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Full Length Research Paper

Antioxidant and antibacterial activities of leaves and branches extracts of *Tecoma stans* (L.) Juss. ex Kunth against nine species of pathogenic bacteria

Mohamed Z.M. Salem[1], Yousry M. Gohar[2], L.M. Camacho[3], Nader A. El-Shanhorey[4] and A.Z.M. Salem[5*]

[1]Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Egypt.
[2]Botany Department, Microbiology Section, Faculty of Science (El-Shatby), Alexandria University, Alexandria, Egypt.
[3]Facultad de Medicina Veterinaria y Zootecnia, Carretera Altamirano – Iguala Km 3 CP 40660 Cd. Altamirano Guerrero, México.
[4]Department of Botanical Gardens Research, Horticultural Research Institute (ARC), Alexandria, Egypt.
[5]Facultad de Medicina Veterinaria, Universidad Autónoma del Estado de México, México.

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***Tecoma stans* of the Bignoniaceae family, is an important medicinal plant. Different extracts of the leaves and branches of *T. stans* were evaluated as antibacterial potential against the growth of some human bacterial strains using the disc diffusion and minimum inhibitory concentration (MIC) methods as well as the antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Total phenolic and total flavonoid contents were determined. The different extracts of *T. stans* were obtained by successive solvent extraction with methanol and its fractions; ethyl acetate (EtOAc), chloroform ($CHCl_3$), *n*-butanol and aqueous. The methanol, EtOAc and $CHCl_3$ extracts of *T. stans* showed significant effect against the tested bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Sarcina lutea*, *Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens*, *Salmonella typhi*, *Proteus vulgaris* and *Pseudomonas aeruginosa*). The methanol extract of the leaves exhibited the highest amount of total phenolics (50.3±3 mg gallic acid equivalents/g extract) and flavonoids (40.66±5.03 mg catechin equivalents/g extract). The EtOAc fraction of leaves and branches extracts showed the highest total antioxidant activity (%) with 83.4±0.31 and 82.06±0.54%, respectively followed by $CHCl_3$ fraction of leaves extracts (79.17±0.20%). These findings provide scientific evidence to support traditional medicinal uses of *T. stans* and indicate a promising potential for the development of an antibacterial and antioxidant agent from *T. stans* trees.**

Key words: Antioxidant activity, antibacterial activity, total phenolics, total flavonoids, *Tecoma stans*.

INTRODUCTION

Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae) trees are grown in North America and East Asia and wildly distributed in tropical and sub-tropical countries. It is a flowering perennial shrub or small tree; 5 to 7.6 m in height and grows to 25 ft. *T. stans* is not a toxic plant because it is used in Latin America as a remedy for diabetes and moreover for feeding cattle and goats in Mexico and are extensively employed in the Mexican traditional medicine (Susano-Hernández, 1981). Traditional uses of *T. stans* leaves throughout Mexico and central America for diabetes and urinary disorder

*Corresponding author. E-mail: asalem70@yahoo.com. Tel: 00521-722-296-55-42. Fax: 00521-722-180-61-94.

control have been reported (Shapiro and Gong, 2002). In addition, the flowers and barks are used traditionally for the treatment of various cancers as well as antimicrobial activity (Binuti and Lajubutu, 1994). The primary applications of *T. stans* have been found in treating diabetes and digestive problems (Kirtikar and Basu, 2006). In the study of Costantino et al. (2003), the flower infusion can be taken orally for diabetes and stomach pains. Amongst nine plants studied, *T. stans* was found to give the best of inhibition zones against the fungal activity (Gandhi and Ramesh, 2010).

The literature survey reveals that the genus *T. stans* possesses various bioactive compounds such as saponins, flavonoids, alkaloids, phenols, steroids, anthraquinones, tannins, terpenes, phytosterols, triterpenes, hydrocarbons, resins, volatile oil and glycosides (Raju et al., 2011; Binuti and Lajubutu, 1994). Recent studies found that *Tecoma* genus possess various bioactive compounds that are reported to exhibit various pharmacological activities such as antioxidant, antimicrobial and antifungal activities (Karou et al., 2006; Raju et al., 2011; Govindappa et al., 2011).

