which are molecular fragments with unpaired electrons in their outer shell. Free radicals have both harmful and beneficial effects in the body. Antioxidants are substances that can neutralize free radicals by donating hydrogen or electrons to stop chain reactions, thereby preventing chronic diseases like cancer. There is increasing interest in natural bioactive compounds that can act as antioxidants in our diet or as alternatives to synthetic compounds (Zari *et al*., 2021). This study aims to (1) Identify phytochemical compounds in methanolic extracts of leaves and flowers and their fractions, (2) Assess the in vitro antioxidant properties using three different methods, and (3) Evaluate the antitumor activity against breast carcinoma (MCF7) and hepatocellular carcinoma (HePG2) cell lines.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals and Reagents were analytical grade and purchased from Sigma – Aldrich company.

Collecting, preserving plant samples and extraction methods.

Fresh leaves and flowers of *Tecoma Stans* were collected from trees on the campus of Mansoura University. They were cleaned, cut, and dried before being ground into a powder. Approximately 1.5 kilograms of powdered leaves and 1.5 kilograms of powdered flowers were extracted using 20 liters of methanol as a solvent. The maceration method in methanol was used, to extract the bioactive compounds of leaves and flowers for 72 hours, with the solvent changed every 24 hours. The methanolic solvent was then evaporated using a rotary evaporator, resulting in a crude methanol extract. This extract was further divided, and solvents of varying polarity, including petroleum ether, methylene chloride, ethyl acetate, and butanol, were successively used to obtain corresponding fractions.

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Preliminary Phytochemical screening of *Tecoma Stans*.

We conducted phytochemical screening on crude methanolic extracts and their fractions to detect the presence of biologically active compounds.

Detection of alkaloids, flavonoids and saponins.

Demonstrated the presence of alkaloids, flavonoids, saponins, tannins and terpenes in leaves and flower of *Tecoma stance* was carried out as described by Arefin *et al*., (2015).

Detection of Glycosides.

Molisch's reagent was used to detect occurrence of glycosides according the described method by Ashtalakshmi and Prabhakaran, (2015).

Determination of total polyphenol content of *Tecoma Stans*.

The total polyphenol content was determined using the Folin reagent method, following the procedure described by Limmongkon *et al*., (2017).

Determination of total flavonoid content of *Tecoma Stans*.

The flavonoid content was determined using the Aluminum chloride colorimetric method based on the method described by Munhoz *et al*., (2014).

HPLC analysis of methanolic and ethyl acetate extracts of *Tecoma Stans* (leaves and flowers).

HPLC analysis was performed using an Agilent 1260 series with an Eclipse C_{18} column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 ml/min. A linear gradient program was used for the mobile phase: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A); 16-20 min (82% A). Detection was performed at 280 nm using a multi-wavelength detector. The injection volume for each sample was 5 µl, and the column temperature was maintained at 40 °C.

Antioxidant activities

For the antioxidant assays, all fractions were dissolved in 95% methanol at a concentration of 50 mg/100 mL. A series of concentration-dependent dilutions were then prepared. Standard chemicals were used for comparison in all antioxidant assays.

DPPH free radical scavenging activity

The ability of a compound to donate a hydrogen atom was assessed by measuring its scavenging activity against the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, following a procedure based on Lim and Quah (2007).

Reducing power assay

The antioxidant activity of the samples was evaluated by measuring the reduction of Fe^{+3} to Fe^{+2} ions (Debnath *et al.,* 2011).

Total antioxidant capacity

The total antioxidant capacity of all tested extracts and their fractions was determined using the phosphomolybdate method (Umamaheswari *et al*., 2007).

Cytotoxicity against HePG2 and MCF7 human cancer cell lines

The methanolic extracts as well as their EtoAc fractions were prepared by dissolving them in a 1:1 (stock solution) and stored at -20°C in dimethyl sulfoxide (DMSO). Different concentrations of the drug were tested within a range of 50-400 µg/ml. Cytotoxicity against HePG2 and MCF7 cell lines were evaluated using the Sulphorhodamine-B (SRB) staining assay, as outlined by Skehan (1990).

Statistical analysis

The research was conducted using a completely randomized design (CRD) with three replications. Data was analyzed using analysis of variance (ANOVA) and presented as mean ± standard deviation with CoStat Ver. 6.400 software. Mean values were compared using Duncan's multiple range test (DMRT) at a significance level of $P<0.05$. Pearson correlation was employed to assess the relationship between phytochemical compounds and antioxidant assays.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening of *Tecoma Stans* of different extracts.

