Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS
Pharmacy

Unravelling the Antioxidant and Antimicrobial Effect of *Tecoma stans* Extracts

Joseph Ngoupayo[1], Clovis Talla[1*], Yanick Kevin Melogmo Dongmo[2], Noumboue Kouamou Bijou-Lafortune[2], Fabrice Fekam Boyom[2], Emmanuel Mpondo Mpondo[1,3]

[1]Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Cameroon
[2]Antimicrobial Agents Unit, Laboratory for Phytobiochemistry and Medicinal Plants Studies, Department of Biochemistry, Faculty of Science, University of Yaound´e 1, P. O. Box 812, Cameroon
[3]Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Cameroon

DOI: https://doi.org/10.36347/sajp.2024.v13i07.004 | **Received:** 09.07.2024 | **Accepted:** 16.08.2024 | **Published:** 17.09.2024

***Corresponding author:** Clovis Talla
Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Cameroon

Abstract **Original Research Article**

Infectious diseases are a serious public health problem responsible for approximately 13.7 million deaths annually. The emergence of multidrug-resistant bacterial and fungi species highlights the urgent need to search for alternative treatment options. In this regard, medicinal plants traditionally used for the treatment of infections as well as associated symptoms constitute a starting point for the search of endogenous solutions. This study was designed to investigate the antimicrobial and antioxidant properties of *Tecoma stans* extracts. The extracts were prepared by maceration using hydroethanolic solution (30:70, v/v) solvent. Then the obtained extract was fractioned by liquid-liquid partitioning using different solvent with increase polarity (hexane, ethylacetate, n-butanol, and water). The qualitative composition was studied using colorimetric methods. Total phenolic and total flavonoids compounds were determine using Folin Cioclateu and Aluminium Chloride methods respectively. The antioxidant activity was performed targeting DPPH, ABTS scavenging and FRAP assays. The antimicrobial activity was done using microdilution coupled with resazurin based assay. The phytochemical study of *T. stans* hydroethanolic extract revealed the presence of tannins, flavonoids, polyphenols, cardiac glycosides, quinones and coumarins. metabolites. The quantity of polyphenols in *T. stans* hydroethanolic extract was 291,139 ± 3,164 - 882,918 ± 3,154 mgEQ/100 gMS while that of flavonoids was 3,965 ± 0,138 -303,266 ± 1,178 mgEQ/100 gMS. The *T. stans* hydroethanolic total extract displayed no antioxidant activity targeting DPPH and ABTS free radicals at the concentrations (SC_{50} >500). But extracts and fractions showed a good reducing potential (SC_{50} ranging from 7.89 ± 0.61 tio 39.88 ± 1.10 µg/mg). Likewise, *T. stans* hydroethanolic extract exhibited antimicrobial activity with the MIC spanning from 1 mg/mL to 32 mg/mL toward *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniea*, *Klebsiella pneumoniae*, and *Candida albicans*. The present findings provide scientific evidence to support the traditional use of *Tecoma stans* for the treatment of infectious diseases indicating that, this species contains metabolites that can be used for the development of antimicrobial agents.

Keywords: *Tecoma stans*, Phytochemicals, Antioxidant activity, antimicrobial activity.

INTRODUCTION

Infectious diseases are ailments caused by pathogenic agents such as bacteria, viruses, parasites, and fungi. Once they have entered the body these pathogens multiply and disrupt bodily functions. They are a real health problem worldwide, especially in developing countries where they are endemic. Bacterial infections are the second leading cause of death worldwide, after heart disease. Around 13.7 million infection-related deaths were recorded in 2019 [1]. General data on the epidemiology of infections reveals the high prevalence of a certain number of species such as *S. aureus*, *E. coli*, *K. pneumoniae* [2] and *S. pneumoniae* [3] worldwide. In 2022, WHO highlighted a resistance rate of over 50% reported for *Klebsiella Pneumonia* spp. Over 20% of *E. coli* blood isolates were resistant to first-line drugs (ampicillin and cotrimoxazole) and second-line treatment

(fluoroquinolones). Moreover, bloodstream infections caused by *E. coli* increased by 15% between 2017 and 2022. Worldwide antimicrobial resistance levels were 42% for *E. coli* and 35% for *S. aureus* over the same period. [4]. Concerning the fungi, Candida spp. are the most frequently implicated in invasive fungal infections[5]. Approximately 1,565,000 people suffer from Candida bacteremia or invasive candidiasis each year, with 995,000 deaths (63.6%) [6]. Unfortunately, the increasing emergence of strains resistant to approved drugs makes it difficult to control these infections [7] [8]. The multiple drug resistances make the treatment of bacteria and fungi infections more difficult as well as high treatment cost, and the contribution to the deterioration in the general state of health of populations. This emphasizes the need to search alternatives treatment. The entry of pathogens led the production of free radical by human body, which contribute to the reduction of pathogens spread. But the imbalance between free radical and antioxidant contribute to the oxidative stress[9]. The antimicrobial agent which possesses antioxidant properties would be a great advantage in the management of bacterial and Fungal infections. Regarding the traditional medicine, natural sources such as medicinal plants have been used for the treatment of some ailment. It could be the case of *Tecoma stans* which is used in Nkolbisson, Yaounde, for the treatment of skin and gastrointestinal infections. *Tecoma stans*, belonging to *Bignoniaceae* family, is a medicinal plant exploited in several regions for the treatment of some ailment. We can hypothesis that *T. stans* contains active chemical constituents which can use for the development of alternative drugs against bacteria and fungal infections. This study was designed to investigated the antioxidant and antimicrobial properties of *T. stans* as well as it fractions.