It was observed that the extracts of the stem bark generally showed better antimicrobial activity than those of the leaves and some organisms were selectively more sensitive to the extracts than others (Binuti and Lajubutu, 1994). On the other hand, the methanol extract of *T. stans* leaf was reported to possess significant wound healing property (Das et al., 2010). Additionally, the ethanol, methanol and water extracts of *T. stans* have been reported for good antimicrobial effects on some human pathogenic bacteria and antioxidant activity (Govindappa et al., 2011; Senthilkumar et al., 2010).

A new iridoid glucoside, 5-deoxystansioside ($C_{12}H_{24}O_8$, mp 146-47°C) has been isolated from *T. stans* leaves and its structure elucidated by the ^{13}CNMR and ^{1}HNMR spectroscopy (Bianco et al., 1981). The fresh leaves of *T. stans* have been found to contain chrysoeriol, luteolin and hyperoside (quercetin-3-O-beta-D-galoctoside) (Ramesh et al., 1986). The methanol extract of *T. stans* was studied for their antimicrobial activity using a wide range of Gram-positive and Gram-negative bacteria and fungi and the wound healing property of *T. stans* has been attributed to its antimicrobial effects (Binutu and Lajubutu, 1994). Additionally, the quantitative and qualitative analysis of phenolic compounds using UV absorption profile indicated the presence of simple phenolic (Chauhan et al., 2004).

However, studies for antioxidants and antibacterial like phenolic and flavonoids compounds and others are recently gaining more attentions. Therefore, the objective of this research was to analyze the effects of methanol extract and its fractions from leaves and branches of *T. stans* as an antibacterial and/or antioxidant activities against the growth of some human pathogenic bacteria as guide step for bactericidal activates and pharmaceutical applications.

MATERIALS AND METHODS

Plant material

The whole plant of *T. stans* was collected from Antoniadis Garden, Latitude (31.20241811), longitude (29.949546839). Horticultural Research Institute, Alexandria, Egypt, during the month of August 2012. The plant was kindly identified, authenticated and obtained voucher number (Hosam00039) at Egypt barcode of life project by Dr. Hosam O. Elansary Floriculture, Ornamental Horticulture and Garden Design Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Preparation of extracts

The fresh leaves and branches were washed, air-dried under shade at room temperature and then milled into powder to obtain a 40–60 mesh materials. The dried powder (120 g) was extracted by soaking with 150 ml of 80% aqueous methanol. After filtration the residue was processed similarly with the same amount of solvent. The methanol extract was concentrated to dryness under reduced pressure at 45°C with a rotary evaporator, lyophilized and were stored at 4°C until further use.

Five grams from the methanol extract was further fractionated by successive solvent extraction with ethyl acetate (EtOAc fraction), chloroform ($CHCl_3$ fraction) and then with *n*-butanol saturated with water (*n*-BuOH fraction). After organic solvent extraction the remaining aqueous fraction was also used for activity testing (Aq fraction) (Houghton et al., 1998).

To precipitated alkaloids from the $CHCl_3$ fraction, samples of about 1 g from the lyophilized methanol extract were dissolved in 50 ml of 99% methanol and treated with an equal volume of 1% aqueous HCl then the alkaloids were precipitated by drop-wise addition of 10% NH_4OH (Harborne, 1973; Cannell, 1998). The precipitate was collected by centrifugation (5000 rpm at 4°C for 30 min) and washed with 1% NH_4OH. The residue was dissolved in a few drops of $CHCl_3$ to obtain the $CHCl_3$ fraction that was containing the precipitated alkaloids. The solvents were removed under reduced pressure and the extracts were concentrated under vacuum at 40-60°C and the weight of the dried mass was recorded.

Preliminary phytochemical screening

All the dried extracts were dissolved in specific reagents through standard procedures and tested for phytoconstituents using standard methods (Raman, 2006; Khandelwal, 2007; Harborne, 2005).

