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Antiradical and Antibacterial Activities of The Ethanolic Extract of *Terminalia Avicennioides* Guill. and Perr. (Combretaceae) from Chad

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Abstract

Oxidative stress associated with bacterial infections, are recognized as being a key factor in the appearance and complications of many chronic pathologies. To help overcome these difficulties, herbal remedies are one of the alternatives for the development of traditionally improved drugs. The objective of this work is to evaluate the antiradical and antibacterial activities of the ethanolic extract of the bark of the trunk of Terminalia avicennioides. Plant used in traditional medicine in Chad in the treatment of infectious diseases.

The antiradical activity was evaluated by the colorimetric method with DPPH and the antibacterial activity by the disk diffusion method. A phytochemical study was carried out to link the structure to the activity.

The ethanolic extract of the bark of the trunk of Terminalia avicennioides gave a yield of 16.39%. Phytochemical analysis revealed the presence of tannins, flavonoids, saponoside alkaloids, alkaloids, anthocyanins, saponosides and antraquinones; sterols, terpenoids, cardiotonic glycosides and free quinones were absent. The ethanolic extract of Terminalia avicennioides showed free radical scavenging activity by discoloring DPPH from initially purple to yellow. It inhibited the growth of Staphylococcus aureus and Salmonella typhi at the MIC of 0.30 mg/ml and that of Escherichia coli at 1.25 mg/ml with inhibition diameters of 8 mm and 9 mm respectively.

These results show that the ethanolic extract of the bark of the trunk of Terminalia avicennioides would be a potential source of secondary metabolites with antiradical and antibacterial activities allowing the development of new molecules to fight against oxidative stress, cardiovascular diseases and microbial infections.

Keywords: *Terminalia avicennioides;* antiradical; antibacterial; Chad

List of Abbreviations

MIC: Minimum Inhibitory Concentration

DPPH: 2, 2-diphenyl-1-picryl-hydrazyl

MCH: Mother and Child Hospital

WHO: World Health Organization

IRED: Livestock Research Institute for Development

TLC: Thin Layer Chromatographyc

MH: Muller-Hinton

DMSO: Dimethyl Sulfoxyde

E. coli: Escherichia coli

Introduction

Oxygen, a molecule essential to life, is likely to cause harmful effects in the body through the formation of free radicals [1]. In the normal physiological state, there is a balance between the production of free radicals and the antioxidant capacity of the body [2]. But bacterial infections lead to an overproduction of free radicals by the defense cells involved in their elimination, this overproduction in turn leads to the disruption of this balance referred to as oxidative stress [3, 4]. Oxidative stress can cause damage to different molecules such as lipids, DNA, carbohydrates and proteins, thus leading to the development of many pathologies such as inflammation, obesity, cancer, atherosclerosis, diabetes [5, 6].

Microbial infections are diseases due to the development, in humans, of bacteria or yeasts, some species of which are pathogenic [7]. These infectious diseases are the cause of 17 million deaths per year worldwide and represent the cause of 43% of deaths in developing countries [8].

The microorganisms targeted in this study are: *Escherichia coli*, *Staphylococcus aureus* and *Samonella typhi*. These bacteria are pathogenic and nowadays present a real public health problem. They cause recurrent pathologies, including: abscess, furuncle, folliculitis, impetigo, cellulitis, endocarditis, peritonitis, necrotizing pneumonia, bacteremia, meningitis, osteomyelitis, septic arthritis, bone infections, intestinal infections (bloody diarrhea), anemia hemolytic, renal insufficiency, urinary tract infection, pus appendicitis, peritonitis, cholecystitis, septicemia, neonatal, pulmonary, osteo-articular meningitis [9,10].

In Chad, a study conducted at MCH of N'Djamena on the prevalence of *E. coli* in the stools of children aged 0 to 5 years gave a prevalence of *E. coli* by 11.11% [11].

Thus, infectious diseases are a major public health concern. To fight against these infections, the scientific world has discovered many treatments [12]. But their misuse has led to the emergence of resistance [13]. The development of resistance to antibiotics has become over the last decade, at the international level, a major concern in terms of human and animal health [14]. The WHO estimates that more than 500,000 people worldwide are affected by the phenomenon of bacterial resistance [15]. Antibiotic-resistant bacteria killed 33,000 people in Europe in 2015 [16].

