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Phytochemical screening and evaluation of antiradical and antibacterial activities of stem and root barks of *Gardenia ternifolia* Schumach. (Rubiaceae): A plant traditionally used in northern Côte d'Ivoire

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Abstract

Gardenia ternifolia is an Ivorian plant species used to treat tooth decay, hemorrhoidal crises, leprosy, skin diseases and rheumatism. The present study is based on phytochemical screening and evaluation of the antiradical and antibacterial activities of aqueous, hydroethanolic and ethanolic extracts of *G. ternifolia* stem and root barks. Phytochemical screening of secondary metabolites was carried out using tube color reaction methods and Thin Layer Chromatography (TLC). Phytochemical screening revealed the presence of polyphenols (flavonoids, coumarins, tannins), saponins, steroids and terpenoids in *G. ternifolia* stem and root extracts. Phenolic acids were revealed only in stems, while alkaloids were absent in both organs studied. Anti-radical activities were highlighted in both organs by qualitative and quantitative methods with respect to the DPPH free radical. However, quantitative spectrophotometric analysis showed better antioxidant activity in stems than in roots. Antibacterial activity assessed against 05 bacterial strains using the solid-medium diffusion and liquid-medium dilution methods showed that only the aqueous and ethanolic extracts of *G. ternifolia* stems were absolutely bactericidal against the *Staphylococcus aureus* strains tested (*S.a* ATCC 29213, *S.a* 06 UB/22 and *S.a* 27 UB/22). The results obtained could give the stems the ability to treat infections caused by *S. aureus* bacteria.

Keywords: *Gardenia ternifolia;* Phytochemical screening; Antiradical activity; Antibacterial activity; Ivory Coast

1. Introduction

People have always used plants, which are a rich source of natural products, to treat various illnesses [1]. This recourse to traditional medicine, which is worldwide, but particularly in Africa [2], is due to the inaccessibility and unbearable costs of modern medicines and health centers by the majority of populations, and to the resistance of certain pathogens [3]. In addition, some of these so-called conventional treatments have harmful side effects for the human organism [4,5]. With this in mind, this study focused on *Gardenia ternifolia*, a plant in the Ivorian pharmacopoeia traditionally used for the treatment of several pathologies, such as bacterial infections [6], diarrhea [7], high blood pressure [8,9], liver

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necrosis [10] and cancer [11]. These pathologies are mostly caused by microbial infections and excess free radicals in the body. Thus, the aim of the present work is to confirm or refute the traditional use of *G. ternifolia* stems and roots as good antioxidants and antibiotics capable of preventing and/or treating pathologies linked to oxidative stress and bacteria.

2. Material and methods

2.1. Material

2.1.1. Plant material

The plant material consists of *G. ternifolia* stem and root barks. The plant was selected on the basis of an ethnobotanical survey of several traditional medicine practitioners in northern Côte d'Ivoire. The various organs were harvested in February 2022 in the town of Korhogo (9° 27' 28'' North, 5° 37' 46'' West), in the village of Kapékaha. The species was authenticated by botanists at the Peleforo GON COULIBALY University (Korhogo). The various organs were dried for 5 days in a room at room temperature, sheltered from the sun. Finally, the dried organs were crushed in a mortar and sieved to obtain fine powders which were used to prepare the different extracts to be tested.

2.1.2. Laboratory materials and equipment

The laboratory equipment consists of the usual glassware, an electronic balance, a hot plate, a water bath, a JENWAY 7315 spectrophotometer, petri dishes and an incubator.

2.1.3. Reagents and chemical products

Les produits chimiques utilisés sont de qualité analytique. Ils ont été achetés chez Polychimie (Côte d'Ivoire). Pour les tests de chromatographie sur couche mince (CCM), il a été fait usage des chromatoplaques en gel de silice 60 F_{254}, sur support aluminium [12]. Les révélateurs et réactifs employés sont le $FeCl_3$ à 2 %, le KOH à 10 %, la vanilline sulfurique, les réactifs de Dragendorff, de Shinoda et le DPPH.

The chemicals used are of analytical quality. They were purchased from Polychimie (Côte d'Ivoire). For thin-layer chromatography (TLC) tests, we used silica gel 60 F_{254} chromatoplates on an aluminum support [12]. The developers and reagents used were $FeCl_3$(2%), KOH (10%), sulfuric vanillin, Dragendorff reagent, Shinoda reagent and DPPH.

2.1.4. Bacterial strains

Five (05) bacterial strains were used:

- 03 *Staphylococcus aureus* species : *S. aureus* ATCC 29213, *S. aureus* 06 UB/22 and *S. aureus* 27 UB/22 : all collected from infected skin.
- 01 *Pseudomonas aeruginosa* species : *P. aeruginosa* 97 UB/22.
- 01 species of Enterobacteria : *Escherichia coli* 78 UB/22.