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for flavonoids

A portion of the extract was heated with 10 ml of EtOAc over a steam bath for 3 min. The mixture was filtered and 4 ml of the

filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for alkaloids

The extract (0.5 g) was diluted in 10 ml of acid alcohol, boiled and filtered. Diluted ammonia (2 ml) was added to 5 ml of the filtrate. Five milliliter (5 ml) of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for saponins

Sample of extract (0.5 g) was shaken with 2 ml of water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Test for phenolics

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenolics.

Test for steroids

Two milliliter of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Determination of total phenolics

Total phenolic contents (TPCs) were determined with the Folin-Ciocalteau assay according to Singleton et al. (1999) method with minor modification. An aliquot (1 ml) of extracts or a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) was added to a 25 ml volumetric flask, containing 9 ml of distilled deionized water (dd H_2O). A reagent blank using dd H_2O was also prepared. One milliliter of the Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to 25 ml with dd H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm with a UV scanning spectrophotometer (Unico® 1200, Alexandria, Egypt). The data for the TPCs of *T. stans* leaves and branches were expressed as milligrams of gallic acid equivalents (GAE) per gram extract (mg GAE/g extract). All samples were analyzed in triplicates.

Determination of the total flavonoids

The total flavonoids content was measured with an aluminum chloride colorimetric assay (Zhishen et al., 1999). An aliquot (1 ml) of extracts or a standard solution of catechin (20, 40, 60, 80 and 100 mg/l) was added to a 10 ml volumetric flask, containing 4 ml of dd H_2O. 0.3 ml 5% $NaNO_2$ was added to the flask and after 5 min, 0.3 ml 10% $AlCl_3$ was added. At the sixth minute, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with dd H_2O. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510 nm with a UV scanning spectrophotometer (Unico® 1200, Alexandria, Egypt). The data of the total flavonoid contents were expressed as milligrams of catechin equivalents (CE) per gram extract (mg CE/g extract). All samples were analyzed in triplicates.

DPPH radical-scavenging assay

Free radical scavenging activity of the samples was determined using 2,2-diphenyl-1-picrylhydrazyl method (DPPH, Sigma-Aldrich) with some modifications (Elansary et al., 2012). An aliquot of 2 ml of stock solution of 0.1 mM DPPH reagent dissolved in pure methanol was added to a test tube with 2 ml of the sample solution in methanol (200 µg/l). The reaction mixture was mixed for 10 s and left to stand in fibber box at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer (Unico® 1200, Alexandria, Egypt). The decrease of the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. Total antioxidant activity (TAA %) was expressed as the percentage inhibition of the DPPH radical and was determined by the following formula:

$$TAA (\%) = (A_{control} – A_{sample}/A_{control}) \times 100$$

Where TAA is the total antioxidant activity; $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The control contained 2 ml of DPPH solution and 2 ml of methanol. The measurements of DPPH radical scavenging activity were carried out for three replicates.

Bacterial cultures and growth conditions

The antibacterial activity was carried out on the extracts with concentration of 2000 µg/ml against the Gram-positive bacteria; *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 4698, *Sarcina lutea* ATCC 9341 and *Staphylococcus aureus* ATCC 6538 and the Gram-negative bacteria; *Escherichia coli* ATCC 8739, *Serratia marcescens* ATCC 13880, *Salmonella typhi* ATCC 6229, *Proteus vulgaris* ATCC 6509 and *Pseudomonas aeruginosa* ATCC 9027. Nutrient agar (NA) medium was used for the maintenance of the tested bacterial organisms. Mueller Hinton agar (MHA) was used in all bioassays applying the disc diffusion method.

Disc diffusion method

Sensitivity of the bacterial strains to the different extracts was determined by the Kirby-Bauer disc diffusion susceptibility test (NCCLS, 1997). A suspension of the tested bacteria [1 ml of 10^5 colony forming units (CFU/ml)] was spread on the solid media plates. Filter paper discs (4 mm in diameter) were loaded with 20 µl of the tested extract and placed on the inoculated plates and, after staying at 4°C for 2 h, the plates were incubated at 37°C for 24 h. The diameters of the inhibition zones (IZs) were measured in millimetres. Control discs were impregnated with 10 µl of dimethyl sulfoxide (DMSO, Sigma-Aldrich) solution. The IZs obtained were compared with a positive control (25 µl of gentamicin 10 µg/disc) and for the negative control, discs were saturated with 20 µl of DMSO. The experiment was done in triplicate.

