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Anti radical and antibacterial activities of the ethanolic extract bark of *Anogeissus leiocarpus* (Guill. Et Perr) from Chad

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Abstract
Introduction: Oxidative stress and bacterial infections are a public health problem in Chad. To reduce these problems, plant-based medicines were used to improve the development of traditionally drugs for infection treatments. The objective of this study was to evaluate the antiradical and antibacterial activities from ethanolic extract of *Anogeissus leiocarpus*.
Material and Methods: In this study we used a stem bark of *Anogeissus leiocarpus* who used by different traditional practitioners in Chad for urinary infections treatment and diarrheal diseases caused by different types of bacteria (*S. aureus*, *E. coli*, *S. typhi*). Also the antiradical activity was evaluated by the method of DPPH colorimetric and the antibacterial activity by the method of disk diffusion. A phytochemical study was carried out to link the structure to the activity.
Results: Our result showed a rate of 13.2% of ethanolic extract. The phytochemical analysis revealed the presence of flavonoids, tannins, saponosides, alkaloids, sterols, terpenoids and anthocyanins. Also the ethanolic extract of *Anogeissus leiocarpus* revealed an antiradical activity by the decolorization of DPPH from an initial purple color to yellow. This extracts inhibited the growth of *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* at the minimum inhibitory concentrations (MIC) of 1.25 mg/ml, 2.5 mg/ml and 5 mg/ml respectively.
Conclusion: Our result showed that the ethanolic extract bark of *Anogeissus leiocarpus* could be a potential provider of secondary metabolites with antiradical and antibacterial activities, allowing the development of a new molecules to fight against oxidative stress, cardiovascular diseases, and microbial infections.

Keywords: *Anogeissus leiocarpus*, antiradical, antibacterial and Chad

Introduction
Many infection diseases contracted by humans are related to bacterial infections [1]. Among this number, gastroenteritis are among the most serious infectious diseases in humans [2]. It is estimated that between 11 and 21 million people infected by Typhoid Fever each year and between 128,000 and 161,000 die from it [3]. Pathologies that vary greatly in location and severity are caused by *Staphylococcus aureus*, one of the most common microorganisms responsible for nosocomial and community infections [4]. In Chad, the study conducted on the biochemical profile and phenotype resistant of bacteria responsible for nosocomial infections in the Urology and Traumatology departments of the National Reference University Teaching Hospital (NRUTH) of N'Djamena, revealed that *Staphylococcus aureus* and *E. coli*. *Staphylococcus aureus* are responsible for 18% and 19% of patients infections respectively in the Trauma and Urology departments of the same hospital, and the *E. coli* species was responsible for 60% of nosocomial infections in Urology and 7% in Traumatology [5].
During bacterial infections, the immune system was activated to protect the organism using certain phagocytic cells (macrophages, neutrophils) which produce free radicals by a variety of physiological mechanisms to destroy the bacteria [6]. Free radicals can interact with lipids, carbohydrates, DNA and proteins and their excessive production leads to oxidative stress, which is potentially involved in the development of numerous pathologies such as cancer, inflammation, diabetes, cardiovascular diseases, atherosclerosis, and ageing [7].

Thus, the discovery of antibiotics has been a real relief for mankind but their overuse and inappropriate use in human and animal health for the treatment or prevention of bacterial diseases has created a selection pressure on bacterial populations favoring the emergence of resistant strains towards different families of antibiotics [8].However, there is a concern about the adverse effects of synthetic molecules and the high costs of modern drugs, intended to fight oxidative stress and bacterial infections.

To address this situation, much hope remains in the secrets of medicinal plants, and the emergence of an alternative medicine based on these plants is more than ever on the agenda. Thus, the scientific community has turned to natural substances, particularly medicinal plants, to find new molecules that will not only contribute to the efficient fight against microbial diseases but also to the development of traditional medicine. According to the World Health Organisation (WHO), 80% of the African population uses traditional medicine to meet their health care needs [9, 10].

Indeed, *Anogeissus leiocarpus* was indicated in the treatment of certain diseases such as urinary bilharzia, amoebic dysentery, malaria, burns, pain, trypanosomiasis, helminthiasis, anorexia, constipation, fatigue, eczema, psoriasis, and diarrhea [11, 12, 13]. Thus, to valorize the medicinal plants of the Chadian pharmacopoeia, particularly *Anogeissus leiocarpus* (DC) Guill and Perr traditionally used in the treatment of microbial affections, this study was carried out to evaluate their antiradical and antibacterial activities with a view to formulating traditionally improved phyto medicines.