MATERIAL AND METHODS

Material

Plant Materials

Both leaves and flowers of *T. stans* were collected on January, 2021 in Nkolbisson, Yaoundé, Mfoundi Department, Center Region (Cameroon). The fresh plant species were identified in National Herbarium of Cameroun by M. Nana by comparison with the specimen deposit under the number 33215 HNC. Once in the laboratory of Pharmacognosy and Pharmaceutical Chemistry, the plants materials were pretreated (washing, cutting) and airdried at room temperature (28-30° C) within 14 days. The dried plants materials were ground into fine powder and kept at room temperature for the further studies.

Bacteria and fungi strains

Klebsiella pneumonia, *Escherichia coli*, 02 strains *Staphylococcus aureus*, *Candida albicans*, provided by Bei resources (https://www.beiresources.org/) and clinical isolates *Streptococcus pneumoniae* provide by *Laboratoire SION* and one fungi species were used in this study. Muller Hinton Agar (Sigma Aldrich, German) was used to revived bacteria 24 hours prior assay while Sabouraud dextrose Agar (Sigma Aldrich, German) was used to revived fungi 48 h prior the assay. While Muller Hunton broth and Sabouraud dextrose broth were used for the antimicrobial assay.

Methods

Preparation of extracts, fractions, and stocks solutions.

The extract was prepared by maceration of 1.32 kg of powder in 3.5 liters of hydroethanol solvent system (30/70 v/v) within 48 hours under shaking. At the end of this period, the resulting was filtrated using Wattman N° 1 paper. The filtrate was then concentrated using a rotary evaporator apparatus (Techmel, TT-52). The filtrate extract was air-dried at room temperature to remove the remaining solvent.

The obtained extract was then fractionated by liquid-liquid partitioning using the different solvents with increase polarity hexane (TS_{Hex} fraction), ethyl acetate (TS_{Ac} fraction), and n-butanol ($TS_{n\text{-}But}$ fraction). The crude extract, fractions, aqueous residue (TS_{H2O} fraction), obtained were kept at 4^0C until the further studies. The stocks solutions of each sample (extracts and fractions) were prepared at 100 mg/mL by dissolving 100 mg of sample in 1 mL of absolute dimethyl sulfoxide (DMSO). The reference antibiotic and antifungal were prepared in the same conditions at 1 mg/mL. by dissolving 1 mg of each powder in acidified distilled water (0.4 N).

Phytochemical qualitative screening

The qualitative phytochemical screening tests was done to determine the presence of secondary metabolites such as alkaloids, Quinone [10], polyphenols, Flavonoid, coumarins, cardiac glycosides, tannins[11], saponins [12], Steroid[13], Resin, anthocyanins [14].

Determination of total phenolic contents

The total phenolic content were determined by the Folin Ciocalteu method as described by Vinson *et al.* [15] with slight modifications. Briefly, to the reaction mixture containing 200 µL of extract or fractions (1 mg/mL), 800 µL of freshly prepared Folin Ciocalteu reagent (0,2N) and 2 mL of 7.5% sodium bicarbonate were added. The final mixture was diluted with 7 mL of distilled water and kept in the dark at room temperature for 2 h and the absorbance was read at 765 nm using a spectrophotometer (URIT). The assay was performed in triplicate.

Determination of total flavonoid content

The determination of flavonoid content was done following the protocol described by Dhar *et al.* (2012) [16]. The test was performed in triplicate in the test tubes. Indeed, in a test tube, 0.1 mL of extract and the fractions at 100 mg/mL was added to 0.3 mL of

distilled water followed of 0.03 mL of $NaNO_2$ (5%). After 5 min of incubation, 30 µL of $AlCl_3$ (10%) was added and the mixture was additional incubated for 5 min. The reaction mixture was treated with 0.2 mL of 1 mM NaOH then the volume was complete to 1 mL with distilled water in all tubes and the absorbance was read at 510 nm using a spectrophotometer (URIT).

Assessment of antioxidant activity of extract and fractions

The antioxidant activity of the extract and fractions was assessed by DPPH (2,2-dipheny -1-picrilhydrazyl) and ABTS (2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical tests and the reduction of ferric iron to ferrous iron (FRAP).

Preparation of solutions

Extracts were prepared at 100 mg/mL in 100% DMSO. A 0.02% DPPH solution was prepared with ethanol. The prepared solution was stored in a hermetically in dark. The ABTS cation radical was generated by mixing equal volumes of a 4.9 mM solution of potassium persulphate ($K_2SO_2^8$) and a stock solution of 7 mM ABTS. The resulting solution was stored in a dark at room temperature for 15 hours before use. The Fe^{3+} solution was prepared at 1.2 mg/ml in distilled water. The orthophenantroline solution was prepared at 0.2% ethanol.