Minimum inhibitory concentrations

The MIC measurement to determine antimicrobial activity is a

Table 1. Phytochemical analysis of methanol extract and its fractions from *T. stans* leaves and branches.

Part	Extract	Tannins	Flavonoids	Alkaloids	Saponins	Phenolics	Steroids
Leaves	MCE	+++	+++	+++	+	+++	+
	EtOAc	+++	+++	-	-	+++	-
	$CHCl_3$	-	-	+++	-	-	-
	n-BuOH	-	+	-	+	+	+
	Aq	+	+	-	-	+	+
Branches	MCE	+	++	+++	+	+++	-
	EtOAc	++	++	-	-	+++	-
	$CHCl_3$	-	-	+++	-	-	-
	n-BuOH	-	+	-	+	+	-
	Aq	+	+	-	-	+	-

+++ Strong; ++ medium; + poor; - absence; MCE- methanol crude extract; EtOAc- ethyl acetate fraction; $CHCl_3$- chloroform fraction; *n*-BuOH-*n*-butanol fraction; Aq- aqueous fraction. The measuring was repeated four times and the classification was based on the color intensity of the precipitate.

quantitative method based on the principle of contact of a test organism to a series of dilutions of test substance (van Vuuren, 2008). Assays involving MIC methodology are widely used and an accepted criterion for measuring the susceptibility of organisms to inhibitors (Lambert and Pearson, 2000).

Minimum inhibitory concentrations (MICs) were determined by serial dilution of extracts (100, 250, 500, 1000 and 2000 µg/ml). This was performed in 96-well micro-plates (Eloff, 1998) by filling all wells, with 50 µl sterile Mueller Hinton Broth (MHB) with minor modification. Two wells were used as a sterility and growth control respectively with the sterility control containing only Oxoid® MHB (Sigma-Aldrich), whilst the growth control containing both MHB as well as test organism. After adding 50 µl of the bacterial suspension (10^5 CFU/ml) to each row (except for the sterility control), the micro-plate was covered and incubated at 37°C at 100% relative humidity overnight. The following morning 50 µl of a 0.2 mg/ml solution of *p*-iodonitrotetrazolium violet (Sigma-Aldrich) was added to each well. Inhibition of the growth was indicated by a clear solution or a definite decrease in colour reaction. Extracts used for the determination of MICs were dissolved in 10% DMSO and was made up as a stock solution (2000 µg/ml) with distilled water.

Statistical analysis

The results are expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA) was used to evaluate the significant difference among various treatments with the criterion of $P = 0.05$ (SAS, 2001).

RESULTS AND DISCUSSION

Yield and phytochemical analysis

The methanol extract of leaves and branches of *T. stans* was 26.26 and 18.26%, respectively. The extracts had a dark brown color. The extraction yield of fractionations (w/w %) depending on the ability of the compounds to dissolve in such solvent was with the following order: aqueous > *n*-BuOH > EtOAc > $CHCl_3$ fractions. The yield of the fractions were aqueous-19.88%, *n*-BuOH -14.5%, EtOAc-10% and $CHCl_3$-5.5%, from leaves and aqueous-15.88%, *n*-BuOH -10.5%, EtOAc-5% and $CHCl_3$-4.5%, from branches.

Preliminary phytochemical screening of the *T. stans* (Table 1) is reported to contain tannins flavonoids, alkaloids, phenols and traces of steroids and saponins. *T. stans* growing in Egypt has two alkaloids called tecomine-1 and tecostanine-2 as the main active compounds in *T. stans* (Hammouda et al., 1964; Hammouda and Amer, 1996). Recently, Alonso-Castro et al. (2010) reported that *T. stans* extracts contained more phenolics, flavonoids and alkaloids than other studied species and the chemical characterization of *T. stans* extra resulted in 12±2.1 GAEg/kg of phenolic compounds, 1.2±0.1 quercetin g/kg of flavonoids and 20.5±3.4 atropine g/kg of alkaloids.