Phytochemicals are biologically active compounds generally produced by plants to protect against diseases. The results are listed in Table 1 demonstrates that preliminary qualitative phytochemical analysis revealed that leaves and flowers extracts of *Tecoma Stans*, such as methanol and methylene chloride contained high levels of active compounds such as flavonoids, alkaloids, tannins, saponins and terpenes with varying compound presence across different extracts. In a study conducted by Anburaj *et al.,* (2016) and Bakr *et al.,* (2019), they have identified and isolated approximately 120 compounds from the plant *Tecoma stans*. These compounds include monoterpene, alkaloids, phenolic acids, flavonoids, carotenoids, terpenoids, glycosides, phytosterols, volatile oils, and unsaturated fatty acids.

Table 1. Preliminary Phytochemical screening the different extracts of *Tecoma stans* leaves and Flowers.

Plant part	Extract	Terpenes	Alkaloids	Flavonoids	Tannins	Saponins	Glycosides
leaves	Me-OH	+	++	+++	++	++	++
	Pet. ether	+++	++	+	-	+++	+
	MC	+++	+++	+++	++	+	+
	EtoAc	+++	+	+	++	-	+
	Bu-OH	-	-	+	-	+	+
Flowers	Me-OH	+++	+	++	++	++	++
	Pet. ether	+	+	++	++	+	+
	MC	+++	+	++	+++	++	++
	EtoAc	+++	+	+	+++	+++	++
	Bu-OH	+	+	++	-	-	+

Me-OH = methanolic extract; Pet. ether = petroleum ether fraction; MC = methylene chloride fraction; EtoAc = ethyl acetate fraction; Bu-OH = butanol fraction.

Also, Taher *et al.,* (2016), recorded the presence of monoterpenes, alkaloids, phenolic acids, flavonoids, and fatty acids as the key bioactive components responsible for the therapeutic benefits of the plant. Mathiyazhagan *et al.,* (2023) conducted a phytochemical analysis of bark and flower extracts of *T. Stans* using different solvents (petroleum ether, chloroform, and ethyl acetate). The results indicated that the ethyl acetate extracts of both bark and flower contained a

higher concentration of phytochemicals, including alkaloids, tannins, phenolic compounds, flavonoids, terpenoids, saponins, steroids, and anthraquinones.

Total polyphenol and flavonoid content.

The total polyphenols and flavonoids content in various plants reflect their potential biological activities. Plants rich in flavonoids and polyphenols are known to possess beneficial properties. Polyphenols are classified into different groups of phenolic compounds, with flavonoids comprising a significant portion. Flavonoids include subclasses such as flavones, isoflavones, flavanols, flavanones, and flavans.

Total polyphenols content

In this study, the total polyphenol content of the phenolic compounds in extracts obtained using different polar solvents showed significant differences ($p<0.05$). The highest content of phenolic compounds was found in the ethyl acetate fraction of green leaves (558.48 mg GAE/g D.W.), followed by flowers ethyl acetate fraction (444.33 mg GAE/g D.W.). The lowest content was observed in the Pet.ether fraction of the leaves and followed by the corresponding one in the flowers as 23.07 and 41.18 mg GAE/g D.W., respectively. These findings are consistent with a study conducted by Ganapathy et al., (2015), which confirmed that the methanolic extract of Tecoma leaves had high phenolic content. However, the results obtained in our research also show higher value, for the methanol extract recording 433.89±0.13 mg GAE/g.D. W and as well as brilliant total polyphenolic content for its ethyl acetate fraction recording 558.48 ±0.34 mg GAE/g.D.W.

Table 2. Total polyphenols content of crude extracts of *Tecoma stans* and their fractions.

Extract /fraction	Total polyphenols (mg GAE/g.D. W)	
	Leaves	Flowers
Me-OH	433.89 ±0.13	358.68 ±0.63
Pet. ether	23.07±0.32	41.18 ±0.15
MC	377.26 ±0.58	307.08±0.08
EtoAc	558.48 ±0.34	444.33 ±0.38
Bu-OH	209.75 ±0.30	251.84 ±0.10

ME-OH = methanolic extract; Pet. ether = petroleum ether fraction; MC = methylene chloride fraction; EtoAc = ethyl acetate fraction; Bu-OH = butanol fraction

On the other hand, Govindppa *et al.,* (2011), analyzed the total polyphenolic contents of three solvent fractions of *Tecoma stans* leaves. The aqueous, ethanolic, and methanolic extracts were found to contain values of 177.12, 206.09, and 216.1 mg gallic acid equivalents /g, respectively.