Given the development of resistance and the potential toxicity of synthetic antioxidants, herbal remedies are an alternative [17]. Around the world, interest in traditional medicine is constantly growing. In Africa, the practice of traditional medicine is a common reality, because more than 80% of the population has recourse to traditional medicine [18].

Indeed, *Terminalia avicennioides* Guill & Perr is indicated in the treatment of certain diseases such as gastrointestinal disorders, urinary tract infections, ulcers, amoebic dysentery, hemorrhoids, cavities and malaria [19].

Previous work carried out on the ethanolic, hydro-ethanolic and aqueous extracts of the leaves, barks, twigs, fruits, pericarp, pulp, seeds of *Terminalia catappa* and *Terminalia mantaly*, two species of the same genus and family (Combretaceae) as *Terminalia avicinoides* have revealed that these extracts inhibit the growth of isolates of *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Cryptococcus neoformans* and the reference strain *Candida albicans* NR-29450 [20]. Similarly, aqueous extracts from the leaves and stem barks of *Terminalia mantaly* and *Terminalia superba* are endowed with antiplasmodial activity [21].

Thus, with the aim of enhancing the medicinal plants of the Chadian pharmacopoeia, in particular *Terminalia avicennioides* Guill & Perr, traditionally used in the treatment of microbial diseases, this study was carried out to evaluate their antiradical and antibacterial activities with a view to formulating phytomedicines traditionally improved.

Materials And Methods

Plant material

The plant material used consisted of bark from the trunk of *Terminalia avicennioides* harvested in September at Mont Illi in the Mayo-Kébbi/Est region (Chad). The samples were identified in the IRED herbarium where the specimen was kept under the identification number (9918/HIRED/Tchad). The choice of the plant used was made on the basis of an ethnobotanical study, the barks of which are represented in figure 1.

Figure 1: Terminalia avicinoides trunk bark

Biological Material

The biological material consisted of *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* taken from patients hospitalized at the National Reference University Hospital Center of Chad.

Methods

Preparation of The Ethanolic Extract

Fifty grams (50g) of powder were macerated in 500 ml of 96 ethanol, under constant stirring for 24 hours and were filtered. The filtrate was dried using an oven at a temperature of 40°C until the dry residue (ethanolic extract) was obtained [22].

Extraction yield

The extraction yield was obtained by the following formula:

$$Yield(\%) = \frac{Mass\ of\ crude\ extract\ obtained(g)}{Mass\ of\ plant\ material\ sample(g)} \times 100$$

Phytochemical Screening

The analysis of the chemical composition in secondary metabolites of the ethanolic extract of the bark of the trunk of Terminalia avicennioides for the purposes of substantiating the different activities (anti-radical and antibacterial), was carried out according to the protocols described by Harbon [23]; Odebeye and Sofowara [24]; Trease and Evens [25] Sofowara [26]; Aromede et al., [27] at the Pharmacology and Toxicology Laboratory of the Faculty of Human Health Sciences of the University of N'Djamena (Chad).

Determination of Biological Activities

Determination of Antiradical Activity

The antiradical activity of the ethanolic extract of the bark of the trunk of *Terminalia avicennioides* was evaluated by the colorimetric method with DPPH.

Principle

This test is based on the scavenging of DPPH free radicals by an antioxidant through the transfer of a hydrogen atom. The reduction results in a drop in absorbance due to the change in color of the solution, which changes from purple to yellow [28].

Operating mode

In each well of the plate, we individually introduced 100 μl of the solutions of the ethanolic extract of the bark of the trunk of *Terminalia avicennioides* previously prepared at increasing concentrations (10mg / ml, 20 mg /ml and 40 mg / ml), then we added 1900 μl of DPPH solution (80 μM). The negative control was prepared under the same conditions and instead of the extract we introduced 100 μl of ethanol to each well. After ten minutes of incubation in the dark, the discoloration of the DPPH which changes from purple to yellow in the presence of the extract reflects its antiradical activity.