Other study by Dash et al. (2011) reported that the presence of bioactive components comprising flavonoids (5.70 mg/100 g), alkaloids (5.40 mg/100 g), tannins (0.40 mg/100 g), saponins (0.38 mg/100 g) and phenols (0.10 mg/100 g) and these compounds might be responsible for the medicinal properties of *T. stans.*

Total phenolics and flavonoids content

The Folin-Ciocalteau reagent and expressed in mg gallic acid equivalent (that is, GAE)/100g extract (Table 2) was used to determine the TPCs. The methanol extract had 50.3±3 and 37.66±2.02 mg GAE/g extract from leaves and branches, respectively. TPC was found with high content in leaves EtOAc (44.1±4.72 mg GAE/g extract) followed by branches EtOAc (30.33±1.16 mg GAE/g

Table 2. Total phenolics and flavonoids contents of extracts from *T. stans* leaves and branches and their antioxidant activity as TAA%.

Part	Extract	Total phenolic (mg GAE/g extract)	Total flavonoids (mg CE/g extract)	TAA%
Leaves	MCE	50.3±3^{a}	40.66±2.03^{a}	74.16±0.85^{d}
	EtOAc	44.1±4.72^{b}	35.0±2.18^{b}	83.4±0.31^{a}
	$CHCl_3$	-	-	79.17±0.20^{c}
	n-BuOH	24.33±1.52^{ef}	19.66±2.51^{e}	67.42±0.41^{f}
	Aq	25.33±0.57^{e}	17.66±2.51^{e}	35.74±0.12^{i}
Branches	MCE	37.66±2.02^{c}	30.66±3.21^{c}	72.85±0.31^{e}
	EtOAc	30.33±1.16^{d}	25.33±2.51^{d}	82.06±0.54^{b}
	$CHCl_3$	-	-	66.46±0.51^{g}
	n-BuOH	20.67±1.52^{f}	17±2^{e}	17.52±0.20^{j}
	Aq	23.66±1.52^{ef}	15±2^{e}	38.48±0.31^{h}
P value		< 0.001	< 0.001	< 0.001

All values are mean±SD of three replicates; TAA%: Total antioxidant activity; GAE: Gallic acid equivalents; CE: (+)-catechin equivalents.
MCE- methanol crude extract; EtOAc-ethyl acetate fraction; $CHCl_3$-chloroform fraction; *n*-BuOH-*n*-butanol fraction; Aq-aqueous fraction. Different letters in italics within the column represent statistical differences between the averages of the mean values. Means with the same letter are not significantly different (P < 0.05) according to Duncan's multiple-range test.

extract). The EtOAc fraction had exceptionally high TPC than other fraction, since previous studies concluded that the EtOAc fraction had the phenolic compounds (Cowan, 1999). The measured TPCs were much higher than the measured by Govindappa et al. (2011) which the TPCs observed in methanol with 11.64 mg GAE/g extract. The total flavonoids of extracts from leaves and branches were ranged between 15±2 (aqueous fraction from branches) and 40.66±2.03 (methanol extract from leaves) mg CE/g extract. In previously studies the flavonoids from plant extracts have been found to possess antimicrobial and antioxidants properties (Lin et al., 2008; Lopez-Lazaro, 2009).

Antioxidant activity determined by DPPH method

The antioxidant activity of the methanol extract and its fractions, as measured by the ability to scavenge DPPH free radicals, is observed in Table 2. The percentage inhibition of free radical by *T. stans* extracts due to the significantly reduced color of the DPPH reagent from purple to yellow. The highest antioxidant activity of 200 µg/l extract was observed by the EtOAc fraction from leaves (83.4±0.31%) and branches (82.06±0.54%) followed by $CHCl_3$ fraction from leaves (79.17±0.20%), methanol extract from leaves (74.16±0.85%) and branches (72.85±0.31%).