Materials and Methods

Material

Plant material

The plant material consisted of stem barks of *Anogeissus leiocarpus* who collected in June 2021 in the Province of Mayo Kebbi East of Chad. The samples were identified at the herbarium of the Livestock Research Institute for Development (HLRID) where the samples were conserved under the identification number of 8907/HIRED/T chad). The choice of this plant was made on the base of an ethno botanical study.

Biological material

The biological material consisted of *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* collected from hospitalized patients at the National Reference University teaching Hospital (NRUTH) of N'Djamena.

Preparation of the ethanolic extract

For the ethanolic extract preparation; Fifty grams (50 g) of powder was macerated in 500 ml of 96° ethanol, under constant agitation for 24 h and filtered. The filtrate was dried in an oven at 40 °C until a dry residue or ethanolic extract was obtained [14].

Extraction rate

The extraction rate was obtained by the following formula:

$$\text{Rate (\%)} = \frac{\text{Mass of the crude extract obtained (g)}}{\text{Mass of the plant material sample (g)}} \text{x } 100$$

Phytochemical screening

The analysis of the chemical composition in secondary metabolites of the different extracts was used to justify the different activities (antiradical and antibacterial), according to the protocols described by Harbon [15]; Odebeye and Sofowara [16]; Trease and Evens [17]; Sofowara [18]; Aromede *et al*. [19] at the Laboratory of Pharmacology and Toxicology of the Faculty of Health and Human Sciences of the University of N'Djamena (Chad).

Biological activity determination

Antiradical activity determination

The antiradical activity of the extract was evaluated by the colorimetric method with DPPH (2, 2- diphenyl-1-picrylhydrazyl).

Principle

This test was based on the scavenging of DPPH free radicals by an antioxidant (AH) through the transfer of a hydrogen atom. The reduction results in a decrease in absorbance due to the change in color of the solution from purple to yellow [20].

Procedure

In each plate well, we introduced individually 100 µL of the ethanolic extract solutions of *Anogeissus leiocarpus* barks previously prepared at increasing concentrations (10mg /ml, 20 mg /ml and 40 mg/ml), then we added 1900µL 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 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 (Thin Layer Chromatography) method on the Thin Layer Chromatography (TLC) plate. It consists in:

- Place 5µl of the extract (1/10) on the plate.
- Spray the plate with DPPH;
- Observe the appearance of yellow spots after 3 minutes which characterize the antiradical activity of the extract [12].

Antibacterial activity determination

Antibacterial activity was determined by the using a disc diffusion method and Muller Hinton (MH) medium as described by Hayes and Markovic [21].

Culture medium preparation

The culture medium preparation of Muller Hinton (MH) medium was done under sterile conditions in a laminar flow hood:

- Weigh 38 g of powder and dissolve in 1 liter of distilled water.
- Homogenize the solution.
- Sterilize the homogenized solution by autoclaving at 121 °C for 15 minutes.

Broadcasting method on discs

This method makes it possible to evaluate the antibacterial activity of the ethanolic extract of *Anogeissus leiocarpus* bark on *Escherichia coli* (Gram- bacteria), *Salmonella typhi* (Gram- bacteria) and *Staphylococcus aureus* (Gram+ bacteria). This consists of placing a sterile disc, soaked with the extracts, on a bacterial mat at the very beginning of its growth and measuring the zone where the bacteria have not been able to grow. The diameter (mm) of inhibition, which reflects the antibacterial activity of the extracts, is thus

determined.

Procedure

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

Commercialized discs of Cefoxitin (FOX 30µg/disc), Amoxicillin (AMC 25µg/disc), Imipenem (IPM 10µg/disc), Chloramphenicol (CHL 30µg/disc) were placed as positive controls. The plates were then incubated at 37 °C for 24 h for the bacteria. Measurement of the diameters (mm) of the inhibition zones surrounding the discs containing the test samples was carried out.

Minimum inhibitory concentrations method determination (MIC)

The minimum inhibitory concentration method (MIC) is defined as the lowest concentration capable of inhibiting growth of the bacteria tested [22].

The sensitivity of the bacterial strains was evaluated according to the inhibition diameters obtained according to the protocol of [22] recorded in Table I.

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

Diameter of inhibition	Degree of sensitivity of the germ
$\Delta < 7$ mm	In sensitive
7 mm $\leq \Delta < 8$ mm	Sensitive
8 mm $\leq \Delta < 9$ mm	Some what sensitive
$\Delta \geq 9$ mm	Very sensitive

Statistical analysis

The results of the bacteriological analyses obtained were processed using Excel software for the comparison of averages.