DPPH Scavenging test

The DPPH (2,2-diphenyl- 1-picrilhydrazyl) radical scavenging activity of extracts and fractions was performed according to the protocol of Sakande (2006) [17] with a slight modification. Two-fold dilutions were performed to obtain different concentrations ranging from 500 to 7.8125 µg/mL for the extracts and 50 to 0.78125 µg/mL for the gallic acid, used as a positive control. Next, 25 µL of each dilution was added to the wells of a plate containing 75 µL of DPPH solution prepared at 0.02%. The plates were then incubated for 30 minutes at room temperature in the dark. At the end of the incubation period, the optical densities were read at 517 nm using a spectrophotometer (TECAN 200) against the blank (extracts at different concentrations). The negative control was DPPH without extract. The radical scavenging activity was described in terms of scavenging concentration 50 (SC_{50}), effective concentration 50 (EC50).

ABTS scavenging test

This test $ABTS^+$ cation radical was carried out according to the protocol described by Khan *et al.*, (2012)[18] with some modifications. A series of geometric dilutions of order 2 were performed to obtain concentrations ranging from 500 to 7.8125 µg/mL for the extracts and from 50 to 0.78125 µg/mL for the gallic acid used as a positive control. Next, 25 µL of each dilution was added to the wells of a plate containing 75 µL of 0.175 mM $ABTS^+$ solution. The plates were then incubated for 30 minutes at room temperature in the dark. At the end of the incubation period, the optical densities were read at 734 nm using a spectrophotometer (TECAN 200) against the blank. The negative control was ABTS reagent without extract and the positive control was gallic acid. The extracts were also tested alone under the same conditions to see which extracts fluoresced. The tests were performed in duplicate and the radical scavenging activity was determined as described above.

Ferric ion reducing antioxidant power (FRAP) essay

The Fe^{3+} reduction test was carried out according to the protocol described by Berker *et al.*, (2007) [19] with some modifications. In a 96-well microplate, 25 µl of extract was then introduced into a plate containing 25µL of Fe^{3+} solution. The plates were incubated for 15 min at room temperature in the dark. After incubation, 50 µL of orthophenantroline solution was added and the plates reincubated for 15 min under the same conditions. At the end of this incubation, the optical density of the contents of the wells was read at 505 nm using a plate reader (TECAN M200). The negative control consisted of 25µl methanol + 25µl Fe^{3+} + 50µl orthophenantroline. The positive control was treated under the same conditions as for DPPH and ABTS

Assessment of antibacterial and antifungal activity
Assessment of antibacterial activity

The antibacterial study of the extracts was performed using the microdilution method according to the M07-A9 protocol of the Clinical and Laboratory Standards Institute (CLSI), 2012. The tests were performed in sterile 96-well microplates. Briefly, 128 µL of each extract and the reference ciprofloxacin (1mg/mL) were introduced into 72 µL of MHB. Then, 13 two-fold serials dilutions were achieved to yield final concentrations ranging from 32 to 0.03125 mg/mL and 10 to 0.00976 µg/mL, respectively for samples (extracts and fractions) and ciprofloxacin. Thereafter, 100 µL of bacterial suspension (1×10^6 UFC/mL) were introduced into each well except for those of sterility control. The negative control was made up with culture media and bacteria suspension while the positive control was constituted of culture media, bacteria suspension and ciprofloxacin. The plates were covered and incubated at 37° C for 24 h. At the end of incubation period, 20 µL of freshly prepared resazurin (0.15 mg/mL), followed by the additional incubation in the same condition for 30 min. MICs were defined as the lowest concentration of samples in which no change in resazurin color from blue to pink was observed. The experiment was performed in triplicate with the concentration of DMSO (Sigma–Aldrich, Germany) ≤1 %, which in which it did not affect cell growth.[20]

Assessment of antifungal activity

The antifungal essay of the extracts was performed using the microdilution method according to the M27-A3 protocol of the Clinical and Laboratory Standards Institute (CLSI), 2008. The tests were

performed in sterile 96-well microplates. Briefly, 128 µL of each extract and the reference fluconazole (0.74 µg/mL) were introduced into 72 µL of Sabouraud dextrose Medium. Then, 13 two-fold serials dilutions were achieved to yield final concentrations ranging from 32 to 0.03125 mg/mL. Thereafter, 100 µL of fungal suspension (1×10^4 UFC/mL) were introduced into each well except for those of sterility control. The negative control was made up with culture media and fungi suspension while the positive control was constituted of culture media, bacteria suspension and fluconazol. The plates were covered and incubated at 37° C for 48 h. At the end of incubation period, 20 µL of freshly prepared resazurin (0.15 mg/mL), followed by the additional incubation in the same condition for 30 min. MICs were defined as the lowest concentration of samples in which no change in resazurin color from blue to pink was observed. The experiment was performed in triplicate with the concentration of DMSO (Sigma–Aldrich, Germany) ≤1 %, in which it did not affect cell growth [21].

Statistical analysis

Data were entered using Excel (Microsoft Office, USA) and statistical analysis was performed using Graphpad software (San Diego, California, USA) version 8.0.1. The ANOVA test coupled with Dunett's test was used for comparison between groups. Results were expressed as mean ± standard deviation with significance levels ($p<0.05$) and ($p<0.001$).