While the scavenging activity of methanol extract had 58.92% (Govindappa et al., 2011). Also Marzouk et al. (2006) reported that *T. stans* had a good antioxidant activity. The antioxidant activity of various extracts of aerial parts of *T. stans* showed significant antioxidant activity as measured by DPPH as scavenging reagent. The antioxidant activity of ethanol is higher than methanol and acetone extract (Torane et al., 2011).

Antibacterial activity

Antibacterial activity of methanol extracts of *T. stans* were evaluated by measuring the diameters of zones and MICs values of growth of inhibition on some human pathogenic bacteria (Table 3).

All the tested microorganisms were susceptible to *T. stans* leaves and branches extracts with different degrees. The susceptibility of bacteria to plant extracts, on the basis of inhibition zone diameters, varied according to strains and species (Karou et al., 2006).The presence of tannins, glycosides, triterpenes and steroids as important constituents and these constituents may be responsible for antimicrobial activity as per the reported activities of *T. stans* (Govindappa et al., 2011).

The antibacterial activities of methanol extract and its fractions of *T. stans* gave different zones of inhibition and MICs on the tested bacterial strains (Table 3). The methanol extracts inhibited the growth of all most all the bacterial strains. $CHCl_3$ and EtOAc fractions of methanol extract from leaves and branches of *T. stans* showed the strongest antibacterial activity against Gram-positive and negative bacteria.

The $CHCl_3$ fraction reported to have a MIC value

Table 3. Antibacterial activities of methanol extract and fractions from leaves and branches of *T. stans* observed against the growth of some human pathogenic bacteria.

Part	Extract	*B. subtilis*	*S. aureus*	*E. coli*	*S. marcescens*	*P. aeruginosa*	*P. vulgaris*	*S. typhi*	*M. luteus*	*S. lutea*
L	MCE	15.66±1.52 (500)	15.66±1.52 (500)	22±1 (250)	14.66±0.8 (<100)	11.33±1.67 (1000)	13 (1000)	12.33±1.3 (1000)	17.3±0.9 (250)	n.a. (>2000)
	EtOAc	19±1 (250)	18±1 (250)	18±1 (500)	11.33±1.15 (500)	n.a. (>2000)	12±1 (1000)	14.33±0.9 (1000)	15.6±0.8 (500)	13±1 (500)
	$CHCl_3$	20.33±1.52 (<100)	22.33±0.59 (<100)	18.66±2.47 (500)	11.33±0.89 (1000)	13±1 (<100)	11.33±1.12 (500)	15±1 (1000)	12.3±0.9 (1000)	12.3±0.5 (1000)
	BuOH	13.66±1.23 (500)	7.66±0.57 (1000)	17.33±1.89 (500)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)	11±0.8 (1000)	n.a. (>2000)	n.a. (>2000)
	Aq	n.a. (>2000)	n.a. (>2000)	12 (1000)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)	6.3±0.9 (1000)	16.6±0.7 (500)
B	MCE	16.66±1.12 (1000)	12.33±0.23 (<100)	16.66±1.53 (500)	14.33±1.52 (<100)	7 (1000)	13 (1000)	16.66±1.4 (250)	13.6±1.2 (500)	12.3±0.58 (500)
	EtOAc	15±1 (500)	14.66±1.56 (1000)	15.66±1.34 (500)	10±1 (<100)	8.33±0.12 (1000)	12.34±1.46 (250)	13.33±0.7 (1000)	13.6±0.6 (500)	18±1 (250)
	$CHCl_3$	21±1 (500)	13±1 (1000)	17.66±0.57 (500)	17.33±1.15 (<100)	n.a. (>2000)	14.38±1.13 (250)	16.6±0.8 (500)	n.a. (>2000)	12.6±0.8 (500)
	BuOH	n.a. (>2000)	7.66±0.89 (1000)	23.33±1.45 (<100)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)	10.33±0.55 (1000)	n.a. (>2000)	n.a. (>2000)
	Aq	n.a. (>2000)	n.a. (>2000)	12 (500)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)	n.a. (>2000)
DMSO		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Gentamicin*		25	23	22	24	25	30	35	23	25