This method of revealing the antiradical activity was confirmed by the TLC method on the plate. It consists of: (i) depositing 5μl of the extract (1/10) on the plate; (ii) spray the plate with DPPH; (iii) observe the appearance of yellow spots after 3 minutes which characterize the antiradical activity of the extract [29].

Determination of Antibacterial Activity

Antibacterial activity was determined by the disc diffusion method using MH medium as described by Hayes and Markovic [30].

Preparation of The Culture Medium

The MH medium was prepared under sterile conditions under a laminar flow hood: (i) weigh 38 g of powder and dissolve in 1 liter of distilled water; (ii) homogenizing the solution; (iii) sterilize the homogenized solution in an autoclave at 121°C for 15 minutes.

Method of Distribution on Discs

This method makes it possible to evaluate the antibacterial activity of the ethanolic extract of the bark of the trunk of *Terminalia avicennioides* on *Escherichia coli* (gram- bacterium), *Salmonella typhi* (gram-bacterium) and *Staphylococcus aureus* (Gram+ bacterium). It consists of placing a sterile disc, soaked in the extract, on a bacterial mat at the very beginning of its growth and measuring the area where the bacteria have not been able to develop. The diameter (mm) of inhibition which reflects the antibacterial activity of the extracts is thus determined.

Operating Mode

20 mg of the ethanolic extract was dissolved in 1ml of DMSO. Dilution series were prepared. Indeed, each of the discs of sterile Wattman paper N ⊠ 3 and 6 mm in diameter was impregnated with 20 µl of the extract of Terminalia avicennioides at decreasing concentrations of C1=20mg/ml, C2=10mg/ml, C3= 5mg/ml, C4=2.5mg/ml, C5=1.25mg/ml, C6=0.6mg/ml. The inoculum used is 108 CFU/ml. This test was repeated 3 times.

Disks of Cefoxitin (FOX at 30 µg/disk) marketed, Amoxicillin (AMC 25 µg/disk), Imipenem (IPM 10 µg/disk), Chloramphenicol (CHL 30 µg/disk) were placed as positive controls. The plates were then incubated at 37°C for 24 hours for bacteria. The diameters (mm) of the zones of inhibition surrounding the discs containing the samples to be tested were measured.

Method For Determining Minimum Inhibitory Concentrations

The minimum inhibitory concentration is defined as the lowest concentration capable of inhibiting the growth of the bacteria tested [31].

The sensitivity of the bacterial strains was evaluated according to the inhibition diameters obtained according to the protocol recorded [31] in Table 1.

Inhibition diameters	Degree of germ sensitivity
Δ ⊠ 7 mm	Insensitive (Resistant)
7 mm ≤ Δ ⊠ 8 mm	Sensitive
8 mm ≤ Δ ⊠ 9 mm	Quite sensitive
Δ ≥ 9 mm	Very sensitive

Table 1: Standard used for reading the results of antibiogram tests on plant extracts.

Statistical Analysis

The bacteriological analysis results obtained were processed using Excel software for the comparison of means.

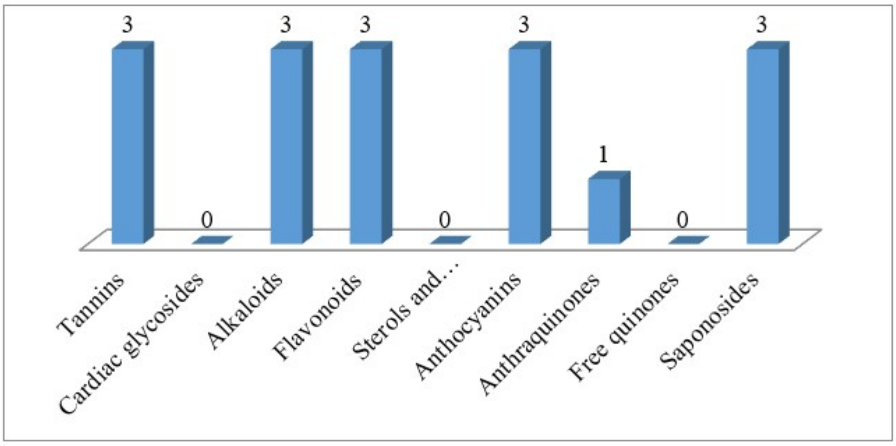
Results

Yield of Ethanol Extraction

The ethanolic extract of the bark of the trunk of *Terminalia avicennioides* gave a yield of 16.39%.