All these bacterial strains come from the Natural Substances Antibiotics and Anti-Infectious Microorganisms Surveillance Unit (ASSURMI) in the Bacteriology and Virology Department of the Institut Pasteur of Ivory Coast (IPCI).

2.2. Methods

2.2.1. Extractions

Maceration and decoction extractive techniques were used to obtain organic and aqueous extracts, respectively, from the two *G. ternifolia* organs studied.

Aqueous extracts

Aqueous extracts were obtained according to the method used in the work of Zirihi *et al*., [13]. A 10 g mass of powder from each organ was mixed with 100 mL of distilled water. The decoctions were obtained after 30 min at temperatures varying progressively up to 120 °C. After filtration, the various decoctions were placed in an oven at 50 °C for 4 days to obtain the aqueous crude extracts. These extracts were used to evaluate the free radical scavenging, by spectrophotometric, in tubes and antibacterial activities.

Ethanolics extracts

Following the method used in the work of Zirihi *et al.* [13], 10 g of powder from each organ (stems and roots) of *G. ternifolia*. was macerated in 100 mL of ethanol for 24 hours. After filtration, the different macerates were placed in an oven at 50°C for 4 days. The crude ethanolic extracts were then used to evaluate their free radical scavenging in tubes, by spectrophotometry activities and antibacterial activities.

Hydroethanolics extracts

A 10 g mass of each organ powder was macerated in 100 mL of ethanol/water mixture (70mL / 30 mL) for 24 h. After filtration, the macerates were placed in an oven at 50°C for 2 h to remove the ethanol. The extract obtained is kept for 24 h in the refrigerator at 4°C for precipitation of lipophilic compounds. After decantation, the hydro-ethanolics extracts were used to evaluate phytochemical screening in tubes and antioxidant activity (tube and spectrophotometry), and to prepare selectives extracts [14].

Extracts selectives

A 15 mL volume of each hydro-ethanolic extract was depleted by successive fractionations with (3 × 10 mL) hexane (C_6H_{14}), dichloromethane (CH_2Cl_2) and ethyl acetate (AcOEt). The various selectives organics fractions are concentrated in the oven and have been used for phytochemical screening and to assess antioxidant activity on TLC plates [14],

2.2.2. Phytochemical screening

Color reaction tests

The search for polyphenols, flavonoids, coumarins, saponins, sterols and polyterpenes was carried out by means of color tests in test tubes following the analytical techniques described in the work of Ladyguina *et al.,* [15]; Dohou *et al.,* [16]; Békro *et al.,* [17].

- Detection of saponins : Foam test

A 2 g mass of ground dry plant material is boiled in 100 mL distilled water for 30 min (or 20 min after initial boiling). After cooling and filtration, the solution is made up to 100 mL with distilled water. From this stock solution, 10 tubes (1.3 cm internal diameter) are prepared with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mL; the volume in each tube is readjusted to 10 mL with distilled water. Each tube is shaken vigorously in the horizontal position for 15 sec. After 15 min rest in vertical position, the height (in cm) of the persistent foam is recorded. If it is less than 1 cm in all tubes, the foam index (Im) sought is less than 100. If it is 1 cm in one of the tubes, then the foam index is calculated by the following formula:

$$\mathrm{Im} = \frac{1\,000}{\mathrm{N°tube}}$$

The presence of saponins in the plant is confirmed with an index greater than or equal to 100 [16].

- Polyphenols detection

A few drops of a 2% (w/v) aqueous iron (III) chloride solution ($FeCl_3$) were added to 2 mL of crude hydroethanolic extract. The appearance of a blue-black or green-black coloration indicates the presence of polyphenols [15,17].

- Flavonoid detection: Shinoda test

5-7 drops of concentrated HCl and 2-5 Mg chips are added to 2 mL of crude hydroethanolic extract. In the presence of flavonoids, a pink-orange coloration is observed after 3 to 5 min. To accelerate the reaction and enhance color, the reaction mass is heated in a water bath for 2-3 min [15,17].

- Coumarin detection : Potassium hydroxide (KOH) test

10 drops of 10% (w/v) alkaline methanolic KOH solution are added to 3-5 mL of plant extract. The mixture is heated in a water bath. Next, 5-10 mL of distilled H_2O are added and the reaction mass is vigorously stirred. The resulting solution is neutralized with 10% (v/v) HCl until an acidic solution is obtained. If cloudiness or precipitation is observed, the presence of coumarins is confirmed [15,17].

- Sterol and polyterpene detection

An aliquot of hydroethanolic crude extract is dissolved in 1 mL acetic anhydride ($CH_3CO_3CCH_3$) in a test tube. Next, 0.5 mL of concentrated sulfuric acid (H_2SO_4) is slowly poured over the walls of the test tube. The appearance of a violet coloration, turning blue and then green, indicates a positive reaction [17].

Phytochemical screening on TLC plates

The search for tannins, phenolic acids and alkaloids was carried out using TLC plate tests following the methods described by Ladyguina *et al.,* [15], Georgievskii *et al.,* [18], Dawson *et al.,* [19], Mamyrbékova-Békro *et al.,* [20] and Kabran [21].