Additionally, Taher *et al.,* (2016) reported the total polyphenol content in the crude extract and its fractions as follows: methanolic extract (230.3 mg GAE/g. D. W), methylene chloride fraction (102.49 mg GAE/g. D. W) ethyl acetate fraction (279.41 mg GAE/g. D. W), and butanol fraction (232.0 mg GAE/g. D. W).

Total flavonoids contents

The total flavonoid contents varied significantly ($p<0.05$) among the tested extracts and fractions, it could be noticed from Table (3), that leaves extract and its fractions of Tecoma stans had higher values of total flavonoids than the corresponding samples of the flowers. The highest flavonoid values found in leaves ethyl acetate fraction (162.68 mg QE/g D.W) followed by the crude methanolic extract of the same part of the plant (138.95 mg QE/g D.W). Likewise, ethyl acetate fraction and the crude extract recorded the highest total flavonoid contents in flowers as 87.59 and 46.13 mg QE/g D.W, respectively. While pet ether fractions of flowers and leaves recorded the lowest total flavonoid contents as 12.61 and 45.88 mg QE/g D.W, respectively. On the other hand, Ganapathy et al., (2015) reported higher results compared to ours, confirming that the methanolic extract of the leaf is the richest in flavonoids, with a value of 324.80 mg QE/g. Additionally, the ethyl acetate extract of the leaf recorded 189.51 mg QE/g.

Table 3. Total flavonoids contents of crude extracts of *Tecoma stans* and their fractions.

Extract /fraction	Total flavonoids (mg QE/g D.W.)	
	Leaves	Flowers
Me-OH	138.95±0.32	46.13 ±0.08
Pet. ether	45.88 ±0.55	12.61 ±0.38
MC	110.68±0.31	35.37 ±0.57
EtoAc	162.68 ±0.35	87.59 ±0.32
Bu-OH	64.75 ±0.57	28.4±0.32

ME-OH = methanolic extract; Pet. ether = petroleum ether fraction; MC =methylene chloride fraction; EtoAc = ethyl acetate fraction; Bu-OH = butanol fraction.

Our results agreement with Gonçalves et al., (2022) who analyzed the total flavonoid content in ethanol extracts and fractions obtained from T. stans flowers. The dichloromethane and ethyl acetate fractions showed the highest levels of phenolic compounds, with the dichloromethane fraction also containing significant amounts of flavonoids. The total flavonoid content ranged from 6.00 to 25.20 mg QE/mg.

Also, Taher *et al*., (2016) found that the total flavonoid content varied in different extracts: methanolic extract had 51.91 mg QE/mg, methylene chloride had 39.21 mg QE/mg, ethyl acetate had 59.91 mg QE/mg, and butanol had 45.75 mg QE/mg.

Generally, the differences in reported total flavonoid and polyphenol contents in the literature can be attributed to some factors such as the plant part used, growing conditions, plant age at harvest, solvent choice, and extraction method (Deng *et al.,* 2014).

Identification and quantification of Phenolic and flavonoid compounds using HPLC technique.

HPLC analysis of *Tecoma stans* Methanolic extract.

The HPLC technique was utilized to analyze phenolic and flavonoid compounds in methanolic extracts of flowers and leaves, as well as their EtOAc fractions, for qualitative and quantitative purposes. The methanolic extract of *Tecoma* plant leaves and flowers contains 19 compounds derived from hydroxybenzoic acid, phenols, and flavonoids. Coumaric acid was the most abundant compound in both leaves (8270.03 µg g-1) and flowers (1158.93 µg g-1).

Vanillic acid was not detected in either flowers or leaves (Table 4). The levels of ferulic acid, gallic acid, and naringenin in leaves methanolic extract ranged between 1052.08- 2628.51 µg g-1. Considerable amount of rutin was found in methanolic extract of both leaves and flowers as 844.35 and 928.35, respectively. While in the study by Taher et al., (2016), they found that rutin was the predominant polyphenol in tecoma stans leaves in amount of 112.7 mg / 100 g D.W. Additionally, Abisha et al., (2020) identified different phenolic in tecoma stans such as caffeic, chlorogenic, vanillic, o-ceramics, and sinapic acids as well as other primary and secondary plant metabolites including sugars such as fructose, glucose, sucrose, and xylose, triterpenoids like ursolic and oleanolic acids, α-amyrine, β-sitosterol.