L: Leaves; B; Branches; IZ: Inhibition Zone (Values are expressed as mean±SD including disc diameter of 5 mm at 2000 μg/ml); MIC: Minimum inhibitory concentration; values are given inter parenthesis as μg/ml. n.a. Not active; n.t. Not tested. *Values are presented in mm and each disc received 10 μg. MCE- methanol crude extract; EtOAc-ethyl acetate fraction; $CHCl_3$-chloroform fraction; BuOH-*n*-butanol fraction; Aq-aqueous fraction.

against the growth of *B. subtilis* at <100 μg/ml of leaves and branches with IZ values of 20.33±1.52 mm and 21±1 mm, respectively at 2000 μg/ml. Additionally, at concentration of 500 μg/ml, methanol extract from leaves and branches inhibited the growth of *B. subtilis*. The MICs value against the growth of *S. aureus* at <100 μg/ml were presented by leaves $CHCl_3$ fraction (22.33±0.59 mm) and branches methanol extract (12.33±0.23 mm). The *n*-BuOH fraction of branches showed the most active extract against the growth of *E. coli* with MIC <100 μg/ml and IZ 23.33±1.45 mm. Additionally, it can be observed that *E. coli* is sensitive to all the extract tested even the aqueous fraction. The highest antibacterial activity of extracts against the growth of *S. marcescens* was reported by methanol (14.33±1.52 mm) and $CHCl_3$ fraction (17.33±1.15 mm) of branches and methanol extract of leaves (14.66±0.8 mm) at MIC <100 μg/ml. Methanol extract (11.33±1.67 mm) and $CHCl_3$ fraction

(13±1 mm) of leaves demonstrated MIC against *P. aeruginosa* at 1000 and <100 µg/ml, respectively.

At 250 µg/ml, the $CHCl_3$ (14.38±1.13 mm) and EtOAc (12.34±1.46 mm) fractions of *T. stans* branches and at 500 from leaves (11.33±1.12 mm) achieved the best MIC against *P. vulgaris*. The MIC of 250 µg/ml of methanol extract (16.66±1.4 mm) and 500 µg/ml of $CHCl_3$ fraction (16.6±0.8mm) of branches was reached against the growth of *S. tephii*. The highest MIC value (250 µg/ml) was achieved by methanol extract of leaves (17.3±0.9 mm) against the growth of *M. luteus*. On the other hand, the highest MIC value (250 µg/ml) was observed by EtOAc fraction from branches (18±1 mm) against *S. lutea* growth. All the IZs reported in this study were compared with gentamicin (10µg/disc) as a standard antibiotic against the growth of the studied bacterial strains and some values of extracts nearly reached the values of IZs gentamicin.

The methanolic crude extracts and its fraction ($CHCl_3$ and EtOAc) of leaves and branches of *T. stans* inhibited the growth of such Gram-negative bacteria that cause majority of diarrheal diseases and which usually display above average resistance to most antibiotics and non-antibiotics antibacterial agents (Abubakar, 2009). These bacteria which have several virulence factors also have intrinsic resistance from a restrictive outer membrane barrier and trans-envelope multidrug resistance pumps (Winstanley et al., 1997).

The efficacy of the extracts could be due to the secondary metabolites, such as alkaloids, phenolic compounds and saponins. The tree can be used to source for antibacterial drugs for treating the infections caused by susceptible Gram-negative bacteria. Although *T. stans* was found to contain alkaloids and phenolic compounds with pronounced antibacterial activities against a wide range of microbial pathogens. Aqueous fraction from leaves and branches of *T. stans* did not exhibit MIC against any Gram-positive and Gram-negative bacteria at any lower concentrations and could be attributed to the presence of poly and oligosaccharides as a water-soluble compounds (Cowan, 1999).