Using capillaries, 2 µL of each selective extract is deposited as a dot 0.5 cm from both edges of the chromatographic plate. The TLC plates are then placed in the migration tank containing the migration solvents (developing agents).

After development, chromatograms were visualized using visible light developers. Colorations appearing as spots are recorded and frontal ratios (Rf) calculated.

2.2.3. Estimation of antioxidant power

DPPH tube screening of ethanolics extracts

The method used for this test is that proposed by Popovici et *al.* [22].

In a 0.5 mL volume of extract solution, 1.5 mL of violet DPPH is added, and the positive reaction is reflected by the appearance of a yellow coloration in the medium after 15 min incubation.

DPPH TLC screening of selectives extracts

The TLC screening for antioxidant capacity used is that developed by the method described by Takao *et al.,* [23].

A 10 µL volume of each plant extract solution is deposited on a chromatoplate (silica gel 60 F254, on aluminum support (Merck)), which is then placed in a chromatography tank saturated with migration solvent. After development, chromatograms are dried and then developed with an ethanolic solution of DPPH (0.2 mg/mL). After 30 min of optimal time, extract constituents with potential free radical scavenging activity are revealed as pale-yellow spots on a violet background.

Assessment of antioxidant activity against DPPH by spectrophotometry

The antioxidant potential of the extracts was assessed using the method of Blois [24].

DPPH was solubilized in absolute EtOH to obtain a solution with a concentration of 0.3 mg/mL. Different concentration ranges (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL) of the extract are prepared in absolute EtOH. 2.5 mL plant extract and 1 mL ethanolic DPPH solution are added to dry, sterile tubes. After shaking, the tubes are placed in a dark place for 30 min. The absorbance of the mixture is then measured at 517 nm against a blank consisting of 2.5 mL pure EtOH and 1 mL DPPH solution. The positive reference control is ascorbic acid (vitamin C). DPPH inhibition percentages are calculated according to the formula :

$$I(\%) = (A_b - A_e) / A_b \times 100$$

- **I** : inhibition percentage
- $\mathbf{A_b}$: absorbance of blank
- $\mathbf{A_e}$: absorbance of sample

The concentrations required to trap 50% (IC50) of DPPH are determined from the graphs showing the percentage inhibition of DPPH as a function of extracts or vitamin C concentrations.

2.2.4. Study of the antibacterial activity of aqueous and ethanolic extracts

The antibacterial study was carried out in 4 stages:

- The first was to perform a sterility test on the plant extracts. To 10 mL of thioglycholate broth is added 0.1 g of each plant extract to be tested, and the mixture is incubated at 37 °C for 24 h. The agar plate was then inoculated with the broth. The plate containing the nutrient agar was incubated under the same conditions. The substance is declared sterile if no colonies are visible on the agar [25] ;
- The second step was to select the bacteria. To ensure reliable results, the strains must be young. This involved streaking with a calibrated loop at 2 µL on Mueller Hinton agar, followed by incubation at 37°C for 24 h. The young bacteria, after growth, are ready to be plated. After growth, the young bacteria are used for the experiment ;
- The third step was to determine the antibacterial efficacy of the extracts, using the method described by Wiegand et *al*. [26] and Biyiti et *al.* [27]. The dishes were first inoculated with the bacterial strains. Then, wells are dug in the culture media, into which the extracts to be tested are added at a concentration of 50 mg/mL. Once dry, the media are incubated at 37 °C for 24 h. The presence of a zone of inhibition greater than 08 mm in diameter indicates that the extract has antibacterial activity ;
- The fourth step was to determine the MIC and BMC according to Dosso & Faye-kette [28]. These biological parameters were used to verify the bactericidal or bacteriostatic properties of the extracts.

2.2.5. Statistical analysis

Analyses of the measurements obtained during the various manipulations were carried out using EXCEL software. It was used to plot the various diagrams and also to determine the IC_{50} parameter for each extract.

3. Results

3.1. Yields

Maceration and decoction are the two extraction methods chosen for this study. They have been used to extract a large number of organic compounds. These are the techniques most commonly used by healers and practitioners of traditional medicine to extract active plant ingredients from medicinal recipes.

The yield values obtained after the various extractions of *G. ternifolia* are given in Table 1. Yields of extracts obtained by maceration and decoction range from 4.94±1.12% to 43.79±0.95% for ethanolic and aqueous extracts of *G. ternifolia* roots respectively.