Table 4. HPLC analysis of *Tecoma Stans* methanolic extract.

Compound	Chemical class	Concentration (µg g^{-1})	
		Leaves	Flowers
Cinnamic acid	Hydroxybenzoic acids	37.40	7.18
Chlorogenic acid	Hydroxycinnamic acids	336.61	247.91
Vanillic acid	Hydroxybenzoic acids	0.00	0.00
Caffeic acid	Hydroxycinnamic acids	435.13	58.33
Syringic acid	Hydroxybenzoic acids	26.46	193.42
Coumaric acid	Hydroxycinnamic acids	8270.03	1158.93
Ferulic acid	Hydroxycinnamic acids	2628.51	687.83
Gallic acid	Hydroxybenzoic acids	1052.08	776.05
Ellagic acid	Hexahydroxydiphenlic acids	0.00	198.32
Methyl gallate	Ester of phenolic acids	80.49	34.76
Catechol	Hydroxylated phenols	2.63	176.39
kaempferol	Flavonols	178.98	520.60
Quercitin	Flavonols	91.00	811.00
Apigenin	Flavones	0.00	44.26
Hesperetin	Flavanones	151.00	0.00
Naringenin	Flavanones	2365.43	693.98
Rutin	Flavonol glycoside	844.35	928.35
Catechin	Flavanols	153.49	28.44
Daidzein	Isoflavonoids	103.07	0.00

HPLC analysis of EtoAc fraction of Tecoma stans.

The analysis of ETOAC extract from *Tecoma* plant leaves and flowers, as shown in table 5, revealed 18 compounds, including derivatives of hydroxybenzoic acid, phenols, and flavonoids. Gallic acid was the most abundant compound in the leaves EtOAc, with a concentration of 2957.62 µg g^{-1}, while chlorogenic acid dominated in the flowers EtOAc fraction at 3375.03 µg g^{-1}. Both EtOAc fractions devoid pyrocatechol.

Table 5. HPLC analysis of *Tecoma stans* EtoAc fraction

Compound	Chemical class	Concentration (µg g^{-1})	
		Leaves	Flowers
Cinnamic acid	Hydroxybenzoic acids	37.40	7.18
Chlorogenic acid	Hydroxycinnamic acids	2016.13	3375.03
Vanillic acid	Hydroxybenzoic acids	0.00	0.00
Caffeic acid	Hydroxycinnamic acids	2363.62	58.33
Syringic acid	Hydroxybenzoic acids	363.68	2422.13
Coumaric acid	Hydroxycinnamic acids	980.66	287.23
Ferulic acid	Hydroxycinnamic acids	557.18	239.64
Gallic acid	Hydroxybenzoic acids	2957.62	2725.28
Ellagic acid	Hexahydroxydiphenlic acids	589.63	11.63
Methyl gallate	Ester of phenolic acids	596.27	563.97
kaempferol	Flavonols	64.69	8.01
Quercitin	Flavonols	505.37	796.23
Pyro catechol	Hydroxylated phenols	0.00	0.00
Hesperetin	Flavanones	58.80	120.88
Naringenin	Flavanones	573.99	460.71
Rutin	Flavonol glycoside	1235.70	1704.86
Catechin	Flavanols	210.01	1749.61
Daidzein	Isoflavonoids	240.50	162.48

It was observed that certain bioactive molecules detected by HPLC in the EtoAc fraction were present in higher amounts compared to the crude methanol extract. Variations in the ratio of bioactive compounds and the presence or absence of specific compounds in Methanol extract and EtoAc fraction can be attributed to factors such as growing conditions, maturity at harvest, plant part used, solvent type, and extraction method (Deng *et al.,* 2014).

Biological activity:

This section will explore the various biological activities of Tecoma stans, including antioxidant, cytotoxic, anti-inflammatory, and apoptosis activities.

Antioxidant activity.

Various methods and mechanisms are commonly employed to assess antioxidant activity. Oxidation involves the transfer of electrons from one substance to an oxidizing agent. The main mechanisms for antioxidant activity include radical scavenging, which can be evaluated by DPPH or estimating reductive capacity by reducing power and total antioxidant capacity assays to determine the effectiveness of samples under investigation.

Reducing power assay

An antioxidant is a molecule that can prevent the oxidation of reactive oxygen species (ROS) in the body, which can damage cells. Various methods are used to determine the antioxidant activity of plants and their components. The key mechanisms include radical scavenging, metal chelation, and reduction activity (Sokamte *et al.,* 2019). Three common methods for evaluating antioxidant properties are the reducing power assay, total antioxidant capacity, and DPPH radical scavenging assay. These methods were utilized to assess the antioxidant properties of *Tecoma stans* extracts. The reducing power assay is a primary test that classifies components as electron donors, making them potential antioxidants (Glucin, 2015).