RESULTS

Phytochemical qualitative screening

The qualitative phytochemical screening tests allowed us to determine the presence of many secondary metabolites. The results are presented in the table 1 below:

Table 1: Phytochemical screening of *T. stans*

Family	Results				
	TS_{ET}	TS_{H2O}	$TS_{n\text{-}but}$	TS_{Ac}	TS_{Hex}
Alkaloids	-	-	-	-	-
Polyphenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	-	+	-	-
Anthocyanins	-	-	-	-	-
Betacyans	-	-	-	-	-
Phlobotanins	-	-	-	-	-
Quinons	+	+	+	+	+
Coumarins	+	+	+	+	+
Saponins	-	-	-	-	-
Cardiacs glycosids	+	+	+	-	-
Resins	-	-	-	-	-

-: Absent; +: Present

TS_{Hex}: hexanic fraction of *T. stans*; TS_{Ac}: ethylacetate fraction of *T. stans*; $TS_{n\text{-}But}$: n-Butanol fraction of *T. stans* TS_{H2O}: aqueous fraction of *T. stans*.

We noticed the presence of polyphenols, flavonoids, coumarins and quinones in all samples. Likewise, alkaloids, saponins, resins, anthocyanins, phlobotannins, and betacyanins are absent from all samples. The rest of the metabolites are variably present in the different extracts depending on the fractionation solvents. In fact, cardiacs glycosids are present in total extract, aqueous and n-butanolic fractions, but absent in hexanic and ethylacetate fractions. Oxalates were present in total extract and aqueous fraction, but absent in all others fractions. Tanins were present in total extract and n-butanolic fraction, but absent in all others fractions.

Determination of total phenols and flavonoids

The levels of phenolic compounds and flavonoids in the crude hydroethanol extract and fractions were obtained from the linear regression equation for gallic acid ($y= 0.0032 x$; $R^2 = 0.9975$) and expressed as mg GAE/g (Table 3).

Total phenol content

The total phenolic content was determined using the Folin ciocalteu method. The results we obtained show that all the samples tested (total extract and fractions) have a high concentration of phenolic compounds with significantly different levels ($p<0.05$).

Thus, we observe that the total extract has the highest phenolic compound content, i.e. 882.918 ± 3.154 mg EAG/l00gMS. It was followed by the n-butanol fraction 614,978 ± 1.826 mgEAG/100gMS, the aqueous fraction 399.789 ± 1.827 mgEAG/100gMS, the total extract 373.417 ± 3.164 mgEAG/100gMS and the hexane fraction 291,139 ±3.164 mgEAG/100gMS as shown in table 1. We noted that all these results were significantly different. The n-butanol fraction presented the better total phenolic compounds content.

Total flavonoid content

The results obtained from the assays of the *T. stans* samples were significantly different ($p<0.05$) as shown in table 1. The n-butanolic fraction had the highest flavonoid content (303.266 ± 1.178 mgEQ/100gMS), followed by the ethyl acetate fraction (73.220 ±0.165 mgEQ/100gMS), then the aqueous fraction (63,613 ± 0.901 mgEQ/100gMS) then the total extract (17.416 ± 0.442 mgEQ/100 gMS) and finally the hexane fraction (3.965 ± 0.138 mgEQ/100 gMS). Consequently, n-butanol fraction presented the highest total flavonoids content.

Table 2: Total phenolic and flavonoid compounds in *T. stans*

Sample codes	Flavonoid content (mgEQ/100 gMS)	Phenolic compound content (mgEAG/100 gMS)
TS_{ET}	$17,416 \pm 0,442^{i}$	$882,918 \pm 3,154^{d}$
TS_{HeX}	$3,965 \pm 0,138^{j}$	$291,139 \pm 3,164^{i}$
TS_{Ac}	$73,220 \pm 0,165^{f}$	$373,417 \pm 3,164^{h}$
$TS_{n\text{-}But}$	$303,266 \pm 1,178^{b}$	$614,978 \pm 1,826^{f}$
TS_{H2O}	$63,613 \pm 0,901^{g}$	$399,789 \pm 1,827^{g}$

Values that do not have the same letter are significantly different, Tukey Test

T. stans TS_{Hex}: hexane fraction *T. stans* TS_{Ac}: acetate fraction *T. stans* $TS_{n\text{-}But}$ *T. stans*$_{n\text{-}Butanol}$ fraction *T. stans* TS_{H2O}: aqueous fraction *T. stans*

Anti-oxidant activity

Anti-oxidant activity was assessed using two different, complementary methods. We measured the free radical scavenging capacity (anti-free radical activity) using DPPH and ABTS tests, and the reducing power using the FRAP test of the antioxidant substances present in our various samples. The DPPH test results showed that for *T. stans*, the acetate fraction (SC50= 182,95 ± 0.95 µg/mL) showed significantly ($p<0.05$) better free radical scavenging activity than the n-butanol fraction (SC50=213.20± 14.70 µg/mL). The other extracts showed low activity, the scavenging concentration 50 was more than the highest tested concentration.

The ABTS essay of *T. stans* extracts and fractions showed that, the acetate fraction (SC50= 119.55 ± 1.75 µg/mL) showed better free radical scavenging activity than the aqueous fraction (SC50= 170.35 ± 5.25 %g/mL) and the difference was significant ($p<0.05$). These results were significantly different from positive control that was gallic acid. Scavenging concentration of the total extract and n-butanol extract was higher than the tested concentrations.