Table 1 Yields of differents *G. ternifolia* extracts

		GTT Aq	**GTT Et**	**GTT H-Et**	**GTR Aq**	**GTR Et**	**GTR H-Et**
	r_1 (%)	17.1548	10.08	23.172	45.222	4.1536	12.315
Yields	r_2 (%)	17.4268	12.0044	20.845	42.4048	6.6256	11.068
	r_3 (%)	16.8184	15.484	26.928	43.7536	4.0464	13.001
	r_{moy} (%)	**17.13**	**12.52**	**23.65**	**43.79**	**4.94**	**12.13**
Standards deviations		0.21	1.97	2.19	0.95	1.12	0.71

3.2. Phytochemical screening

3.2.1. Phytochemical screening in tubes

Detection of saponins (foam index)

The presence of saponins in *G. ternifolia* stem and root barks was determined by Dohou *et al*, [16], based on the calculation of the moss index (Im) with values above 100 (Table 2).

Table 2 Detection of saponins (Moss index)

	Foam height (cm)										
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**Im**
GTT	0.7	2.7	2.9	3.6	4.2	4.8	5.2	5.9	6.2	7.0	500
GTR	0.2	0.7	3.2	3.3	3.4	5.0	6.2	7.4	8.0	8.8	333.33

Detection of polyphenols, flavonoids, coumarins, sterols and polyterpenes

The results of phytochemical screening for polyphenols, flavonoids, coumarins, sterols and polyterpenes in hydroethanolics extracts of *G. ternifolia* stem and root bark are shown in Table 3. They are interpreted as follows: (+) presence and (-) absence.

Table 3 Detection of polyphenols, flavonoids, coumarins and sterol-terpenes

	Polyphenols	**Flavonoids**	**Coumarins**	**Sterols and terpenes**
GTT	+	+	+	+
GTR	+	+	+	+

3.2.2. Phytochemical screening by TLC

The various secondary metabolites sought were identified using the following migration or developing solvents:

- Hexane / ethyl acetate (5 : 0.375 ; V/V) for hexane extracts ;
- Dichloromethane / ethyl acetate / acetic acid (CH_3COOH) (1 :4 :1 ; V/V/V) for dichloromethane and acetate-ethyl extracts;

Test results for alkaloids (Table 5), tannins and phenolic acids (Table 4) are given below. Tannins are present in the stems and roots of *G. ternifolia*. Phenolic acids are only present in the stems, while alkaloids are absent from both organs.

Table 4 Detection of tannins and phenolic acids in CH_2Cl_2/AcOEt/ CH_3COOH (1 :4 :1) (V/V/V) developer

Extracts	**R_f (Color) : *Possible compound***
GTT H-Et	0.91 (gray) : tannin ; 0.12 (green) : phenolic acid ; 0.00 (gray) : tannin
GTR H-Et	0.10 (gray) : tannin

Table 5 Detection of alkaloids in CH2Cl2/AcOEt/C6H14/CH3COOH (1,2, 1, 0,1) (V/V/V/V) developer

Extracts	**R_f (Color) : *Possible compound***
GTT H-Et	No alkaloid identified
GTR H-Et	No alkaloid identified

3.2.3. Summary of phytochemical screening

Table 6 summarizes the results obtained from the qualitative detection of secondary metabolites in tubes and on TLC plates.

Table 6 Summary table of phytochemical screening of secondary metabolites

	Polyph.	**Flav.**	**Coum.**	**Ste.**	**Terp.**	**Sap.**	**Alka.**	**Tan.**	**A. phe.**
GTT	+	+	+	+	+	+	-	+	+
GTR	+	+	+	+	+	+	-	+	-

(+) ; présence ; (-) : absence ; Polyph : Polyphenols ; Flav. : Flavonoids ; ste. : Sterols ; Terp. : Terpenes ; Coum. : Coumarins ; Sap : Saponins ; Alka. : Alcaloids ; A. phe : Phenolic acid.

3.3. Antioxidants activities

3.3.1. Antioxidant activity in tubes

After addition of DPPH solution to aqueous, ethanolic and hydro-ethanolics extracts of *G. ternifolia* stems and roots, a yellow coloration was observed, indicating the presence of antioxidant activity.

3.3.2. Antioxidant activity by TLC

The same migration solvents used to detect secondary metabolites by TLC were used to assess antioxidant potential by TLC. The results of the antioxidant activity of the extracts towards the DPPH radical are shown in Tables 7 and 8. The compounds that showed antioxidant activity by appearing yellow on a violet background include the tannins and phenolic acids detected during phytochemical screening by TLC.

Table 7 Phytocompounds scavenge DPPH in the developer CH_2Cl_2/AcOEt/CH_3COOH (1 ,4, 1) (V/V/V)

Extracts	**R_f (Color) : *Possible compound***
GTT H-Et	0.91 (yellow) : *tannin* ; 0.57 (yellow) : NI ; 0.46 (yellow) : NI ; 0.37 (yellow) : NI ; 0.31 (yellow) : NI ; 0.25 (yellow) : NI ; 0.19 (yellow) : NI ; 0.12 (yellow) : *phenolic acid* ; 0.06 (yellow) : NI ; 0.00 (yellow) : NI
GTR H-Et	0.38 (yellow) : NI ; 0.26 (yellow) : NI ; 0.21 (yellow) : NI ; 0.11 (yellow) : *tannin* ; 0.05 (yellow) : NI ; 0.00 (yellow) : NI

NI : unidentified compound.