On the other hand, the presence of alkaloids in *T. stans* have been shown to possess an antimicrobial and antioxidant activities (Erdemoglu et al., 2007; Maiza-Benabdesselam et al., 2007). In our study, the $CHCl_3$ fraction which contains the precipitated alkaloids was found to own a potential activity against the tested bacterial strains. For example, the values of IZs were 20.33±1.52 and 22.33±0.59 mm against the growth of *B. subtilis* and *S. aureus*, respectively as affected by the $CHCl_3$ fraction of leaves at concentration of 2000 µg/ml with MIC <100 µg/ml.

The action mechanism of alkaloids is attributed to their ability to intercalate with DNA (Phillipson and O'Neill, 1987). Furthermore, the most susceptible bacterium was *E. coli* and the most resistance was *P. aeruginosa* and might be due to their cell wall structure and outer membrane. Our results suggest that Gram-positive bacteria are generally more sensitive to the extracts from studied trees. This was consistent with previous studies on other spices (Arora and Kaur, 1999).

In the study of Muthu et al. (2012) the strong antimicrobial activities were observed in the methanolic extracts against tested bacteria *(P. fluorescens*, *Clavibacter michiganensis* sub sp. *michiganensis*, *Xanthomonas axanopodis* pv. *malvacearum*, *S. aureus, E. coli, P. aeruginosa* and *Klebsiella pneumonia*) and the phytochemical screening showed that the different solvent extracts of *T. stans*, the tannin, flavonoids, phenol, alkaloids, steroids, anthraquinones and saponins were present in all solvent extracts. Senthilkumar et al. (2010) reported extracts of *T. stans* having antibacterial activity on human pathogenic bacteria. Moreover, Al-Azzawi at al. (2012) reported that the whole alcoholic and aqueous extract of *T. stans* exhibited the antibacterial activity and isolated tecomine, where the growth of *E. coli* and *B. subtilis* was inhibited at different concentrations. On the other hand, Facey et al. (1999) reported that the *E. coli, S. aureus, P. mirabilis* and *P. aeruginosa* showed resistant to extracts from *T. stans* (inhibition zone <10 mm).

The mechanisms responsible for phenolic toxicity (methanol extract and EtOAc fraction) to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987). High TPC values found in methanol extracts and EtOAc fraction plays the role of phenolic compounds in contributing these activities and possess potent antioxidants (Adesegun et al., 2009) and antimicrobial (Alcaraz et al., 2000). Fractionation of the weakly active crude extracts results in more active antibacterial fractions. For instance, the methanol extract from leaves did not show any activity against *S. lutea* at 2000 µg/ml and the EtOAc fraction divided from the methanol extract presented a good activity (13±1 mm of IZ and MIC value of 500 µg/ml).

Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial and anticarcinogenic activities (Cook and Samman, 1996). Flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides in the food systems (Roedig-Penman and Gordon, 1998). On the other hand, the bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, hydrogen donors and singlet oxygen quenchers (Pietta, 2000). The presence of tannins in the extracts may explain its potent bioactivities known to possess potent antioxidants (Zhang and Lin, 2008). The saponins from plant extracts already have antioxidant activity (Gulcin et

al., 2004).

Conclusion

The present study indicated that the extracts from leaves and branches of *Tecoma stans* (L.) Juss. ex Kunth (Bignoniaceae) grown in Egypt had a potent antibacterial and antioxidant activities. These findings provide scientific evidence to support traditional medicinal uses of *T. stans* and indicate a promising potential for the development of an antibacterial and antioxidant agent from *T. stans* trees.

This medicinal tree appears interesting in its efficacy as potential sources of new antibacterial and antioxidant drugs in the pharmaceuticals industry. We found strong antibacterial and antioxidant activities in the methanol extract and ethyl acetate and chloroform fractions of *T. stans*. These extracts showed significantly antibacterial activity against all Gram-positive and Gram-negative bacteria. Strong antioxidant activities were presented in the methanol extract and ethyl acetate fractions. These activities could be due to strong occurrence of compounds such as flavonoids, tannins, alkaloids and saponins.

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