Phytochemical Screening

Phytochemical analysis of the ethanolic extract of *Terminalia avicennioides* trunk bark revealed the presence of the secondary metabolites listed in figure 2.



Legend: (0) Completely absent; (1) Weakly positive; (3) Frankly positive.

Figure 2: Phytochemical analysis of the ethanolic extract of *Terminalia avicennioides*

At the end of the phytochemical analysis, the ethanolic extract of the bark of the trunk of *Terminalia avicennioides* is rich in tannins, alkaloids, flavonoids, saponins, anthocyanins, anthraquinones and cardiotonic glycosides, free quinones and sterols are absent in this extract.

Scavenging Activity

The antiradical activity was carried out by the DPPH colorimetric method of the ethanolic extract of the bark of the trunk of Terminalia avicennioides at increasing concentrations and compared to the negative control after 10 minutes of incubation is represented by Figure 3.

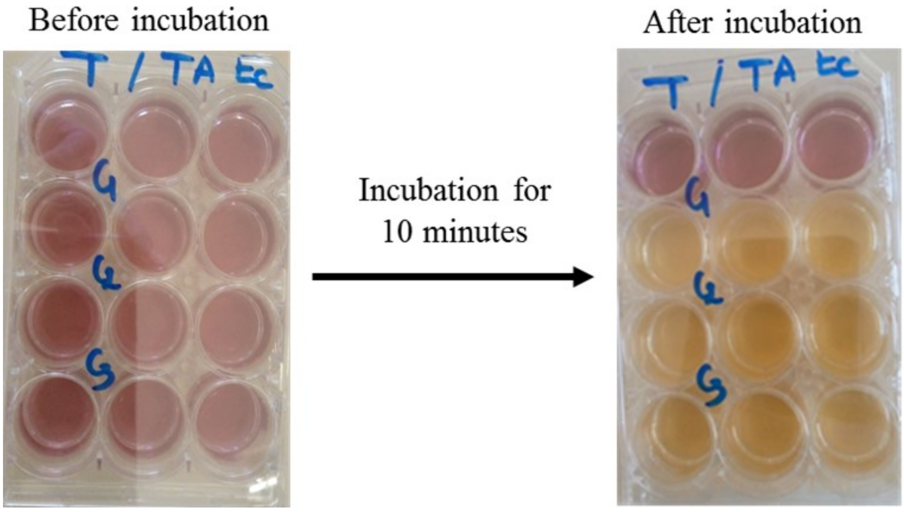


Figure 3: Revelation of the antiradical activity of the ethanolic extract of *Terminalia avicennioides*

This antiradical activity observed in Figure 3 was confirmed by the TLC method on the thin layer chromatography plate shown in Figure 4.

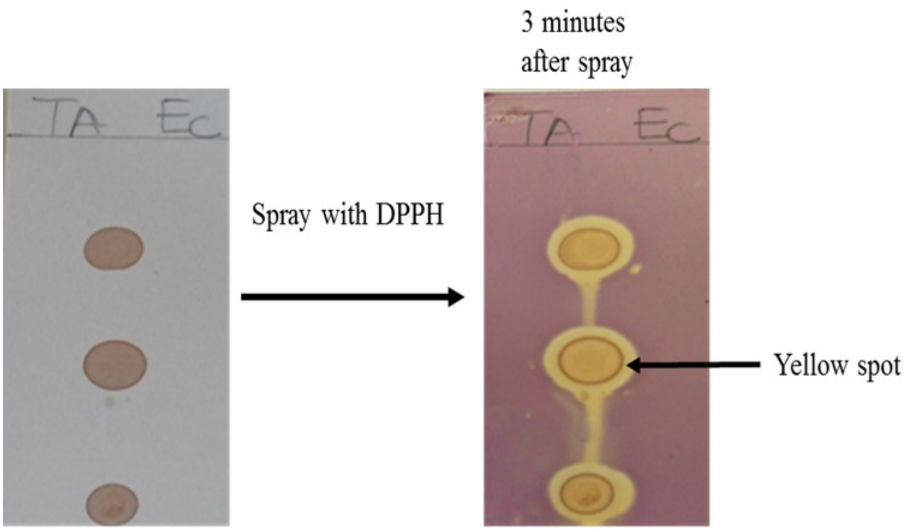


Figure 4: Thin layer chromatography plate (confirmation test by TLC method).