Table 8 Phytocompounds scavenge DPPH in the developer CH_2Cl_2/AcOEt/C_6H_{14}/ CH_3COOH (1 ,2 ,1, 0,1)

Extracts	**R_f (Color) : *Possible compound***
GTT H-Et	0.94 (yellow) : NI ; 0.81 (yellow) : NI ; 0.69 (yellow) : NI ; 0.04 (yellow) : NI
GTR H-Et	0.94 (yellow) : NI ; 0.85 (yellow) : NI ; 0.10 (yellow) : NI ; 0.05 (yellow) : NI

NI : unidentified compound.

3.3.3. Antioxidant activity spectrophotometry of aqueous, ethanolics and hydroethanolics crude extracts

This method is scientifically more accurate than the qualitative methods performed in tubes and by TLC.

Inhibition percentages of aqueous, ethanolics and hydro-ethanolics crude extracts and vitamin C

The differents percentages of DPPH inhibition by aqueous, ethanolics and hydro-ethanolics extracts and vitamin C taken as the reference molecule are shown in Figures 1 and 2. The differents extracts show an anti-free radical potential whatever the extract concentration. Inhibition percentages for stem bark ranged from 43.133±1.027% for the aqueous extract to 98.689±0.109% for the ethanolic extract, while those for roots varied from 32.481±1.988% for the aqueous extract to 97.542±0.262% for the hydroethanolic extract. Vitamin C levels ranged from 63.913±0.131% to 99.705±0.000% at the same concentration ranges.

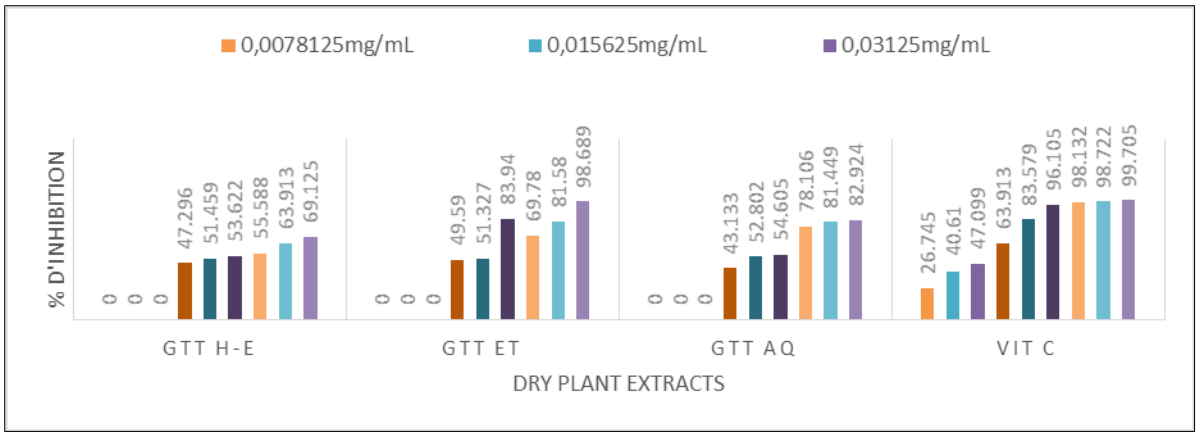

Figure 1 DPPH inhibition by hydroethanolic, ethanolic and aqueous extracts of GTT

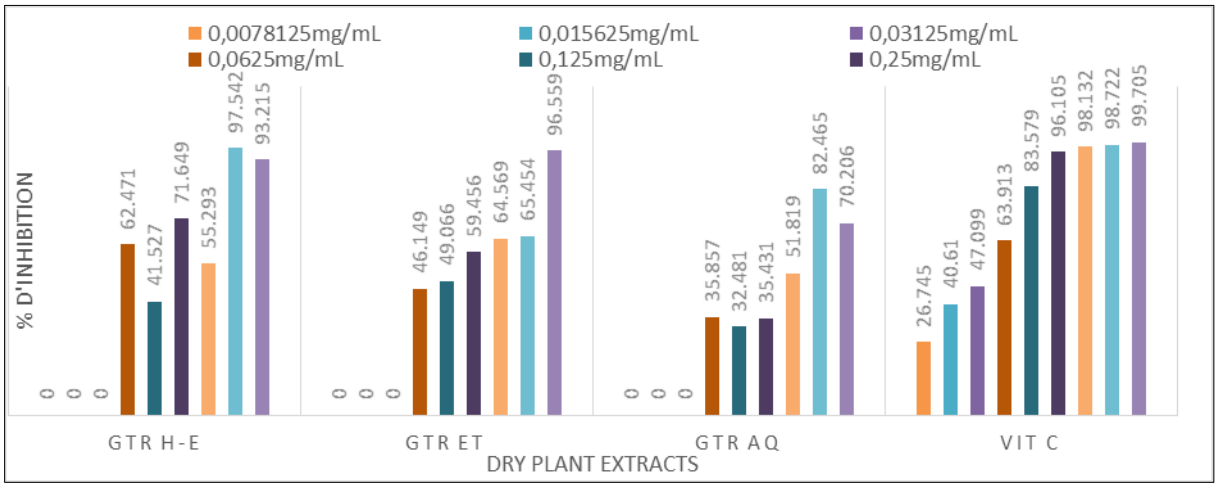

Figure 2 DPPH inhibition by hydroethanolic, ethanolic and aqueous extracts of GTR