The ferric reducing antioxidant power test of the *T. stans* extract and fractions showed that the total extract had the highest activity (SC50= 7.89 ± 0.61 µg/ ml). This result was similar to that of n-butanol fraction (SC50= 9.63 ± 0.21 µg/ ml), their comparison showing no significant difference ($p>0.05$) between them. This was followed by the acetate fraction that has presented high activity than aqueous extract (SC_{50}= 12.82 ± 0.90 µg/ ml). The aqueous extract had significantly ($p<0.05$) the lowest reducing power (SC50= 39.88 ± 1.10 µg/ ml). All these results were significantly different from the positive control that was Gallic acid.

Table 3. Free radical scavenging by DPPH and ATBS and reducing power of *T. stans*

SC50 in µg/mL			
Sample code	**ABTS**	**DPPH**	**FRAP**
TS_{ET}	>500	213.20 ± 14.70^{c}	7.89 ± 0.61^{g}
TS_{Ac}	119.55 ± 1.75^{d}	182.95 ± 0.95^{d}	12.82 ± 0.90^{f}
$TS_{n\text{-}But}$	>500	>500	9.63 ± 0.21^{g}
TS_{H2O}	170.35 ± 5.25^{c}	>500	39.88 ± 1.10^{b}
Gallic acid	10.11 ± 0.154^{e}	31.21 ± 0.59^{e}	1.18 ± 0.02^{h}

Means that do not share a same letter are not significantly different, Tukey Test

$TS_{ET:}$ hydrocthanolic extract of *T. stans T. stans* TS_{Ac} : acetate fraction *T. stans* $TS_{n\text{-}But}$, TS_{H2O} fraction *T.stans,* TS_{H2O} : aqueous fraction *T.stans,* SC50 : scavenging concentration 50.

Minimum Inhibitory Concentration (MIC) of the hydroethanolic extract and fractions of *T. stans* by the microdilution method in liquid medium

The antimicrobial activities of the extract and the different fractions (hexane, acetate, n-butanol and water) and the findings are presented in Table 4 below.

Table 4: MIC (mg/mL) of *T. stems* extract and fractions on five bacterial strains and one yeast strain

Extract/strain	EC ATCC 25922	KP NR 4189	SA ATCC 12600	SA ATCC 700698	Spi	CA ATCC 14516
$TS_{n\text{-}but}$	32	>32	2	1	16	16
TS_{ET}	1	1	1	1	1	8
TS_{H20}	32	>32	4	2	32	>32
TS_{Ac}	1	1	1	1	0.5	1
TS_{Hex}	>32	32	8	32	32	>32
CP (ug/mL)	0.019	/	0.15	10	0.039	> 10

EC: Escherichia coli, KP: Klebsiella pneunoniea; SA: Staphylococcus aureus; Spi: Streptococcus pneumoniea-, CA: Candida albicans. $TS_{ET:}$ hydrocthanolic extract of *T. stans T. stans* TS_{Ac} : acetate fraction *T. stans* $TS_{n\text{-}But}$, TS_{H2O} fraction *T.stans*, TS_{H2O} : aqueous fraction *T.stans*, EC: *E. coli*, KP: *K. pneumonia*, SA: *S. aureus*, Spi: *S. pneumonia*, CA: *C. albicans*, ATCC: American Type Culture Collection

The experiment was conducted according to microdilution technics. The different samples showed variable levels of activity depending on the strains tested.

The total extract and the fractions of *T. stans* were active on the genus tested at different concentrations. MICs ranged from 0.5 mg/mi to 32 mg/ml, the total extract, n-butanol and water fractions were active on all strains except *K. pneumoniea*, which showed no sensitivity at the concentrations tested. The lowest MIC of the samples was obtained on *Steptococcus pneumoniae* (0.5 mg/ml) by the acetate fraction, which was active only on Gram + germs. The hexane fraction was active on all strains except *E. coli*, which showed no sensitivity at the concentrations tested. The different results of *T. stans* extracts show low activity against gram-negative germs, with MICs above the concentrations tested (MIC > 32 mg/ml and MIC > l mg/ml) for *K. pneumoniea* and *E. coli.* For gram-positive strains, MICs ranged from 0.5 to 32 mg/ml. The lowest concentrations were obtained on the *Streptococcus pneumoniae* strain with the acetate fraction (0.5 mg/ml). For S. *aureus* ATCC 12600, the lowest MIC was obtained with the acetate fraction (1 mg/ml) and the hexanic fraction had the highest MIC (8 mg/ml). Concerning *S. aureus* ATCC 700698, the lowest MIC was obtained by the acetate and n-butanol fractions (1 mg/ml), and the highest MIC was obtained with hexanic fraction (32 mg/ml) (Table 3). For the yeast strain, MICs ranged from 1 mg/ml for the acetate fraction to more than 32 mg/ml that was the highest concentration tested. As shown in table 3, acetate fraction exhibited the lowest concentration, followed by total extract (8 mg/ml) and n-butanol extract (16 mg/ml).

DISCUSSION

Many plants in developing countries are used by the population to resolve their health problems. But these plants do not have scientific proof to validate the traditional use. So, this study aimed to investigate the antioxidant and antimicrobial properties of the hydroethanolic extract of *T. stans* as well as it fractions.