This assay relies on the reduction of the ferric ferricyanide complex to ferrous in the presence of antioxidants (Bajpai *et al*., 2017). Table 6 demonstrates that all tested extracts displayed varying degrees of reducing power, which increased with higher concentrations. The results, measured as absorbance values at 700 nm, revealed that leaves EtoAc fraction at 4000 µg/g^{-1} exhibited the highest reducing power, with the highest absorbance value (3.800) followed by the equivalent fraction in *Tecoma stans* flowers (3.741). In contrast, the Pet. ether showed the lowest reducing power, with values of 1.683 for *Tecoma stans* leaves and 1.250 for *Tecoma stans* flowers, at 4000 µg/g^{-1}. The lower reducing power of the pet. ether extract compared to the EtoAc extract may be due to the lower levels of hydrophilic phenolic compounds such as gallic acid, caffeic acid, ferulic acid, and p-coumaric acid, which are more readily available in the ferric reduction system (Barapatre *et al.,* 2016).

Table 6. Reducing power assay of *Tecoma stans* fractions

Plant	Extract	Concentration (µg g^{-1})						
		62.5	125	250	500	1000	2000	4000
Leaves	Me-OH	0.240 ± 0.001	0.412±0.001	0.698±.001	0.971 ±0.001	1.017±0.001	1.915±0.002	2.815±0.002
	Pet.ether	0.098 ± 0.001	0.217±0.001	0.453±.001	0.546 ±0.001	0.882±0.001	1.111±0.001	1.683±0.001
	MC	0.130±0.001	0.238±0.001	0.469±0.001	0.738 ±0.001	0.979±0.001	1.525±0.001	2.106±0.002
	EtoAc	0.618 ± 0.001	0.911±0.002	1.021±0.001	1.994±0.002	2.401±0.001	2.911±0.001	3.800±0.001
	Bu-OH	0.115 ± 0.001	0.221±0.001	0.415±0.002	0.669 ±0.002	0.891±0.002	1.21±0.002	2.011±0.001
Flowers	Me-OH	0.299±0.002	0.484±0.001	0.769±0.001	0.995 ±0.001	1.691±0.001	2.221±0.001	3.014±0.002
	Pet.ether	0.191±0.001	0.298±0.001	0.585±0.002	0.897 ±0.001	1.002±0.002	1.098±0.002	1.250±0.001
	MC	0.211±0.001	0.412±0.001	0.679±0.001	0.984 ±0.002	1.047±0.001	1.915±0.001	2.122±0.001
	EtoAc	0.302±0.001	0.579±0.001	0.849±0.003	1.002 ±.002	1.729±0.002	2.319±0.002	3.741±0.001
	Bu-OH	0.234±0.001	0.397±0.001	0.611±0.002	0.832 ±0.001	1.012±0.002	1.887±0.001	2.113±0.002

ME-OH = methanolic extract; Pet.ether = petroleum ether fraction; MC = methylene chloride fraction; EtoAc = ethyl acetate fraction; Bu-OH = butanol fraction. All values represent mean ± standard error (SE). Different letters in the same column are significantly different according to Tukey's HSD test at P< 0.05 for each concentration point.

Total antioxidant capacity (TAC)

Total antioxidant capacity is a reliable method for assessing the overall antioxidant activity of plant extracts. It measures the ability of antioxidants in the extracts to reduce phosphate-molybdenum (VI) to a green molybdenum complex (V). The results are compared to ascorbic acid, a reference antioxidant, and expressed as dry extract mgAAE/g. The results from the table 7 indicate that the antioxidant capacity of leaves ethyl acetate fraction was the highest as 442.13 mg AAE/g dry fraction followed by flowers ethyl acetate fraction with the value of 394.91 mg AAE/g dry fraction. In contrast, the Pet.ether fraction from both the leaves and flowers had the lowest values of 101.02 mg AAE/g dry extract and 119.02 mg AAE/g dry extract, respectively.

Table 7. Total antioxidant capacity of various extracts on Tecoma Stans using a concentration of 500µg/ml.