Determination of IC50s for aqueous, ethanolic, hydro-ethanolic and vitamin C crude extracts

The 50% inhibitory concentration (IC_{50}) represents the concentration of an extract that results in a 50% loss of DPPH activity [29]. The lower the IC_{50}, the greater the antioxidant activity of the extract.

The IC_{50} values for aqueous and organic extracts in the table below range from 0.07833 mg/mL to 0.45013 mg/mL, while that for vitamin C is 0.03664 mg/mL.

Table 9 Summary table of IC_{50} values (mg/mL) for various extracts

Extraits	GTT H-Et	GTT Et	GTT Aq	GTR h-e	GTR Et	GTR Aq	Vit C
IC_{50}(mg/mL)	0.10310	0.07833	0.10689	0.35582	0.13624	0.45013	0.03664

3.4. Antioxidants activities

3.4.1. Sterility of aqueous and ethanolic crude extracts

The sterility test consisted in checking that the extracts were not contaminated. No colonies were observed on any of the agar plates after 24 hrs. As a result, the extracts studied did not appear to show any signs of contamination.

3.4.2. Antibacterial activity of aqueous and ethanolics crude extracts in liquid media

The results of bacterial sensitivity to aqueous and ethanolics extracts of *G. ternifolia* stems and roots and to antibiotics (Figure 3) are reported in Table 10. Inhibition disc diameters for aqueous, ethanolic and hydro-ethanolic extracts ranged from 06.0±0.0 to 13.7±0.4 mm, and for antibiotics from 6±0.0 to 23.0±0.7 mm.

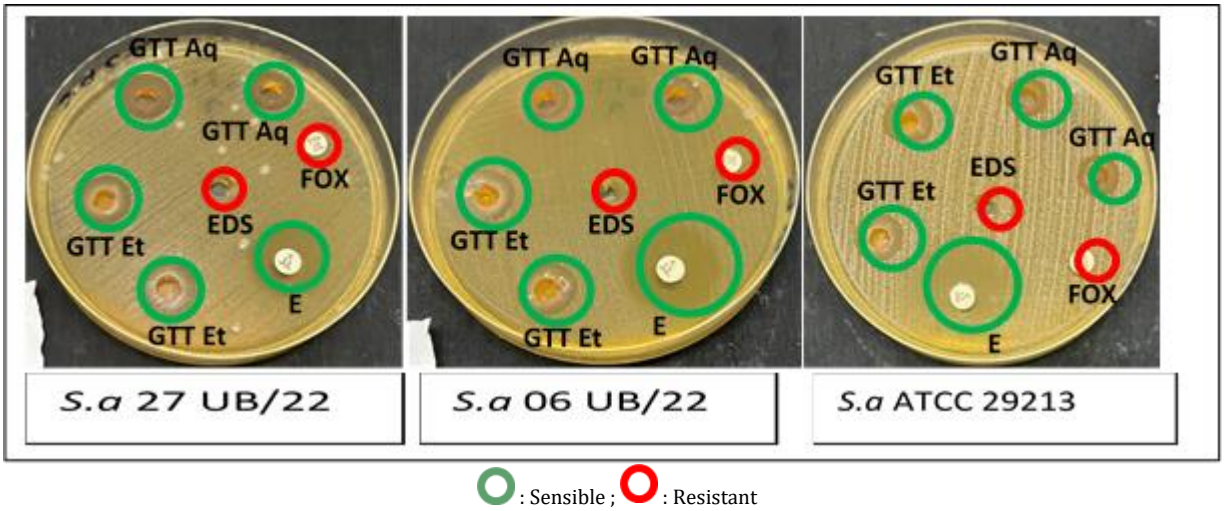

Figure 3 Sensitivity testing of study extracts and antibiotics against bacteria

Table 10 Inhibition diameters (mm) of bacteria against aqueous and ethanolics extracts and reference antibiotics

	G.T.T		G.T.R								
	H_2O	**EtOH**	**H_2O**	**EtOH**	**FOX**	**E**	**CRO**	**TCC**	**IPM**	**CIP**	**EDS**
S. A_1	10,3	12,7	06,0	06,0	06,0	23,0					06,0
S. A_2	11,7	13,00	06,0	06,0	06,0	20,0					06,0
S. A_3	13,7	13,7	06,0	06,0	06,0	18,3					06,0
E. C.	06,0	06,0	06,0	06,0			23,7	06,0			06,0
P. A	06,0	06,0	06,0	06,0					06,0	28,0	06,0