The results of the phytochemical screening made it possible to highlight the presence of tannins, polyphenols, flavonoids, quinones, coumarins, cardiotonic glycosides. These results are similar to those of Anburaj *et al.* (2016)[22]. On the other hand, some secondary metabolites such as alkaloids, which was found the present by Govindappa *et al.* (2011) of alkaloids in extracts from the trunk of *T. stans* [23] where absent in our extract. We also noted the absence of other metabolites (saponins, resins, anthocyanins, phlobotannins, and betacyanins). Though, several other families of compounds such as glycosides, saponins, tannins, alkaloids and sugars were found in methanolic extracts of *T. stans* [24]. All this can be explained by environnemental factors including the place of harvesting, the part of the plant used and the season of the harvest. In fact plants interact with the environment for their survival, and are influenced by environmental factors including biotic and abiotic stimulants that regulate biosynthesis of secondary metabolites [25]. Plants of the same species that grow in different environments may have different secondary metabolite concentrations[26].

The results of the polyphenol content that we obtained are different from those obtained by Vandana *et al.* (2011) [27] on ethanolic extracts of the aerial parts of *T. stans*, where they obtained the total phenol content of 60.238 mg/g [28]. Similarly, these results differ from those of Mohamed *et al.* (2013) who found a polyphenol content of 50.3±3 mg GAE/g DM in the ethanolic extract of leaves[29]. The flavonoid content was above that found by Vandana *et al.* (2011) (6.545 mgGAE/l00g) [27]. In addition, Mohamed et *al* (2013) found a higher flavonoid content (40.66±5.03 mg GAE/g) in the leaves and branches of *T. stans* [29]. This would be justified by the part of the plant used, namely the leaves of *T. stans.* Such a high proportion of flavonoids in plants could be explained by the fact that they are synthesized in greater quantities to protect the aerial parts of plants from oxidation caused by solar radiations. The stronger the radiation, the higher the flavonoid content [30-31], this could explain the difference in flavonoid content in the bark and leaves, with a higher value in the leaves.

The extraction methods used would also explain these different results. In fact, we obtained the different fractions for our work by the fractionation of the total hydroethanol extract, whereas the extracts studied in the

aforementioned works were obtained by direct extraction from the different powders. The high content of total phenols and flavonoids is linked to extraction using a highly polar solvent. As a result, the secondary metabolites present are mainly found in the most polar fractions (n-butanol and water).

Concerning the antioxidant activity, plants extracts are mixtures of several compounds, with different functions, polarities and chemical behaviors. This chemical complexity of the extracts could lead to scattered results depending on the test used [32]. Therefore, an approach with multiple analyzes to evaluate the antioxidant potential of the extracts would be more informative and even necessary. So, the various samples of *T. stans* was tested using anti-free radical activity (DPPH, ABTS) and reducing activities (FRAP) to determine their antioxidant activity. Our findings outlined that the extract and fractions were effective. This activity of the extracts and fractions can be explained by the presence of phenolic compounds and flavonoids in the plant. However, we saw that, some sample exhibit no activity, although containing good concentration of polyphenols. This implies that polyphenols are not the only ones responsible for the effectiveness of the samples. In fact, it has been reported that apart from phenolic compounds, antioxidant activity could be due to other bioactive compounds in the extracts such as vitamins, some sugars [33]. So, the results we obtained suggest that there is no link between the activity and the content of phenolic compounds and flavonoids in the plant. Moreover, antioxidant activity also depends on the chemical structure of antioxidants and the interaction between them and other plant constituents [34, 23].

Evaluation of antibacterial activity showed that all microbial strains were sensitive to the samples tested at different concentrations. These results revealed activity on both Gram- and Gram+ bacteria, except *K. pneumonia* and *E. coli*. These results differ from those obtained by Govindappa *et al* [23] who demonstrated that the methanolic extract of *T. stans* leaves was active on *Escherichia coli.* With regards to *C. albicans*, the results obtained showed an antifungal activity of the various extracts at various concentrations (8 to 16 mg/ml). These results are in line with those of Al-Azzawi *et al* (2022), who showed the activity of *T. stans* leaf and root extracts on *C. albicans* [35]. The antimicrobial activity observed could be correlated with the chemical composition of the plants studied, which contain some secondary metabolites such as polyphenols, flavonoids and tanins [36]. Indeed, Deeni and Sadiq (2003) showed that the presence of these families of compound may explain the antimicrobial activity [37]. A study carried out by Gonsalves *et al* (2021) also showed that the flavonoids present in *Tecoma stans* had significant inhibitory activity against Gram-positive and Gram-negative strains of bacteria [38]. Thus, the antimicrobial (antibacterial and antifungal) activity of plants could be due to the presence of these secondary metabolites [37].

CONCLUSION

Tecoma stans belongs to the Bignoniaceae family and is a plant used in Cameroon to treat various ailments. In this study, the antioxidant and antimicrobial activities of this plant were assessed. The results revealed that *T.stans* extracts have DPPH and ABTS free radical scavenging and reducing properties, justifying their antioxidant activity. In addition, extracts of this plant have shown both antibacterial and antifungal activity, which could explain its use in Cameroonian folk medecine, for the treatment of wounds and gastroenteritis. Further studies will be conducted to investigate the specific compounds that may be responsible of these observed activities.