Results

Extraction rate

According to the ethanolic extract stem bark of *Anogeissus leiocarpus* a rate of 8.9% was found in this study.

Phytochemical screening

The phytochemical analysis of the ethanolic extract of *Anogeissus leiocarpus* bark revealed the presence of the secondary metabolites listed in Table II.

Table 2: Phytochemical analysis of the ethanolic extract of *Anogeissus leiocarpus*

Secondary metabolites	Ethanolic extract of *Anogeissus leiocarpus*
	Results
Tannins	+++
Cardiotonic heterosides	-
Alkaloids	+++
Flavonoids	+++
Stérols and terpenoids	+++
Anthocyanins	++
Anthraquinones	-
Free quinones	-
Saponosides	+++

+++: Abundant;++: Less abundant, +: Low; -: Absent

After a photochemical analysis, the ethanolic extract of *Anogeissus leiocarpus* was rich in tannins, alkaloids, flavonoids, saponosides, anthocyanins, sterols and steroids; cardiotonic heterosides, anthraquinones and free quinones are absent in this extract.

Antiradical activity

The antiradical activity revealed by the DPPH colorimetric method of the ethanolic extract of *Anogeissus leiocarpus* at increasing concentrations (C1 = 5 mg/ml, C2 = 10 mg/ml and C3 = 20 mg/ml) compared to the negative control after 10 minutes of incubation in the dark, is presented in figure 1.

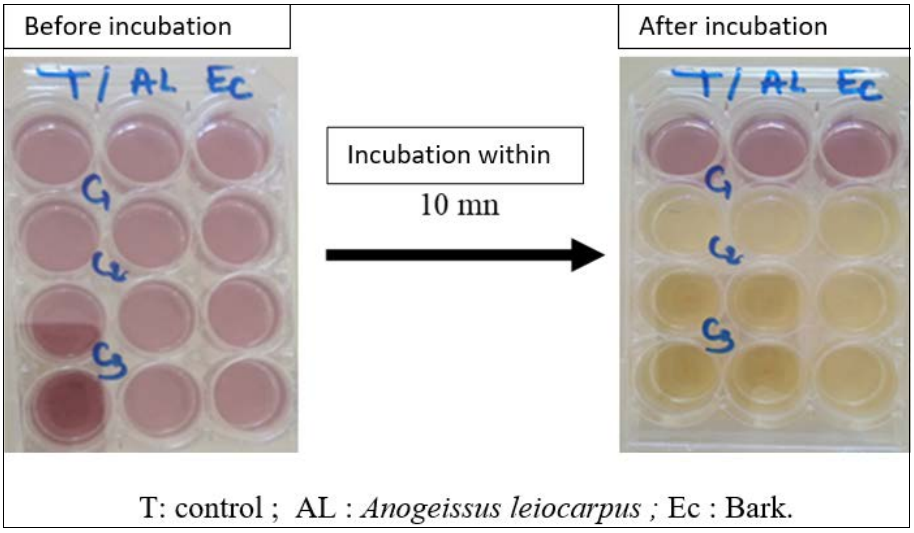

Fig 1: Revealing the anti-free radical activity of *Anogeissus leiocarpus*

From figure 1, it can be seen that after ten minutes of incubation, the DPPH solution, initially purple, turns yellow in the presence of the ethanolic extract of *Anogeissus leiocarpus* at concentrations C1 = 5 mg/ml, C2 = 10 mg/ml) and C3 = 20 mg/ml; characteristic of the antiradical activity of this extract, which was able to trap the free radicals of DPPH. This observed antiradical activity was confirmed by the TLC (Thin Layer Chromatography) method on the Thin Layer Chromatography (TLC) plate shown in figure 2

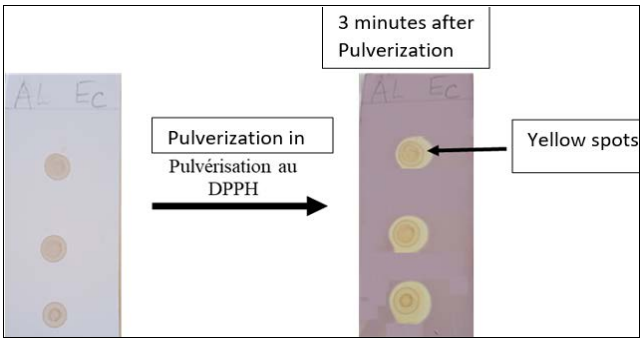

Fig 2: Thin layer chromatography plate (TLC confirmation test)

From figure 2, we can see the apparition of yellow spots after three minutes of DPPH spraying, characteristic of the antiradical activity of the ethanolic extract of *Anogeissus leiocarpus* stems.