G.T.T: *Gardenia ternifolia* trunk bark; G.T.R: *Gardenia ternifolia* root bark; H_2O: water; EtOH: ethanol; FOX: cefoxitin; E: erythromycin; CRO: ceftriaxone; TCC: ticarcillin+clavulanic acid; IPM: imipenem; CIP: ciprofloxacin; EDS: sterilized distilled water; S. A1: *Staphylococcus aureus* ATCC 29213; S. A2: *Staphylococcus aureus* 06 UB/22; S. A3: *Staphylococcus aureus* 27 UB/22; E. C.: *Escheichia coli* 78 UB/22, and P. A.: *Pseudomonas aeruginosa* 97 UB/22

3.4.3. Antibacterial activity in liquid medium

Antibacterial activity in liquid medium was used to determine the MIC and BMC antibacterial parameters of the extracts showing activity on the bacterial strains used. These were the aqueous and ethanolics extracts of *G. ternifolia* stem bark for the three *Staphylococcus aureus* strains used in this study.

Figure 4 provides information on the techniques used to determine antibacterial parameters. The values obtained and associated interpretations are given in Table 11.

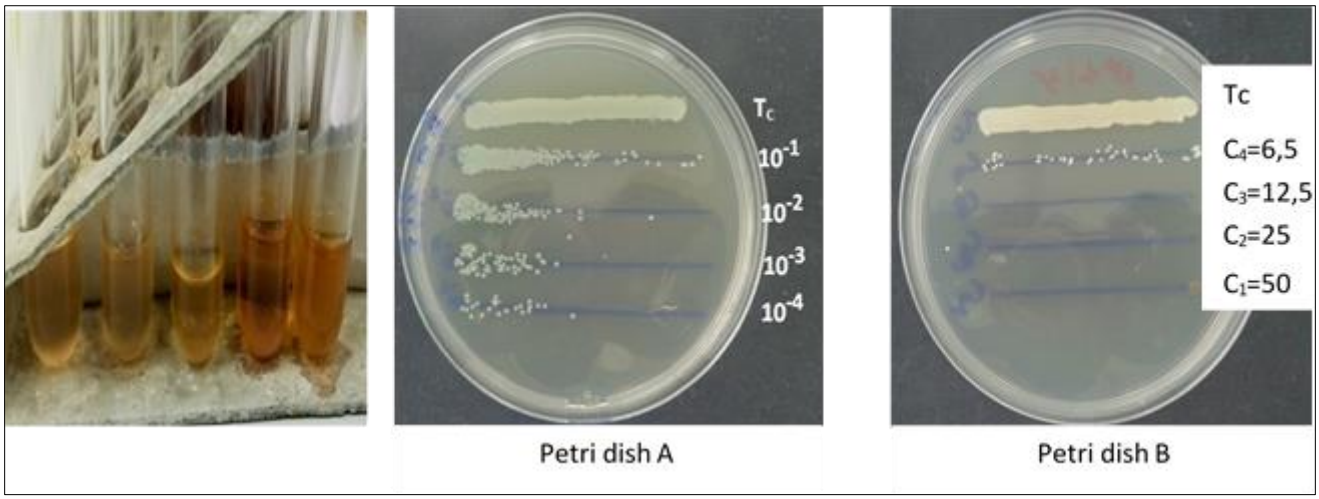

Figure 4 Determination of MICs and MBCs for aqueous and ethanolic extracts of *G. ternifolia* stems

Table 11 Antibacterial parameters of extracts on tested strains

Extracts	Strains	MIC mg/mL	MBC mg/mL	MBC/MIC	Interpretation
GTT H_2O	*S.a ATCC 29213*	6.25	6.25	1	Absolute bactericide
	S.a 06 UB/22	6.25	6.25	1	Absolute bactericide
	S.a 27 UB/22	6.25	6.25	1	Absolute bactericide
GTT EtOH	*S.a ATCC 29213*	6.25	6.25	1	Absolute bactericide
	S.a 06 UB/22	6.25	6.25	1	Absolute bactericide
	S.a 27 UB/22	6.25	6.25	1	Absolute bactericide

4. Discussion

The scientific work carried out falls within the scope of the valorization of plant species from the Ivorian flora. This study involved phytochemical screening and evaluation of the antiradical and antibacterial activities of *G. ternifolia* stem and root barks, with a view to confirming or invalidating their traditional uses as antioxidants and antibiotics capable of preventing and/or treating various pathologies.

Extractions by maceration and decoction were carried out using distilled water, ethanol and the distilled water/ethanol mixture, depending on the method chosen. Organic compounds are more extractable with the water/ethanol solvent mixture in stem barks (23.65±2.19%), while in root barks, distilled water presents the best extraction yield with a value of 43.79±0.95%. Overall, the various yields are relatively high, justifying the widespread use of these different extraction techniques.