ACKNOWLEDGEMENTS

Our acknowledgments go: To the Laboratory of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medecine and Biomedical Science, University of Yaounde I, for receiving us in their working group.

To the Laboratory for Phytobiochemistry and Medicinal Plants Studies, Department of Biochemistry, Faculty of Science, University of Yaounde I, Cameroon for their great support in the implementation of this work.

REFERENCES

1. Ikuta, K. S., Swetschinski, L. R., Aguilar, G. R., Sharara, F., Mestrovic, T., Gray, A. P., ... & Dhingra, S. (2022). Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *400*(10369), 2221-2248.
2. Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., ... & Zorzet, A. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet infectious diseases*, *18*(3), 318-327.
3. Wahl, B., O'Brien, K. L., Greenbaum, A., Majumder, A., Liu, L., Chu, Y., ... & Knoll, M. D. (2018). Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. *The Lancet Global Health*, *6*(7), e744-e757.
4. OMS (2022) Un rapport met en avant une progression de la résistance aux antibiotiques dans les infections bactériennes chez l'être humain et la nécessité de disposer de données plus solides. Organisation Mondiale de la Santé. https://www.who.int/fr/news/item/09-12-2022-report-signals-increasing-resistance-to-antibiotics-in-bacterial-infections-in-humans-and-need-for-better-data

5. Dannaoui, É. (2013). Résistance des Candida aux antifongiques: détection et mécanismes. *revue francophone des laboratoires*, *2013*(450), 71-77.
6. Denning, D. W. (2024). Global incidence and mortality of severe fungal disease. *The Lancet Infectious Diseases*.
7. Muteeb, G., Rehman, M. T., Shahwan, M., & Aatif, M. (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, *16*(11), 1615.
8. World Health Organization. www. who. int/en/news-room/fact-sheets/detail/antimicrobial-resistance.
9. Romain, C., Clément, O., Sismeiro, D., Louis, M. (2016) Inter pharma ; infectiologie (édition 2016) - Romain Courseau, Clément Ourghanlian, Diane Sismeiro, Mathilde Louis - Vernazobres Grego - Grand format - Lamartine PARIS. 2nd ed. Vernazobres Grego; 2016 [cited 2024 Feb 26]. https://www.lamartine.fr/livre/9782818314265-inter-pharma-infectiologie-edition-2016-romain-courseau-clement-ourghanlian-diane-sismeiro-mathilde-louis/
10. Sobottka, A. M., Tessaro, E., Silva, S. M. D., Pedron, M., & Seffrin, L. T. (2021). Polyphenol content and antioxidant potential of Allophylus edulis (A. St.-Hil. et al.) Hieron. ex Niederl. and Cupania vernalis Cambess.(SAPINDACEAE). *Revista Árvore*, *45*, e4507.
11. Youl, O., Moné-Bassavé, B. R. H., Yougbaré, S., Yaro, B., Traoré, T. K., Boly, R., ... & Hilou, A. (2023). Phytochemical Screening, Polyphenol and Flavonoid Contents, and Antioxidant and Antimicrobial Activities of Opilia amentacea Roxb.(Opiliaceae) Extracts. *Applied Biosciences*, *2*(3), 493-512.
12. Mursaliyeva, V. K., Sarsenbek, B. T., Dzhakibaeva, G. T., Mukhanov, T. M., & Mammadov, R. (2023). Total content of saponins, phenols and flavonoids and antioxidant and antimicrobial activity of in vitro culture of Allochrusa gypsophiloides (Regel) Schischk compared to wild plants. *Plants*, *12*(20), 3521.
13. Gadouche, L., Alsoufi, A. S. M., Pacholska, D., Skotarek, A., Pączkowski, C., & Szakiel, A. (2023). Triterpenoid and steroid content of lipophilic extracts of selected medicinal plants of the Mediterranean region. *Molecules*, *28*(2), 697.
14. Pal, L., Dwivedi, V., Gupta, S. K., Saxena, S., Pandey, A., & Chattopadhyay, D. (2023). Biochemical analysis of anthocyanin and proanthocyanidin and their regulation in determining chickpea flower and seed coat colour. *Journal of Experimental Botany*, *74*(1), 130-148.
15. Vinson, J. A., Hao, Y., Su, X., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *Journal of agricultural and food chemistry*, *46*(9), 3630-3634.
16. Dhar, P., Tayade, A. B., Saurav, S. K., Chaurasia, O. P., Srivastava, R. B., & Singh, S. B. (2012). Antioxidant capacities and phytochemical composition of Hippophae rhamnoides L. leaves methanol and aqueous extracts from trans-Himalaya. *Journal of Medicinal Plants Research*, *6*(47), 5780-5788.
17. SAKANDE, J., SAWADOGO, M., PALE, E., LOMPO, M., NIKIEMA, J., KABRE, E., ... & GUISSOU, I. (2006). Etude de l'effet anti-oxydant d'extraits d'inflorescences mâles de Borassus aethiopum Mart (Arecaceae): Anti-oxidant activity of Borassus aethiopum male inflorescences extracts. *Sciences de la Santé*, *29*(1 et 2).
18. Khan, R. A., Khan, M. R., Sahreen, S., & Ahmed, M. (2012). Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill. *Chemistry Central Journal*, *6*, 1-7.
19. Berker, K. I., Güçlü, K., Tor, İ., & Apak, R. (2007). Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. *Talanta*, *72*(3), 1157-1165.
20. CLSI, (2012) Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., M02-A11 Vol. 32 No. 1
21. Rex, J., Alexander, B., Andres, D., Arthinton-Skaggs, B., Brown, S., Chaurvedi, V., ... & Ostrosky-Zeichner, L. (2008). Clinical and Laboratory Standards Institute: M27-A3 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. *Approved Standard—*.
22. Anburaj, G., Marimuthu, M., Rajasudha V. & Manikandan, R. (2016) Phytochemical screening and GC-MS analysis of ethanolic extract of *Tecoma stans* (Family: Bignoniaceae) Yellow Bell Flowers. *Journal of Pharmacognosy and Phytochemistry* 2016; 5(4): 172-175
23. Sadananda, T. S., Jeevitha, M. K., Pooja, K. S., & Raghavendra, V. B. (2011). Antimicrobial, Antioxidant Activity and Phytochemical Screening of Tecoma stans (L.) Juss. ex Kunth. *Journal of Phytology*, *3*(3).
24. Galano, A., Mazzone, G., Alvarez-Diduk, R., Marino, T., Alvarez-Idaboy, J.R. & Russo, N. (2016) Food Antioxidants: Chemical Insights at the Molecular Level. *Annu Rev Food Sci Technol* 7, 335–352 https://doi.org/10.1146/annurev-food-041715-033206
25. Zhi-lin, Y., Chuan-chao, D. & Lian-qing, C. (2007) Regulation and accumulation of secondary metabolites in plant-fungus symbiotic system. *African Journal of Biotechnology* 6 https://www.ajol.info/index.php/ajb/article/view/57436
26. Radusiene, J., Karpaviciene, B. & Stanius, Ž. (2012) Effect of External and Internal Factors on Secondary Metabolites Accumulation in St. John's Worth. *Botanica Lithuanica* 18 https://doi.org/10.2478/v10279-012-0012-8