Antibacterial activity of ethanolic extract of *Anogeissus leiocarpus*

The growth inhibition of *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* induced by the ethanolic extract of *Anogeissus leiocarpus* at different concentrations was presented in figure 3.

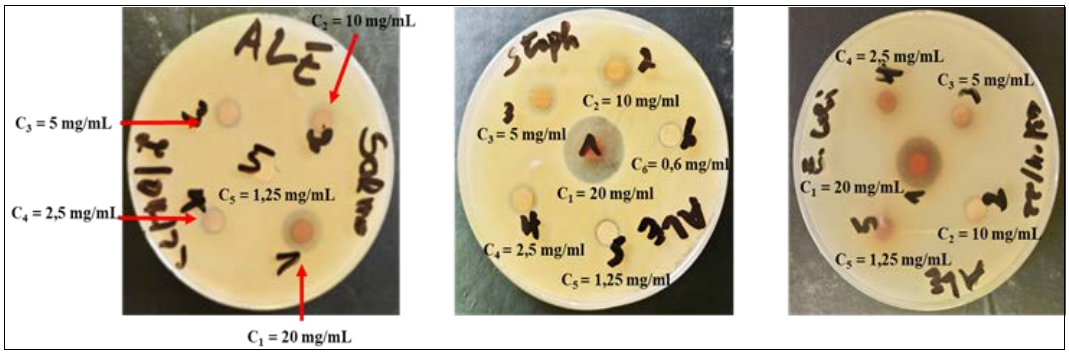

Fig 3: Inhibition areas of the ethanolic extract against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*

The inhibition areas (diameters) of the growth of these bacteria, expressed in millimetres (mm) as a function of the concentration of ethanolic extract of *Anogeissus leiocarpus* stem bark are given in Table III.

Table 3: Growth inhibition diameters of bacteria and degree of sensitivity

Concentrations (mg/ml)	Inhibition diamètres (mm)			Degree of sensitivity of the germ [22]	
	S. aureus	*E. coli*	*S. typhi*		
20	18±0,1	14±0,1	12±00	S	
10	11±00	8±00	9±00	S	
5	9±0,1	8±00	8± 00	S	
2,5	8±00	6±00	8±00	I (*E. coli*)	S (*S. typhi*)
1,25	8 ± 0,2	6±00	6±0,1	S (*S. aureus*)	I (*E. coli* et *S. typhi*)

S: Sensitive and I: Un-sensitive

Based on the diameters obtained, the ethanolic extract of *Anogeissus leiocarpus* stem bark inhibits the growth of *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* at minimum inhibitory concentrations (MICs) of 1.25 mg/ml, 2.5 mg/ml and 5mg/ml respectively [22].

Reference molecule activity

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 *Anogeissus leiocarpus* are shown in figure 4.

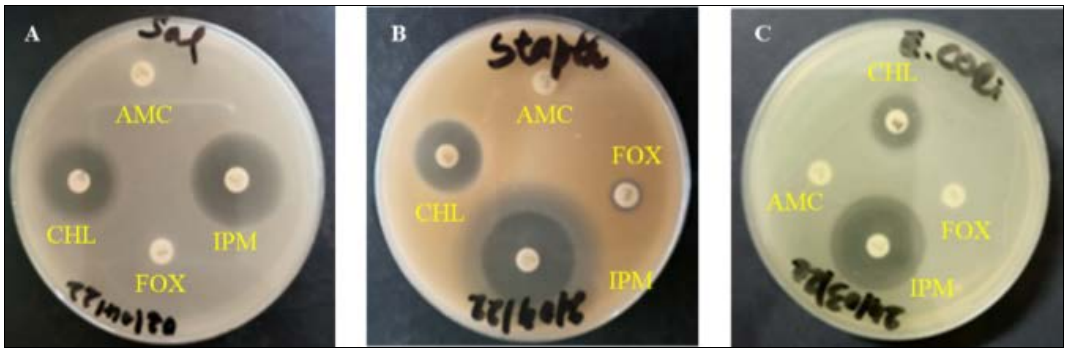

Fig 4: antibiogram of the three strains used

The zones (diameters) of growth inhibition of *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* expressed in millimetres (mm) are given in Table IV.