Figure 4 shows the appearance of yellow spots after three (3) minutes of DPPH spraying, characteristic of the antiradical activity of the ethanolic extract of *Terminalia avicennioides*.

Antibacterial Activities of The Ethanolic Extract of *Terminalia Avicinoides*

The inhibition of the growth of *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* induced by the ethanolic extract of the bark of the trunk of *Terminalia avicennioides* at different concentrations, the results of which are expressed in diameters of inhibition (mm), is illustrated in the Figure 2.

It follows from Figure 2 that the ethanolic extract of the bark of the trunk of *Terminalia avicennioides* inhibited the growth of *Staphylococcus aureus* and of *Salmonella typhi* at an MIC of 0.30 mg/ml and that of *Escherichia coli* at 1.25 mg/ml with inhibition diameters of 8 mm and 9 mm respectively.

Antibacterial Activity of Reference Molecules

The results of the antibiogram of these three bacteria using four synthetic antibiotics (Amoxicillin, Chloramphenicol, Imipenem and Cefoxitin) tested under the same conditions as the ethanolic extract of Terminalia avicennioides are shown in Table 2.

Inhibition diameters (mm) / Sensitivity			
Strength (mg)	***Staphylococcus aureus***	***Escherichia coli***	***Salmonella typhi***
20	22±0,00 (S)	19±0,5 (S)	17±1,00(S)
10	16±0,50 (S)	15±0,15 (S)	14±0,2(S)
5	14±1,00 (S)	13±1,67 (S)	12±1,5(S)
2.5	12±1,67(S)	10±0,00 (S)	11±0,00(S)
1,25	10±0,00 (S)	**9±1,00** (S)	10±1,67(S)
0,62	9±1,5 (S)	6±0,57 (R)	8±1,57(S)
0,31	**8±1,00** (S)	6±0,20 (R)	**8±0,00(S)**

(S): sensitive and (R): resistant

Table 2: Growth inhibition diameters of the strains by the ethanolic extract of Terminalia avicennioides bark.

Table 3 shows that *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* are sensitive to Ceftriaxone, Imipenem and Chloramphenicol. Imipenem is the most active antibiotic with a diameter of 40 mm on *Staphylococcus aureus*. The strains of *Escherichia coli* and *Salmonella typhi* were resistant to the antibiotic Amoxicillin, i.e. a diameter of 6 mm. Amoxicillin only acts on *Staphylococcus aureus*.

Inhibition diameters (mm)				
Antibiotic discs	**Disc load (µg)**	***S. aureus***	***E. coli***	***S. typhi.***
Ceftriaxone	30	17 (S)	24 (S)	24 (S)
Imipenem	10	40 (S)	30 (S)	26 (S)
Chloramphenicol	30	20 (S)	22 (S)	22 (S)
Amoxicillin	25	12 (S)	6 (R)	6 (R)

(S): sensitive; (R): resistant; S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; S. typhi : Salmonella typhi.

Table 3: Different diameters of reference molecules

Discussion

The ethanolic extract of the bark of the trunk of Terminalia avicennioides gave a yield of 16.39%. This result corroborates with the work of Musa et al in 2016 who obtained (18.8%) with the ethanolic extract of the bark of Terminalia avicennioides harvested in the village of Karaukaraw in Nigeria [32].