27. Arbale, V., Lavate, S.M., Jadhav, R.B., Kamble, G.S. & Deshpande, N.R. (2011) Evaluation of phenol and flavonoid content from aerial parts of *Tecoma stans*. *International Journal of Pharmacy and Pharmaceutical Sciences* 3, 126–127
28. Sowemimo, A., Samuel, F. & Fageyinbo, M.S. (2013) Anti-inflammatory activity of *Markhamia tomentosa* (Benth.) K. Schum. Ex Engl. ethanolic leaf extract. *Journal of Ethnopharmacology* 149, 191–194 https://doi.org/10.1016/j.jep.2013.06.020
29. Mohamed, Z.M.S., Yousry, M.G., L M, C., Nader, A.E.S. & Salem, A Z M. (2013) Antioxidant and antibacterial activities of leaves and branches extracts of *Tecoma stans* (L.) Juss. ex Kunth against nine species of pathogenic bacteria. *Afr. J. Microbiol. Res.* 7, 418–426 https://doi.org/10.5897/AJMR12.2274
30. Fethi, R. (2017) Coumarines à intérêt thérapeutique : Synthèse et contrôle analytique
31. El-Chaghaby, G.A., Ahmad, A.F. & Ramis, E.S. (2014) Evaluation of the antioxidant and antibacterial properties of various solvents extracts of Annona squamosa L. leaves. *Arabian Journal of Chemistry* 7, 227–233 https://doi.org/10.1016/j.arabjc.2011.06.019
32. Öztürk, M., Aydoğmuş-Öztürk, F., Duru, M.E. & Topçu, G. (2007) Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. *Food Chemistry* 103, 623–630 https://doi.org/10.1016/j.foodchem.2006.09.005
33. Musaa, N.C. Phytochemical study and evaluation of antimicrobial activity of methanolic leaf extract of *Tecoma stans* (L.) Juss Ex Kunth (Bignoniaceae)
34. Nimse, S.B. & Pal, D. (2015) Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv.* 5, 27986–28006 https://doi.org/10.1039/C4RA13315C
35. Al-Azzawi, A., Al Dibsawi, A., Talath, S., Wali, A.F. & Sarheed, O. (2022) Method Development: The Antioxidant and Antifungal Activity of the Isolated Component from the Ethanolic Extract of *Tecoma stans* Leaves Using Flash Chromatography https://doi.org/10.3390/separations9100317
36. Khattab, A., Awad, N.E., Fadeel, D.A. & Fadel, M. (2022) Reviewing the reported pharmacognostic and pharmacological investigations on *Tecoma stans* Juss. ex Kunth. *J Herbmed Pharmacol* 12, 25–40 https://doi.org/10.34172/jhp.2023.03
37. Deeni, Y. & Sadiq, N. (2002) Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. *Journal of ethnopharmacology* 83 https://doi.org/10.1016/s0378-8741(02)00244-1
38. Gonçalves, T. P. R., Parreira, A. G., dos Santos Zanuncio, V. S., de Souza Farias, K., da Silva, D. B., & dos Santos Lima, L. A. R. (2022). Antibacterial and antioxidant properties of flowers from Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae). *South African Journal of Botany*, *144*, 156-165.