Phytochemical screening carried out qualitatively in tubes and by TLC with extracts from *G. ternifolia* stem and root barks revealed the presence of polyphenols, flavonoids, coumarins, saponin tannins, sterols and terpenes in both organs. Phenolic acids were only revealed in the stems, while alkaloids were absent from both plant organs. These results are in line with those of work carried out on the same species but harvested in other countries. Indeed, work carried out in Senegal by Diouf [30] showed the presence of saponins, reducing compounds, sterols, triterpenes and polyphenolic substances such as tannins, flavonoids, coumarins and anthocyanins in *G. ternifolia* roots. Other researchers have identified certain flavonoids and sterols in roots [31,32].

The antioxidant activity of *G. ternifolia* root and stem bark extracts was assessed in tubes and by TLC, and may be partly explained by the presence of these secondary metabolites. Indeed, numerous studies have already demonstrated the

antioxidant properties of these compounds [33,34]. With regard to antioxidant activity by spectrophotometry, vitamin C shows the best antioxidant activity, with an IC_{50} of 0.03664 mg/mL. However, the antioxidant activity of *G. ternifolia* stem and root extracts is not negligible compared with that of vitamin C. A comparison of IC50 values shows that IC_{50} (GTT Et) < IC_{50} (GTT H-Et) < IC_{50} (GTT Aq) and IC_{50} (GTR Et) < IC_{50} (GTR H-Et) < IC_{50} (GTR Aq). Thus, for both organs, ethanolic extracts show better antioxidant activity than hydro-ethanolics extracts, and even better than aqueous extracts. Moreover, stems are generally more active than roots.

Evaluation of antibacterial activity showed that only the aqueous and ethanolic extracts of *G. ternifolia* stems had inhibition diameters greater than 10 mm, and only on the three *Staphylococcus aureus* tested. Thus, according to Biyiti [27], these extracts are effective on all three *Staphylococcus aureus* bacteria. However, the observed inhibition diameters indicate that the ethanolic stem extract is more active than its aqueous extract.

As for the antibiotics tested, only erythromycin (E), ceftriaxone (CRO) and ciprofloxacin (CIP) were active on associated bacteria, with inhibition diameters ranging from 18.33±0.44 to 28.0±0.0mm. In contrast, imipenem (IPM), ticarcillin+clavulanic acid (TCC) and cefoxitin (FOX) had no activity on the bacteria tested. This shows that *G. ternifolia* stems have superior antibacterial activity to some of these reference antibiotics. This antibacterial activity observed in stems could be justified by the combined action of the active secondary metabolites contained in this organ. Indeed, the antibacterial activities of said secondary metabolites have also been published by Osho [35], Lunga *et al*., [36] and Ngbolua *et al*., [37].

Antibacterial activity in liquid medium was used to determine MICs and BMCs, the antibacterial parameters needed to interpret the bacteriological properties of the various extracts tested. The aqueous and ethanolic extracts of *G. ternifolia* stems were absolutely bactericidal against all *S. aureus* strains (*S.a* ATCC 29213, *S.a* 06 UB/22 and *S.a* 27 UB/22) tested. This suggests that *G. ternifolia* stems could be actively used against *S. aureus.*

5. Conclusion

The aim of this work was to confirm or invalidate the use of *G. ternifolia* stems and roots as a good antioxidant and/or antibiotic that would be capable of traditionally treating various pathologies.

The results of phytochemical screening of the various extracts, carried out by color reaction in tubes and by TLC, highlighted the presence of polyphenols, flavonoids, coumarins, tannins, saponins, sterols and terpenes in *G. ternifolia* stems and roots. Phenolic acids were found only in the stems. Alkaloids, on the other hand, were absent in both organs studied.

The antioxidant activity towards DPPH of the 02 organs was assessed using qualitative methods (tube and TLC) and a quantitative method (spectrophotometry). These organs were found to have a good antioxidant profile. Also, quantitative spectrophotometric tests of antioxidant capacity showed that vitamin C has a better antioxidant capacity (IC_{50} = 0.03664 mg/mL) than that of the plant's stems and roots. However, the antioxidant activity of stem and root bark is not negligible. Nevertheless, the antioxidant activity of stem bark is better than that of root bark.

Antibacterial activity was assessed against 05 bacterial strains using the solid diffusion and liquid dilution methods. The results showed that only the aqueous and ethanolic extracts of *G. ternifolia* stems were absolutely bactericidal, and only against the *Staphylococcus aureus* strains tested.

These antioxidant and antibacterial properties are due to the synergistic action of secondary metabolites present in these two organs. Thus, the stems could be used as an antioxidant and antibiotic against *Staphylococcus aureus* alone. As for the roots, they could only be used as an antioxidant. However, it is imperative to prove the innocuousness of these two organs in order to consider producing a phytomedicine for all social classes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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