Extract / Fraction	Concentration (mg AAE/g)	
	leaves	Flowers
Me-OH	281.02	255.47
Pet.ether	101.02	119.02
MC	245.75	272.41
EtoAc	442.13	394.91
Bu-OH	201.30	183.52

***Expressed as mg AAE/g dry extract**

DPPH radical scavenging assay.

The radical scavenging activity of BHT and the samples under investigation was assessed using the DPPH radical scavenging assay. This assay is commonly used to evaluate antioxidant properties due to its short testing time compared to other methods. The stable free radical DPPH· has a deep violet color that changes to yellow (DPPH-H) upon reduction, which is measured by a decrease in absorbance at 517nm (Barapatre *et al.,* 2016). The samples were tested for their ability to scavenge DPPH radicals, and the results were expressed as IC_{50} values. A lower IC_{50} value indicates higher antioxidant activity. Among the investigated samples, the ethyl acetate fraction from *Tecoma* leave (as shown in Table 8) exhibited the most potent DPPH radical scavenging activity with an IC_{50} value of 26.25 µg/ml, outperforming the standard antioxidant compound BHT, which had an IC_{50} value of 145.72 µg/ml.

Table 8. DPPH assay of various extracts of *Tecoma Stans* (µg/ml).

Plants	Conc	BHT	Me-OH	Pet. ether	MC	EtoAc	Bu-OH
leaves	5	6.19	12.49	5.22	9.10	13.92	6.16
	10	8.07	25.80	7.08	15.80	28.51	10.79
	20	12.10	33.20	14.44	26.77	42.17	16.33
	30	17.66	42.70	26.32	37.48	53.82	28.92
	40	23.18	56.89	36.81	50.60	69.34	44.18
	50	26.12	63.45	52.33	60.24	75.23	55.89
	IC50	145.72	35.87	48.86	39.85	26.25	44.51
Flowers	5	6.19	8.68	3.15	6.57	9.91	4.94
	10	8.07	23.64	10.96	15.61	24.50	12.57
	20	12.10	37.48	17.51	26.33	38.15	19.26
	30	17.66	46.99	26.07	38.67	49.80	27.70
	40	23.18	53.81	39.16	44.81	54.33	40.26
	50	26.12	60.67	51.04	56.92	65.08	53.67
	IC50	145.72	36.40	49.59	43.49	34.62	48.73

Additionally, the flowers also demonstrated significant radical scavenging activity. The EtOAc extract showed a radical scavenging activity of 34.62 µg/ml, while the Pet.ether extract from flowers had an IC50 value of 49.59 µg/ml, indicating weaker DPPH activity. The Pet.ether fraction from leaves exhibited a radical scavenging activity of 48.86 µg/ml.

In a previous study by Govindappa et al., (2011), different extracts of Tecoma stans, including ethanol, methanol, and water, demonstrated significant antioxidant effects through DPPH radical scavenging assays. Other studies have also reported on the antioxidant activity of T. stans. Mohamed et al. (2013), evaluated the DPPH free radical scavenging activity of the ethyl acetate fraction of T. stans leaves and branches at a concentration of 200 mg/mL, with IC50 values of 83.4 and 82.06, respectively. These results were higher than those observed in our study. The differences in antioxidant profiles among various plant extracts underscore the need for employing multiple antioxidant methods to carefully evaluate the antioxidant properties of phytochemical compounds. A comprehensive assessment of antioxidant capacity needs the use of diverse antioxidant assays (Gong et al., 2012).

EtoAc, and Bu-OH fractions of *Tecoma stans* were found to possess strong antioxidant properties, likely attributed to the presence of polyphenolic compounds such as flavonoids, tannins, phenolic acids. The antioxidant activity of these fractions was comparable to the standard BHT. These results support the traditional medicinal uses of *Tecoma stans* and suggest its potential as a source for developing antioxidant agents. The *in vitro* findings indicate that this medicinal plant shows promise as a potential source of novel antioxidant drugs (Govindappa *et al.,*2011).

Cytotoxic effect of Tecoma Stans extract

Cancer is a prevalent disease worldwide and a leading cause of death. Treatment options include chemotherapy, hormone therapy, biological therapy, and targeted therapy (American Cancer Society, 2011), leading to drug resistance, which is a major task in cancer treatment (Holohan *et al.,* 2013). Studies have shown that plant-derived compounds have clinical significance and can be developed into effective drugs against cancer. Bioactive compounds have garnered attention from researchers as a potential solution to the challenges associated with chemotherapy.