To link the structure to the activity, the phytochemical analysis of the ethanolic extract of the bark of the trunk of Terminalia avicennioides harvested in Chad revealed the presence of tannins, alkaloids, flavonoids, saponosides, anthocyanins, anthraquinones and the absence of glycosides. cardiotonics, free quinones and sterols, also reported by the work of Musa et al., in 2016 in Nigeria [32]. Similarly, studies conducted by Abdoulaye et al., in 2008 in Burkina Faso showed that the Combrétaceae family is rich in these secondary metabolites known to have antibacterial properties [33]. However, we noted the absence of cardiotonic glycosides, sterols and free quinones, results similar to the work of Hamidu et al in 2018 in Nigeria [34]. The richness of the ethanolic extract of the bark of the trunk of Terminalia avicennioides in these secondary metabolites constitutes a potential of biomolecules endowed with antiradical and antibacterial properties [35].

Regarding the antiradical activity, this study showed that the ethanolic extract of the bark of the trunk of Terminalia avicennioides has an antiradical activity by the colorimetric method with DPPH. This activity could be explained by the presence in the extract of secondary metabolites such as tannins, flavonoids, anthocyanins which are phenolic compounds with powerful antiradical properties as described in the literature by Ebrahimzadeh et al., 2010 [35, 36]. These results are in agreement with those obtained by Rebaya et al., in 2016 who reported a significant correlation between the polyphenol content and the antiradical effect of Cistus salvifolius extract [37].

This study conducted links the structure to the activity because the ethanolic extract of Terminalia avicennioides rich in phenolic compounds is an antiradical and antibacterial potential [38].

The ethanolic extract of the bark of the trunk of Terminalia avicennioides inhibited the growth of Staphylococcus aureus, Salmonella typhi and Escherichia coli whose MIC varies between 0.31 and 1.25 mg/ml. This antibacterial activity obtained is due to its richness in tannins, alkaloids, flavonoids, saponins, anthocyanins and anthraquinones as reported by the work of Yimta et al., in 2014 and Mahudro et al., in 2020 [35, 39]. Similarly, Famen et al., in 2020 found an MIC of between 0.06 and 0.512mg/ml but with the ethanolic extract of stem bark on Salmonella typhi [40]. Mann et al., in 2014 who found an MIC of between 0.1 and 10μg/ml of methanolic extracts of leaves, stems and roots on the strains Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis, Klebsiella preumonea and Escherichia coli [41]. These differences could be explained by the parts used, the strains of bacteria tested and the solvents used for the extraction.

As far as reference molecules are concerned, the most active antibiotic is imipenem with 40 mm on Staphylococcus aureus and the least active is amoxicillin with an inhibition diameter of 6 mm on strains of Escherichia coli and Salmonella typhi. Despite the great resistance of these strains to amoxicillin, they were sensitive to the ethanolic extract with inhibition diameters of 8 mm on Salmonella typhi and 9 mm on Escherichia coli corresponding to minimum inhibitory concentrations of 0.31 mg/ ml and 1.25 mg/ml respectively. The ethanolic extract of the bark of the trunk of Terminalia avicennioides could be exploited for the research of antimicrobial biomolecules.

Flavonoids are good inhibitors of sortases (enzymes found in the cytoplasmic membrane of Gram-positive bacteria which catalyze all surface proteins, eg adhesins and internalins) and only trace amounts of Rutin inhibit sortases A and B. Indeed, all S. aureus bacteria treated with Rutin showed decreased binding to fibrinogen, one of the host ligands to which bacteria attach upon infection [42]. Flavonoids inhibit the release of virulence factors from this bacterium. Epigallocatechin inhibits the secretion of coagulase and α-toxin [42].

Steroids are said to have antibacterial and antifungal properties. The correlation between membrane lipids and sensitivity to steroid compounds indicates the mechanism by which steroids specifically associate with membrane lipids and exert their action by causing leakage from liposomes [43].

Conclusion

This study allowed us to highlight the richness in secondary metabolites of the ethanolic extract of *Terminalia avicennioides* trunk bark in phenolic compounds such as flavonoids, tannins and anthocyanins which are probably responsible for the antiradical and antibacterial activities observed on strains of *Staphylococcus aureus*; *Salmonella typhi* and *Escherichia coli*. The ethanolic extract of the trunk bark of *Terminalia avicennioides* could be considered as sources of biomolecules fighting against oxidative stress and bacterial infections.

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