Table 4: Results of the antibiogram

Antibiotics (µg)	Diamètres d'inhibition (mm)		
	S. aureus	***E. coli***	***S. typhi***
Amoxicillin (25 µg/disc)	00±00	00±00	00±00
Chloramphenicol (30 µg/disc)	22±0,1	20±0,3	22±0,1
Imipenem (10 µg/disc)	40±0,1	30±0,1	26±0,1
Cefoxitin (30 µg/disc)	00±00	00±00	00±00

The study shows that bacterial strains (*Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*) were susceptible to the effects of (Imipenem and Chloramphenicol) and resistant to Amoxicillin and Cefoxitin.

Discussion

The result of ethanolic extract stem bark of *Anogeissus leiocarpus* gave a rate of 8.9%. This result was slightly higher from that obtained by Sale *et al.* in 2020 in Nigeria who worked on ethanolic extract of root bark of the same species and found a rate of (5%) [23], this differences could be explained by the fact that the concentration of secondary metabolites is variable depending on the organs of the plant according to Bruneton in 1999 [24].

The phytochemical study of the ethanolic extract of *Anogeissus leiocarpus* stem bark revealed the presence of alkaloids, tannins, flavonoids, anthocyanins, sterols and terpenoids, and saponosides. These results corroborate with those of Sanogo *et al.* in 2016 [11] in Côte d'Ivoire who obtained alkaloids, tannins, flavonoids, anthocyanins, sterols and terpenoids, saponosides, but our results was differ from those obtained by Mann *et al* in 2008 [12] in Nigeria who noted the absence of tannins in the stem bark of *Anogeissus leiocarpus*; and that could be explain by the difference in the harvesting period and edaphic factors [24].

The free radical scavenging activity of the ethanolic extract of *Anogeissus leiocarpus*, revealed by the DPPH colorimetric method, is due to the presence of flavonoids in *Anogeissus leiocarpus* extracts. These results corroborate with the study of Le *et al.* in 2007 [25] who showed that flavonoid-rich extracts have antiradical properties; and similarly to the result of Ebrahimzadeh *et al.* in 2010 [26] who revealed that tannins, flavonoids, anthocyanins, leuco-anthocyanins and quinones are phenolic compounds with potent anti-free radical properties that act by scavenging the radicals produced during cellular metabolism. These free radical scavengers may activate the immune system and reduce the risk of cancer and degenerative diseases [27].

The antibacterial activity of ethanolic extract stem barks of *Anogeissus leiocarpus* revealed that this extract inhibited the growth of *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* at minimum inhibitory concentrations (MICs) of 1.25 mg/ml, 2.5 mg/ml and 5mg/ml respectively. The inhibition of bacterial growth was reported to be due to a synergistic effect between the different phytochemical groups present in the ethanolic extract of *Anogeissus leiocarpus* stem bark, consisting of alkaloids, tannins, flavonoids, saponosides, sterols and steroids. These results corroborate with those of Koudoro *et al.* in 2015 [28] Sanogo *et al.* in 2016 [11] who revealed that extracts rich in alkaloids, tannins, flavonoids and saponosides possess antibacterial activity. In fact Sanogo *et al.* in 2016 [11] showed that *Escherichia coli* and *Salmonella typhi* are resistant to *Anogeissus leiocarpus* extract with inhibition diameters of 6 mm [22]. These results are in concordance with several study on medicinal plant extracts where some Gram-negative bacteria shows a higher resistance to plant extracts than Gram-positive bacteria [29-31]. This was explained by the presence of an envelope that includes a lipopolysaccharide-rich cell membrane and a cell wall, which limits the access of antimicrobial agents to their target in bacterial cells, as the antimicrobial agents are in contact with the cell envelope unlike Gram-positive bacteria that are unprotected against external agents [5, 8].

Furthermore, some antibiotics (Imipenem and Chloramphenicol) showed higher antibacterial activities than the tested plant substances, with higher inhibition diameters. This could be explained by the fact that these antibiotics were isolated, pure molecules of known concentrations, whereas the ethanolic extract stem bark of *Anogeissus leiocarpus* is an unpurified mixture of active substances from secondary metabolism [31]. Bacterial strains (*Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*) were found to be resistant to Amoxicillin and Cefoxitin although they are reference molecules compared to the ethanolic extract stem bark of *Anogeissus leiocarpus* which inhibited the growth of these strains at certain minimum concentrations, suggesting its potential for secondary metabolites that may exhibit antimicrobial properties.

Conclusion

The present study allowed us to highlight the richness of ethanolic extracts stem bark of *Anogeissus leiocarpus* in compounds such as flavonoids, tannins, anthocyanins would be responsible for the antiradical and antibacterial activities observed on the different strains studied. Ethanolic extracts of the stem bark of *Anogeissus leiocarpus* could be a potential source of natural biomolecules to fight against oxidative stress and bacterial infections.

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