The sulforhodamine B (SRB) assay is used to determine cell density by measuring cellular protein content. This method has been optimized for toxicity screening of compounds on adherent cells in a 96-well format (Skehan *et al.,* 1990). The crude methanolic extract and EtoAc fraction of Tecoma stans leaves and flowers showed significant cytotoxic effects at concentrations ranging from 50 to 400 µg mL^{-1}, exhibiting strong inhibitory activity against hepatocellular carcinoma (HePG2) and breast carcinoma (MCF7), (Table 9 and 10). The leaves methanolic extract of *T. stans* leaves and flowers exhibited dose-dependent cytotoxicity against HePG2 and MCF7 cell lines, with cytotoxicity levels reaching up to 56.3% and 58,5 %, respectively. In the same manner, flowers methanolic extract showed cytotoxicity percentages of 58.6 and 60.2 for HePG2 and MCF7, respectively. The IC_{50} values of leaves methanolic extract against HePG2 and MCF7 were determined to be 196.8 µg mL^{-1}, and 193.00 µg mL^{-1}, respectively (Table 9). Also, flowers methanolic extract showed comparable IC_{50} values against HePG2 (182.4µg mL^{-1}) and MCF7 (175.0 µg mL^{-1}). Thus, flavonoid-enriched extracts demonstrated dose-dependent cytotoxicity on HePG2 and MCF7 cell lines. The flavonoid content can

induce apoptosis by activating endonuclease enzymes, causing double-strand breaks in cancer cell DNA, leading to DNA fragmentation and cell death. Additionally, necrosis-based cell death may occur due to ATP depletion, hypoxia, and other factors, resulting in cell inflammation and death (Neerugatti et al., 2016)

Similarly, both ethyl acetate fractions of *T. stans* exhibited significant cytotoxicity against HePG2 and MCF-7 cell lines in concentration dependent manner (Table 10).

Among all tested samples, flowers ethyl acetate fractions excitingly showed the highest antitumor activity with lowest IC_{50} values of 95.4 and 90.5 μgmL^{-1} against HePG2 and MCF-7 cell lines, respectively. While, leaves ethyl acetate fraction showed IC_{50} values comparable to those of both tested methanolic extracts against HePG2 (184 μgmL^{-1}) and MCF-7 (170.6 μgmL^{-1}), (Table 10).

Table 9. Cytotoxicity activity of *Tecoma stans* MeOH extract on HePG2 and McF7 cell line by SRB assay.

Plant	Concentration (µg /ml)	HEPG2	MCF7
Tecoma stans Leaves	50	30.6 ± 0.34	37.5 ± 0.28
	100	43.5 ± 0.28	48.2 ± 0.69
	200	50.8 ± 0.39	51.8 ± 0.53
	400	56.3 ± 0.63	58.5 ± 0.95
	IC_{50}	196.8	193
Tecoma stans flowers	50	28.6 ± 0.17	31.9 ± 0.26
	100	41.8 ± 0.24	40.8 ± 0.58
	200	54.8 ± 0.67	57.1 ± 0.63
	400	58.6 ± 0.82	60.2 ± 0.69
	IC_{50}	182.4	175

Table 10. Cytotoxicity activity of *Tecoma stans* EtoAc extract on HePG2 and McF7 cell line by SRB assay.

Plant	Concentration (µg /ml)	HEPG2	MCF7
Tecoma stans Leaves	50	44.1 ± 0.51	41.2 ± 0.24
	100	49.9 ± 0.58	51.4 ± 0.28
	200	54.3 ± 0.32	58.6 ± 0.44
	400	62.2 ± 0.69	66.8 ± 0.75
	IC_{50}	184	170.6
Tecoma stans flowers	50	45.8 ± 0.29	48.9 ± 0.63
	100	52.4 ± 0.34	55.2 ± 0.41
	200	60.0 ± 0.54	63.4 ± 0.56
	400	70.2 ± 0.69	72.4 ± 0.88
	IC_{50}	95.4	90.5

Mathiyazhagan *et al.,* (2023) found that the ethyl acetate fraction of *T. stans* bark and flowers also showed cytotoxicity against the MCF-7 breast cancer cell line, with IC_{50} values of 208.50µgmL−1and207.38µgmL−1, respectively. The mechanism of action involves enhancing antioxidant capacity, inactivating carcinogens, inhibiting cell proliferation, inducing cell cycle arrest and apoptosis, and regulating immune responses (Hariharan and Dharmaraj, 2020).

The results of the SRB assay in Table 10 indicate that the Ethyl acetate extract of *Tecoma stans* flowers exhibits higher cytotoxic activity against HePG2 and MCF7 cancer cell lines compared to the methanolic extract of *Tecoma stans* flowers. These findings are consistent with previous reports that have demonstrated the superior cytotoxic activity of *Tecoma stans* (Jayachandran *et al.,* 2017). *Tecoma stans* MeOH and EtOAc extracts demonstrated significant dose-dependent inhibition of proliferation and viability in hepatocellular carcinoma (HePG2) and breast carcinoma (MCF7) cells, with the EtOAc extract showing higher cytotoxic activity than the MeOH extract.

Previous studies have shown the cytotoxic effects of *Tecoma stans* extracts, but the specific mechanisms underlying these effects are not fully understood. In this study, Methanolic extracts of *T. stans* obtained from leaves and flowers and their EtoAc fractions exhibited moderate cytotoxicity against HePG2 and MCF7 cells, with a dose-dependent increase in cytotoxicity.

CONCLUSION

Based on the previous analyses, it was found that Tecoma stans extracts have high levels of phenols and flavonoids, which contribute to their antioxidant and antitumor properties. The ethyl acetate (EtoAc) extract, followed by the methanol extract, contained significant amounts of active bioactive compounds.

These phytochemicals showed promising antioxidant activity by acting as effective radical scavengers and reducing agents, potentially responsible for the observed antioxidant effects. These results suggest the potential use Tecoma stans plants could be used as antioxidant agents. This study provides scientific evidence of the biological activity of methanolic and ethyl acetate extracts of *Tecoma stans*.

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الصورة الفيتو كيمائية ـ مضادات الأكسدة والنشاط المضادة للأورام في مستخلصات الأوراق والزهور لنبات التيكوما

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الملخص

تركز هذه الدراسة على تحليل الفينولات الفلافونيدات الكلية، ومضادات الأكسدة، والنشاط المضادة للأورام في مستخلصات الأوراق والزهور في نبات التيكوما. المحتوى الفينولي الكلي (TPC)؛ تم تحديده باستخدام كاشف Folin-Ciocalteu، وتراوحت نسبه المركبات الفينولية من ٠,٣٢ ± ٢٣,٠٧ إلى ٠,٣٤ ± ٥٥٨,٤٨ (ملجرام GAE) / جرام من الوزن الجاف. تراوح المحتوي الفلافونيدى الكلي (TFC) من ٠,٣٨ ± ١٢,٦١ إلى ٠,٣٥ ± ١٦٢,٦٨ (مجم QE) / جرام D.W. تم تحديد وتقدير إجمالى ١٩ مركبًا فينوليًا:١١ حمضًا فينوليًا و٨ فلافونيدات، باستخدام التحليل الكروماتوجرافي السائل عالي الأداء (HPLC). وكان لحمض الكيومريك أعلى قيمة للمركب الفينولي تبلغ 8270.03 ميكروجرام$^{-1}$ و1158.93 ميكروجرام$^{-1}$ في المستخلص الميثانولي للأوراق والزهور على الترتيب. وكان النارنجينين 2365.46 ميكروجرام$^{-1}$ هو الفلافونويد السائد في مستخلص الميثانول لأوراق التيكوما. تم تحديد وتقدير إجمالي ١٨ مركبًا فينوليًا و٧ فلافونيدات، باستخدام التحليل الكروماتوجرافي السائل عالي الأداء (HPLC) في مستخلص خلات الإيثايل. وسجل حمض الفانيلك أعلى قيمة للمركب الفينولي تبلغ5379.38 ميكروجرام$^{-1}$ وكان الروتين 1704.86 ميكروجرام$^{-1}$ هو الفلافونويد السائد في مستخلص خلات الإيثايل لزهور التيكوما. أظهر مستخلص خلات الإيثايل لنبات التيكوما أعلى كفاءة في جميع طرق مضادات الأكسدة التي تم اختبارها. لقد وجد أنه أكثر فعالية مقارنة بباقي المستخلصات MC وMeOH وEtoAc. كما أظهر مستخلص خلات الإيثايل لنباتات التيكوما تأثيرات سامة للخلايا بتركيزات تتراوح من ٥٠ إلى ٤٠٠ ميكروجرام مل$^{-1}$، خاصة ضد سرطان الثدي (MCF7)، وسرطان خلايا الكبد (HepG2). تشير هذه النتائج إلى أن نبات التيكوما التي تم تحليلها هي مصدر طبيعي للمركبات النشطة بيولوجيا مع خصائص مضادة للأكسدة ومضادة للأورام.